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SOLUTION AND SOLID-PHASE FORMATION OF GLYCOSIDIC LINKAGES

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(57) Claim

1. A method of forming multiple regioselective glycosidic linkages in a single step comprising:

(a) treating a bifunctional first glycoside (FG) in an organic solvent with an effective amount of an activating agent (AG), said FG having (i) an anomeric sulfoxide substituent, and (ii) glycosyl accepting and glycosyl donating characteristics that are made manifest in the presence of the AG; and

(b) allowing multiple glycosylation reactions to proceed such that two or more glycosidic bonds linking three or more glycosides are formed regioselectively in a single step.

27. A method of forming a glycosidic linkage on a solid phase comprising:

(a) exposing, in an organic solvent, a glycosyl acceptor (GA) bound to a solid support to a glycoside (G) having an activated anomeric sulfoxide group, said GA having glycosyl accepting characteristics

.../2

and said G having glycosyl donating characteristics; and

(b) allowing a glycosylation reaction to proceed such that a glycosidic bond is formed which links said GA to the anomeric carbon of said G.

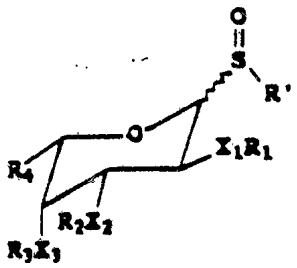
28. A method of forming a 1,2-*trans* glycosidic linkage on a solid phase comprising:

(a) exposing, in an organic solvent, a glycosyl acceptor (GA) bound to a solid support to a glycoside (G) having an activated anomeric sulfoxide group and a neighboring participating group (NPG) at C-2, said GA having glycosyl accepting characteristics and said G having glycosyl donating characteristics; and

(b) allowing a glycosylation reaction to proceed such that a glycosidic bond is formed which links said GA to the anomeric carbon of said G and which bond is *trans* to said NPG.

38. A chemical composition comprising:

(i) a glycoside of the formula (I), having potential glycosyl accepting and glycosyl donating characteristics,



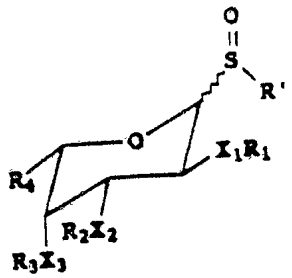
(I)

in which X_1, X_2, or X_3 independently can be a hydrogen, nitrogen, oxygen, or sulfur atom but not all hydrogen; R' and R_4 can be an aromatic or aliphatic group comprising 1-25 carbon atoms; R_1, R_2, or R_3 independently can each be an H, aromatic or aliphatic group comprising 1-25 carbon atoms, except that when X_1, X_2, or X_3 is H, then the

corresponding R_1, R_2, or R_3 is not present; said aromatic or aliphatic groups of R', R_1, R_2, R_3, or R_4, optionally, further comprising nitrogen, oxygen, phosphorus, silicon, or sulfur atoms; and

(ii) an activating agent capable of eliciting the manifestation of both the glycosyl accepting and glycosyl donating characteristics of said glycoside.

39. A glycoside of the formula (II), having potential glycosyl accepting and glycosyl donating characteristics,



(II)

in which X_1, X_2, or X_3 independently can be a hydrogen, nitrogen, oxygen, or sulfur atom but not all hydrogen; R' and R_4 can be an aromatic or aliphatic group comprising 1-25 carbon atoms; R_1, R_2, or R_3 independently can each be an H, aromatic or aliphatic group comprising 1-25 carbon atoms, except that when X_1, X_2, or X_3 is H, then the corresponding R_1, R_2, or R_3 is not present; said aromatic or aliphatic groups of R', R_1, R_2, R_3, or R_4, optionally, further comprising nitrogen, oxygen, phosphorus, silicon, or sulfur atoms; and provided that when R_3 is not H, the R_3-X_3 bond is susceptible to bond scission under the same conditions used to activate the anomeric sulfoxide group, thus allowing for the manifestation of both the glycosyl accepting and glycosyl donating characteristics of said glycoside.

41. A glycosidic library comprising a mixture of oligosaccharides formed by the method of claim 1, 2, 5, 6, 13, 14, 26, or 35.

47. A catalytic method of forming a glycosidic linkage comprising:

(a) providing a mixture comprising a glycosyl donor (GD), bearing an anomeric sulfoxide group, and a glycosyl acceptor (GA), having glycosyl accepting characteristics, in an organic solvent under anhydrous conditions;

(b) cooling said mixture below ambient temperature;

(c) adding to said mixture a catalytic amount of an activating agent (AG) effective to activate said anomeric sulfoxide group of said GD;

(d) allowing a catalytic reaction to proceed under conditions effective to provide the formation of a glycosidic linkage between said GD and said GA.

73. A library of glycosidic compounds comprising a mixture of oligosaccharides of varying sequence or length, each oligosaccharide of said mixture having at least one glycosidic linkage that is obtained through the activation and condensation of a glycosyl donor bearing an anomeric sulfoxide group on a solid phase.

78. An oligosaccharide comprising three or more glycosidic residues prepared by the method of claim 1, 27, 28 or 30.

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(54) Title: SOLUTION AND SOLID-PHASE FORMATION OF GLYCOSIDIC LINKAGES

(57) Abstract

The invention relates to methods that permit the rapid construction of oligosaccharides and other glycoconjugates. Methods for forming multiple glycosidic linkages in solution in a single step are disclosed. The invention takes advantage of the discovery that the relative reactivity of glycoside residues containing anomeric sulfoxides and nucleophilic functional groups can be controlled. In another aspect of the invention, the reactivity of activated anomeric sugar sulfoxides is utilized in a solid phase method for the formation of glycosidic linkages. The methods disclosed may be applied to the preparation of specific oligosaccharides and other glycoconjugates, as well as to the preparation of glycosidic libraries comprising mixtures of various oligosaccharides, including glycoconjugates, which can be screened for biological activity.

SOLUTION AND SOLID-PHASE FORMATION OF GLYCOSIDIC LINKAGES

1. Field Of The Invention

The present invention relates generally to methods that permit the rapid construction of oligosaccharides and other glycoconjugates. More particularly, the present invention relates to methods for forming multiple glycosidic linkages in solution in a single step. The present invention takes advantage of the discovery that the relative reactivity of glycoside residues containing anomeric sulfoxides and nucleophilic functional groups can be controlled. In another aspect of the present invention, the reactivity of activated anomeric sugar sulfoxides is utilized in a solid phase method for the formation of glycosidic linkages. The methods disclosed may be applied to the preparation of specific oligosaccharides and other glycoconjugates, as well as to the preparation of glycosidic libraries comprising mixtures of various oligosaccharides, including glycoconjugates, which can be screened for biological activity.

2. Background of the Invention

2.1. General Background

The oligosaccharide chains of glycoproteins and glycolipids play important roles in a wide variety of biochemical processes. Found both at cell surfaces and circulating in biological fluids, these glycosidic residues act as recognition signals that mediate key events in normal cellular development and function. They are involved in fertilization, embryogenesis, neuronal development, hormonal activities, inflammation, cellular proliferation, and the organization of different cell types into specific tissues. They are also involved in intracellular sorting and secretion of glycoproteins as well as in the clearance of plasma glycoproteins from circulation.

In addition to their positive role in the maintenance of health, oligosaccharides are also involved in the onset of disease. For instance, oligosaccharides on cell surfaces function as receptors for viruses and toxins, as well as more benign ligands. Modified cell surface carbohydrates have been implicated in tumorigenesis and metastasis. The oligosaccharide structures that mediate inflammation and help prevent infection can, when produced at excessive levels, stimulate the development of chronic inflammatory disease. (Some references on the roles of oligosaccharides produced by eukaryotes in health and disease include: Hakomori *TIBS*, **1984**, 45; Feizi et al. *TIBS*, **1985**, 24; Rademacher et al. *Annu. Rev. Biochem.* **1988**, 57, 785; Feizi *TIBS*, **1991**, 84; Dennis and Laferte *Cancer Res.* **1985**, 45, 6034; Fishman *J. Membr. Biol.* **1982**, 69, 85; Markwell et al. *PNAS USA*, **1981**, 78, 5406; Wiley and Skehel *J. Annu. Rev. Biochem.* **1987**, 56, 365; Kleinman et al. *PNAS USA*, **1979**, 76, 3367; Walz et al. *Science* **1990**, 250.)

Although bacteria do not produce the same types of oligosaccharides or other glycoconjugates as eukaryotes, procaryotes nevertheless produce a wide variety of glycosylated molecules. Many such molecules have been isolated and found to have antitumor or antibiotic activity. Bacterially produced glycosylated molecules having potential therapeutic utility include chromomycin, calicheamicin, esperamicin, and the ciclamycins. In all these cases, the carbohydrates residues have been shown to be important to biological activity. However, the precise functions of the carbohydrate residues are not well understood and there is no understanding of structure-activity relationships.

Because of their diverse roles in health and disease, oligosaccharides have become a major focus of research. It is widely accepted that the development of technology to 1) detect and 2) block or otherwise regulate some of the abnormal functions of

oligosaccharides would lead to significant improvements in health and well-being. Moreover, it should be possible to exploit some of the normal functions of oligosaccharides (e.g., various recognition processes) for other purposes, including drug delivery to specific cell types. In addition, it may be possible to develop new antitumor agents from synthetic glycosylated molecules reminiscent of glycosylated bacterial antitumor agents.

There are ongoing efforts to develop products related to oligosaccharides, including diagnostic kits for detecting carbohydrates associated with various diseases, vaccines to block infection by viruses that recognize cell surface carbohydrates, drug delivery vehicles that recognize carbohydrate receptors, and monoclonal antibodies, which recognize abnormal carbohydrates, for use as drugs. The timely development of these and other carbohydrate-based biomedical products depends in turn on the availability of technology to produce oligosaccharides and other glycoconjugates rapidly, efficiently, and in practical quantities for basic and developmental research.

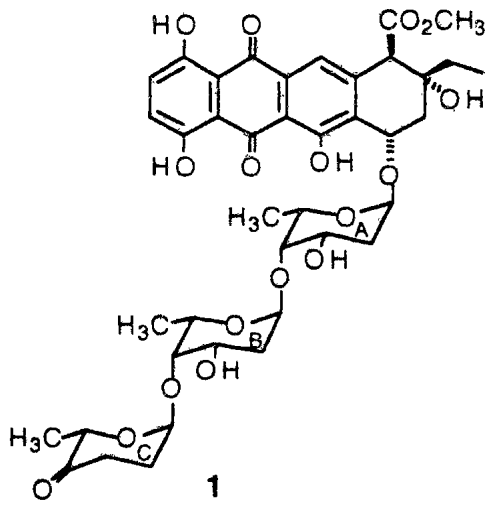
In particular, there is a need for methods that permit the rapid preparation of glycosidic libraries comprising mixtures of various oligosaccharides or other glycoconjugates which could then be screened for a particular biological activity. It has been shown, for example, that screening of mixtures of peptides is an efficient way of identifying active compounds and elucidating structure-activity relationships. There are numerous ways to generate chemically diverse mixtures of peptides and determine active compounds. See, for example, Furka et al. *Int. J. Peptide Protein Res.* **1992**, 37, 487; Lam et al. *Nature* **1991**, 354, 82; Houghten *Nature* **1991**, 354, 84; Zuckermann et al. *Proc. Natl. Acad. Sci. USA* **1992**, 89, 4505; Petithory *Proc. Natl. Acad. Sci. USA*, **1991**, 88, 11510; Geyse *Proc. Natl. Acad. Sci. USA*, **1984**, 81,

3998; Houghten *Proc. Natl. Acad. Sci. USA*, **1985**, 82, 5131; Fodor *Science* **1991**, 251, 767. We are not aware of effective methods to generate diverse mixtures of oligosaccharides and other glycoconjugates for screening purposes.

2.2. Anthracyclines

Ciclamycin 0 (**1**, below), an anthracycline antibiotic isolated from *Streptomyces capoamus*, possesses high inhibitory *in vitro* activity against experimental tumors. This drug is comprised of the aglycone ε-pyrromycinone and a trisaccharide. See, Bieber et al. *J. Antibiot.* **1987**, *40*, 1335. The trisaccharide contains two repeating units of 2-deoxy-L-fucose (A, B) and one unit of the keto sugar (C), L-cinerulose. All the sugars are connected to each other through a 1-4 axial linkage.

Although ciclamycin was discovered almost thirty years ago, little is understood about its function because insufficient quantities are available from natural sources. Consequently, the best way to obtain ciclamycin in large quantites, and the only way to obtain its analogs, is through chemical synthesis.



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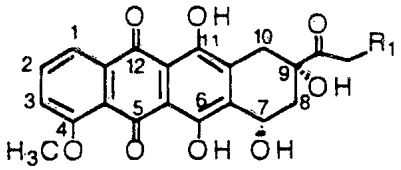
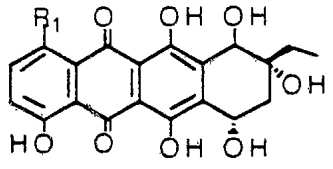
The aglycone of ciclamycin, ε-pyrromycinone, can be obtained by deglycosylation of other readily available antibiotics, such as marcellomycin, musettamycin and cinerubin. Efficient strategies exist in the literature for coupling the trisaccharide to the aglycone. See, for example, Kolar et al. *Carbohydr. Res.* **1990**, *208*, 111. However, methods for the construction of the trisaccharide suffer from limitations of overall ease and efficiency.

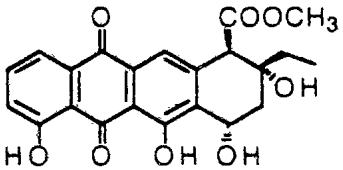
Anthracycline antibiotics occur as intermediates in the metabolism of several *Streptomyces* species. They are potent chemotherapeutic drugs that have been used extensively in the treatment of various solid tumors and leukemias. See, Arcamone, F. *Doxorubicin Anticancer Antibiotics* ; Academic Press: New York, **1981**. The aglycone of all anthracyclines consists of a tricyclic quininoid system with a functionalized cyclohexane moiety. Various substitution patterns frequently encountered among the aglycones are outlined, below.



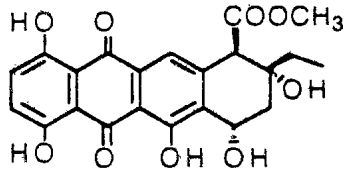
Daunomycinone (R_1=H)
Adriamycinone (R_1=OH)



β-Rhodomycinone (R_1=H)
1-OH-β-Rhodomycinone

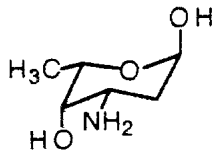


Aklavinone

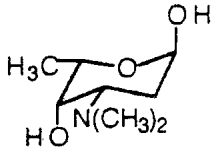


ε-pyrromycinone

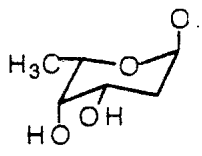
A common feature of all anthracycline antibiotics is an oligosaccharide residue attached to the C-7 hydroxyl group of the aglycone. The sugar residue at this position can be a mono, di or trisaccharide. The most frequently encountered sugars include daunosamine, rhodosamine, 2-deoxy-L-fucose and L-cinerulose.



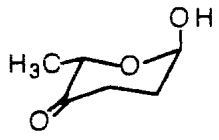
α-L-Daunosamine



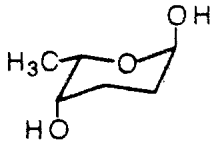
α-L-Rhodosamine



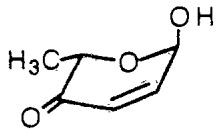
α-L-Deoxyfucose



α-L-Cinerulose



α-L-Rhodinose



α-L-Aculose

On the basis of several studies conducted on the anthracycline antibiotics daunomycin, adriamycin, and aclacinomycin, it has become increasingly clear that the oligosaccharide components of these natural DNA binders play an important role in DNA binding and recognition. See, Bieber et al, *supra*. However, little is known about the actual function of the sugars, in part because it is difficult to selectively modify these drugs. The first chemical synthesis of ciclamycin 0 was accomplished by S. J. Danishefsky and coworkers. See, Suzuki et al. *J. Am. Chem. Soc.* **1990**, *112*, 8895.

2.2.1. Synthesis of 2-Deoxy Oligosaccharides

Complex glycoconjugates like anthracyclines and aureolic acids are of considerable scientific and pharmaceutical interest and have been applied extensively in cancer chemotherapy. A common structural feature in these compounds is the presence of 2-deoxy

oligosaccharides. Indeed, several types of alpha- and beta-2-deoxy glycosides are frequently found in naturally occurring bioactive molecules. In addition to the aureolic acid antibiotics and anthracycline antibiotics, there can be found cardiac glycosides, avermectins, erythromycins, and the enediyne antibiotics. The efficient construction of these 2-deoxy glycosides, particularly 2-deoxy-ß-glycosides, has been a long-standing problem in carbohydrate chemistry. Controlling the ß stereoselectivity in 2-deoxy sugars is difficult because there can be no stereo-directing anchimeric assistance from the C-2 position.

In general, the specific therapeutic effect of these drugs is thought to be caused by the aglycone, while the sugars are thought to be responsible for regulating the pharmacokinetics. It is hoped that by modifying the carbohydrate moiety, it is possible to increase the efficacy and also decrease the cytotoxicity of these drugs.

The development of sugar analogs requires good synthetic methods for the construction of 2-deoxy oligosaccharides. Unfortunately, glycosylation methods available for synthesis of 2-deoxy oligosaccharides are generally unsatisfactory. Since 2-deoxy glycosyl donors lack a substituent at the C-2 position, they are unstable. They decompose rapidly in most glycosylation reactions, thereby resulting in poor yields of glycosides.

In fact, one of the better existing methods for constructing 2-deoxy oligosaccharides, the glycal method, circumvents this problem by *not actually using* 2-deoxy glycosyl donors *directly*. This procedure, which is one of the most widely used glycosylation methods for constructing 2-deoxy glycosides, involves a two-step process. In the first step, a 1,2-anhydro sugar (glycal) is treated with a suitable electrophile, E^+, to form a 1,2-onium intermediate. Nucleophilic attack from

the opposite side affords the glycoside, with 1,2-*trans*-configured bonds. In the second step, the substituent at C-2 is removed to form the desired 2-deoxy glycoside.

2.3. Solution Methods for Obtaining Oligosaccharides

There are currently two general ways to obtain oligosaccharides. The first is by isolation from natural sources. This approach is limited to naturally occurring oligosaccharides that are produced in large quantities. The second way is through enzymatic or chemical synthesis. The variety of oligosaccharides available through enzymatic synthesis is limited because the enzymes used can only accept certain substrates. Chemical synthesis is more flexible than enzymatic synthesis and has the potential to produce an enormous variety of oligosaccharides. The problem with chemical synthesis has been that it is extremely expensive in terms of time and labor. This problem is a consequence of the way in which the chemical synthesis of oligosaccharides has been carried out to date.

Oligosaccharides are formed from monosaccharides connected by glycosidic linkages. In a typical chemical synthesis of an oligosaccharide, a fully protected glycosyl donor is activated and allowed to react with a glycosyl acceptor (typically another monosaccharide having an unprotected hydroxyl group) in solution. The glycosylation reaction itself can take anywhere from a few minutes to days, depending on the method used. The coupled product is then purified and chemically modified to transform it into a glycosyl donor. The chemical modification may involve several steps, each single step requiring a subsequent purification. (A "single step" is defined as a chemical transformation or set of transformations carried out in a "single" reaction vessel without the need for intermediate isolation or purification steps.) Each purification is time consuming and can result in

significant losses of material. The new glycosyl donor, a disaccharide, is then coupled to another glycosyl acceptor. The product is then isolated and chemically modified as before. It is not unusual for the synthesis of a trisaccharide to require ten or more steps from the component monosaccharides. In one recent example, the fully protected trisaccharide side chain of an antitumor antibiotic called ciclamycin 0 was synthesized in 14 steps with a 9% yield based on the component monosaccharides. See, Suzuki et al, *supra*. Thus, the time and expense involved in the synthesis of oligosaccharides has been a serious obstacle to the development of carbohydrate drugs and other biomedical products.

One way to increase the speed and efficiency of oligosaccharide synthesis is to develop methods that permit the construction of multiple glycosidic linkages in a single step. Before the present discovery, the applicants are unaware of a one-step method which involves the regioselective formation of multiple glycosidic bonds and which provides a rapid, efficient and high yield process for the production of oligosaccharides.

2.4. Solid-Phase Synthesis of Oligosaccharides

Besides reducing the number of steps involved in the synthesis of oligosaccharides, one can also increase the speed and efficiency of a synthetic process by eliminating the need for isolation and purification. Theoretically, elimination of the need for isolation and purification could be achieved by developing a solid-phase process for the synthesize of oligosaccharides.

Due to the magnitude of the potential advantages of solid-phase synthesis, there have been previous attempts to synthesize oligosaccharides on a solid phase. Solid-phase methods for synthesis make isolation and purification unnecessary because excess

reagents and decomposition products can simply be washed away from the resin-bound product. This advantage translates into an enormous savings in terms of time, labor, and yield. (The advantages of solid-phase methods over solution methods for the synthesis of peptides and nucleic acids have been amply demonstrated. These advantages would, of course, extend to a solid-phase synthesis of oligosaccharides. For the solid-phase synthesis of peptides, see, for example, Barany, G. and Merrifield, R. B. 1980, in *The Peptides*, Gross, E. and Meienhofer, J. Eds., Academic Press, New York, Vol 2, pp. 1-284.)

As far back as 1971, Frechet and Schuerch outlined the requirements for solid-phase oligosaccharide synthesis. See, Frechet and Schuerch *J. Am. Chem. Soc.* **1971**, 93, 492. First, the resin must be compatible with the reaction conditions. Second, the solid support must contain appropriate functionality to provide a link to the glycosidic center (or elsewhere), which link is inert to the reaction conditions but can be easily cleaved to remove the oligosaccharide upon completion of the synthesis. Third, appropriate protecting group schemes must be worked out so that particular hydroxyls can be selectively unmasked for the next coupling reaction. The other hydroxyls should be protected by "permanent" blocking groups to be removed at the end of the synthesis. Fourth, the glycosylation reactions should be efficient, mild, and go to completion to avoid failure sequences. Fifth, the stereochemistry of the anomeric centers must be maintained during the coupling cycles and should be predictable based on the results obtained in solution for any given donor/acceptor pair. Sixth, cleavage of the permanent blocking groups and the link to the polymer must leave the oligosaccharide intact.

Unfortunately, although it has been generally accepted that solid-phase oligosaccharide synthesis is a desirable goal, and although Frechet and Schuerch (as

well as others) were able to outline a strategy for solid-phase oligosaccharide synthesis, no one, before the present discovery, had been able to implement such a strategy. In previous attempts to synthesize oligosaccharides on insoluble resins, the coupling yields were low and the stereochemical control was inadequate, particularly for the construction of ß-glycosidic linkages (i.e., 1,2-*trans* glycosidic linkages in which the glycosidic bond at the anomeric position of the sugar is *trans* to the bond bearing the sugar substituent at C-2).

These problems have been attributed to the fact that reaction kinetics on the solid phase are slower than they are in solution. See, Eby and Schuerch, *Carbohydr. Res.* **1975**, *39*, 151. The consequence of such unfavorable kinetics is that most glycosylation reactions, which may work reasonably well in solution, simply do not work well on a solid phase both in terms of stereochemical control and yield. Thus, for example, Frechet and Schuerch found that two glycosylation reactions, which both involve the displacement of an anomeric halide in the presence of a catalyst, gave predominantly the ß-anomer (i.e., the 1,2-*trans* product) in solution but gave mixtures on the solid phase. Frechet and Schuerch concluded that it would be necessary to use neighboring group participation to form ß-glycosidic linkages on the solid phase.

Again, however, it has been found that neighboring participating groups (NPGs) frequently *deactivate* glycosyl donors to the point that existing glycosylation methods could not be adapted to the solid phase. Frequently, glycosyl donors would decompose in the resin mixture before glycosylation can take place. See, Eby and Schuerch, *supra*. In some instances the resin has also been known to decompose due to the harshness of the conditions required for glycosylation.

Furthermore, for many ester-type NPGs, there is a significant problem with acyl transfer from the glycosyl donors to the free glycosyl acceptors on the resin. This side reaction caps the resin and prevents further reaction.

Frechet has reviewed the problems encountered in trying to implement a strategy for solid-phase oligosaccharide synthesis. See, Frechet, *Polymer-supported Reactions in Organic Synthesis*, p. 407, P. Hodge and D. C. Sherrington, Eds., John Wiley & Sons, 1980. He has concluded that solid-phase oligosaccharide synthesis is not yet competitive with solution synthesis "due mainly to the lack of suitable glycosylation reactions."

There have been some efforts to overcome the unfavorable reaction kinetics associated with solid-phase reactions by using soluble resins. In the best example to date Douglas et al. used a soluble polyethylene glycol resin with a succinic acid linker and achieved 85-95% coupling yields using a glycosylation method known for over 80 years (the Koenigs-Knorr reaction) with excellent control of anomeric stereochemistry. See, Douglas et al. *J. Am. Chem. Soc.* **1991**, 113, 5095. Soluble resins may have advantages for some glycosylation reactions because they offer a more "solution-like" environment. However, step-wise synthesis on soluble polymers requires that the intermediate be precipitated after each step and crystallized before another sugar residue can be coupled.

Moreover, several additions of the same glycosylating reagent are typically required to push a reaction to completion. In the above case, for example, Douglas et al. had to repeat the same coupling reaction five times to achieve a high yield. Each repetition requires a precipitation step to wash the reagents away. Product may be lost with each precipitation step. In addition, repeated precipitations make the process very

time-consuming. Thus, the soluble resin approach to oligosaccharide synthesis fails to provide all the potential advantages associated with solid phase synthesis using insoluble resins.

A new method for glycosylation involving anomeric sugar sulfoxides was reported by Kahne and co-workers. See, Kahne et al. *J. Am. Chem. Soc.* **1989**, 111, 6881. The anomeric sugar sulfoxides were activated with equimolar amounts of triflic anhydride in the presence of a hindered base. The triflic anhydride-activated glycosyl donors proved to be quite reactive in solution and could be used to glycosylate extremely unreactive substrates under mild conditions. However, this report was limited to solution reactions, and there was no suggestion that solid-phase reactions could be carried out with any degree of utility.

Thus, the state of the art underscores the prevailing and unfullfilled need for a glycosylation method which provides for the rapid, efficient, and high yield synthesis of oligosaccharides. Moreover, an efficient synthesis of oligosaccharides on the solid phase has not been demonstrated which provides all the previously mentioned advantages of solid-phase methods.

3. Summary Of The Invention

The present invention provides methods for constructing multiple glycosidic linkages in solution using anomeric sugar sulfoxides as glycosyl donors and for constructing sequential glycosidic linkages on the solid phase, with control over the stereochemical configuration of the anomeric bond. Thus, depending upon the selected conditions and starting materials, α- or ß-anomers can be produced on the solid phase using anomeric sugar sulfoxides as glycosyl donors. The methods of the present invention may be applied to the preparation of specific oligosaccharides or glycoconjugates or to the preparation of mixtures of

various oligosaccharides or glycoconjugates for the creation of glycosidic libraries that can subsequently be screened to detect compounds having a desired biological activity.

The present invention also relates to the discovery that the activation of anomeric sulfoxides with catalytic quantities of an activating agent provides very good yields of condensation product under very mild conditions. Preferably, the activating agent is a strong organic acid, such as trifluoromethanesulfonic or "triflic" acid (TfOH), p-toluenesulfonic acid (TsOH) or methanesulfonic acid (MsOH), most preferably, TfOH. In particular, it has been found that for the construction of 2-deoxy glycosides, the catalytic glycosylation procedure described herein is considered the method of choice. A preferred embodiment of this aspect of the invention, involving the synthesis of 2-deoxy glycosides via the triflic acid-catalyzed glycosylation, is described in greater detail, below.

Other objects of the present invention will be apparent to one of ordinary skill on consideration of the present disclosure.

4. Brief Description Of The Figures

FIG. 1 illustrates a method of synthesizing the protected trisaccharide of ciclamycin 0 in one step from the component monosaccharides.

FIG. 2 illustrates a process of forming homopolymers of 2-deoxy fucose in one step.

FIG. 3 illustrates a process of synthesizing mixtures of glycoconjugates having biological activity, including potential DNA binding activity. The glycoconjugates so produced can subsequently be screened (e.g., for DNA binding activity) to evaluate the preferred length and the preferred sugar residues of the oligosaccharide portion of the glycoconjugate based on the activity being tested.

FIG. 4 illustrates methods of forming and removing two exemplary types of linkages from a solid support (e.g., polystyrene resin).

FIG. 5 illustrates an apparatus used to carry out solid phase oligosaccharide synthesis.

FIG. 6 illustrates the general scheme for synthesis of a ß-linked disaccharide on the solid phase.

FIG. 7 illustrates the general scheme for synthesis of an α-linked disaccharide on the solid phase.

FIG. 8 illustrates the general scheme for synthesis of a trisaccharide on the solid phase.

FIG. 9 is a ^1H NMR spectrum of monosaccharide **1** of FIG. 1.

FIG. 10 is a ^1H NMR spectrum of monosaccharide **2** of FIG. 1.

FIG. 11 is a ^1H NMR spectrum of monosaccharide **3** of FIG. 1.

FIG. 12 is a ^1H NMR spectrum of trisaccharide **5** of FIG. 1.

FIG. 13 presents an expanded region of the ^1H NMR spectrum of **5**, in which the anomeric protons of the trisaccharide are labeled.

FIG. 14 is a ^1H NMR spectrum of disaccharide **4** of FIG. 1.

FIG. 15 represents a scheme for the synthesis of ciclamycin 0.

FIG. 16 represents a scheme for the synthesis of a trisaccharide.

FIG. 17 represents a scheme for the synthesis of selected disaccharides.

5. Detailed Description Of The Invention

What follows is a detailed description of the preferred embodiments of the present invention.

5.1. Definitions

Activating agent: A chemical agent that on addition to a glycosyl sulfoxide reacts with the anomeric sulfoxide group, thus rendering the anomeric carbon susceptible to nucleophilic attack. In the case of bifunctional sugars or glycosidic residues, the activating agent is also able to deprotect a blocked nucleophilic group under the same conditions used to activate the anomeric sulfoxide group.

Acid scavenger: A chemical agent such as any base that sequesters protons, thereby minimizing side reactions that are promoted by acidic conditions.

Sulfenic acid scavenger: A chemical agent such as methyl propiolate that specifically sequesters sulfenic acid, typically resulting in the formation of an unreactive monophenyl sulfoxide. In the absence of a sulfenic acid scavenger, sulfenic acid reacts with itself to form diphenyl disulfide monosulfoxide and water. Water interferes with the glycosylation reaction.

Bifunctional: The characteristic of a sugar or glycosidic residue to be able to function on activation both as a glycosyl donor and a glycosyl acceptor under the conditions of the single-step process of the present invention.

Biological activity: Any activity displayed by a compound or molecule which has potential physiologic, pharmacologic, diagnostic, or therapeutic applications.

Carbohydrate receptor: Any molecule that binds any carbohydrate. Typically the molecule is a macromolecule such as a protein or DNA.

Glycoconjugate: Any compound or molecule that is covalently bound to a glycosidic residue.

Glycoside: Any sugar containing at least one pentose or hexose residue in which the anomeric carbon bears a non-hydrogen substituent. Typically, the non-hydrogen substituent is a heteroatom, such as nitrogen, oxygen, phosphorus, silicon or sulfur.

Glycosyl acceptor: Any compound that contains at least one nucleophilic group which, under the conditions of the single-step process of the present invention, is able to form a covalent bond with the anomeric carbon of a *glycosyl donor*. As referred to herein, a *glycosyl acceptor* is any sugar or glycoconjugate that contains unprotected hydroxyl, amino, or mercapto groups or such groups that are blocked by protecting groups that can be removed in situ, i.e., under the conditions of the single-step process of the present invention.

Glycosyl donor: A sugar or glycosidic residue that bears a sulfoxide group at the anomeric carbon, which group on activation renders the anomeric carbon susceptible to attack by the nucleophilic group of a glycosyl acceptor to form the glycosidic linkage.

Glycosidic libraries: A mixture of oligosaccharides of varying sequences which can be subjected to a screening procedure to identify compounds or molecules that exhibit biological activity. Such libraries may also include various glycoconjugates.

Monofunctional glycosyl acceptor: A glycosyl acceptor as in the definition above, with the additional provision that the capacity to act as a glycosyl donor at the same time (i.e., under the conditions of the single step process of the present invention) is specifically excluded.

Monofunctional glycosyl donor: A glycosyl donor as in the definition above, with the additional provision that the capacity to act as a glycosyl acceptor at the same time (i.e., under the conditions of the single step process of the present invention) is specifically excluded.

Monofunctional glycosyl unit: A sugar that is *either* a glycosyl acceptor *or* a glycosyl donor but does not have the capacity to function as both upon activation under the conditions of the single step process of the present invention.

Oligosaccharides: A glycosidic residue having three or more monosaccharide units joined by glycosidic linkages.

Potential glycosyl acceptor: Any compound containing at least one nucleophilic group which is potentially able to form a covalent bond with the anomeric carbon of a glycosyl donor.

Single step reaction: A single step reaction is defined as a chemical transformation or set of transformations carried out in a "single" reaction vessel without the need for intermediate isolation or purification steps (i.e., a one-step or one-pot reaction).

Temporal protecting group: A blocking or protecting group that can be removed in situ, preferably, but not necessarily, under the same conditions used to activate an anomeric sulfoxide group.

5.2. General Methods

The following general methods have been divided into two main categories: the first concerns solution reactions involving the formation of multiple glycosidic bonds and the second relates to the synthesis of oligosaccharides in which the growing oligomer is bound to a solid support.

5.3. Formation Of Multiple Glycosidic Linkages In Solution

One or more glycosyl donors having alkyl or aryl sulfoxides at the anomeric position and one or more glycosyl acceptors having one or more free hydroxyls and/or other nucleophilic groups (e.g., amines) and/or silyl ether protected hydroxyls are combined in a

reaction vessel. The resulting mixture may include both monofunctional glycosyl donors and glycosyl acceptors as well as bifunctional glycosyl units, i.e., saccharides that can function simultaneously as glycosyl donors and acceptors. However, in order to form more than one glycosidic linkage (i.e., to produce a trisaccharide or longer product), at least one of the reactants must be a bifunctional glycosyl unit.

The glycosyl acceptors and donors may be blocked by a suitable protecting group, including, but not limited to, ether, ester, acetamido, or thioester protecting groups, at one or more positions. However, it is understood that an ester (or acetamido or thioester) protecting group at C-2 of a glycosyl donor will influence the stereochemical outcome of glycosylation, resulting in a 1,2-*trans* glycosidic bond.

The mixture of glycosyl donors and acceptors is dissolved under anhydrous conditions in a non-nucleophilic solvent, including, but not limited to, toluene, ether, tetrahydrofuran (THF), methylene chloride, chloroform, propionitrile, or mixtures thereof. It has been found that the choice of solvent influences the stereochemical outcome of glycosylation for reactions in which neighboring group participation is not involved. In general, for a given donor/acceptor pair, the use of a non-polar solvent, such as toluene, results in the formation of a higher percentage of alpha isomer, while the use of a more polar solvent, such as propionitrile, results in formation of a higher percentage of the beta anomer.

The reaction is initiated by the addition of an effective amount of an activating agent. In a particular embodiment of the present invention, 0.5 equiv. of triflic anhydride, plus 1.5 equiv. base (as an acid scavenger), are added to the reaction mixture. (Equivalents are relative to glycosyl sulfoxide.) A catalytic amount of triflic acid (e.g., <0.05 equiv.) can also be used, preferably along with excess sulfenic

acid scavenger (e.g., ca. 20 equiv. of methyl propiolate). It has been found that catalytic triflic acid is preferred when the reaction mixture contains 2-deoxy glycosyl donors or when one of the glycosyl acceptors in the reaction is a silyl ether. On the other hand, triflic anhydride is preferred when maximum reactivity of the glycosyl donors is important. However, it should be noted that the moderately basic conditions that obtain with the use of triflic anhydride are not effective to deprotect certain silyl ethers (e.g., t-butylsilyl ethers). Moreover, although the use of triflic anhydride plus 2,6-ditert-butyl-4-methyl pyridine will result in the in situ deprotection of trimethylsilyl ethers, the use of triflic anhydride plus a stronger base (such as Hunig's base) will not. Thus, both activating agents can be used in reactions involving a bifunctional glycosyl unit containing a silyl ether protected hydroxyl, although triflic anhydride only works under a specific set of conditions (choice of base, choice of silyl protecting group). Otherwise, the two activation methods are usually interchangeable.

The methyl propiolate or other sulfenic acid scavenger and/or activated molecular sieves may be added to the reaction either before or just after the addition of activating agent. Sulfenic acid scavengers significantly improve the yield of glycosylation when catalytic triflic acid is used as the activating agent.

The reaction is normally carried out at low temperature (preferably in the range of about -78 °C to as low as bout -100 °C) but may be allowed to proceed at higher temperatures, in some cases as warm as room temperature.

The reaction is quenched by the addition of aqueous bicarbonate and extracted. The reaction mixture may then be subjected to a purification procedure and/or the product(s) deprotected if necessary. The procedure may be used to construct specific oligosaccharides or

mixtures of various oligosaccharides or other glycoconjugates for screening for biological activity.

In particular embodiments of the present invention, it has been discovered that the reactivity of different glycosyl donors may be modulated by manipulating the chemical structure and electronic nature of the anomeric sulfoxide. Such manipulation is due, in part, to the finding that the rate-limiting step in the glycosylation reaction is activation of the sulfoxide by the action of the activating agent. It was subsequently shown that the reactivity of the glycosyl sulfoxides can be influenced by manipulating the nucleophilicity of the sulfoxide oxygen.

Generally, the more nucleophilic the sulfoxide oxygen, the faster the glycosylation reaction. Thus, electron-donating substituents on the R' group attached to the sulfoxide increase the nucleophilicity of the sulfoxide oxygen and speed up the rate of the reaction. By contrast, electron-withdrawing groups decrease the nucleophilicity of the sulfoxide oxygen and slow down the reaction. For example, perbenzylated glucosyl *p*-methoxyphenyl sulfoxide reacts faster than the corresponding unsubstituted phenyl sulfoxide, while perbenzylated glucosyl *p*-nitrophenyl sulfoxide reacts slower than the corresponding unsubsituted phenyl sulfoxide.

The ability to influence the nucleophilicity of different sulfoxides and hence to manipulate the reactivity of different glycosyl donors has been exploited in particular embodiments of the present invention. For example, this ability permits sequential glycosylations to take place in solution, as illustrated in FIG. 1.

In yet other embodiments of the present invention, multiple glycosidic linkages are formed in solution using silylated glycosyl acceptors. Silyl ethers are excellent glycosyl acceptors when catalytic triflic acid is the activating agent and trimethylsilyl

ethers work well as glycosyl acceptors when triflic anhydride is the activating agent and 2,6-ditert-butyl-4-methyl-pyridine is the base. However, they must be unmasked in order to couple. (Hence the requirement for slightly acidic conditions in the glycosylation reaction when silyl ethers are used as glycosyl acceptors.) Because silyl ethers must be unmasked in order to couple, they react more slowly than unprotected alcohols. In this manner, it has been demonstrated that one can modulate the reactivity of two otherwise similar glycosyl acceptors by selective use of silyl protecting groups.

In selected embodiments of the present invention, the distribution of the length of the oligosaccharides or the glycosidic residues of the glycoconjugates produced can be influenced by varying the ratio of monofunctional glycosyl acceptors and monofunctional glycosyl donors to bifunctional glycosyl units in the reaction mixture. For example, it has been shown that higher ratios of monofunctional glycosyl acceptors to bifunctional glycosyl units in the reaction mixture lead to shorter length polymers. The total concentration of reactants also influences the length distribution. (*See*, Sections 6.6 and 6.8 and FIGS. 2 and 3, below.)

In particular embodiments of the present invention, it may be desirable to include only two or three different types of sugars in the reaction mixture and to manipulate the reactivity of the glycosyl donors and acceptors so that a specific oligosaccharide is produced. An example of this procedure is given in Sections 6.1. and 6.6, below.

Yet in other embodiments of the present invention, it may be desirable to include several different types of sugars in the reaction mixture in order to generate a chemically diverse mixture of oligosaccharides or glycoconjugate products for the creation of libraries that may be screened for

biological activity. An example of such a method is illustrated in Section 6.6 and FIG. 3, below.

The chemical diversity can be influenced by manipulation of the number of different sugars included in the mixture. The chemical diversity will also be a function of the order in which different glycosyl donor/acceptor pairs react. The order in which different donor/acceptor pairs react will depend, in turn, on the relative reactivity of different donor/acceptor pairs. The relative reactivity of different/donor acceptor pairs can be manipulated in various ways, as already described above (e.g., by manipulating the structure of the sulfoxide groups used and by protecting some glycosyl acceptors with silyl ethers to slow down the rate at which they react).

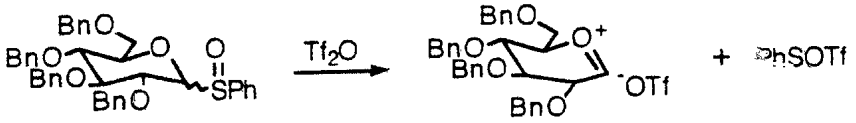
Other factors that influence the relative reactivity of glycosyl donors and acceptors, such as the presence of electronegative protecting groups on the sugar rings or the presence of steric hindrance can also be exploited. See, e.g., Binkley *Modern Carbohydrate Chemistry*, Marcel Dekker, Inc: New York, 1988; also, Paulsen *Angew. Chem. Int. Ed. Engl.* **1982**, 22, 156. Hence, potentially many factors can be taken into account in the implementation of the disclosed method of forming multiple glycosidic linkages to produce chemically diverse mixtures.

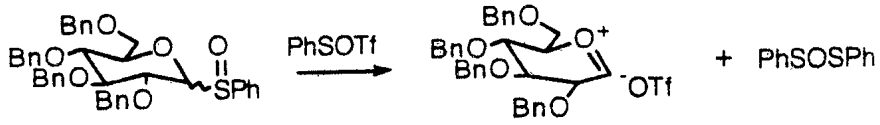
5.4. Catalytic Activation of Anomeric Sulfoxides

Elsewhere in this disclosure, the triflic anhydride activation of anomeric sulfoxides was described, and the mechanism of this glycosylation reaction discussed. Triflic anhydride reacts with the sulfoxide to form a trifloxy sulfonium salt that is extremely reactive.

In the presence of base, half an equivalent of triflic anhydride was sufficient to activate a full equivalent of sulfoxide. The phenyl

trifluromethanesulfenate (PhSOTf) generated during the course of the reaction evidently activated the remaining 0.5 equivalent of the sugar sulfoxide (See, below). Indeed, others have used sulfonate esters to activate thioglycosides. For example, Ogawa et al. use phenyl selenium triflate to activate phenyl and alkyl thioglycosides. See, Ito and Ogawa *Tetrahedron Lett.* **1987**, *28*, 2723.





In the absence of base, however, less than 0.05 equivalents of triflic anhydride activated a full equivalent of sulfoxide. Because the trifloxy phenyl sulfonate (PhSOTf) and triflic anhydride combined did not amount to more than 0.1 equivalent, some other species generated in the reaction was activating the sulfoxide in a catalytic cycle. It was reasoned that the catalyst in question was triflic acid (TfOH). TFOH has been used by others to activate other glycosyl donors. See, e.g., most recently, Lonn *Glycoconjugate J.* **1987**, *4*, 117; Mootoo et al. *J. Am. Chem. Soc.* **1989**, *111*, 8540; Evans et al. *J. Am. Chem. Soc.* **1990**, *112*, 7001; and Veeneman et al. *Tetrahedron Lett.* **1990**, *31*, 1331. Furthermore, it was discovered that only catalytic amounts are typically required because the acid is regenerated in the reaction.

To determine whether TfOH can activate anomeric sulfoxides, the following experiment was conducted using perbenzylated glucose sulfoxide **1** as the glycosyl donor and the C-6 primary alcohol **2a** as the glycosyl acceptor (See, scheme below).

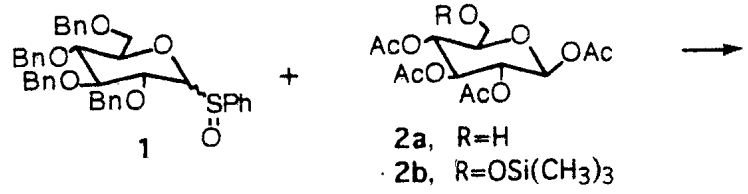
The sulfoxide **1** (1.5 equivalents) was treated with triflic acid (0.05 equivalents) at -78 °C in methylene chloride. This step was followed by the addition of the nucleophile (1.0 equivalent) to the reaction. All of the sulfoxide was consumed to form product, indicating that triflic acid in catalytic amounts activates anomeric sulfoxides.

5.4.1. Mechanism

Although not wishing to be limited by theory, the following mechanistic interpretation is offered for the benefit of the interested reader. The stereochemical outcome for glycosylation using the catalytic triflic acid method was identical to that obtained from the stoichiometric triflic anhydride method. It was surmised that both reactions proceed via the same reactive intermediate, i.e., an oxonium ion or tight ion pair.

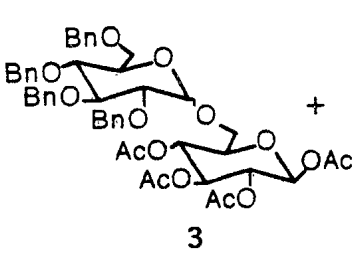
With the TFOH method, however, the yield for the desired disaccharide **3** was low. A significant amount of lactol, **4**, and the 1,1-dimer, **5**, were produced as by products (See, below). The nature of these byproducts indicated to the present applicant that water was present in the reaction. In particular, if an

oxonium ion is trapped by water, a lactol will be formed. If the anomeric lactol then traps another oxonium ion, a 1,1-dimer of the glycosyl donor would be formed.

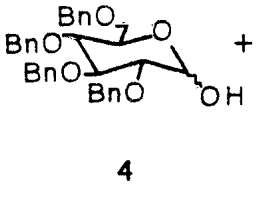


1

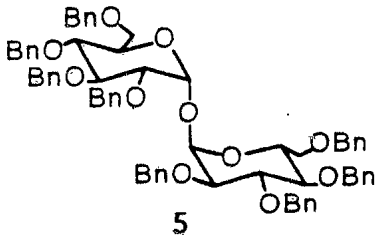
2a, R=H
2b, R=OSi(CH$_3$)$_3$



3



4

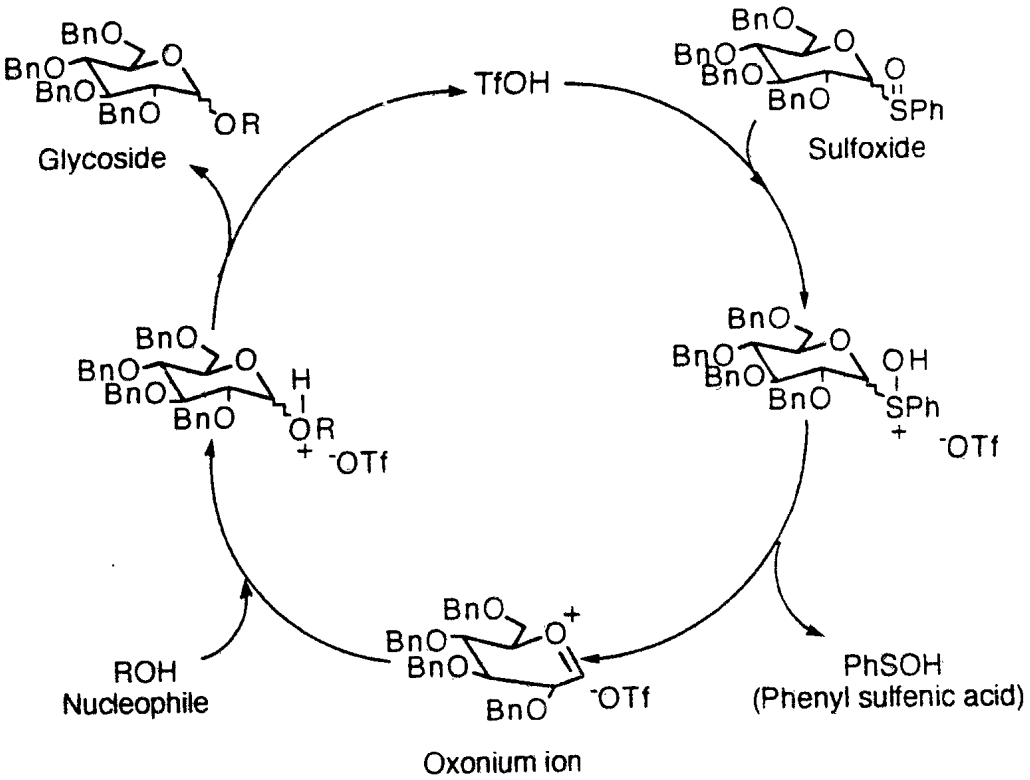


5

	1	2		3	4	5
A.	1.5 eq	1.0 eq	Tf_2O, base	55%	25%	9%
B.	1.5 eq	1.0 eq	TfOH	35%	41%	15%
C.	1.5 eq	1.0 eq	TfOH, HC≡CCOOMe	60%	23%	5%

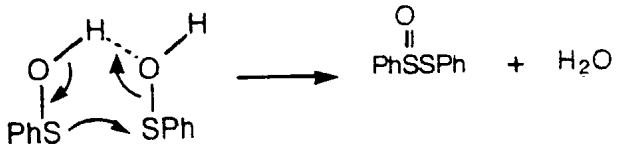
R=H with Tf_2O; R=Si(CH$_3$)$_3$ with TfOH

To prevent the formation of water, the glycosylations were conducted under scrupulously anhydrous conditions, using activated molecular sieves. Despite these precautions however, formation of byproducts, accounting for 40% of the mass balance, was observed. This last observation suggested that water was somehow being formed during the course of the reaction, possibly from the disproportionation of phenyl sulfenic acid.



As illustrated in the scheme, above, the first step of the catalytic cycle is protonation of the sulfoxide to form a sulfonium salt. The sulfonium salt then ejects phenyl sulfenic acid (PhSOH) to form an oxonium ion or a tight ion pair. The nucleophile traps the oxonium ion, subsequently regenerating TfOH. In every catalytic cycle, one molecule of sulfoxide forms product and generates one molecule of phenyl sulfenic acid as byproduct.

Sulfenic acids are a class of organo sulfur compounds that have eluded isolation because of their instability. They have high reactivity as both electrophiles and nucleophiles. Sulfenic acids readily undergo disproportionation to thiosulfinate esters and water. The postulated mechanism for disproportionation, incorporating their dual electrophilic/nucleophilic character, is illustrated below.



5.4.2. Addition of Scavengers for Sulfenic Acids

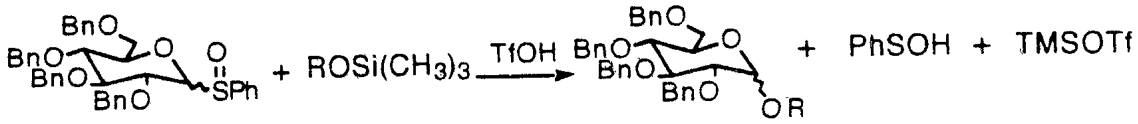
Sulfenic acids readily add to electron deficient alkenes and alkynes to form vinyl sulfoxides. Thus, it may be possible to trap these compounds with a sulfenic acid scavenger before they self condense. Examples of alkenes and alkynes frequently used to trap sulfenic acids include methyl propionate, methyl propiolate, styrene, and dimethyl dicarboxylate. The above compounds were screened as potential scavengers, and methyl propiolate was found to be the most effective.

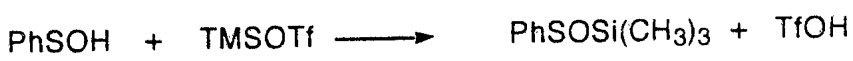
In a typical reaction, **1** and **2** were allowed to react with TfOH in the presence of methyl propiolate (20 equivalents). The yield for the reaction improved from 35% (in the absence of methyl propiolate) to 45% (in the presence of methyl propiolate). Although the reaction yield improved, significant quantities of **4** and **5** were still produced. Hence, still further ways to prevent the disproportionation of sulfenic acid were sought.

5.4.3. Use of Silyl Ethers as Nucleophiles

It was reasoned that the use of silyl ether protected alcohols as nuclephiles could further minimize the buildup of water. The silyl ethers could react under mild reaction conditions to produce the desired disaccharide condensation product, TMSOTf and phenyl sulfenic acid. The sulfenic acid could then be expected to react with TMSOTf to form the silyl phenyl sulfonate

($PhSOSi(CH_3)_3$), thereby regenerating triflic acid (See, below). It was reasoned that because silylated sulfenates are much more stable than sulfenic acids and can be expected not to disproportionate as readily, silylated sulfenates could help minimize water build-up. See, Nakamura *J. Am. Chem. Soc.* **1983**, *105*, 7172.





Accordingly, perbenzylated glucose sulfoxide **1** (1.5 equivalents) was treated with triflic acid (0.05 equivalents) in methylene chloride at -78 °C. The silyl ether of the nucleophile (**2b**) was added to the reaction. After work-up the desired trisaccharide **3** was isolated as the major product in 60% yield. (See, first scheme in Section 5.4.1.) Thus, by using the silyl ether of the nucleophile, the reaction yield improved dramatically, i.e., from 35% to 60%.

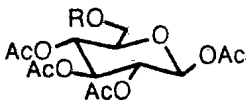
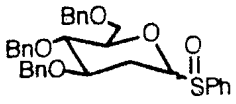
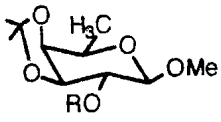
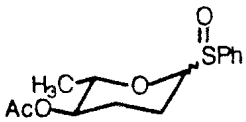
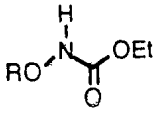
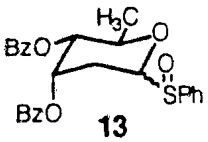
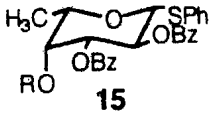
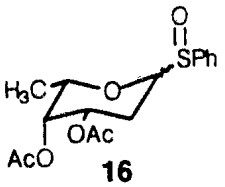
5.4.4. Application of the Catalytic Method for the Synthesis of 2-Deoxy Oligosaccharides

In subsequent investigations the scope of the catalytic triflic acid method for activating sulfoxides was explored. Table I shows a comparison of the catalytic triflic acid and stoichiometric triflic anhydride methods for glycosylation using a range of 2-deoxy glycosyl sulfoxides as glycosyl donors.

2-Deoxy glycosyl sulfoxides are notoriously unstable and tend to give low yields of coupled product (See, Table I). The stoichiometric triflic anhydride method for activating sulfoxides does not always give good results with 2-deoxy glycosyl donors. The

catalytic triflic acid method however, works very well, presumably because the mild reaction conditions minimizes decomposition of 2-deoxy sulfoxides. In fact, the use of triflic acid improves the glycosylation yields by at least 50% for all the cases examined.

TABLE I.. Synthesis of 2-Deoxy Glycosides

Entry	Glycosyl acceptor[a]	Glycosyl donor	Glycoside	Yield Tf_2O	Yield TfOH	Ratio (α: β)
1.	 **2**	 **6**	**7**	50%	88%	5:1
2.	 **8**	**6**	**9**	40%	75%	>20:1
3.	**2**	 **10**	**11**	40%	85%	1:2
4.	 **12**	 **13**	**14**	57%	80%	1:2
5.	 **15**	 **16**	**17**	35%	60%	>20:1

[a]R= H, with triflic anhydride; R= $OSi(CH_3)_3$, with triflic acid.

As can be seen from the results listed in Table I, the yields obtained from the catalytic glycosylation method are comparable to the best yields reported in the literature for the various glycal methods. Furthermore, the catalytic TfOH can be used to activate sulfoxides even in the presence of acid sensitive functional groups (Table I, Entry 2).

5.5. Aspects of the Catalytic TfOH and the Stoichiometric Tf_2O Methods for Activating Sulfoxides

The two glycosylation methods complement each other. The catalytic triflic acid method is advantageous when the sulfoxide is unstable. The reaction conditions are mild; therefore, decomposition of glycosyl donors is minimized. Furthermore, side reactions such as triflation or sulfenylation of the nucleophile, which can result in decreased yields for glycosylation, do not occur with the catalytic TfOH method. However, the catalytic triflic acid method for activating sulfoxides is also significantly slower and requires slightly higher temperatures (-78 °C to -30 °C) compared to the triflic anhydride method. Additionally, the catalytic method is not efficient when electron-withdrawing protecting groups are present on the glycosyl donor.

The stoichiometric Tf_2O glycosylation method, on the other hand, works extremely well for glycosyl donors with electron-withdrawing protecting groups. It may be the best glycosylation method available when neighboring group participation is used to obtain stereoselectivity.

An important point to note is that neither triflic acid nor triflic anhydride activate anomeric phenyl sulfides under the reaction conditions used (Table I, Entry 5). Since anomeric sulfides can be readily converted to the sulfoxides under extremely mild conditions (mCPBA, CH_2Cl_2, -78 °C to 0 °C) both methods lend themselves readily to iterative strategies for oligosaccharide synthesis.

It has, thus, been shown that anomeric sulfoxides can be activated for glycosylation with a catalytic quantity of a strong organic protic acid, such as triflic acid. The glycosylation reaction proceeds

under very mild conditions and offers the following advantages: (i) decomposition of sulfoxides is minimal under the reaction conditions; and (ii) problems of triflation and sulfenylation of the nucleophile are eliminated. These advantages are significant, especially in the context of solid phase oligosaccharide synthesis where sulfenylation or triflation of the nucleophile can result in the capping of a growing oligosaccharide chain on a resin and, hence, termination of the synthesis.

This catalytic method complements the triflic anhydride method, and is especially useful for the construction of 2-deoxy oligosaccharides. In fact, the catalytic triflic acid method has been employed in an efficient construction of the 2-deoxy trisaccharide of ciclamycin 0, as described elsewhere in the present disclosure. Finally, we have demonstrated that neither triflic anhydride nor triflic acid activates anomeric phenyl sulfides under the reaction conditions used; therefore, both methods readily lend themselves to iterative strategies for oligosaccharide synthesis.

5.6. Application of the Single-Step Glycosylation Method to the Synthesis of an Anthracyline Antibiotic

We have found that the order of reactivity of different anomeric phenyl sulfoxides can be controlled by varying the substituents at the para position of the phenyl ring. Consequently, we have succeeded in synthesizing ciclamycin 0 trisaccharide to be synthesized stereoselectively in 25% yield from the component monosaccharides in one step. The synthetic approach is outlined in FIG. 15.

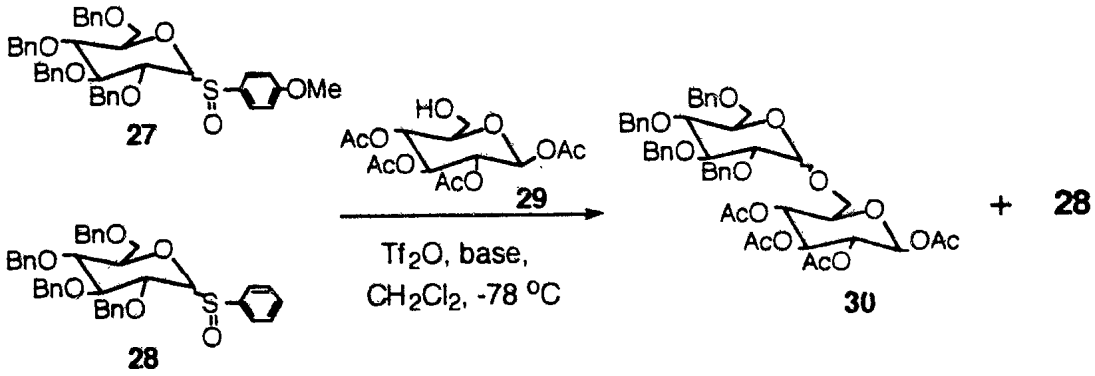
Salient features of the synthesis include employing a catalytic triflic acid glycosylation method to construct all the 2-deoxy glycosidic bonds stereoselectively. Also, the trisaccharide bears a phenyl sulfide at the anomeric center of the A ring.

Anomeric phenyl sulfides are stable ("disarmed") to the conditions that activate anomeric sulfoxides for glycosylation. They can be readily oxidized under mild conditions. Thus the sulfoxide glycosylation method lends itself well to an iterative strategy for oligosaccharide synthesis. The sulfide on the A ring of the ciclamycin trisaccharide was oxidized to the corresponding sulfoxide with mCPBA and then coupled to the aglycone.

It has been discovered that the rate limiting step of the sulfoxide-mediated glycosylation reaction is triflation of the sulfoxide. The reactivity of phenyl sulfoxides can therefore be modulated by varying the substituents in the para position of the phenyl ring. The observed reactivity trend is as follows:

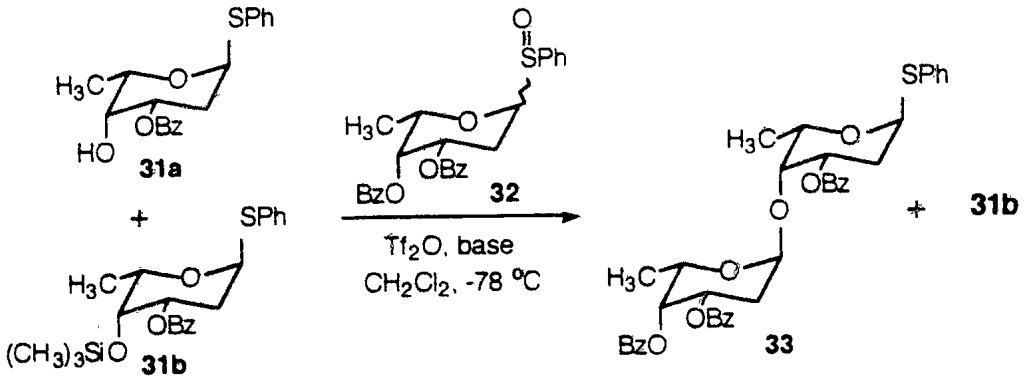
$$p\text{-OMe} > p\text{-H} > p\text{-}NO_2$$

Hence, when perbenzylated glucose para-methoxy phenyl sulfoxide **27** (2.0 eq) and perbenzylated glucose phenyl sulfoxide **28** (2.0 eq) were premixed together in CH_2Cl_2 and treated with triflic anhydride (1.0 eq), base (2.0 eq) and the nucleophile **29** (2.0 eq) at -78 °C, it was observed by TLC that the para-methoxy phenyl sulfoxide was activated *selectively*.



The products isolated after chromatography included the disaccharide (80%) and unreacted phenyl sulfoxide (<60% yield). However, when the same reaction was conducted in the presence of *excess* triflic anhydride, both the sulfoxides **27** and **28** were activated, presumably in a sequential manner, to give the glycosylated product.

Moreover, additional competition experiments revealed that the relative reactivity of glycosyl acceptors (nucleophiles) can also be manipulated. Thus, perbenzoylated 2-deoxy fucose phenyl sulfoxide **32** (2.0 eq), the nucleophile **31a** (1.0 eq), the silyl ether **31b** (1.0 eq), and the base (2,6-di-*tert*-butyl-4-methyl pyridine, 2.0eq) were premixed in CH_2Cl_2 and cooled to -78 °C. This reaction mixture was then treated with triflic anhydride (1.0 eq). The reaction was followed by TLC which indicated that the sulfoxide **32** and the nucleophile **31a** were consumed (to form the disaccharide in 60% yield) while the silyl ether **31b** remained unreacted.

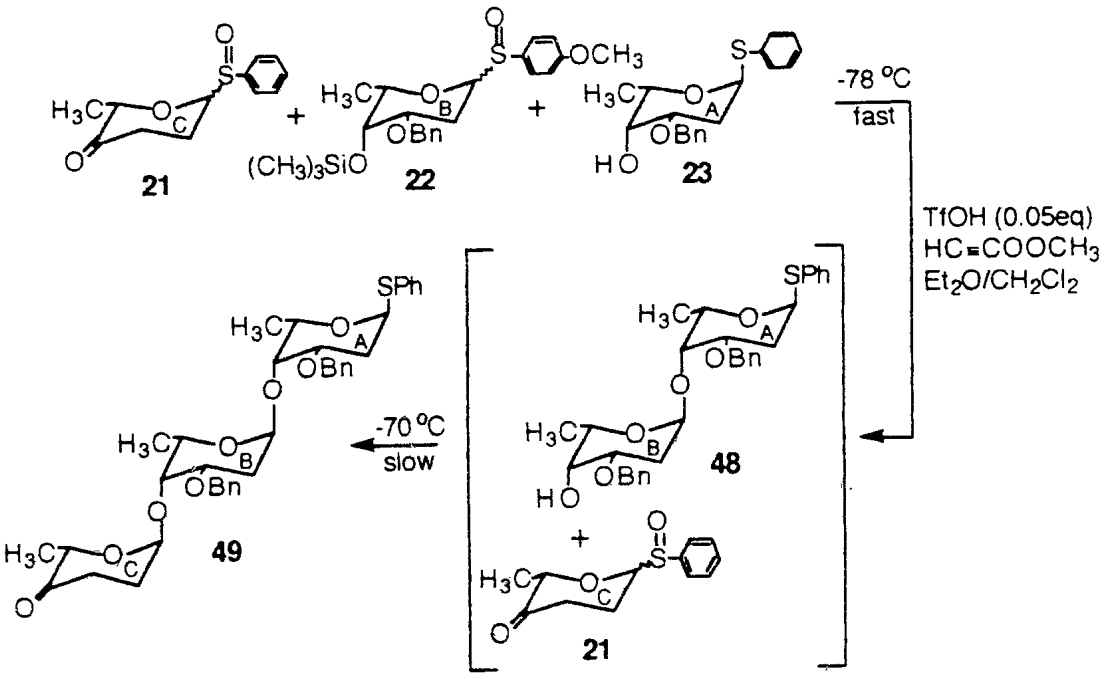


In another experiment, an excess of the sulfoxide **32** (5.0 equiv) was used; in this case, the nucleophile **31a** was consumed first followed by the silyl ether **31b**. Thus, silyl ethers react more slowly than unprotected alcohols as glycosyl acceptors, presumably, because they must first be unmasked.

Having demonstrated that the ability to manipulate the reactivity of both the glycosyl donors *and* the glycosyl acceptors, the synthesis of the ciclamycin trisaccharide was pursued according to FIG. 15. It was hoped that the p-methoxy phenyl sulfoxide B would be activated first, allowing it to react with the C-4 alcohol of A to form the AB disaccharide. Then the phenyl sulfoxide C would be activated and couple with the AB disaccharide to form the desired trisaccharide ABC.

5.6.1. One-Step Synthesis of the Trisaccharide

The sulfoxides **21**, and **22** and the nucleophile **23** were premixed and dissolved in a 1:1 mixture of ether-methylene chloride at -78 °C. Methyl propiolate (20 eq), followed by a catalytic quantity of triflic acid, (0.05 eq) were added to this solution. The reaction was stirred at -70 °C for half an hour and then quenched by pouring it into a saturated sodium bicarbonate solution.



The desired trisaccharide **49** was the major product isolated in 25% yield after flash chromatography. No other trisaccharides were isolated from the reaction mixture. The only other significant coupled product isolated was the disaccharide **48**, the precursor to the trisaccharide. The reaction had taken place in a sequential manner as hoped with the p-methoxy phenyl sulfoxide B becoming activated first and then reacting with the free C-4 hydroxyl of nucleophile A to form the AB disaccharide (**48**). Subsequently, the phenyl sulfoxide C is activated and reacts with the disaccharide AB to form the desired trisaccharide ABC (**49**).

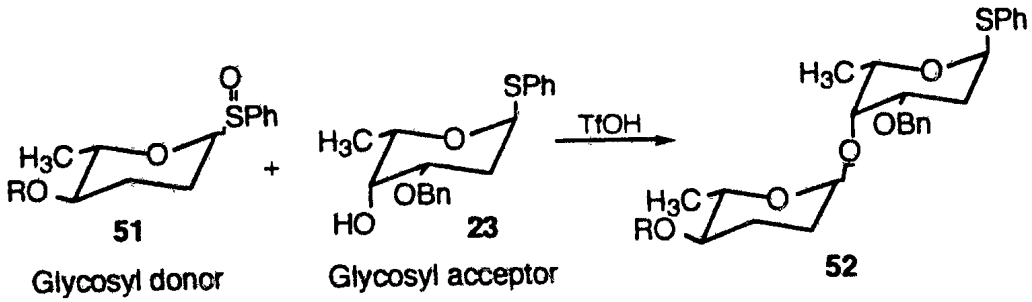
In keeping with the proposed mechanism, when the same reaction is conducted at -100 °C (hexane-liquid nitrogen bath), the products isolated are the silyl ether ~~47~~ of the disaccharide AB (60% yield) along with the unactivated sulfoxide C. Performing the experiment at low temperature, thus, confirms the stepwise nature of the reaction.

The yield of the one step glycosylation is limited not by any undesired cross coupling but by the instability of the glycosyl donors, particularly the keto sulfoxide C, which decomposes readily at room temperature even in the absence of activating agent. Indeed, less than 5% of the disaccharide from the cross coupling of phenyl sulfoxide **21** and free alcohol **23** was detected even though **21** is present in excess; *no* disaccharide from the cross coupling of **21** and **22** was detected. The presence of the ketone functional group in the pyranose ring may contribute to the instability of this sulfoxide.

In an effort to increase the overall yield for the glycosylation, the use of a suitably protected form of the keto sulfoxide was explored.

5.6.2. Improving the BC Coupling Yields

Since the AB disaccharide **48** and the nucleophile **23** are structurally very similar, the nucleophile **23** was chosen as a model compound for the glycosyl acceptor in the glycosylation reaction with C. The precursor to the keto sulfide, the C-4 equatorial alcohol, was chosen as the glycosyl donor. The effect of different protecting groups at the C-4 center were examined.



51 Glycosyl donor **23** Glycosyl acceptor **52**

The use of a suitably protected C-4 alcohol of the glycosyl donor improved the yield for the glycosylation dramatically (40-60%). For all of the cases examined, however, there was loss of

stereochemical control at the anomeric center, as illustrated in Table II, immediately below.

TABLE II. Effect of Protecting Groups at C-4

Protecting group R (51)	*Yield of disaccharide (52)*	*Ratio α:β*
CH_3CO	40%	1:2
$pMeOC_6H_5CH_2$	60%	1:2
TBDMS	60%	1:1
TBDPS	60%	1:1

The presence of a suitably protected C-4 *axial* hydroxyl on the C ring was also examined. In this case, the desired α stereoselectivity was obtained; however, two additional steps following glycosylation were required. These include deprotection of the C-4 alcohol followed by oxidation of the axial alcohol to the ketone. These additional steps result in decreasing the overall yield. Thus, although a 25% yield for the one step synthesis of the trisaccharide appears modest, lack of further functional group manipulations makes the synthesis efficient.

5.6.3. Coupling of Trisaccharide to the Aglycone ε-Pyrromycinone

The trisaccharide has an anomeric phenyl sulfide on the A ring. This sulfide was oxidized to the sulfoxide using mCPBA. The aglycone ε-*pyrromycinone* **15** (1.0 eq) and the trisaccharide sulfoxide 49 (3.0 eq) were dissolved in a 1:1 mixture of ether-methylene chloride and cooled to -78 °C. Methyl propiolate (20 eq) was added to the reaction mixture, followed by a catalytic quantity (0.05 eq) of triflic acid.

A TLC taken soon after the addition of triflic acid indicated the presence of a new spot just above the aglycone. After work up and purification by chromatography, this new compound was identified by NMR

spectroscopy to be the aglycone coupled to the trisaccharide (See Figure 15 (compound 25)) The J_{H-H} coupling constant of 3.0 Hz for the anomeric proton was consistent with α stereochemistry of the glycosidic linkage.

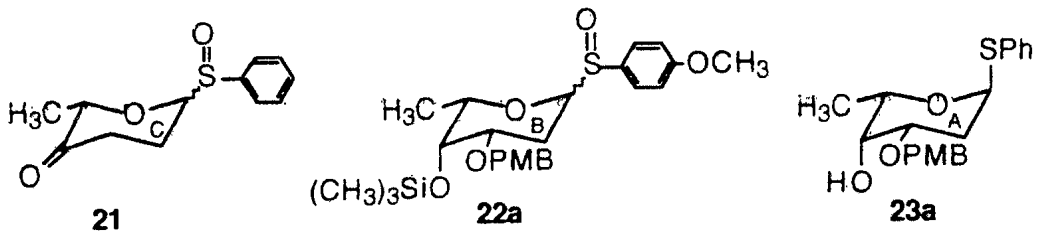
5

5.6.4. Deprotection of Ciclamycin

To complete the synthesis of ciclamycin 0 the removal of the benzyl ether protecting groups on the A and B ring was required. The benzyl ethers were removed by hydrogenolysis using $Pd(OH)_2$ on carbon as the catalyst. Unfortunately, under these reaction conditions, in addition to the benzyl ethers, the aglycone also gets cleaved. In retrospect, this was not surprising since the C-7 hydroxyl of the aglycone to which the sugar is attached resembles a benzyl ether. Thus, to obtain the intact ciclamycin 0, hydrogenolysis conditions could not be used. To circumvent this problem, the protecting groups on the sugar rings A and B needed to be changed.

Para methoxy benzyl ethers can be readily cleaved under mild oxidative conditions using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). See, for example, Ikemoto and Schreiber *J. Am. Chem. Soc.* **1990**, *112*, 9657; Horita et al. *Tetrahedron*, **1986**, *42*, 3021; Oikawa et al. *Tet. Lett.* **1984**, *25*, 5393; and *Carbohydrates*, Ed. Collins, P. M. Chapman and Hall: New York, **1987**. Thus, a 1:1 mixture of marcellomycin (which bears a trisaccharide at the C-7 position of the aglycone) and the A ring (with p-methoxy benzyl ether protecting group at C-3) were treated with an excess of DDQ. Under these reaction conditions only the p-methoxy benzyl ether on the A ring was

hydrolyzed while the marcellomycin remained intact. Based on this result, the protecting groups on the ciclamycin trisaccharide may preferrably be changed from benzyl to p-methoxy benzyl ethers.



PMB=para-methoxybenzyl

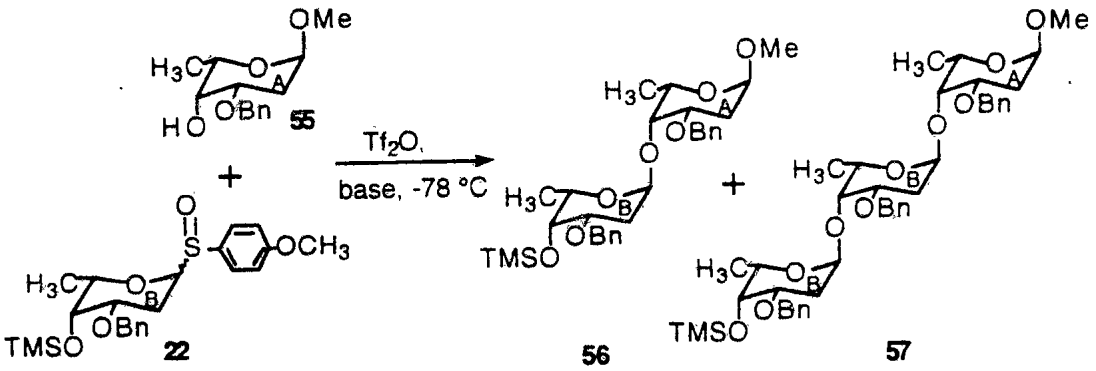
The monosaccharides **21**, **22a** and **23a** can be used to synthesize the desired ciclamycin trisaccharide in one-step (20% yield) following the usual procedure. The trisaccharide sulfide was oxidized to the sulfoxide with mCPBA and then coupled to the aglycone using the catalytic triflic acid glycosylation method.

The deprotection of the coupled product **54a** required the removal of the para-methoxy benzyl ethers. The coupled product **54a** (1 mg) was treated with DDQ in CH_2Cl_2 and stirred at room temperature for 10 hours. The reaction proceeded cleanly to give ciclamycin 0 quantitatively.

5.7. Rapid Synthesis of Oligosaccharides Through Controlled Polymerization: Constrained Libraries of 2-Deoxy Fucose Homopolymers

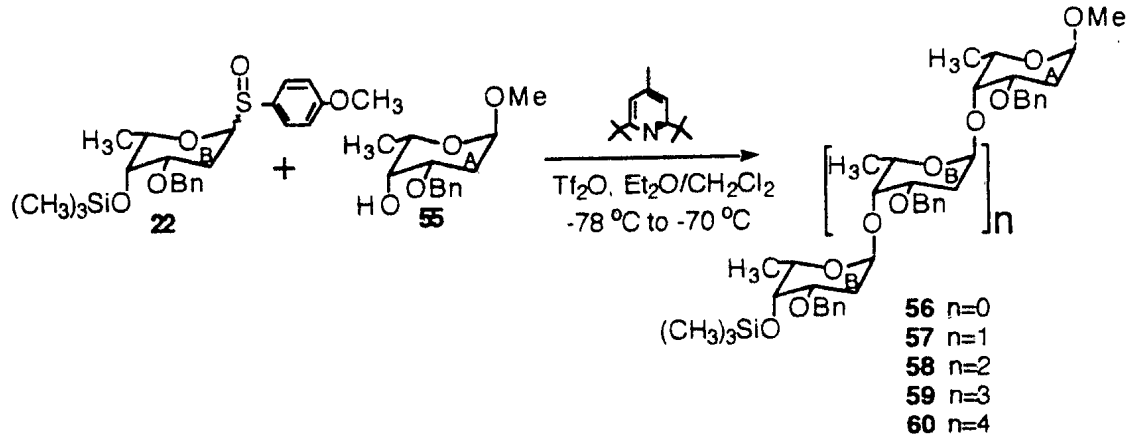
The 2-deoxy fucose sulfoxide B used for the one step synthesis of the ciclamycin trisaccahride is a bifunctional sugar. It contains a leaving group at the anomeric center (p-methoxy phenyl sulfoxide) and can serve as a glycosyl donor. In addition, it has a silyl ether at the C-4 center and can also serve as a glycosyl acceptor.

The most common 2-deoxy sugars found in bioactive natural products are 2,6-dideoxy sugars. They frequently occur as dimers or trimers attached to an aglycone. Given our success with the synthesis of a complicated trisaccharide in one step, we wondered if it was possible to extend this idea for the rapid assembly of oligosaccharides through controlled polymerization. The bifunctional 2,6-dideoxy B ring presented an opportunity to explore the possibility of synthesizing homopolymers in a single reaction.



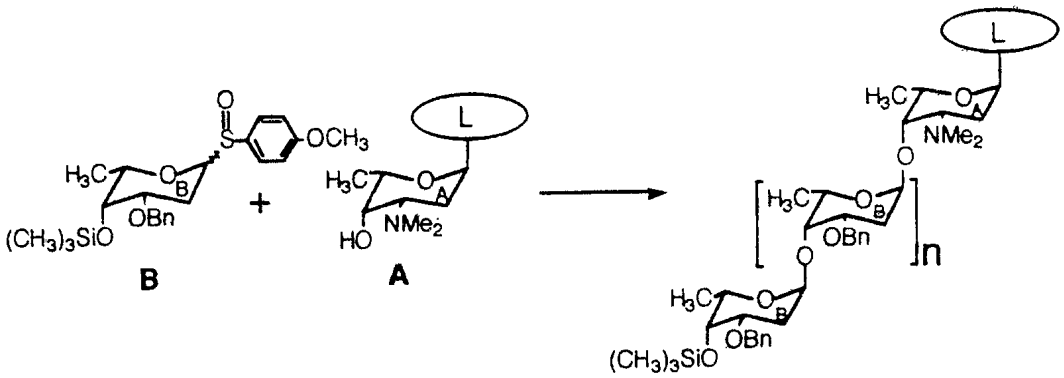
The sulfoxide **22** (2.0 eq) and the nucleophile **55** (1.0 eq) were premixed in 1:1 mixture of ether-methylene chloride and cooled to -78 °C. The reaction mixture was treated with base (2.0 eq) and triflic anhydride (1.0 eq) (See, above). A TLC of the reaction taken soon after indicated the presence of two new spots related to product. After chromatography two products were isolated and characterized by NMR spectroscopy. These products were the AB disaccharide **56** (45% yield) and the ABB trisaccharide **57** (20% yield). The J_{H-H} coupling constants for the anomeric protons were consistent with α stereochemistry for all the glycosidic linkages. This result indicated that homopolymers of 2-deoxy fucose can be formed stereoselectively in one step.

To determine whether higher order polymers of 2-deoxy fucose could be obtained, the number of equivalents of the sulfoxide B (**22**) used in the glycosylations was increased.



When 5.0 equivalents of B and 1.0 equivalent of A were used, a statistical mixture of di, tri, tetra, penta and hexasaccharides was obtained in a single reaction, as noted above. Thus, using the sulfoxide method it is possible to rapidly synthesize a mixture of homopolymers of 2-deoxy fucose through controlled polymerization.

The sulfoxide glycosylation method is powerful and versatile. It can be used to rapidly synthesize a complicated trisaccharide like ciclamycin from component monosaccharides in a single reaction. This strategy could be extended to synthesize a hexasaccharide from the component disaccharides in one step. In addition, by using bifunctional sugars it is possible to synthesize a mixture of homopolymers (of varying chain length) in a single step with the sulfoxide method. Thus far, we have only examined 2-deoxy fucose as a substrate for the controlled polymerization reactions. Nevertheless, this strategy could in principle be extended to include other bifunctional substrates as well.



By using a combination of the one-step and controlled polymerization strategy it is possible to very rapidly synthesize libraries of oligosaccharides with various aglycones attached, as shown above. These oligosaccharide libraries can be screened for DNA binding, for example, by using DNA affinity chromatography. Such a study will help elucidate the features in oligosaccharides that confer DNA binding.

The sulfoxide glycosylation method is rapid, flexible and efficient for the construction of oligosaccharides using conventional approaches. The reactivity of glycosyl donors can be modulated by varying the substituent at the para position of the phenyl ring. Electron-donating substituents accelerate the rate of the reaction relative to the unsubstituted case, while electron withdrawing groups decelerate the rate of the reaction. The reactivity of glycosyl acceptors can be modulated as well. Silyl ethers react more slowly in glycosylations than free alcohols. This permits the controlled formation of two or more glycosidic linkages in a single reaction. This strategy was employed to synthesize the ciclamycin 0 trisaccharide stereoselectively from component monosaccharides in a single step. Furthermore, bifunctional sugars can be used to synthesize libraries of oligosaccharides. The sulfoxide method is thus a

versatile glycosylation method that allows the rapid synthesis of oligosaccharides through controlled oligomerization.

5.8. Formation Of Glycosidic Linkages On The Solid Phase

A potential glycosyl acceptor is attached to an insoluble support (hereafter termed the resin) through a linkage that can be readily cleaved at the end of the synthesis using conditions that do not damage glycosidic linkages. The resin may be any insoluble polymer that swells in organic solvents and has sites for attaching the glycosyl acceptor. Preferred resins include, but are not limited to, polystyrene resins, such as the Merrifield resin, and PEG-derivatized polystyrene resins, such as the TentaGell resins.

The type of linkage depends on the type of functional sites available on the polymer phase and on the glycosyl acceptor. Because polystyrene-based resins can be readily functionalized with chloromethyl subsitituents, the linkage is typically a benzyl ether, formed by nucleophilic displacement of a benzyl chloride on the resin with a free hydroxyl on the glycosyl acceptor. Alternatively, a benzyl ester can be used which is formed by nucleophilic displacement of a benzyl chloride on the resin with the salt of an acid on the glycosyl acceptor. (FIG. 4) Both types of linkages can be readily hydrolyzed at the anomeric carbon of the glycosyl acceptor by treating the resin with Hg(II) compound. Alternatively, the ester linkage can be hydrolyzed by methanolysis as is done for ester linkages to resins in peptide synthesis. The Hg(II) method is preferred for treating aliquots of the resin to monitor the extent of reaction. The Hg(II) method is also preferred when the lactol of the completed oligosaccharide is desired as a final product. The methanolysis method is preferred when the sulfide of the

completed oligosaccharide is desired as a final product (FIG. 4).

The potential glycosyl acceptor may be any molecule having one or more potentially reactive nucleophiles, including potentially reactive hydroxyls, amines, and/or thiols, provided that it also has a suitable site for attachment to the resin. A potentially reactive nucleophile is a free nucleophile or a nucleophile with a temporal protecting group that can be removed readily once the glycosyl acceptor is attached to the resin. The potential glycosyl acceptor may also have permanently protected nucleophiles, which are nucleophiles that cannot be deprotected under the conditions that are used to remove the temporal protecting groups. The potential glycosyl acceptor may be a sugar or some other nucleophile-bearing molecule, including, but not limited to, steroids, amino acids or peptides, polar lipids, polycyclic aromatic compounds, and the like. Protecting group schemes for sugars that permit selective protection and deprotection at any position are well known. See, e.g., Binkley, above.

Following attachment to the resin, the potentially reactive nucleophile is selectively deprotected, if necessary, and the derivatized resin is lyophilized overnight and stored in a desiccator until use. The resin is then preferably placed in a specially designed reactor vessel with a glass frit. Any openings are sealed, e.g., with rubber septa (*See*, e.g., FIG. 5). There may be many variations on the general apparatus. However, the important features can be enumarted as follows:

a) An inlet for the addition of solvent and dissolved reagents to the reaction chamber and which is suitable for maintaining an anhydrous atmosphere; (In the apparatus shown, a rubber septum over a cup-shaped opening permits the addition of solvent and dissolved reagents by canula or syringe needle while preventing exposure of the reaction chamber to the outside air. In

a preferred embodiment of the reaction vessel, this inlet is also equipped with a T-connector or similar adapter which allows the inlet to double as a vent for releasing inert gas, such as nitrogen or argon, to prevent the build up of excess pressure within the apparatus.)

b) A reaction chamber for holding the resin and reagent solution which is equipped with a frit or filter of such coarseness or porosity so that unbound components, such as unreacted dissolved reagents, but not resin, can be washed from the reaction chamber;

c) A port, located on the side of the frit which is opposite to the inlet side, for introduction of an inert gas; the gas passes through the frit, thus agitating the reaction mixture, and settles over the reaction mixture, thus maintaining an anhydrous atmosphere inside the reaction chamber. (As evident from FIG. 5, the argon or nitrogen passes through the resin from below, opposing the flow of solvent through the frit and agitating the resin simultaneously. In a preferred embodiment, this port is equipped with a T-connector or similar adapter to allow the port to be attached to an aspirator for removal of solvent under vacuum.)

It should also be noted that the configuration of the apparatus is such that the apparatus up to the level of most of the reaction chamber can be immersed in a cooling bath. Hence, below the frit, the apparatus may be in a U-shape, as shown in FIG. 5, so that the gas port can be positioned above the cooling medium.

Next, an inert gas, such as argon or nitrogen, preferably argon, is passed through the resin for about 1 hour. The resin is then suspended in 3-5 mL anhydrous solvent including but not limited to toluene, ether, THF, methylene chloride, chloroform, propionitrile, or mixtures thereof). From the discussion in the previous section, it is understood

that the choice of solvent will influence the stereochemical outcome of glycosylation for reactions in which neighboring group participation is not involved. The argon flow is adjusted to agitate the resin gently and prevent solvent from draining through the frit.

A glycosyl sulfoxide is then dissolved under anhydrous conditions in 2-4 mL anhydrous solvent and transferred by canula to the reactor vessel containing the resin. The glycosyl sulfoxide may also have protecting groups present elsewhere in the molecule. If the saccharide chain is to be further extended, the glycosyl sulfoxide must also have at least one temporal protecting group. Typically the glycosyl sulfoxide is added in 2-4-fold excess relative to the amount of glycosyl acceptor on the resin.

Depending on the activation method used to initiate the glycosylation reaction, a non-nucleophilic base, such as 2,6-di-t-butyl-4-methyl pyridine or Hunig's base (diisopropyl ethylamine), may be dissolved with the glycosyl sulfoxide or added to the vessel containing the resin. When used, the base is preferably present in a slight excess relative to the amount of glycosyl sulfoxide added.

The reactor vessel containing the resin is then immersed in a cold bath at -78 °C. To activate the glycosyl donors for reaction, either 0.05 equiv. (i.e., catalytic) triflic acid or 0.5 equiv. of triflic anhydride diluted in a large volume of anhydrous solvent is added to the reaction mixture under anhydrous conditions. The molar equivalents are measured relative to the amount of glycosyl sulfoxide used. Also, dilution in a large volume means that the volume of the neat activating agent is diluted at least 100-fold by the addition of the appropriate volume of solvent (e.g., 1 μL of neat activating agent is added to at least 99 μL of solvent before addition to the donor).

The addition of activating agent may be carried out, for example, with the aid of a canula.

Other activating agents suitable in the present method include, but are not limited to, an alkyl- or arylsilyl triflate (e.g., trimethylsilyl triflate), an alkyl- or arylsulfenyl triflate, and an alkyl- or arylselenenyl triflate. If protons are generated in the reaction (as when 0.5 equiv. of triflic anhydride is used to activate the sulfoxide), an acid scavenger must be present in the resin mixture. Moreover, unless the activating agent is used in catalytic amounts (e.g., <0.1 equiv relative to glycosyl sulfoxide), the activating agent must be diluted about 100-fold or more prior to addition. It has been discovered with triflic anhydride that dilution is critical, triflation of the glycosyl acceptors on the resin thus being avoided.

Next, the resin is gently agitated by the flow of argon. Typically the reaction is allowed to continue for approximately 30 minutes after which the resin is rinsed repeatedly to remove byproducts and unreacted glycosyl donor. If desired, the reaction may be monitored by removing aliquots of resin, rinsing the resin to remove reagents, and then hydrolyzing the linkage to the resin. Alternatively, if the glycosyl acceptor is a sugar which is attached to the resin via a sulfide derivative linked to the anomeric carbon, the link to the anomeric carbon may be hydrolyzed with a Hg(II) compound. Hydrolysis by Hg(II) is preferred for monitoring the extent of the glycosylation reaction.

The products and the progress of the reaction may be analyzed by thin layer chromatography using standards for comparison. For example, after Hg(II) hydrolysis of an aliquot from the reaction mixture, the soluble products are analyzed by TLC. The absence of the monosaccharide residue that was bound to the resin is taken as an indication that the reaction has proceeded to completion.

To obtain the products, the resin is typically rinsed repeatedly with methylene chloride followed by methanol (preferably, about 10 cycles). The

coupling may be repeated if necessary to drive the reaction to completion. Otherwise, if the saccharide chain is to be further extended, temporal protecting groups are next removed, the resin rinsed repeatedly to remove reagents, and another glycosyl sulfoxide residue added as before.

Upon completion of the synthesis and rinsing to remove reagents, the disaccharide, oligosaccharide or glycoconjugate is removed from the resin. The product may then be purified and/or deprotected if desired. Alternatively, the disaccharide, oligosaccharide or glycoconjugate may be used while still attached to the resin in screening procedures to elucidate biological activity.

Strategically, mixtures of oligosaccharides can also be produced by solid phase synthesis and screened for biological activity. To produce mixtures, more than one different type of glycosyl sulfoxide is added to the resin at one or more cycles of the synthesis. It may be desirable to vary the sugars at only one position in the synthesis to probe the structural requirements at that position. In this way, structure-activity relationships can be rapidly evaluated in cases where both a particular carbohydrate and its receptor are known. Alternatively, it may be desirable to vary the sugars at several positions in the synthesis, producing a complex mixture that can be screened for binding to various receptors. In either case, if activity is detected, the active compound(s) can be identified using methods similar to those used in the peptide field for identifying active peptides from mixtures produced by solid phase synthesis. See, for example, Furka et al. *Int. J. Peptide Protein Res.* **1992**, 37, 487; Lam et al. *Nature* **1991**, 354, 82; Houghten, R. A. *Nature* **1991**, 354, 84; Zuckermann et al. *Proc. Natl. Acad. Sci. USA* **1992**, 89, 4505; Petithory *Proc. Natl. Acad. Sci. USA*, **1991**, 88, 11510; Geyse et al. *Proc. Natl. Acad. Sci. USA*, **1984**, 81, 3998;

Houghten *Proc. Natl. Acad. Sci. USA*, **1985**, 82, 5131; Fodor et al. *Science* **1991**, 251, 767.

6. Examples

The following specific examples are provided to better assist the reader in the various aspects of practicing the present invention. As these specific examples are merely illustrative, nothing in the following descriptions should be construed as limiting the invention in any way. Such limitations are, of course, defined solely by the accompanying claims.

6.1. Synthesis Of The Ciclamycin 0 Trisaccharide In A Single Step

FIG. 1 illustrates one embodiment of the process for forming multiple glycosidic linkages in solution in which a specific trisaccharide, the ciclamycin 0 trisaccharide, is synthesized stereospecifically in protected form in a single step from the component monosaccharides. The monosaccharides **1**, **2**, and **3** are combined in a ratio of 3:2:1, as shown (417 mg, 1.812 mmol; 541 mg, 1.2 mmol; and 165 mg, 0.604 mmol, respectively). Water is then removed from the mixture by distillation of the azeotrope from anhydrous toluene. (This drying step is carried out by dissolving the sugar mixture in toluene (ca. 30 mL) and removing the toluene on a rotary evaporator under vacuum. The anhydrous sugar mixture is then used directly or stored under inert gas until needed.)

The anhydrous sugar mixture is next dissolved in 20 mL of anhydrous methylene chloride in a 50 mL flame dried flask. Then, 20 mL of freshly distilled diethyl ether containing 20 equivalents of methyl propiolate is added. (The propiolate ester is used to scavenge the sulfonic acid that is produced in the reaction.) The solution is then cooled to -78 °C. A catalytic amount of triflic acid (5.3 μL, 0.05 eq.) is then added dropwise, and the reaction is allowed to warm

from -78 to -70 °C over a period of 45 minutes and then quenched with saturated $NaHCO_3$. The biphasic mixture is then extracted with CH_2Cl_2 (3 x 15 mL). The organic extracts are combined, dried over anhydrous Na_2SO_4 and concentrated. The major product, trisaccharide **5**, is isolated in 25% yield, based on monosaccharide **3**, after extraction with ethyl acetate and flash chromatography on silica gel (20% ethyl acetate/petroleum ether).

The ^1H NMR spectra of trisaccharide **5** are shown in FIGS. 12 and 13. The stereoselectivity achieved is a function of the donor-acceptor pairs and the glycosylation conditions (solvent, temperature). We have found that catalytic triflic acid does not anomerize glycosidic linkages at an appreciable rate below -30 °C. No other trisaccharide is produced. Indeed, the only other significant coupled product detected from the reaction is disaccharide **4** (Scheme 1 of FIG. 1, 15% yield; ^1H NMR, FIG. 14), the precursor to the trisaccharide **5**. Less than 5% of the disaccharide from the cross coupling of phenyl sulfoxide **1** and free alcohol **3** is detected even though **1** is present in excess; *no* disaccharide from the cross coupling of **1** and **2** is detected.

Thus, the yield of trisaccharide **5** in the reaction is not limited by any undesired cross coupling. However, the instability of the glycosyl donors, particularly keto sulfoxide **1**, which decomposes readily at room temperature even in the absence of activating agent, can affect the yield.

The products of the reaction indicate that glycosylation takes place in a sequential manner, with p-methoxy phenyl sulfoxide **2** activating faster than phenyl sulfoxide **1**, and C-4 alcohol **3** reacting faster than C-4 silyl ether **2**. Consistent with this sequence, if the reaction is quenched at -100 °C, *only* the silyl ether of disaccharide **4** can be isolated (60%).

Thus, the products of the one step reaction described above illustrate the principle established by the present invention; i.e. that the reactivity of both glycosyl donors and glycosyl acceptors can be manipulated effectively so that glycosylation takes place in a sequential manner to produce a desired oligosaccharide in a single step.

Finally, it should be noted that the trisaccharide (**5**) produced in the one step reaction has an anomeric phenyl sulfide on the A ring. Anomeric phenyl sulfides are stable ("disarmed") to the conditions that activate anomeric phenyl sulfoxides for glycosylation, but they can be readily oxidized under mild conditions. See, Mootoo et al. *J. Am. Chem. Soc.* **1988**, *110*, 5583; Veeneman and van Boom *Tet. Lett.* **1990**, *31*, 275; and Mehta and Pinto *Tet. Lett.* **1991**, *32*, 4435. Thus, the sulfoxide glycosylation reaction also lends itself well to an iterative strategy for oligosaccharide synthesis. See, Friesen and Danishefsky *J. Am. Chem. Soc.* **1989**, *111*, 6656; Halcomb and Danishefsky *J. Am. Chem. Soc.* **1989**, *111*, 6661; Mootoo et al. *supra*; Veeneman and van Boom, *supra*; and Mehta and Pinto, *supra*; Nicolaou et al. *J. Am. Chem. Soc.* **1984**, *106*, 4189; Mootoo et al. *J. Am. Chem. Soc.* **1989**, *111*, 8540; Barrett et al. *J. Am. Chem. Soc.*, **1989**, *111*, 1392. The ciclamycin trisaccharide **5** is oxidized to the corresponding sulfoxide in 80% yield (1.2 eq. mCPBA, CH_2Cl_2. -78 °C to -50 °C, 2hr) and is ready for coupling to the ciclamycin chromophore.

Monosaccharide **1** is prepared from L-rhamnose in 60% overall yield (^{1}H NMR; FIG. 9). See, Martin et al. *Carbohydr. Res.* **1983**, *115*, 21. Monosaccharides **2** (^{1}H NMR; FIG. 10) and **3** (^{1}H NMR; FIG. 11) are prepared from **L**-fucose with overall yields of 47% and 52%, respectively. See, Giese et al. *Angew Chem. Int. Ed. Engl.* **1987**, *26*, 233.

According the another method of the present invention, the preparation of individual starting materials, their orchestrated condensation to form the trisaccharide of interest, and their subsequent coupling to an aglycone are described in greater detail, below.

6.1.1. Phenyl-3-O-benzoyl-2,6-dideoxy-1-thio-α-L-galactopyranoside (31a)

The compound **37** (1.41 g, 5.9 mmol) is dissolved in dichloromethane and cooled to -78 °C under argon. Sodium hydride (566 mg, 23.6 mmol) is added to this solution. There is brisk effervescence. After ten minutes, benzoyl chloride (2.05 mL, 17.7 mmol) is added to the solution. The reaction is stirred at -78 °C for 1/2 hour, gradually warmed -60 °C and then quenched by pouring into saturated solution of $NaHCO_3$. The resulting solution is extracted with CH_2Cl_2 (3 x 50 mL); the organic layers are combined, dried over anhydrous Na_2SO_4 and concentrated under vacuum. Flash chromatography (20% ethyl acetate-petroleum ether) yields the product as a white solid (1.6 g, 70%). R_f = 0.3 (20% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 8.3 (m, 2H), 7.8-7.2 (m, 8H), 5.85 (d, J=5.94 Hz, 1H, H-1), 5.55 (m, 1H, H-3), 4.7 (q, J=6.60 Hz, H-5), 4.1 (bs, 1H, H-4), 2.78 (dt, J=5.93, 12.86 Hz, 1H, $H\text{-}2_{ax}$), 2.33 (dd, J=4.95, 13.19 Hz, $H\text{-}2_{eq}$), 2.12 (bs, 1H, OH), 1.41 (d, J=6.60 Hz, 3H, CH_3).

6.1.2. Phenyl-3,4-O-benzoyl-2,6-dideoxy-1-thio-α-L-galactopyranoside (32a)

This compound is synthesized from phenyl-3,4-O-diacetyl-2,6-dideoxy-1-thio-α-L-galactopyranoside **36** in the following two step sequence: (i) $NaOMe/CH_3OH$, amberlite IR(120) plus ion-exchange resin; and (ii) Benzoyl chloride, pyridine. R_f (TLC) = 0.3 (30% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 8.1-7.7(m, 6H), 7.5-7.2 (m, 9H), 5.71 (m, 1H, H-3), 5.61 (bs, 1H, H-4), 5.48 (d, J=5.28 Hz, 1H), 4.33 (q, J=6.27 Hz, 1H, H-5), 2.45 (dt, J=3.63, 12.54 Hz, 1H, H-2_{ax}), 2.17 (dd, J=4.95, 12.54 Hz, 1H, H-2_{eq}), 1.25 (d, J=6.27 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 169.49, 164.88, 161.88, 134.11, 130.77, 130.00, 129.28, 129.13, 128.56, 128.44, 128.28, 128.12, 127.83, 83.33, 69.93, 67.81, 65.74, 53.22, 30.62, 16.17.

6.1.3. Phenyl-3,4-O-benzoyl-2,6-dideoxy-1-sulfinyl-α-L-galactopyranoside (32)

The sulfide **32a** (900 mg, 1.99 mmol) is dissolved in dichloromethane and cooled to -78 °C (acetone/dry-ice bath) under argon. mCPBA (483 mg, 2.80 mmol) is added and the reaction is stirred at -78 °C for one hour. The reaction is gradually warmed to 0 °C over a period of two hours and then quenched by pouring into saturated solution of $NaHCO_3$. The resulting biphasic mixture is extracted with CH_2Cl_2 (3 x 25 mL). The organic layers are combined, washed with brine and dried over anhydrous Na_2SO_4. Flash chromatography (40% ethyl acetate-petroleum ether) provides the sulfoxide **32** as a white crystalline solid (690 mg, 80% yield). R_f (TLC) = 0.4 (40% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 8.1-7.65 (m, 6H), 7.5-7.2 (m, 9H), 5.9 (m,

1H, H-3), 5.68 (d, J=2.64 Hz, 1H, H-4), 4.71 (d, J=5.61 Hz, 1H, H-1), 4.63 (q, J=6.27 Hz, 1H, H-5), 2.80 (dd, J=5.28, 14.19 Hz, 1H, H-2_{eq}), 2.55 (dt, J=5.94, 12.53 Hz, 1H, H-2_{ax}), 1.27 (d, J=6.27 Hz, 3H, CH_3).

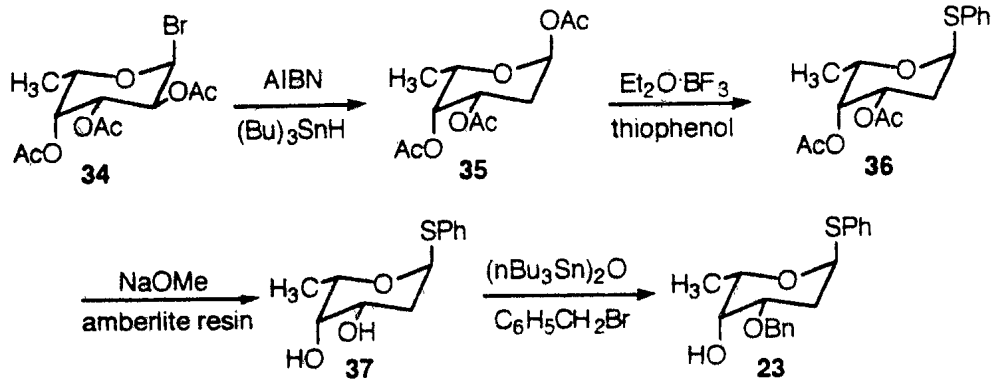
6.1.4. 3,4-O-Benzoyl-2,6-dideoxy-galacto-pyranosyl-α-(1→4)-phenyl-3-O-benzoyl-2,6-dideoxy-1-thio-α-L-galactopyranoside (33)

The sulfoxide **32** (177 mg, 0.4 mmol), nucleophile **31a** (80 mg, 0.2 mmol), silyl ether **31b** (93 mg, 0.2 mmol) and base (82 mg, 0.4 mmol) are premixed and azeotroped 3 times with toluene. To a 25 mL flame-dried flask under argon is added freshly distilled CH_2Cl_2. The azeotroped reactants are dissolved in 10 mL CH_2Cl_2 and added to the flask, which is then cooled to -78 °C. After 10 minutes triflic anhydride (33.6 μL, 0.2 mmol) is added. The reaction is followed by TLC (30% ethyl acetate-petroleum ether). The TLC indicates that the nucleophile **31a** has reacted completely while the silyl ether **31b** remains unreacted. The reaction is gradually warmed to -60 °C over a period of two hours and then quenched by pouring into a saturated solution of $NaHCO_3$. The resulting mixture is extracted with CH_2Cl_2 (3 x 15 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. Flash chromatography (30% ethyl acetate-petroleum ether) gives the disaccharide **33** as a white crystalline solid (85 mg, 60% yield). R_f = 0.4 (30% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 8.1-7.9 (m, 6H), 7.6-7.1 (m, 14H), 5.80 (d, J=5.28 Hz, 1H, H-1), 5.74 (m, 1H, H-3), 5.47 (m, 1H, H-3'), 5.43 (d, J=1.98 Hz, H-4), 5.21 (d, J=1.98 Hz, H-4'), 4.54 (q, J=6.27 Hz, H-5), 4.39 (q, J=6.60 Hz, 1H, H-5'), 4.20 (d, J=2.31 Hz, 1H, H-an), 2.96 (dt, J=5.61, 12.87 Hz, 1H, H-2'), 2.20 (m, 3H), 1.28 (d, J=6.60 Hz, 3H, CH_3), 0.47 (d, J=6.27 Hz, 3H, CH_3). ^{13}C

NMR ($CDCl_3$, 67.5 MHz) δ 165.95, 165.90, 165.69, 134.94, 133.42, 133.11, 132.92, 131.06, 129.91, 129.88, 129.85, 129.76, 129.69, 129.57, 128.91, 128.58, 128.42, 128.23, 127.01, 98.93, 83.79, 75.33, 70.34, 70.10, 67.60, 67.53, 65.71, 30.98, 30.21, 17.41, 16.08.

6.2. Synthesis of the A Ring

The synthesis of the A ring starts with L-fucose, and is accomplished in six steps with an overall yield of 60%, according to the scheme, below.



6.2.1. 1,3,4-O-Tri-O-acetyl-2,6-dideoxy-α-L-galactopyranoside (35)

To a solution of tetra-*O*-acetyl-fucose (5.0 g, 15.05 mmol) in 10 mL of glacial acetic acid is added 15 mL hydrobromic acid (30 %) in a dropwise manner and the resulting solution stirred at room temperature. After two hours the reaction is complete. Work up is done under anhydrous conditions by pouring the reaction mixture into a flask containing 25 g of anhydrous sodium carbonate (Na_2CO_3) suspended in 200 mL of carbon tetrachloride. The resulting mixture is stirred at room temperature for 45 minutes and filtered. The procedure is repeated with the filtrate. The resulting solution is then concentrated under vacuum to afford crude

bromide **34** (5.2 g, 80 %). This is used without further purification in the next step.

To a two-liter three-necked flask, equipped with a reflux condenser and an addition funnel, is added one liter of freshly distilled benzene. The fucose bromide **34** (5.2 g, 12.17 mmol) is dissolved in benzene (15 mL) and added to the flask. The resulting mixture is heated to refux. AIBN (200 mg, 1.21 mmol) is added to this solution. After 30 minutes the tributyl tin hydride (4.91 mL, 18.25 mmol) in benzene (100 mL) is added dropwise to the reaction mixture over a period of 16 hours via the addition funnel. At the end of this time, the reaction mixture is then cooled to room temperature and concentrated under vacuum. Flash chromatography (25% ethyl acetate-petroleum ether) affords the product **35** (2.5 g, 81 %) as a crystalline white solid. R_f (TLC) = 0.3 (25% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 6.24 (d, J=2.64 Hz, 1H, H-1), 5.22 (m, 1H, H-3), 5.17 (t, J=0.66 Hz, 1H, H-4), 4.11 (q, J=5.94 Hz, 1H, H-5) 2.13 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.10 (d, J=6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$ 67.9 MHz) δ 170.13, 169.65, 168.82, 91.51, 68.87, 66..93, 65.86, 28.37, 20.60, 20.42, 16.14, 16.01.

6.2.2. Phenyl-3,4-O-diacetyl-2,6-dideoxy-1-thio-α-L-galactopyranose (36)

Compound **35** (2.5 g, 9.11 mmol) and thiophenol (1.12 mL, 10.94 mmol) are dissolved in dichloromethane (100 mL) and cooled under argon to -78 °C. $Et_2O{\cdot}BF_3$ (5.6 mL, 45.5 mmol) is added dropwise via a syringe to the solution. The reaction is kept at low temperature for one hour and then gradually warmed to 0 °C and quenched by pouring it into saturated aqueous $NaHCO_3$. The resulting biphasic mixture is extracted with CH_2Cl_2 (3 x 50 mL); the organic layers are combined, dried over

anhydrous Na_2SO_4 and concentrated. Flash chromatography of the crude material (15 % ethyl acetate-petroleum ether) gives the sulfide **36** as a white solid (2.1 g, 71% yield). R_f (TLC) = 0.3 (15% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 7.5-7.2 (m, 5H), 5.73 (d, *J*=5.61 Hz, 1H, H-1), 5.29 (m, 1H, H-3), 5.23 (bs, 1H, H-3), 4.56 (q, *J*=6.6 Hz, 1H, H-5), 2.49 (dt, *J*=5.94, 12.87 Hz, 1H, H-2_{ax}), 2.38 (s, 3H, OAc), 2.06 (m, 1H, H-2_{eq}), 1.99 (s, 3H, OAc), 1.13 (d, *J*=6.6 Hz, 3H, CH_3). ^{13}C NMR for α anomer of sulfide ($CDCl_3$, 67.9 MHz) δ 170.51, 169.87, 159.54, 134.33, 124.54, 114.54, 84.58, 69.69, 67.18, 65.51, 55.22, 20.79, 20.60, 16.34.

6.2.3. Phenyl-2,6-dideoxy-1-thio-α-L-galactopyranoside (37)

The compound **36** (2.1 g, 6.48 mmol) is dissolved in methanol (50 mL) and sodium methoxide (420 mg, 7.78 mmol) is added to the solution. The reaction is stirred at room temperature for one hour. A TLC (40% ethyl acetate-petroleum ether) taken at the end of this time indicates that the reaction is complete. The solution is neutralized with amberlite IR (120) plus ion-exchange resin (1.0 g). The solution is filtered through a fritted funnel, washed with ethyl acetate and concentrated to afford the diol **37**. This compound is used without further purification in the next step.

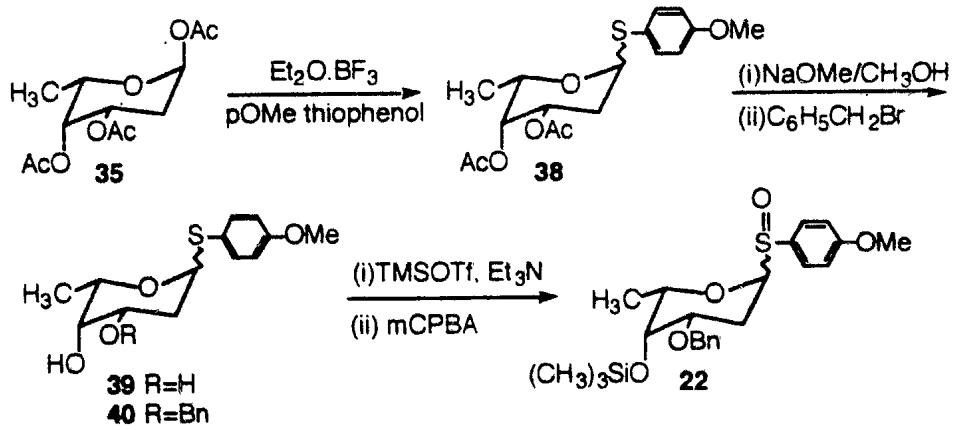
6.2.4. Phenyl-3-O-benzyl-2,6-dideoxy-1-thio-α-L-galactopyranoside (23)

A solution of **37** (923 mg, 3.84 mmol) and dibutyl tin oxide (956 mg, 3.84 mmol) in benzene (60 mL) is heated to reflux in a flask fitted with a Dean-Stark apparatus. After 15 hours the reaction mixture is cooled to room temperature and tetrabutylammonium bromide (1.24 g, 3.84 mmol) is added followed by benzyl

bromide (5 mL, 8.4 mmol). The resulting mixture is refluxed further for two hours, then cooled to room temperature and concentrated under vacuum. Flash chromatography on the crude product (15% ethyl acetate-petroleum ether) affords the sulfide **23** as a white solid (1.10 g, 90 % yield). R_f (TLC) = 0.5 (30% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) α anomer δ 7.40-7.41 (m, 10H), 5.57 (d, *J*=5.61 Hz, 1H, H-1), 4.49 (s, 2H), 4.18 (q, *J*=6.6 Hz, 1H, H-5), 3.74 (m, 1H, H-3), 3.70 (d, *J*=3.63 Hz, 1H, H-4), 2.20(dt, *J*=5.61, 12.5Hz, 1H, H-2), 2.14 (bs, 1H, OH), 1.95 (m, 1H, H-2'), 1.17 (d, *J*=6.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 137.72, 135.20, 130.76, 128.84, 128.52, 127.92, 127.67, 126.83, 83.93, 73.514, 70.13, 68.46, 66.83, 30.67, 16.61. HRMS *m/e* 330.1290 (M^+) calcd for $C_{19}H_{22}O_3S$ 330.1290.

6.3. Synthesis of the B ring

The synthesis of the B ring is accomplished as follows:



6.3.1. 4-Methoxyphenyl-3,4-O-diacetyl-2,6-dideoxy-1-thio-L-galactopyranoside (38)

To a solution of **35** (1.95 g, 7.14 mmol) in distilled dichloromethane (100 mL) is added 4-methoxy thiophenol (1 mL, 8.56 mmol) and the resulting mixture

is cooled to -78 °C. To this is added $Et_2O \cdot BF_3$ (4.4 mL, 35.70 mmol) dropwise. The reaction mixture is stirred a low temperature for 1/2 hour then gradually warmed to -30 °C and quenched by pouring into a saturated solution of $NaHCO_3$. The resulting mixture is extracted with CH_2Cl_2 (3 x 30 mL). The organic extracts are combined, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The crude product is purified by flash chromatography (20% ethyl acetate-petroleum ether) to afford the sulfide **38** (2.12 g, 85 %). R_f (TLC) = 0.4 (20% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 7.40 (d, 2H), 6.8 (d, 2H), 5.51 (d, *J*=5.61 Hz, 1H, H-1), 5.26 (m, 1H, H-3), 5.19 (t, *J*=2.97 Hz, 1H, H-4), 4.54 (q, *J*=6.6 Hz, 1H, H-5), 3.77 (s, OCH_3), 2.32(dt, *J*=5.61, 12.80 Hz, 1H, H-2), 2.12 (s, 3H), 2.02 (dt, *J*=4.62, 12.80 Hz, 1H, H-2'), 1.98 (s, 3H), 1.07 (d, *J*=6.6 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 170.51, 169.87, 159.54, 134.33, 124.54, 114.54, 84.58, 69.69, 67.18, 65.51, 55.22, 30.24, 20.79, 20.60, 16.34; HRMS *m/e* 354.1140 (M^+), calcd for $C_{17}H_{22}O_6S$ 354.1137.

6.3.2. 4-Methoxyphenyl-2,6-dideoxy-1-thio-L-galactopyranoside (39)

To a solution of **38** (1.0 g, 2.82 mmol) in methanol (100 mL) is added sodium methoxide (183 mg, 3.38 mmol). The reaction mixture is stirred at room temperature for two hours and then neutralized by adding Amberlite resin (1.0 g). The reaction mixture is filtered and concentrated under vacuum to afford the diol **39** (760 mg, 100 %). This is used without further purification in the next step. R_f (TLC) = 0.1 (50% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) α anomer δ 7.35 (d, 2H), 6.80 (d, 2H), 5.41 (d, *J*=5.28 Hz, 1H, H-1), 4.41 (q, *J*=6.6 Hz, 1H, H-5), 3.95 (m, 1H,

H-3), 3.76(s, OCH_3), 3.65 (d, J=2.64 Hz, 1H, H-4), 2.01(m, 2H, H-2, 2'), 1.23 (d, J=6.6 Hz, 3H, CH_3).

6.3.3. 4-Methoxyphenyl-3-O-benzyl-2,6-dideoxy-1-thio-L-galactopyranoside (40)

A solution of **39** (2.13 g, 7.90 mmol) and dibutyl tin oxide (1.96 g, 7.90 mmol) in benzene (200 mL) is heated to reflux in a flask fitted with a Dean-Stark apparatus. After 15 hours the reaction mixture is cooled to room temperature and to it is added tetrabutyl ammonium bromide (2.54 g, 7.90 mmol) followed by benzyl bromide (2.82 mL, 23.7 mmol). The resulting mixture is refluxed further for two hours, then cooled to room temperature and concentrated under vacuum. Flash chromatography on the crude product (15% ethyl acetate-petroleum ether) affords the sulfide **40** as an oil (2.5 g, 88 %). R_f (TLC) = 0.25 (20% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 7.5-7.25 (m, 7H), 6.81 (d, J=8.91 Hz, 2H), 5.49 (d, J=5.61 Hz, 1H, H-1), 4.6 (s, 2H), 4.32 (q, J=6.59 Hz, 1H, H-5), 3.85 (m, 1H, H-3), 3.82 (d, J=3.3 Hz, 1H, H-4), 3.77 (s, OCH_3), 2.25 (dt, J=5.94, 12.8 Hz, 1H, H-2), 2.2 (bs, OH), 1.72 (m, 1H, H-2'), 1.27 (d, J=6.59 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 159.25, 137.66, 134.03, 128.33, 127.70, 127.52, 124.98, 114.37, 84.81, 73.40, 69.87, 68.35, 66.55, 55.06, 30.24, 16.47. HRMS m/e 360.1385 (M^+), calcd for $C_{23}H_{24}O_4S$ 360.1396.

6.3.4. 4-Methoxyphenyl-3-O-benzyl-2,6-dideoxy-1-thio-4-O-(trimethylsilyl)-L-galactopyranoside (41)

A solution of **40** (1.4 g, 3.88 mmol) and triethylamine (1.62 mL, 11.64 mmol) in dichloromethane (100 mL) is cooled to -78 °C under argon. To this solution is added TMSOTf (825 µl, 4.27 mmol) dropwise. The reaction is stirred at low temperature for 30 minutes and then quenched by pouring it into a solution of saturated $NaHCO_3$. The resulting mixture is extracted with CH_2Cl_2 (3 x 30 mL). The organic extracts are combined, dried over anhydrous Na_2SO_4 and concentrated. Flash chromatography (10% ethyl acetate-petroleum ether) afforded the product **41** (1.3 g, 83 %) as an oil. R_f (TLC) = 0.85 (15% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 7.32 (d, *J*=8.58 Hz, 2H), 7.28 (m, 5H), 6.79 (d, *J*=8.91 Hz, 2H), 5.52 (d, *J*=5.28 Hz, 1H, H-1), 4.55 (d, *J*=0.99 Hz, 2H), 4.21 (q, *J*=6.60 Hz, 1H, H-5), 3.79 (bs, 1H, H-4), 3.76 (s, OCH_3), 3.66 (m, 1H, H-3), 2.32 (dt, *J*=5.61, 12.54 Hz, 1H, H-2), 1.97 (m, 1H, H-2'), 1.14 (d, *J*=6.59 Hz, 3H, CH_3), 0.11 (s, 9H, TMS). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 138.19, 133.67, 128.15, 127.46, 127.39, 125.55, 114.32, 85.16, 74.26, 71.05, 70.16, 67.76, 55.03, 30.11, 16.99, 0.5. HRMS *m/e* 432.1808 (M^+), calcd for $C_{23}H_{32}O_4SSi$ 432.1790

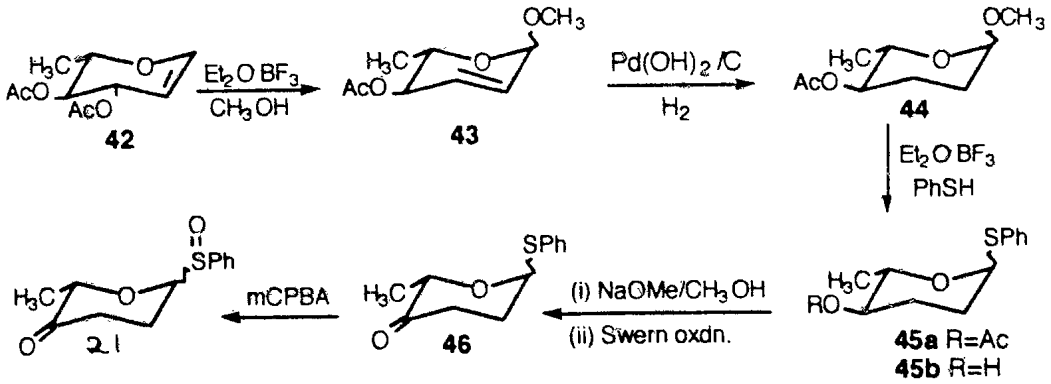
6.3.5. 4-Methoxyphenyl-3-O-benzyl-2,6-dideoxy-1-sulfinyl-4-O-(trimethylsilyl)-L-galactopyranoside (22)

To a solution of the sulfide **41** (402 mg, 0.93 mmol) in dichloromethane (60 mL) is added an excess of solid sodium bicarbonate (1.0 g) and the resulting mixture cooled to -78 °C. To this suspension is added mCPBA (193 mg, 1.11 mmol) and the resulting mixture is stirred at low temperature for 30 minutes. The temperature of the reaction mixture is gradually raised

to 0 °C over a period of one hour and then quenched by pouring into a saturated solution of $NaHCO_3$. The resulting mixture is extracted with CH_2Cl_2 (3 x 30 mL) the organic layers combined, dried over anhydrous Na_2SO_4 and concentrated under vacuum. Flash chromatography (30% ethyl acetate-petroleum ether) affords the sulfoxide **22** (400 mg, 96 %) as a white solid. R_f (TLC) = 0.4 (30% ethyl acetate-petroleum ether). ^1H NMR (270 MHz, $CDCl_3$) δ 7.51 (d, J=8.58 Hz, 2H), 7.4-7.2 (m, 5H), 6.97 (d, J=8.58 Hz, 2H), 4.62 (d, J_{AB}=11.87 Hz, 1H), 4.55 (d, J_{AB}=11.88 Hz, 1H), 4.47 (d, J=5.27 Hz, 1H, H-1), 4.02 (q, J=6.6 Hz, 1H, H-5), 3.91 (m, 1H, H-3), 3.84 (bs, 1H, H-4), 3.83 (s, OCH_3), 2.63 (dd, J=4.62, 13.86 Hz, 1H, H-2), 2.02 (dt, J=5.61, 13.85 Hz, 1H, H-2'), 1.12 (d, J=6.26 Hz, 3H, CH_3), 0.09 (s, 9H, TMS).

6.4. Synthesis of the C ring

The C ring is prepared as follows:



6.4.1. Methyl-4-O-acetyl-2,3,6-trideoxy-L-erythro-hexopyranoside (44)

To a solution of Methyl-4-O-acetyl-2,3,6-trideoxy-L-*erythro*-hex-2-enopyranoside **43** (2.0 g, 0.01 mmol) (See, Martin et al. *Carbohydr. Res.* **1983**, *115*, 21) in benzene is added the catalyst $Pd(OH)_2/C$ (200 mg) and the resulting suspension is shaken in a Parr shaker under H_2 (50 psi). After two hours the reaction mixture is filtered through Celite and concentrated under vacuum to give **44** (2.0 g, 100% yield). This is used in the next step without further purification. R_f (TLC) = 0.5 (20% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 4.63 (s, 1H, H-4), 4.52 (bt, 1H, H-1), 4.32 (q, J=6.27 Hz, 1H, H-5), 3.22 (s, OCH_3), 2.02 (s, 3H, OAc), 1.9 (m, 1H, H-3'), 1.85-1.65 (m, 3H, H-2, 2', 3), 1.02 (d, J=6.27 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 169.14, 96.78, 72.96, 65.85, 53.70, 28.68, 23.69, 20.29, 17.34. HRMS *m/e* 187.0971 (M^+) calcd for $C_9H_{15}O_4$ 187.0970.

6.4.2. Phenyl-4-O-acetyl-1-thio-2,3,5-trideoxy-ß-L-arabino-hexapyranoside (45a)

To a solution of **44** (2.3 g, 12.23 mmol) and thiophenol (1.5 mL, 14.67 mmol) in dichloromethane (100 mL) cooled to -78 °C is added $BF_3.OEt_2$ (4.5 mL, 36.69 mmol) dropwise. The reaction is stirred at low temperature for 30 minutes, gradually warmed to -60 °C, and quenched by pouring into a saturated solution of $NaHCO_3$. The resulting mixture is then extracted with CH_2Cl_2 (3 x 25 mL); the organic layers are combined, dried over anhydrous Na_2SO_4 and concentrated under vacuum. Flash chromatography (15% ethyl acetate-petroleum ether) affords the sulfide **45a** (3.0 g, 92 %) as a white solid. R_f (TLC) = 0.4 (15% ethyl acetate-

petroleum ether). ^1H NMR (270 MHz, $CDCl_3$) δ 7.55-7.2 (m, 5H), 5.52 (d, *J*=4.95 Hz, 1H, H-4), 4.59 (dt, *J*=4.62, 10.23 Hz, 1H, H-1), 4.30 (m, 1H, H-5), 2.2-2.1 (m, 2H, H-3, 3'), 2.09 (s, 3H, OAc), 1.9-1.75 (m, 2H, H-2, 2'), 1.17 (d, *J*=5.95 Hz, 3H, CH_3). HRMS *m/e* 266.0970 (M^+) calcd for $C_{14}H_{18}O_3S$ 266.0977.

6.4.3. Phenyl-4-O-hydroxy-1-thio-2,3,5-trideoxy-ß-L-arabino-hexapyranoside (45b)

To a solution of **45a** (3.0 g, 11.27 mmol) in methanol (100 mL) is added sodium methoxide (365 mg, 6.76 mmol). The reaction mixture is stirred at room temperature for two hours and then neutralized by adding Amberlite resin (2.0 g) and stirred for 15 minutes. The reaction mixture is filtered and concentrated under vacuum to afford the alcohol **45b** (2.52 g, 100 %). This is used without further purification in the next step. R_f (TLC) = 0.2 (25% ethyl acetate-petroleum ether). ^1H NMR (270 MHz, $CDCl_3$) δ 7.5-7.2 (m, 5H), 5.49 (d, *J*=4.62 Hz, 1H, H-1), 4.1 (m, 1H, H-4), 3.3 (m, 1H, H-5), 2.2-1.6 (m, 4H, H-2, 2',3, 3'), 1.2 (d, *J*=5.49 Hz, 1H, CH_3).

6.4.4. Oxidation to Keto Sulfide (46)

A solution of oxalyl chloride (5.5 mL, 11.16 mmol) in dichloromethane (150 mL) is treated with DMSO (1.5 mL, 22 mmol) at -78 °C. After 10 minutes a solution of the alcohol **45b** (2.27 g, 10.15 mmol) in dichloromethane (15 mL) and triethylamine (7 mL, 50.75 mmol) is added to the reaction mixture. The reaction mixture is stirred at low temperature for 30 minutes, then warmed to 0 °C and quenched by pouring it into a solution of $NaHCO_3$. The resulting mixture is extracted with CH_2Cl_2 (3 x 30 mL); the organic layers combined, dried over anhydrous Na_2SO_4 and concentrated under vacuum. Flash chromatography (15% ethyl acetate-

petroleum ether) afforded the ketone **46** (2.20 g, 97 %) as a pale yellow oil. R_f (TLC) = 0.35 (15% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 500 MHz) δ 7.65-7.20 (m, 5H), 5.52 (t, *J*=6.59, 6.96 Hz, 1H, H-1), 4.49 (q, *J*=6.6 Hz, 1H, H-5), 2.65-2.40 (m, 3H, H-3, 3', 2), 1.93 (m, 1H, H-2'), 1.22 (d, *J*=6.59 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 209.58, 134.40, 131.49, 128.91, 127.35, 82.78, 71.54, 34.98, 28.85, 14.63. HRMS *m/e* 222.0718 (M^+) calcd for $C_{12}H_{14}O_2S$ 222.0715.

6.4.5. Oxidation of Keto Sulfide to Sulfoxide (21)

To a solution of the sulfide **46** (418 mg, 1.88 mmol) in dichloromethane (40 mL) is added an excess of solid sodium bicarbonate (1.0 g) and the resulting mixture cooled to -78 °C. To this solution is added mCPBA (455 mg, 2.63 mmol) and the reaction mixture stirred at low temperature for 30 minutes. The temperature of the reaction mixture is slowly raised to 0 °C and then quenched by pouring into a saturated solution of $NaHCO_3$. The resulting mixture is extracted with CH_2Cl_2 (3 x 30 mL); the organic layers are combined, dried over anhydrous Na_2SO_4 and concentrated under vacuum. Flash chromatography (40% ethyl acetate-petroleum ether) affords the sulfoxide **21** (380 mg, 85 %) as a pale yellow oil. R_f (TLC) = 0.25 (40% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 500 MHz,) δ 7.65-7.42 (m, 5H), 4.64 (t, *J*=5.93 Hz, 1H, H-1), 4.59 (q, 1H, H-5), 2.80-2.40 (m, 3H, H-3, 3', 2), 1.80 (m, 1H, H-2'), 1.29 (d, *J*=6.93 Hz, 3H, CH_3).

6.4.6. 3-O-Benzyl-2,6-dideoxy-4-O-(trimethylsilyl)-α-L-galacto-pyranosyl-(1→4)-phenyl-3-O-benzyl-2,6-dideoxy-1-thio-α-L-galatopyranoside

The sulfoxide **22** (350 mg, 0.78 mmol) and nucleophile **23** (129 mg, 0.39mmol) are premixed and azeotroped together three times with distilled toluene. Freshly distilled diethyl ether (9 mL) is added to a flame dried flask under argon and cooled to -78 °C. The premixed reactants are dissolved in 6 mL of distilled dichloromethane and added to the flask. This is followed by the addition of Hunig's base (136 μL, 0.78 mmol). After stirring for 5 minutes triflic anhydride (65.6 μL, 0.19 mmol) is added to the reaction. The reaction is followed by TLC (10% ethyl acetate-petroleum ether). The reaction is warmed to -70 °C and quenched by pouring into a saturated $NaHCO_3$ solution. The resulting solution is extracted with CH_2Cl_2 (3 x 15 mL); the organic layers are combined and dried over anhydrous Na_2SO_4. The solution is concentrated under vacuum and purified by flash chromatography (5% ethyl acetate-petroleum ether) to give the product dissaccharide as an oil (95 mg, 40% yield). R_f (TLC) = 0.8 (10% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 7.45-7.10 (m, 10H), 5.68 (d, J=5.28 Hz, 1H), 5.07 (bs, 1H), 4.67 (d, J_{AB}=12.54 Hz, 1H), 4.55 (d, J_{AB}=12.54 Hz, 1H), 4.53 (s, 2H), 4.23 (q, J=6.60 Hz, 1H), 4.17 (q, J=6.60 Hz, 1H), 3.85 (d, J=2.64 Hz, 1H), 3.73 (m, 2H), 3.68 (d, J=1.32 Hz, 1H), 2.33 (dt, J=6.93, 12.54 Hz, 1H), 2.02 (m, 1H), 1.19 (d, J=6.60 Hz, 3H), 0.89 (d, J=6.27 Hz, 3H), 0.06 (s, 9H). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 138.63, 138.29, 135.32, 130.93, 128..90, 128.85, 128.59, 128.49, 128.20, 127.61, 127.55, 127.32, 126.89, 84.39, 74.26, 73.79, 73.76, 71.14, 70.25, 68.10, 67.43, 31.72, 29.54, 17.37, 17.23, 16.65, 0.63.

6.4.7. 3-O-Benzyl-2,6-dideoxy-α-L-galactopyranosyl-(1→4)-phenyl-3-O-benzyl-2,6-dideoxy-1-thio-α-L-galatopyranoside (48)

The product disaccharide from the previous section (70 mg, 0.11 mmol) is dissolved in freshly distilled THF. Tetrabutylammonium fluoride (500 μL, 5 mmol) is added to the solution. The reaction is complete in one hour. Work up is done by pouring the reaction mixture in $NaHCO_3$ solution and extracting (3 x 15 mL) with THF. The organic layers are combined and concentrated under vacuum. The product **48** is used without further purification in the next step. R_f (TLC) = 0.4 (25% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 7.5-7.2 (m, 10H), 5.69 (d, J=5.27 Hz, 1H), 5.03 (d, J=2.97 Hz, 1H), 4.66 (d, J_{AB}=12.54 Hz, 1H), 4.60 (d, J_{AB}=11.55 Hz, 1H), 4.56 (d, J_{AB}=11.22 Hz, 1H), 4.50 (d, J_{AB}=11.22 Hz, 1H), 4.27 (q, J=6.93 Hz, 1H), 4.22 (q, J=6.60 Hz, 1H), 3.90 (m, 1H,), 3.86 (bd, J=2.31 Hz, 1H), 3.74 (m, 1H), 3.72 (bs, 1H), 2.12 (bs, OH), 1.91 (m, 2H), 1.19 (d, J=6.60 Hz, 3H), 1.01 (d, J=6.60 Hz, 3H). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 138.10, 138.01, 135.22, 130.86, 128.83, 128.46, 128.40, 127.79, 127.67, 127.63, 127.39, 126.88, 99.15, 84.30, 74.84, 73.64, 73.21, 70.34, 70.07, 68.30, 67.97, 65.86, 31.65, 29.83, 17.34, 16.62.

6.4.8. ABC trisaccharide (49)

The sulfoxide **21** (60 mg, 0.22 mmol) and the nucleophile **48** (61 mg, 0.11 mmol) are premixed and azeotroped three times with distilled toluene. Freshly distilled dichloromethane (1 mL) and diethylether (5 mL) are added to a flame dried flask and cooled under argon to -78 °C. The premixed nucleophile and sulfoxide are dissolved in dichloromethane (3 mL) and added to the

cooled flask. This is followed by the addition of Hunig's base (40 μL, 0.22 mmol). After five minutes the triflic anhydride (18.5 μL, 0.11mmol) is added to the flask. The reaction is stirred for two hours between -78 and -70 °C. The reaction is then quenched by pouring into a solution of saturated $NaHCO_3$. The reaction mixture is extracted with CH_2Cl_2 (3 x 15 mL), the organic layers combined and dried over anhydrous Na_2SO_4. The solution is concentrated under vacuum and purified by flash chromatography (20% ethyl acetate-petroleum ether) to give the trisaccharide **49** (18 mg, 25% yield).

6.5. One Step Synthesis of the Ciclamycin 0 Trisaccharide

The sulfoxides **21** (417 mg, 1.812 mmol, 3.0 eq), **22** (541 mg, 1.2 mmol, 2.0 eq) and the nucleophile **23** (165 mg, 0.604 mmol, 1.0 eq) are premixed and thoroughly dried by azeotroping three times with distilled toluene. The starting materials are then dissolved in freshly distilled CH_2Cl_2 (20 mL) and added to a 50 mL flame dried flask under argon. To this reaction mixture is added 20 mL of freshly distilled Et_2O followed by methyl propiolate (9.06 mmol, 15 eq). The flask is cooled to -78 °C using an acetone/dry ice bath. After 5 minutes, triflic acid (5.3 μL, 0.06 mmol, 0.05 eq) is added to the reaction mixture dropwise. The reaction is followed by TLC (20% ethyl acetate-petroleum ether). The reaction mixture is slowly warmed to -70 °C over a period of half an hour and then quenched by pouring it into a saturated solution of $NaHCO_3$ (30 mL). The resulting biphasic mixture is extracted with CH_2Cl_2 (3 x 15 mL). The combined organic extracts are dried over anhydrous Na_2SO_4 and concentrated. Flash chromatography (20% ethyl acetate-petroleum ether) provides the trisaccharide **49** (99 mg 25 %) as a

colourless oil. R_f (TLC) = 0.2 (20% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 500 MHz,) δ 7.45-7.20 (m, 15 H), 5.67 (d, *J*=4.85 Hz, 1H), 5.07 (d, *J*=2.64 Hz, 1H), 4.98 (t, *J*=3.36 Hz, 1H), 4.66 (d, *J*=1.32 Hz, 1H), 4.19 (q, *J*= 6.6 Hz, 1H), 3.88 (m, 1H), 3.84 (bs, 1H), 3.73 (m, 1H), 2.52 (m, 1H), 2.28 (m,1H), 2.23-2.02 (m, 2H), 1.19 (d, *J*=6.60 Hz, 3H), 0.90 (d, *J*=5.27 Hz, 3H), 0.88 (d, *J*=6.60 Hz, 3H). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 211.13, 139.13, 138.94, 135.74, 131.74, 131.63, 129.20, 128.69, 128.60, 127.87, 127.81, 127.72, 127.69, 127.31, 99.57, 98.22, 84.80, 75.79, 74.98, 74.69, 74.19, 73.40, 71.85, 70.45, 68.44, 67.59, 34.25, 31.88, 31.09, 29.89, 17.48, 17.35, 14.93.

6.5.1. <u>Ciclamycin trisaccharide (49a)</u>

The sulfoxides **21** (190 mg, 0.8 mmol, 3.5 eq), **22a** (230 mg, 0.48 mmol, 2.0 eq) and the nucleophile **23a** (85 mg, 0.24 mmol, 1.0 eq) are premixed and thoroughly dried by azeotroping three times with distilled toluene. The starting materials are then dissolved in freshly distilled CH_2Cl_2 (5 mL) and added to a 25 mL flame dried flask under argon. To this reaction mixture is added 5 mL of freshly distilled Et_2O followed by methyl propiolate (4.8 mmol, 20 eq). The flask is cooled to -78 °C using an acetone/dry ice bath. After 5 minutes, triflic acid (5.3 μL, 0.06 mmol, 0.05 eq) is added to the reaction mixture dropwise. The reaction is followed by TLC (20% ethyl acetate-petroleum ether). The reaction mixture is slowly warmed to -70 °C over a period of half an hour and then quenched by pouring it into a saturated solution of $NaHCO_3$ (30 mL). The resulting biphasic mixture is extracted with CH_2Cl_2 (3 x 15 mL). The combined organic extracts are dried over anhydrous Na_2SO_4 and concentrated. Flash chromatography (30% ethyl acetate-petroleum ether) provides the

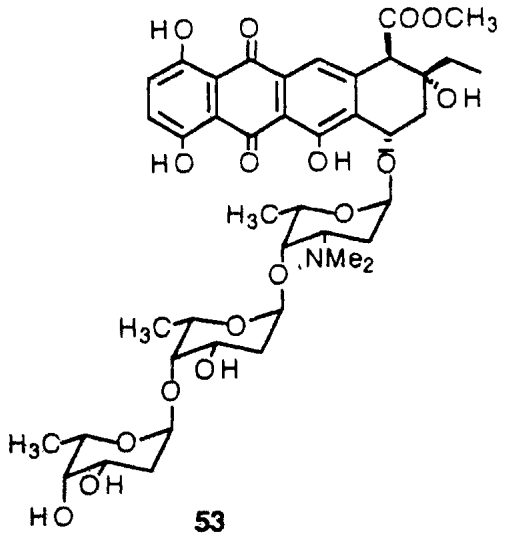
trisaccharide **49a** (35 mg 20 %) as a colourless oil. R_f (TLC) = 0.3 (30% ethyl acetate-petroleum ether).

6.5.2. Oxidation of the sulfide to the sulfoxide

The trisaccharide sulfide **49a** (20 mg, 0.027 mmol) is dissolved in 15 mL of freshly distilled CH_2Cl_2 taken in a 25 mL flask. To this solution is added solid $NaHCO_3$ (500 mg) followed by mCPBA (7.8 mg, 0.045 mmol). The reaction is followed by TLC (40% ethyl acetate-petroleum ether). The reaction mixture is slowly warmed to -60 °C and quenched by pouring into a saturated solution of $NaHCO_3$. The resulting biphasic mixture is extracted with CH_2Cl_2 (3 x 10 mL); the organic layers are combined and dried over anhydrous Na_2SO_4. The trisaccharide sulfoxide **50a** (PMB-protected trisaccharide sulfoxide) is obtained as an oil (19 mg). It is used without purification for glycosylation.

6.5.3. Degradation of Marcellomycin to Obtain the Aglycone ε-Pyrromycinone

Marcellomycin, **53**, an anthracycline antibiotic isolated from bohemic acid complex, has the same aglycone ε-pyrromycinone as ciclamycin (See, below). (The marcellomycin is a generous gift from Bristol-Myers Squibb Company.) The aglycone can be obtained by removing the marcellomycin trisaccharide by acid hydrolysis.



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The drug (75 mg) is refluxed in methanolic HCl (25 mL, 0.1 N) for two hours. At the end of this Marcellomycin (75 mg, 0.175 mmol) is dissolved in methanolic HCl (25 mL, 0.1N) and refuxed at 50 °C for two hours. At the end of this time the reaction mixture is concentrated under vacuum and purified by preparative thin layer chromatography (15% methanol-chloroform). The aglycone ε-pyrromycinone is isolated as a bright red solid (21 mg, 54% yield).

6.5.4. Coupling of the trisaccharide to the aglycone

The PMB-protected trisaccharide sulfoxide **50a** (19 mg, 25 μmol) ε-pyrromycinone (6 mg, 14.15 μmol) and stilbene (2.5 mg, 14 μmol) are premixed and azeotroped three times with distilled toluene. Freshly distilled ether (2 mL) is added to a 15 mL flame dried flask and cooled under argon to -78 °C. The azeotroped reactants are dissolved in distilled dichloromethane (3 mL) and added to the flask. After 10 minutes, triflic anhydride (0.118 μl, 0.7 μmol) is added to the flask and the

reaction followed by TLC (15% ethyl acetate-petroleum ether). The reaction mixture is gradually warmed to -50 °C and quenched by pouring into a saturated solution of $NaHCO_3$. The resulting mixture is extracted with dichloromethane (3 x 5 mL); the organic layers are combined and dried over anhydrous Na_2SO_4. The solution is concentrated under vacuum and purified by flash chromatography (15% ether-methylene chloride followed by 10% methanol-methylene chloride). The product is isolated as a bright red solid (0.75 mg, 16% yield).

6.6. One-Pot Synthesis Of Homopolymers Of Different Lengths

FIG. 2 illustrates another aspect of the present invention which allows the synthesis of "homopolymers" of different lengths. Hence, alpha-linked homopolymers of 2-deoxy fucose with different length distributions are produced by mixing in separate flasks different ratios of the bifunctional sulfoxide 4-methoxy phenyl-3-O-benzyl-4-O-trimethylsilyl-2-deoxy-1-sulfinyl-α-L-fucopyranoside, **B**, with the monofunctional glycosyl acceptor methyl-3-O-benzyl-2-deoxy-α-L-fucopyranoside, **A**, and the base 2,6-di-t-butyl-4-methylpyridine (2 equivalents relative to sulfoxide). The Table, below, indicates the reactant ratio that is used for each of the experiments 6.6.1-6.6.5. The mixtures are first dried thoroughly by azeotropic distillation from toluene (preferably, three times, as above).

The mixtures are then each dissolved in 2.5-5 mL anhydrous methylene chloride and added to separate 25 mL flame dried flasks under argon. To each reaction mixture is added an equal volume of freshly distilled diethyl ether. The flasks are next cooled to -78 °C using an acetone/dry ice bath. After 5 minutes, a methylene chloride solution of triflic anhydride (1.0 equiv relative to **B**) is added dropwise to the reaction

mixtures. The reactions are monitored by thin layer chromatography using 15% ethyl acetate/petroleum ether as the eluant.

After warming to -70 °C over a period of about half an hour, the reaction mixtures are quenched with saturated solution of $NaHCO_3$ (approximately 30 mL each). Each of the resulting biphasic mixtures is extracted with methylene chloride (3 x 15 mL). The organic extracts are combined, dried over anhydrous Na_2SO_4 and concentrated. Flash chromatography (1:5 ethyl acetate/petroleum ether) is used to isolate the glycosylated products from each reaction. The length distribution of "homopolymers" produced is found to vary with the ratio of **A** to **B** and also with the total concentration of reactants in the reaction mixture, as shown in the Table III, below.

TABLE III

Relative Amounts Of Various "Homopolymers" Produced
As A Function Of Molar Ratios Of Reactant And Total Concentration

Entry	A:B (ratio)	[A+B] (mmol/mL)	AB (%)	AB^2 (%)	AB^3 (%)	AB^4 (%)	AB^5 (%)
6.6.1	1:1	0.088	40	-	-	-	-
6.6.2	1:2	0.083	45	20	-	-	-
6.6.3	1:3	0.096	60	30	8.7		
6.6.4	1:5	0.050	50	30	8.0	1.5	
6.6.5	1:3	0.233	30	40	17	8.4	1.7

AB = A-B (^{1}H NMR, given below)
AB^2 = A-B-B (^{1}H NMR, given below)
AB^3 = A-B-B-B (^{1}H NMR, given below)
AB^4 = A-B-B-B-B (^{1}H NMR, given below)
AB^5 = A-B-B-B-B-B (^{1}H NMR, given below)

More specifically, the results of the reaction described above, may be obtained by premixing the sulfoxide (320 mgs, 0.714 mmol, 3.0 eq), nucleophile (60 mgs, 0.238 mmol, 1.0 eq) and base (147 mgs, 0.714 mmol, 3 eq). The resulting mixture is then dried by azeotropic distillation three times from toluene. The

reactants are then dissolved in 2.5 ml of distilled CH_2Cl_2 and added to a 25 ml flame-dried flask under argon. The flask is then cooled to -78 °C and 2.5 ml of Et_2O is added to the reaction. (The concentration of the sulfoxide is approximately 0.144 mmol/ml.) After 10 min Tf_2O (1.5 eq, 0.357 mmol, 60 µl) is added. The reaction is maintained at -78 °C for 1/2 hour, then allowed to warm to -70 °C and stirred at this temperature for an additional 1/2 hour.

The reaction is quenched by pouring the reaction mixture into sat. $NaHCO_3$ solution, followed by extraction 3 times with CH_2Cl_2, drying over anhydrous Na_2SO_4 and concentration. Purification is accomplished by flash chromatography: 15% EA/PE; 20% EA/PE. The structures of the various oligosaccharides are supported by the proton NMR data (270 MHz, $CDCl_3$), in which the non-terminal B's are labeled X, X_1, or X_2, etc.:

AB disaccharide: ^1H NMR δ 5.08, bs, 1H, H1B; 4.81, d, 1H, H1A; 4.7-4.5, 4H benzyl methylenes; 4.2, q, 1H, H5B; 3.9-3.7, m, 5H, H3A, H4A, H4B, H3B, H5A; 3.3, s, 3H, methoxy; 1.8-2.1, 4H, H2A, H2B; 1.21, d, 3H, H6A (methyl); 0.9, d, 3H, H6B (methyl) ppm.

AXB trisaccharide: ^1H NMR δ5.06, s, 1H, H1B; 5.01, s, 1H, H1X; 4.82, d, 1H, H1A; 4.75-4.45, m, 6H benzyl methylene; 4.21, q, 2H, H5X, H5B; 3.91-3.64, m. 7G, H3A, H3X, H3B, H5A, H4A, H4X, H4B; 3.29, s, 3H, Al methoxy; 2.08-1.80, d, 3H, H6A; 1.23, d, 3H, H6A (methyl) δ0.90, 3d, 6H, H6X, H6B (methyls) ppm.

AX_1X_2B tetrasaccharide: ^1H NMR δ 5.27, d, 1H, H1B; 4.99, d, 2H, $H1X_1$, $H1_2$; 4.81, d, 1H, H1A; 4.72-4.45, m, BH benzyl methylenes; 4.2, m, 3H, $H5X_1$, $H5_2$, H5B; 3.90-3.62, m. 9H, H5A, H4A, $H4X_1$, $H4X_2$, H4B, H3A, $H3X_1$, $H3X_2$, H3B;3.29, s, 3H; 2.05-1.80, 8H, 2-deoxy,

CH2A, $CH2X_1$, $CH2X_2$, CH2B; 1.22, d, 3H, H6A (methyl); 1.9-1.8, 9H, $H6X_1$, $H6X_2$, H6B (methyls) ppm.

$AX_1X_2X_3B$ pentasaccharide: ^1H NMR δ 5.06, s, 1H, H1B; 4.95, s, 3H, $H1X_1$, $H1X_2$, HX; 4,79, 1H, s, H1A; 4.7-4.4, m, 10H, benzyl methylenes; 4.15, m, 4H, $H5X_1$, $H5X_2$, $H5X_3$,H5B; 3.3, s, 3H, A1 methoxy; 1.2, 3H, d, H6A (methyl); 0.85, 12H, m, $H6X_1$, $H6X_2$, $H6X_3$, H6B (methyls) ppm.

$AX_1X_2X_3X_4B$ hexasaccharide: ^1H NMR δ5.05, s, 1H, H1B; 4.98, s, 4H, HX_1, $H1X_2$, $H1X_3$, $H1X_4$; 4.7-4.4, 12H, benzyl methylenes; 4.2-4.1, 5H, $H5X_1$, $H5X_2$, $H5X_3$, $H5X_4$, H5B; 2.1-1.8, m, 12H, H2A, $H2X_1$, $H2X_2$, $H2X_3$, $H2X_4$, H2B; 1.2, d, 3H, H6A (methyl); 0.9-0.8, m, 15H, $H6X_1$, $H6X_2$, $H6X_3$, $H6X_4$, H6B (methyls) ppm.

6.7. Controlled Polymerization Reactions

According to another method of the present invention, a controlled polymerization reaction is performed as follows:

The sulfoxide **22** (260 mg, 0.58 mmol), nucleophile **53** (73 mg, 0.29 mmol), and base 2, 6-di-tert-butyl-4-methyl pyridine (118 mg, 0.58 mmol) are premixed and azeotroped three times with distilled toluene. To a 25 mL flame dried flask is added freshly distilled diethyl ether (5 mL) and cooled under argon to -78 °C. The azeotroped reactants are dissolved in distilled dichloromethane (5 mL) and added to the flask. After 10 minutes triflic anhydride (48.5 μL, 0.29 mmol) is added to the flask. The reaction is stirred at -70 °C for 45 minutes and then quenched by pouring into a saturated solution of $NaHCO_3$. The resulting biphasic mixture is extracted with CH_2Cl_2 (3 x 15 mL); the organic layers are combined and dried over anhydrous Na_2SO_4. The solution is concentrated under vacuum and

purified by flash chromatography (15% ethyl acetate-petroleum ether). The AB disaccharide **54** (70 mg, 45%) and the ABB trisaccharide **55** (40 mg, 20%) are isolated as oils. AB dissacharide: R_f (TLC) = 0.4 (15% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz,) δ 7.4-7.2 (m, 10H), 5.06 (bs, 1H, H_{an}), 4.81 (d, J=2.97 Hz, 1H, H-1'), 4.68 (d, J_{AB}=12.53 Hz, 1H), 4.54 (s, 2H), 4.50 (d, J_{AB}=10.89 Hz, 1H), 4.16 (q, J=6.26 Hz, 1H, H-5), 3.9 (m, 1H, H-3), 3.8 (s, 1H, H-4), 3.75 (q, J=5.94 Hz, 1H, H-5'), 3.70 (m, 1H, H-3'), 3.67 (d, J=1.65 Hz, 1H, H-4), 3.27 (s, 3H, OCH_3), 2.1 (m, 3H, H-2's), 1.8 (dd, J=4.62, 12.21 Hz, 1H, H-2_{ax}), 1.21 (d, J=6.6 Hz, 3H, CH_3), 0.9 (d, J=6.27 Hz, 3H, CH_3), 0.06 (s, 9H). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 138.61, 128.24, 128.14, 127.57, 137.32, 127.27, 127.19, 99.27, 98.89, 74.17, 73.78, 72.91, 71.13, 70.22, 70.04, 67.28, 66.80, 54.67, 30.77, 29.51, 17.51, 17.21, 0.58.

6.8. One-Pot Synthesis Of Glycoconjugates With Potential DNA Binding Activity

The strategy for forming multiple glycosidic linkages in solution can be used to synthesize in the same reaction several glycoconjugates with potential DNA binding activity. Depending on the situation, the glycoconjugates can be separated and screened individually for DNA binding activity or they can be screened as mixtures. For example, a mixture of glycoconjugates, each comprised of a potential DNA intercalator and an oligosaccharide side chain, and differing one from another only in the length of the oligosaccharide side chain, are synthesized as in Example 6.6, but using a 4:1 ratio of bifunctional donor to glycosyl acceptor. Specifically, the 2-deoxy fucosyl sulfoxide derivative **B** (908 mg, 1.90 mmol), the glycosyl acceptor **A** (294 mg. 0.48 mmol), and 2,6-ditert-butyl-4-methyl pyridine (779 mg, 3.80 mmol) are

combined, dried by azeotropic distillation three times from toluene and then dissolved in 10 mL of a 1:1 mixture of ether/methylene chloride (freshly distilled solvents). The solution is transferred to a flame dried flash under argon. The flask is cooled to -78 °C using an acetone/dry ice bath. After 5 minutes, 161.1 μL (0.96 mmol) triflic anhydride is added dropwise to the reaction mixture. The reaction is slowly warmed to -70 °C over a period of half an hour and then quenched by pouring into a saturated solution of $NaHCO_3$ (30 mL). The mixture is extracted with methylene chloride (3 x 15 mL). The combined organic extracts are dried over anhydrous Na_2SO_4, filtered, and the solvent removed under vacuum. The reaction is dissolved in 10 mL of wet methylene chloride and treated with excess dichlorodicyanoquinone (DDQ) at room temperature for 1 hour to remove the *p*-methoxy benzyl ether protecting groups. The solvent is then removed under vacuum and the components are separated by flash chromatrography on silica gel.

Their relative affinities for DNA are evaluated to determine the preferred length of the oligosaccharide side chain. Affinity chromatography can be used to identify oligosaccharides that bind to particular receptors. For example, a mixture of compounds is passed over a column containing a solid support to which is attached a receptor of interest (or ligand, if the mobile phase contains a mixture of potential receptors). Compounds that bind to the receptor are retained on the column longer than compounds that do not. Compounds can be fractionated according to their affinity for the receptor.

Thus, receptors that bind carbohydrates can be attached to the solid support. Carbohydrate receptors may be comprised of DNA (double or single stranded), RNA, protein, oligosaccharides, or other molecules. Methods to attach nucleic acids, proteins,

and oligosaccharides to solid supports for use in affinity chromatography have been described. See: (a) *Template Chromatography of Nucleic Acids and Proteins*, Schott, H. Marcel Dekker, Inc.: New York, 1984; (b) *Glycoconjugates: Composition, Structure and Function*, Allen, H.J.; Kisailus, E.C., Eds. Marcel Dekker: NY 1992 (and references therein). (NOTE: Retention times can also be used to quantitate affinities for single compounds passed down the affinity column.)

In another example, glycosyl acceptor **A** (FIG. 3) is premixed with 2,3-*p*-methoxy benzyl-4-trimethylsilyl rhamnosyl sulfoxide **C** (FIG. 3) and allowed to react under conditions (e.g., temperature, solvent, concentration, donor/acceptor ratio) identical to those described above. After workup and removal of the p-methoxy benzyl protecting groups with DDQ, as above, the mixture of glycoconjugates is separated by flash chromatography on silica gel and the relative affinities of the different compounds for DNA are determined. The glycoconjugates produced by the above-described methods are compared with respect to their abilities to bind to DNA. In this way, the effects of different sugars on DNA binding affinity can be compared to identify preferred sugars.

The glycosyl acceptor **A** in FIG. 3 is made by glycosylating a suitably protected juglone derivative (obtained by the procedures of Inhoffen et al. *Croatica Chem. Acta.* **1957**, *29*, 329; Trost et al. J. Am. Chem. Soc. **1977**, *99*, 8116; and Stork and Hagedorn *J. Am. Chem. Soc.* **1978**, *100*, 3609) with compound **B** (FIG. 3) using Tf_2O-Hunig's base CH_2Cl_2/ether (1:1) at low temperature. After a standard workup (including extraction, as described in the other Examples herein) and removal of solvent, the product mixture is dissolved in methylene chloride and treated with excess tetrabutylammonium fluoride at 0 °C. The solvent is

then removed in vacuo and the product isolated by flash chromatography.

The general process described above may also be applied to the synthesis of mixtures of glycoconjugates containing several different sugars. In this case, two or more bifunctional glycosyl donors are used in the reaction. After deprotection, the resulting mixture of glycoconjugates can be screened for DNA binding activity by passing it down a DNA affinity column. Compounds can be fractionated according to their retention times on the affinity column. Compounds with long retention times can be isolated and identified using standard methods for structure elucidation.

6.9. Additional Embodiments Illustrating the Catalytic Glycosylation Method

The following examples relate to glycosylation methods mediated by catalytic amounts of sulfonic acid, including reactions involving silylated glycosyl acceptors.

6.9.1. 1,2,3,4-Tetra-O-acetyl-6-O-(trimethylsilyl)-D-glucopyranose (2b)

The following procedure is typical for all silylations of the nucleophile. The alcohol **2a** (600 mg, 1.85 mmol, 1.0 eq) and triethyl amine (775 μL, 3.43 mmol, 3.0 eq) are dissolved in distilled methylene chloride and cooled to -78 °C under argon. Trimethyl silyl triflate (394 μL, 2.03 mmol, 1.1 eq) is added to the reaction mixture. The reaction is followed by TLC. After 30 minutes the reaction is quenched by pouring into a saturated solution of $NaHCO_3$. The resulting biphasic mixture is extracted with CH_2Cl_2 (3 x 25 mL). The organic layers are combined and dried over anhydrous Na_2SO_4. Purification by flash chromatography affords the silyl ether **2b** (700 mg, 90%) as a white solid. R_f

(TLC) = 0.6 (40% ethyl acetate-petroleum ether). ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.69 (d, *J*=8.25 Hz, IH, H-1), 5.25 (t, *J*=9.24 Hz, IH, H-3), 5.15 (t, *J*=9.24 Hz, 1H, H-4), 5.12 (t, *J*=8.24, 9.25 Hz, IH, H-2), 3.8-3.7 (m, 3H, H-5, H-6,6'), 2.1-2 (m, 12H, OAc), 0.5 (s, 9H, TMS).

6.9.2. α,α-1,1-Dimer of Perbenzylated Glucose (5)

The perbenzylated glucose 1,1-dimer is identified, as follows: R_f (TLC) = 0.8 (25% ethyl acetate-petroleum ether). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.4-7.1 (m, 40H), 5.19 (d, *J*=3.30 Hz, 1H), 5.16 (d, J_{AB}=11.55 Hz, 1H), 5.02 (d, J_{AB}=10.89 Hz, 1H), 4.97 (d, J_{AB}=12.21 Hz, 1H), 4.90 (d, J_{AB}=10.89 Hz, 1H), 4.89 (d, J_{AB}=10.89 Hz, 1H), 4.83 (d, J_{AB}=10.89 Hz, 1H), 4.82 (d, *J*=3.30 Hz, $1H_{an}$), 4.81 (d, J_{AB}=11.54 Hz, 1H), 4.75 (d, J_{AB}=12.21 Hz, 1H), 4.69 (d, J_{AB}=12.21 Hz, 1H), 4.59 (bs, 2H), 4.55 (d, J_{AB}=11.88 Hz, 1H), 4.52 (d, J_{AB}=10.88 Hz, 1H), 4.49 (bs, 2H), 4.34 (d, J_{AB}=12.21 Hz, 1H), 4.21 (m, 1H), 4.15 (t, *J*=9.24, 9.47 Hz, 1H), 3.82 (t, *J*=9.24, 9.9, Hz, 1H), 3.7-3.45 (m, 7H).

6.9.3. Synthesis of 3,4,6-Tri-O-benzyl-2-deoxy-D-glucopyranosyl-(1→6)-1,2,3,4-tetra-O-acetyl-ß-D-glucopyranose (7)

The following procedure is typical of all glycosylation reactions run under acid catalyzed conditions using TfOH: The sulfoxide **6** (140 mg, 0.258 mmol, 1.5 eq) and the nucleophile **2** (69 mg, 0.172 mmol, 1.0 eq.) are premixed and thoroughly dried by "azeotroping" 3 times with distilled toluene. The starting materials are then dissolved in 8 mL of freshly distilled CH_2Cl_2 and added to a 25 mL flame dried flask under argon. The flask is cooled to -78 °C using an

acetone-dry ice bath. Methyl propiolate (230 μL, 2.58 mmol, 15 eq) is added to this solution as a sulfenic acid scavenger. After the solution is stirred at -78 °C for 2 minutes, triflic acid (1.1 μL, 0.0129 mmol, 0.05 eq) is added. The reaction is followed by TLC (25% ethyl acetate-petroleum ether). The reaction mixture is slowly warmed to -30 °C over a period of 1 hour, and then quenched by pouring it into a saturated solution of $NaHCO_3$ (25 mL). The resulting mixture is extracted with CH_2Cl_2 (3 x 15 mL). The organic extracts are combined, dried with anhydrous Na_2SO_4, and concentrated. Flash chromatography (25% ethyl acetate-petroleum ether) provides the disaccharide 7 (116 mg, 88 %) as a white solid. R_f (TLC) = 0.3 (25% ethyl acetate-petroleum ether). ^1H NMR (270 MHz, $CDCl_3$) δ 7.4-7.2 (m, 15H), 5.75 (d, *J*=8.25 Hz, 1H, H-a), 5.32 (t, *J*=9.24 Hz, 1H, H-c), 5.21 (t, J=9.57 Hz, 1H, H-d), 5.20 (t, *J*=8.25, 9.24 Hz, 1H, H-b), 4.99 (d, *J*=2.64 Hz, 1H, H-1), 4.95 (d, JAB=11.21 Hz, IH), 4.71 (bs, 2H), 4.66 (d, J_{AB}= 11.87 Hz, 1H), 4.59 (d, J_{AB}= 11.22 Hz, 1H), 4.55 (d, J_{AB}= 11.88 Hz, 1H), 4.10 (m, 1H, H-e), 3.85-3.50 (m, 7H), 2.36 (dd, *J*=4.94, 12.86 Hz, 1H), 2.15 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 1.95 (s, 3H), 1.70 (m, 1H).

6.9.4. Methyl-6-deoxy-3,4-isopropylidene-2-O-(trimethylsilyl)-ß-D-galactopyranoside (8)

The compound **8** is synthesized from D-fucose by the following three-step sequence: (i) MeOH, H^+; (ii) acetone, H_3PO_4 (cat); (iii) TMSOTf, Et_3N, CH_2Cl_2, -78 °C. Rf (TLC) = 0.5 (15% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) δ 4.03 (d, *J*=8.24 Hz, 1H, H-1), 3.98 (dd, *J*=5.28, 7.26 Hz, 1H), 3.97 (dd, *J*=5.40, 1.98 Hz, 1H), 3.84 (dq, *J*=1.98, 6.6 Hz, 1H, H-5), 3.49 (s, 3H, OCH_3), 3.47 (dd, *J*=7.59, 5.49 Hz, 1H), 1.48 (s, 3H, CH_3), 1.38 (d, *J*=6.6 Hz, 3H, CH_3) 1.31

(s, 3H, CH_3), 0.5 (s, 9H, TMS). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 109.38, 103.70, 80.52, 76.48, 74.45, 68.58, 56.64, 28.02, 26.34, 16.49, 0.31.

6.9.5. 3,4,6-Tri-O-benzyl-2-deoxy-D-glucopyranosyl-α-(1→2)-methyl-6-deoxy-3,4-isopropylidene-ß-D-galactopyranoside (9)

R_f (TLC) = 0.5 (25% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) α anomer δ 7.4-7.15 (m, 15H), 5.30 (d, J=2.97 Hz, 1H), 4.86 (d, J_{AB}=10.89 Hz, 1H), 4.64 (d, J=1.65 Hz, 2H), 4.62 (d, J_{AB}=12.20 Hz, 1H), 4.53 (d, J_{AB}=10.89 Hz, 1H), 4.45 (d, J_{AB}=12.20 Hz, 1H), 4.06 (d, J=8.25 Hz, 1H), 3.70 (t, J=9.56 Hz, 1H), 3.46 (s, OCH_3), 2.02 (ddd, J=0.99, 4.95, 12.87 Hz, 1H), 1.65 (dt, J=3.62, 12.87 Hz, 1H), 1.40 (s, CH_3), 1.36 (d, J=6.6 Hz, 3H), 1.24 (s, 3H). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 138.91, 138.83, 138.32, 128.28, 128.23, 127.92, 127.79, 127.45, 127.42, 127.38, 109.35, 103.95, 97.45, 78.39, 78.20, 77.70, 76.31, 76.12, 74.95, 73.45, 71.73, 70.53, 68.73, 68.48, 56.58, 35.47, 28.08, 26.34, 16.49.

6.9.6. Phenyl-4-O-acetyl-2,3,6-trideoxy-1-sulfinyl-α-L-erythro-hexopyranoside (10)

Compound **10** is synthesized from L-rhamnal in 3 steps. a) H_2 (1 atm), $Pd(OH)_2/C$, C_6H_6; b) $BF_3 \cdot OEt_2$, thiophenol, CH_2Cl_2, -78 °C to -60 °C; and c) mCPBA, CH_2Cl_2, -78 °C to -60 °C. R_f (TLC) = 0.4 (25% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) ß anomer (sulfide) δ 7.5-7.2 (m, 5H), 5.54 (d, J=4.95 Hz, H-4), 4.81 (dd, J=1.98, 12.1 Hz, 1H, H-1), 4.6 (m, 2H, H-3, 3'), 4.3 (m, 1H, H-5), 2.2 (m, 2H, H2,2'), 2.1 (s, 3H, OAc), 1.19 (d, J=5.94 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 67.9 MHz) ß anomer (sulfide) δ 169.80, 130.98, 130.73,

128.58, 128.49, 83.79, 67.15, 29.95, 25.34, 20.82, 18.00, 17.44.

6.9.7. 4-O-Acetyl-2,3,6-trideoxy-L-erythro-hexopyranosyl-α-(1→6)-1,2,3,4-tetra-O-acetyl-ß-D-glucopyranose (11)

R_f (TLC) = 0.3 (25% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) α anomer δ 5.63 (d, J=7.92 Hz, 1H), 5.20 (t, J=9.24 Hz, 1H), 5.19 (t, J=9.24, 9.57 Hz, 1H), 5.09 (d, J=7.91 Hz, 1H), 5.03 (t, J=9.57 Hz, 1H), 4.61 (dd, J=1.98, 8.58 Hz, 1H), 4.33 (ddd, J=4.61, 10.23, 10.23 Hz, 1H), 3.94 (dd, J=3.96, 11.22 Hz, 1H), 3.71 (m, 1H), 3.56 (dd, J=2.96, 11.22 Hz, 1H), 2.05 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.11 (d, J=6.59 Hz, 3H). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 170.19, 170.12, 169.18, 169.15, 168.95, 100.85, 91.79, 73.03, 72.96, 70.34, 68.60, 66.01, 29.54, 26.88, 21.07, 20.76, 20.64, 20.54, 20.50, 18.01, 17.72.

6.9.8. Phenyl-3,4-bis-O-(4-methoxybenzoyl)-1-sulfinyl-D-digitoxose (13)

Compound **13** is synthesized from digitoxose by the following three-step sequence: (i) $pOMeC_6H_4COCl$, pyridine; (ii) thiophenol, $Et_2O{\cdot}BF_3$, CH_2Cl_2; (iii) mCPBA. R_f (TLC) = 0.2 (40% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) α anomer (sulfoxide) δ 8.09 (d, J=8.91 Hz, 2H), 7.87 (d, J=9.24 Hz, 2H), 7.70 (m, 2H), 7.55 (m, 3H), 6.94 (d, J=8.90 Hz, 2H), 6.85 (d, J=8.91 Hz, 2H), 5.84 (q, J=3.31 Hz, 1H), 5.10 (dd, J=2.63, 2.97, 1H), 5.04 (m, 1H), 4.49 (dd, J=1.64, 5.13 Hz, 1H), 3.83 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 1.28 (d, J=5.95 Hz, 3H).

6.9.9. Phenyl-3,4-O-(4-methoxybenzoyl)-ß-D-digitoxosyl-(O)-N-hydroxyethyl carbamate (14)

R_f (TLC) = 0.45 (cc anomer, 40% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) α anomer δ 8.03 (d, *J*=8.91 Hz, 2H), 7.79 (d, *J*=9.24 Hz, 2H), 6.88 (d, *J*=9.24 Hz, 2H), 6.77 (d, *J*=9.01 Hz, 2H), 5.60 (q, 1H), 5.12 (d, *J*=4.62 Hz, 1H), 4.95 (dd, *J*=2.97, 10.23 Hz, 1H), 4.78 (m, 1H), 4.18 (q, 2H), 4.17 (m, 1H), 3.84 (s, OCH_3), 3.79 (s, OCH_3), 2.37 (dd, *J*=3.3, 15.51 Hz, 1H), 2.19 (m, 1H), 1.26 (t, J=6.93 Hz, 3H), 1.25 (d, J=6.27 Hz, 3H). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 165.48, 165.17, 163.53, 163.45, 157.42, 131.97, 131.75, 122.65, 121.92, 113.62, 113.59, 100.69, 100.61, 72.21, 65.90, 63.65, 62.08, 55.40, 32.04, 17.53, 14.44 R_f (TLC) = 0.5 (ß anomer, 40% ethyl acetate-petroleum ether). ^1H NMR ($CDCl_3$, 270 MHz) ß anomer δ 7.92 (d, J=8.91 Hz, 2H), 7.78 (d, *J*=8.91 Hz, 2H), 6.89 (d, *J*=8.91 Hz, 2H), 6.77 (d, *J*=9.24 Hz, 2H), 5.74 (q, 1H), 5.23 (dd, *J*=2.31, 9.23 Hz, 1H), 4.93 (dd, *J*=2.97, 9.23 Hz, 1H), 4.23 (q, 1H), 4.16 (q, 2H), 4.17 (m, 1H), 3.85 (s, OCH_3), 3.79 (s, OCH_3), 2.36 (m, 1H), 2.04 (m, 1H), 1.29 (d, *J*=6.27 Hz, 3H), 1.23 (t, *J*=6.93 Hz, 3H). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 165.10, 164.91, 163.61, 163.55, 131.85, 122.18, 121.83, 113.74, 113.59, 101.77, 72.31, 69.21, 67.41, 62.19, 60.34, 55.44, 55.38, 33.29, 18.01, 14.38.

6.9.10. Phenyl-2,3-O-benzoyl-6-deoxy-1-thio-ß-L-galactopyranoside (15)

The compound **15** is synthesized from L-fucose by the following four-step sequence: (i) Ac_2O, pyridine; (ii) thiophenol, $Et_2O{\cdot}BF_3$; (iii) NaOMe, CH_3OH, amberlite H^+ resin; (iv) C_6H_5COCl, DMAP. R_f (TLC) = 0.6

(40% ethyl acetate-petroleum ether). ^{1}H NMR ($CDCl_3$, 270 MHz) δ 8.0-7.9 (m, 4H), 7.55-7.2 (m, 1H), 5.64 (t, J=9.89 Hz, 1H), 5.27 (dd, J=2.97, 9.9 Hz, 1H), 4.86 (d, J=9.9 Hz, 1H), 4.09 (d, J=2.97 Hz, 1H), 3.87 (q, J=6.27 Hz, 1H), 1.40 (d, J=6.27 Hz, 3H).

6.9.11. Phenyl-3,4-O-acetyl-2,6-dideoxy-1-sulfinyl-L-galactopyranoside (16)

Compound **16** is prepared from 1,3,4-tri-O-acetyl-2-deoxy-α-L-fucose by the following two-step sequence: (i) thiophenol, $Et_2O{\cdot}BF_3$; and (ii) mCPBA. R_f (TLC for a sulfide) = 0.3 (15% ethyl acetate-petroleum ether). ^{1}H NMR ($CDCl_3$, 270 MHz) a sulfide δ 7.5-7.2 (m, 5H), 5.73 (d, J=5.61 Hz, 1H, H-1), 5.29 (m, 1H, H-3), 5.23 (bs, 1H, H-3), 4.56 (q, J=6.6 Hz, 1H, H-5), 2.49 (dt, J=5.94, 12.87 Hz, 1H, H-2_{ax}), 2.38 (s, 3H, OAc), 2.06 (m, 1H, H-2_{eq}), 1.99 (s, 3H, OAc), 1.13 (d, J=6.6 Hz, 3H, CH_3). ^{13}C NMR a sulfide ($CDCl_3$, 67.9 MHz) δ 170.51, 169.87, 159.54, 134.33, 124.54, 114.54, 84.58, 69.69, 67.18, 65.51, 55.22, 20.79, 20.60, 16.34. R_f (TLC for a sulfoxide) = 0.25 (30% ethyl acetatepetroleum ether). ^{1}H NMR α anomer sulfoxide ($CDCl_3$, 270 MHz) δ 7.6-7.3 (m, 5H), 5.43 (m, 1H), 5.26 (bd, J=2.64 Hz, 1H), 4.52 (d, J=5.61 Hz, 1H), 4.32 (q, J=6.6 Hz, 1H), 2.47 (dd, J=5.28, 14.18 Hz, 1H), 2.14 (dt, J=5.61, 14.19 Hz, 1H), 2.10 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.12 (d, J=6.26 Hz, 3H).

6.9.12. 3,4-O-Acetyl-2,6-dideoxy-L-galactopyranosyl-α-(1→4)-phenyl-3,4-O-benzoyl-6-deoxy-1-thio-ß-L-galactopyranoside (17)

R_f (TLC) = 0.4 (30% ethyl acetate-petroleum ether). ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.9 (m, 4H),

7.55-7.25 (m, 1H), 5.55 (t, *J*=10.23 Hz, 1H), 5.20 (dd, *J*=2.96, 10.22 Hz, 1H), 5.12 (m, 1H), 5.02 (bd, *J*=1.98 Hz, 1H), 4.94 (bs, 1H), 4.84 (d, *J*=9.9 Hz, 1H), 4.15 (d, *J*=2.97 Hz, 1H), 3.96 (q, *J*=6.27 Hz, 1H), 3.86 (q, *J*=6.27 Hz, 1H), 2.04 (s, 3H), 2.0 (s, 3H), 1.94 (m, 2H), 1.35 (d, *J*=6.27 Hz, 3H), 0.33 (d, *J*=6.27 Hz, 3H). ^{13}C NMR ($CDCl_3$, 67.9 MHz) δ 170.44, 169.62, 166.05, 164.95, 133.51, 133.36, 133.01, 131.49, 129.78, 129.62, 129.12, 128.65, 128.44, 128.27, 128.18, 99.49, 85.42, 76.86, 75.27, 74.68, 69.56, 67.47, 66.50, 65.27, 29.70, 20.85, 20.56, 17.32, 15.77.

6.10. Synthesis Of A ß-Linked Disaccharide On The Solid Phase

To a 10 mL solution of DMF containing 0.338 g (0.350 mmol) of 2,3,4-tribenzyl-6-tritylgalactose-1-*p*-hydroxy phenythioglycoside cesium acetate (**X**, FIG. 6) is added 0.356 g (0.385 mmol Cl equiv, 1,1 equiv) of Merrifield resin (BACHEM Bioscience). This mixture is agitated with a wrist action shaker for 24 h under argon atmosphere at 75 °C. At this time, the polymer is poured into a tared coarse-fritted Gooch funnel and washed repeatedly with methanol and methylene chloride. The funnel is then dried for 4 h in a lyophilizer jar at 20 milliTorr. A mass change of 0.244 g is recorded, which is calculated to be 85% chemical yield with respect to the cesium salt.

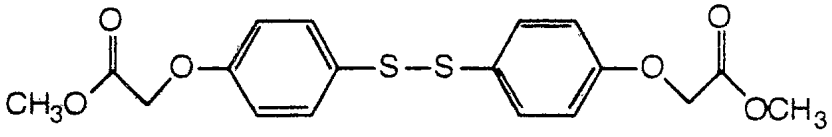
The polymer in the coarse-fritted Gooch funnel is then treated by vacuum filtration at moderate flow rate with 40 mL of 10% trifluoroacetic acid (TFA) in methylene chloride until no yellow color is apparent in the filtrate. (The TFA is used to remove the trityl protecting groups.) The polymer is next washed repeatedly with methanol and methylene chloride. The funnel, together with the resin-linked nucleophile, is then dried for 4 h in a lyophilizer jar at 20 milliTorr before "massing". A mass change of 0.065 g is

subsequently measured, which is calculated to be 83% chemical yield with respect to the cesium salt. The concentration of the resin-linked nucleophile (**resin-X**, FIG. 6) is then calculated to be 0.544 mmol/g.

200 mg (i.e., 0.11 mmol) of derivatized resin is lyophilized overnight in the reaction vessel and then purged for 1 hour with argon. The resin is then suspended in 5 mL CH_2Cl_2. 4 equivalents (0.44 mmol) of 2-pivaloyl-3,4-benzyl-6-*p*-methoxy benzyl galactosyl phenyl sulfoxide (**Y**, FIG.6) and 6 equivalents (0.66 mmol) 2,6-di-t-butyl-4-methyl pyridine are dissolved in 5 mL methylene chloride and added by canula to the reactor vessel. The mixture is agitated gently by argon flow for 30 minutes at room temperature and then the reactor vessel is immersed in a cold bath and allowed to cool to -60 °C. 2 equivalents (0.22 mmol) of triflic anhydride diluted one hundred fold (v/v) in methylene chloride are added slowly (over 15 minutes) to the reaction vessel. The resulting reaction mixture is gently agitated for 1 hour.

After the reaction is completed, as indicated by the Hg(II) hydrolysis method and TLC analysis, the solvent and unbound reagents are then drained from the reactor vessel, and the resin mixture is rinsed repeatedly with methanol followed by methylene chloride. Subsequently, the resin mixture is suspended in 15 mL of methylene chloride and then treated with excess $Hg(OCOCF_3)_2$ for 8 hours to cleave the glycosidic linkage to the resin. (Note: only 5 minutes is required to remove sufficient product from the resin to monitor the reaction by TLC analysis.) The solvent is allowed to drain from the resin. Additional solvent is then used to rinse the resin. The filtrates are then combined, extracted three times with water and concentrated by evaporation. The desired ß-linked disaccharide is obtained by flash chromatography on silica gel. No α-linked disaccharide is isolated from the reaction.

Thiosugar **X** in FIG. 6 is prepared from the readily available 1,6-anhydroglucose by treatment with benzyl bromide followed by acidic hydrolysis (H_2SO_4-THF-H_2O), tritylation (trityl chloride-pyridine) of the more reactive C6 primary alcohol, and treatment of the resulting lactol with disulfide **XX** and tri-n-butylphosphine (i.e., standard procedure for making thiophenyl glycosides from lactols). The disulfide **XX** is produced by reacting the disulfide of the readily available 4-hydroxythiophenol with α-bromo-methyl acetate.



Disulfide XX

Sulfoxide **Y** in FIG. 6 is prepared from readily available penta-acetylated galactose using the following sequence of reagents: (1) BF_3/etherate-ethiophenol; (2) hydroxide; (3) acetone-H^+; (4) *p*-methoxy benzyl bromide-sodium hydride; (5) pivaloyl chloride; (6) mCPBA. Each step is well known in the art and the reactions are carried out under the standard conditions. (*See*, list of "Standard References" below in Section 6.15 the disclosures of which are incorporated by reference herein.).

The disaccharide produced from the reaction of **X** and **Y** is subjected to methanolysis (to remove it from the resin) and is characterized by ^1H NMR spectroscope. The relevant data include: ($CDCl_3$) 5.58 ppm (d, J=5.3 Hz, H1 of thiosugar), 5.47 ppm (dd, J=7.9, 10.2 Hz, H2 of C2 pivaloylated sugar), 4.45 ppm (d, partially overlapped, H1 of C2 pivaloylated sugar).

6.11. Synthesis Of An α-Linked Disaccharide On The Solid Phase

The sodium salt of a glycosyl acceptor (**X**, FIG. 7) is attached to the Merrifield resin by the standard method (DMF, 80 °C, 24 h). Following the coupling and rinsing (as described in Example 6.10), the resin is lyophilized and weighed. Loading is calculated at 0.52 mmol/g from the mass gain.

200 mg (i.e., 0.1 mmol) of derivatized resin is lyophilized overnight in the reaction vessel (FIG. 7) and then purged for 1 hour with argon. The resin is then suspended in 5 mL methylene chloride. 4 equivalents (0.4 mmol) of perbenzylated fucosyl sulfoxide **Y** and 6 equivalents of 2,6-di-t-butyl-4-methyl pyridine are dissolved in 5 mL methylene chloride and added by syringe to the reactor vessel. The mixture is agitated gently by argon flow for 30 minutes at room temperature and then the reactor vessel is immersed in a cold bath and allowed to cool to -60 °C. 2 equivalents (0.22 mmol) of triflic anhydride diluted one hundred fold in methylene chloride are added slowly by syringe (over 15 minutes) to the reaction vessel. The reaction is gently agitated for 1 hour. The solvent and unbound reagents are then drained from the reactor vessel, and the resin mixture is rinsed repeatedly with methanol followed by methylene chloride. Subsequently the resin mixture is suspended in 15 mL of methylene chloride and then treated with excess $Hg(OCOCF_3)_2$ for 8 hours to cleave the glycosidic linkage to the resin (only 5 minutes is required to remove sufficient product from the resin to monitor the reaction by TLC analysis). The solvent is allowed to drain from the resin as before. The resin is then rinsed with additional solvent and the filtrates combined, extracted three times with water, and concentrated by evaporation. Flash chromatography on silica gel gives only the desired α-linked disaccharide.

Thiosugar **X** in FIG. 7 is prepared from the readily available corresponding glucosamine by treatment with the following reagents: (1) phthalic anhydride; (2) acetic anhydride; (3) tetrachlorotin-4-hydroxy thiophenol; (4) hydroxide; (5) benzaldehyde-H^+; (6) NaH, under conditions that are standard in the art. (*See*, Section 6.15, below).

Sulfoxide **Y** in FIG. 7 is made from peracetylated fucose by treating the starting material sequentially with BF_3/etherate-thiophenol, followed by hydroxide, followed by benzyl bromide, and then with mCPBA. All these steps are standard and well known in the art.

The disaccharide produced from the reaction of **X** and **Y** following treatment with $Hg(OCOCF_3)_2$ (to remove it from the resin) is characterized by ^1H NMR. Relevant data: $CDCl_3$) 5.6 ppm (apparent t, J=7.6 Hz, H1 of C2 phthalimido sugar), 3.35 ppm (d, J=7.6 Hz, lactol OH, i.e., of phthalimido sugar after hydrolytic removal from resin w/ Hg(II), 4.9 ppm (d, J=2.8 Hz, H1 of fucose derivative).

6.12. Solid Phase Synthesis Of Lewis X Trisaccharide

The sodium salt of a glycosyl acceptor (**X**, FIG. 8) is attached to the Merrifield resin using the standard method (DMF, 80 °C, 24 h). After using the general linking procedure described in detail in Example 6.10, the anhydrous resin is suspended in 5 mL methylene chloride. 4 eq. 2,3,4,6-pivaloylated galactosyl sulfoxide **Y** and 6 eq. base (as above) is dissolved in 5 mL methylene chloride. The reagent solution is then added to the resin, and the reaction mixture is cooled to -60 °C. 2 equivalents of triflic anhydride diluted one hundred fold (v/v) in methylene chloride are then added.

After 30 minutes, the resin is "drained" and rinsed repeatedly with methylene chloride and methanol. The resin is then suspended in 5 mL methylene chloride and cooled to 0 °C. 5 mL of a 1:2 solution of trifluoroacetic acid/methylene chloride is then added, and the resin is agitated gently for 5 hours. The resin is then "drained" and rinsed repeatedly with methylene chloride and methanol. Following a final rinse with anhydrous methylene chloride, the resin is suspended in 5 mL of anhydrous methylene chloride. 4 equivalents of 2,3,4-triethylsilyl fucosyl sulfoxide **Z** and 6 equivalents of hindered base (i.e., that used above) are dissolved in 5 mL methylene chloride and added to the resin. The reaction mixture is cooled to -60 °C. 2 equivalents of triflic anhydride are diluted 100 fold in methylene chloride and added slowly by syringe to the reaction. After agitating gently for 30 minutes, the resin is "drained" and rinsed. The trisaccharide is then removed and isolated from the resin using $Hg(OCOCF_3)_2$, as described above in Example 6.10.

Thiosugar **X** in FIG. 8, is prepared from the readily available glucosamine by treatment with (1) phthalic anhydride; (2) acetic anhydride; (3) tetrachlorotin-4-hydroxy thiophenol; (4) benzyl bromide; (5) hydroxide; (6) benzaldehyde-H^+; (7) chloromethyl methyl ether (MOM chloride); (8) H_20/H^+; (9) pivalyoyl chloride; (10) hydrogenation, $Pd(OH)_2$; (11) NaH. Again, all steps are standard including the conditions for the deprotection of the benzyl protecting group on the 4-hydroxy thiophenyl glycoside, which conditions are typical for debenzylation. In the debenzylation step, no cleavage of the sugar to sulfur bond is observed.

Sulfoxide **Y** in FIG. 8 is prepared by treating perpivaloylated galactose with BF_3/etherate-thiophenol, followed by mCPBA.

Sulfoxide **Z** in FIG. 8 is prepared by treating peracetylated fucose with BF_3/etherate-thiophenol,

followed sequentially by hydroxide, triethylsilyl chloride, and mCPBA.

Similarly, other Lewis blood group sugars are synthesized readily, including, but not limited to, Lewis A and Lewis B.

6.13. Additional Experiments Conducted in the Solid Phase

Referring to FIG. 16, in particular, cesium fluoride (157.2 mg, 1.03 mmol, 1.1 eq.) and Merrifield resin (1 mol. eq. Cl per gram) (1.0975 g, 1.10 mol, 1.1 eq.) are added to a solution of freshly prepared **1** (803.5 mg, 0.94 mmol, 1.0 eq.) in DMF (20 mL). The resulting suspension is shaken mechanically under an argon atmosphere at 60 °C for 24 h. The sugar-derivatized resin is then isolated by vacuum filtration and washed with DMF (3 x 10 mL), methanol (3 x 10 mL) and CH_2Cl_2 (5 x 10 mL) and then dried under vacuum overnight to give resin **2** (1.4738 g, calculated based on mass gain, or 0.34 mmol/g). IR (KBr disc) 1734 cm^{-1} (υ, C=O).

Resin **2** is then washed with 10% trifluoroacetic acid in CH_2Cl_2 (20 mL) to remove the protecting group. Subsequently, the deprotected resin **3** is washed with CH_2Cl_2 (10 x 10 mL) and dried under vacuum overnight. IR (KBr disc) 1734 cm^{-1} (υ, C=O), 3430 cm^{-1} (br, υ, O-H).

To obtain resin **5**, **3** (95.4 mg) is added to a solution of the sulfoxide **4** (122.1 mg, 0.156 mmol, 1.0 eq.) and 2,6-di-tert-butyl-4-methyl-pyridine (98.2 mg, 0.468 mmol, 3.0 eq.) in CH_2Cl_2 (7.0 mL) and the suspension cooled to -78 °C. After 10 mins, triflic anhydride (13.1 μL, 0.078 mmol, 0.5 eq.) in CH_2Cl_2 (5.0 mL) is added dropwise over a period of 20 mins. Ten minutes after the addition is complete, the reaction is

warmed to -60 °C over a period of 20 mins. The reaction is quenched by the addition of saturated $NaHCO_3$. The resin is collected by suction filtration and washed with methanol (2 x 10 mL), water (1 x 10 mL), methanol (1 x 10 mL), and then CH_2Cl_2 (10 x 10 mL). The product resin **5** is then dried under vacuum overnight, after which the glycosylation is repeated.

The product resin **6** is obtained as follows: resin **5** (112.7 mg) is washed with 10% trifluoroacetic acid in CH_2Cl_2 (20 mL). It is then washed with CH_2Cl_2 (10 x 10 mL) and dried under vacuum overnight.

Then resin **7** is obtained from resin **6** as follows: resin **6** (110.7 mg) is added to a solution of sulfoxide **4** (123.5 mg, 0.158 mmol, 1.0 eq.) and 2,6-di-*tert*-butyl-4-methyl-pyridine (99.3 mg, 0.474 mmol, 3.0 eq.) in CH_2Cl_2 (7.0 mL) and the suspension cooled to -78 °C. After 10 mins, triflic anhydride (13.3 μL, 0.079 mmol, 0.5 eq.) in CH_2Cl_2 (5.0 mL) is added dropwise over a period of 20 mins. Ten minutes after the addition is complete, the reaction is warmed to -60 °C over a period of 20 mins. The reaction is quenched by the addition of saturated $NaHCO_3$. The resin is isolated by filtration and washed with methanol (2 x 10 mL), water (1 x 10 mL), methanol (1 x 10 mL), and then CH_2Cl_2 (10 x 10 mL). The product resin **7** is dried under vacuum overnight, after which the glycosylation is repeated.

Finally, compound **8** is obtained from resin **7** as follows: resin **7** (113.6 mg) is washed with 10% trifluoroacetic acid in CH_2Cl_2 (20 mL). It is then washed with CH_2Cl_2 (10 x 10 mL) and suspended in pyridine (10 mL). Acetic anhydride (183.7 μL, 1.932 mmol, 1.0 eq.) and 4-dimethylaminopyridine (4.7 mg, 0.039 mmol, 0.02 eq.) are added to the suspension under argon. The mixture is stirred overnight and then quenched by the addition of methanol (200 μL). The resin is isolated by filtration and washed with methanol

(2 x 10 mL) and CH_2Cl_2 (10 x 10 mL). The free compound **8** is released from the resin by treatment with bis(trifluoroacetato)mercury(II) in wet methylene chloride, as described below, for example, for the synthesis of disaccharide **13**. (Compound **8**) ^1H NMR (500 MHz, $CDCl_3$) δ 4.42 (d, J = 8.06 Hz, H_1"), 4.49 (d, J = 8.06 Hz, H_1'), 6.27 (d, J = 3.67 Hz, H_1). ^{13}C NMR (69 MHz, $CDCl_3$) δ 91.6, 101.0, 101.3 (anomeric carbon atoms).

6.14. Solid Phase Synthesis of Disaccharides 13 and 16

According to the scheme of FIG. 17, the Pht-protected N-acetylglucosamine residue 9 is attached to a solid support, as follows: Merrifield resin (1.4075 g, 1% cross-linked, chloromethylated styrene/divinylbenzene copolymer, approximately 1 mmol Cl/g, Aldrich) is added to a solution of freshly prepared **9** (1.42 g, 2.693 mmol) in DMF (20 mL). The suspension is shaken mechanically at 60 °C under argon for 24 h. The sugar-derivatized resin is isolated by filtration, washed with DMF (5 x 10 mL), methanol (3 x 10 mL) and CH_2Cl_2 (10 x 10 mL), and dried under vacuum overnight to give 1.7466 g of resin-attached **10**. The level of loading is calculated to be 0.414 mmol/g (based on mass gain). IR (KBr) 1670, 1716 cm^{-1} (υ, C=O); 3470 cm^{-1} (υ, C=O).

Resin **10** is coupled to sulfoxide **11**, a fucose unit, to give **12** (α-glycosidic linkage), as follows: Compound **10** (218.4 mg) is added to a solution of sulfoxide **11** (204.1 mg, 0.3766 mmol, 1.0 eq.) and 2,6-di-tert-butylpyridine (236.7 mg, 1.1297 mmol, 3.0 eq.) in CH_2Cl_2 (7 mL). The suspension is cooled to -60 °C and, after 10 mins, a solution of triflic anhydride (31.7 μL, 0.1883 mmol, 0.5 eq.) in CH_2Cl_2 (5 mL) is added dropwise over a period of 20 mins. Ten minutes

after the addition is complete, the reaction is slowly warmed to -30 °C and, after 30 mins, quenched by the addition of saturated aqueous sodium bicarbonate. The resin is isolated by filtration, washed with methanol (2 x 10 mL), water (2 x 10 mL), methanol (10 mL) and CH_2Cl_2 (10 x 10 mL), and dried under vacuum overnight to give **12**.

The final product **13** is obtained as follows: $Hg(OCOCF_3)_2$ (120.5 mg, 0.2824 mmol) and water (a few drops) are added to a suspension of **12** (222.0 mg) in CH_2Cl_2 (20 mL). After 5 h the mixture is filtered through cotton. The filtrate is washed with saturated sodium bicarbonate, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue is purified by flash chromatography (35% EtOAC-pet. ether) to give 33.9 mg of **13** (59% yield) as a colorless solid. This solid could be purified further by RP-HPLC (C-18 column, 60 to 98% MeCN in water over 30 mins), R_T 15.7 mins. ^1H NMR (500 MHz, $CDCl_3$) δ 4.81 (d, J = 3.66 Hz, H1 of fucose unit), 5.59 (d, J = 8.42 Hz, H1 of glucosamine unit). ^{13}C NMR (69 MHz, $CDCl_3$) δ 81.9, 93.3, 101.2 (anomeric carbon atoms).

Similarly, the resin **10** can be coupled to a galactose unit, **14**, to provide resin **15** (ß-glycosidic linkage), as follows: Compound **10** (184.0 mg, 0.414 meq./g) is added to a solution of sulfoxide **14** (208.0 mg, 0.325 mmol) and 2,6, di-tert-butylpyridine (204.3 mg, 0.975 mmol) in CH_2Cl_2 (7 mL). The suspension is cooled to -60 °C and, after 10 mins, a solution of triflic anhydride (31.7 µL, 0.1883 mmol, 0.5 eq.) in CH_2Cl_2 (5 mL) is added dropwise over a period of 20 mins. Ten minutes after the addition is complete, the reaction is slowly warmed to -30 °C and, after 30 mins, quenched by the addition of saturated aqueous sodium bicarbonate. The resin is isolated by filtration, washed with methanol (2 x 10 mL), water (2 x 10 mL),

methanol (10 mL) and CH_2Cl_2 (10 x 10 mL), and dried under vacuum overnight to give **15**. To obtain best results, the glycosylation procedure is repeated twice.

The product is removed from the resin using the Hg(II) reagent. $Hg(OCOCF_3)_2$ (325.0 mg, 0.2824 mmol) and water (a few drops) are added to a suspension of **15** (222.0 mg) in CH_2Cl_2 (20 mL). After 5 h the mixture is filtered through cotton. The filtrate is washed with saturated sodium bicarbonate, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue is purified by flash chromatography (35% EtOAC-pet. ether) to give 19.4 mg of **16** (28% yield). This product could be purified further by RP-HPLC (C-18 column, 60-95% MeCN in H_2O over 30 mins), R_T = 26.1 mins. ^1H NMR (500 MHz, $CDCl_3$) δ 4.69 (d, J = 7.69 Hz, H_1 of galactose residue), 5.32 (dd, J = 7.32, 8.8 Hz, H_1 of glucosamine residue). ^{13}C NMR (69 MHz, $CDCl_3$) δ 93.5, 98.8, 101.7 (anomeric carbons).

6.15. Standard References

Most of the transformations mentioned above (protection: benzylation, benzylidenation, acetonation, esterification, and carbo- or silylethentication of sugars; deprotection: debenzylation, acidic hydrolysis of benzylidenes or acetonates, basic hydrolysis of esters, removal of silyl groups with fluoride or under acidic conditions) are described in Binkley, R. W. *Modern Carbohydrate Chemistry*, Marcel Dekker, Inc.: New York, 1988. Methods to convert lactols or anomeric esters or anomeric esters to thiophenyl groups (to produce thiophenyl glycosides) are well known. See, e.g., Ferrier et al. Carbohydr. Res. **1973**, *27*, 55; Mukaiyama et al. Chem. Lett. **1979**, **487**; Van Cleve Carbohydr. Res. **1979**, *70*, 161; Hanessian et al. *Carbohydr. Res.* **1980**, *80*, C17; Garegg et al. *Carbohydr.*

Res. **1983**, *116*, 162; and Nicolaou et al. *J. Am. Chem. Soc.* **1983**, *105*, 2430.

From the principles established herein, it should be apparent to one of ordinary skill in the art that the ability to manipulate the reactivity of both glycosyl donors and glycosyl acceptors to control the order in which glycosylation takes place can be exploited to synthesize many other oligosaccharides or glycoconjugates rapidly, efficiently and in high yield, under either homogeneous (in solution) or heterogeous (in the solid phase) conditions.

WHAT IS CLAIMED IS:

1. A method of forming multiple regioselective glycosidic linkages in a single step comprising:

(a) treating a bifunctional first glycoside (FG) in an organic solvent with an effective amount of an activating agent (AG), said FG having (i) an anomeric sulfoxide substituent, and (ii) glycosyl accepting and glycosyl donating characteristics that are made manifest in the presence of the AG; and

(b) allowing multiple glycosylation reactions to proceed such that two or more glycosidic bonds linking three or more glycosides are formed regioselectively in a single step.

2. The method of claim 1 in which said FG is treated with the AG in the presence of a second glycoside (SG) different from said FG, said SG being either a glycosyl acceptor or a glycosyl donor.

3. The method of claim 2 in which said SG is a glycosyl donor.

4. The method of claim 2 in which said SG is a glycosyl acceptor.

5. The method of claim 4 in which said three or more glycosides linked by two or more glycosidic bonds include the sequence SG-$(FG)_n$ in which n is an integer greater than or equal to 2.

6. The method of claim 1 or 5 in which more than one type of FG is present during the reaction, such that proportion of said three or more glycosides linked by two or more glycosidic bonds are comprised of different glycosidic residues.

7. The method of claim 2 in which said FG is treated with the AG in the presence of a third glycoside (TG) different from said FG and said SG, said TG being either a glycosyl donor when said SG is a glycosyl acceptor or a glycosyl acceptor when said SG is a glycosyl donor.

8. The method of claim 7 in which said TG is a glycosyl donor.

9. The method of claim 7 in which said TG is a glycosyl acceptor.

10. The method of claim 1, 2, 6, or 7 in which about half an equivalent or less of the AG is used for each equivalent of FG present.

11. The method of claim 10 in which a catalytic amount of the AG is used.

12. The method of claim 7 in which said FG, SG and TG are present in molar ratios in the range of about 1 to 20 : about 1 to 20 : about 1 to 20, respectively.

13. The method of claim 7 in which said three or more glycosides include the sequence TG-$(FG)_n$-SG or SG-$(FG)_n$-TG in which n is an integer greater than or equal to 1.

14. The method of claim 13 in which more than one type of FG is present during the reaction, such that said FG in either of said sequence is comprised of different glycosidic residues.

15. The method of claim 1, 2, 6, or 7 in which the organic solvent is substantially anhydrous.

16. The method of claim 15 in which the organic solvent is selected from the group consisting of a nonpolar aromatic solvent, a cyclic aliphatic ether, an acyclic aliphatic ether, a halogenated aliphatic hydrocarbon, or an aliphatic nitrile.

17. The method of claim 10 in which triflic anhydride is used as the AG together with an effective amount of an acid scavenger.

18. The method of claim 11 in which triflic acid is used as the AG together with an effective amount of a sulfenic acid scavenger.

19. The method of claim 1 in which said FG derives its potential glycosyl accepting characteristics from a nucleophilic group that is free or, if blocked, can be deprotected in situ.

20. The method of claim 19 in which said nucleophilic group is a hydroxyl group.

21. The method of claim 19 in which said nucleophilic group is an amino group.

22. The method of claim 19 in which said nucleophilic group is a sulfuryl group.

23. The method of claim 4 or 9 in which said SG or TG is linked at the anomeric carbon to a biologically active molecule.

24. The method of claim 23 in which said biologically active molecule is selected from the group

consisting of steroids, peptides, lipids, and polycyclic aromatic compounds.

25. The method of claim 23 in which said biologically active molecule interacts with polynucleotides.

26. The method of claim 1 in which said FG is comprised of 2-10 monosaccharide units.

27. A method of forming a glycosidic linkage on a solid phase comprising:

(a) exposing, in an organic solvent, a glycosyl acceptor (GA) bound to a solid support to a glycoside (G) having an activated anomeric sulfoxide group, said GA having glycosyl accepting characteristics and said G having glycosyl donating characteristics; and

(b) allowing a glycosylation reaction to proceed such that a glycosidic bond is formed which links said GA to the anomeric carbon of said G.

28. A method of forming a 1,2-*trans* glycosidic linkage on a solid phase comprising:

(a) exposing, in an organic solvent, a glycosyl acceptor (GA) bound to a solid support to a glycoside (G) having an activated anomeric sulfoxide group and a neighboring participating group (NPG) at C-2, said GA having glycosyl accepting characteristics and said G having glycosyl donating characteristics; and

(b) allowing a glycosylation reaction to proceed such that a glycosidic bond is formed which links said GA to the anomeric carbon of said G and which bond is *trans* to said NPG.

29. The method of claim 28 in which said NPG is a pivaloyl group.

30. A method of forming a disaccharide on a solid phase comprising:

(a) exposing, in an organic solvent, a first glycoside (1G) bound to a solid support to a second glycoside (2G) having an activated anomeric sulfoxide group, said 1G having glycosyl accepting characteristics and said 2G having glycosyl donating characteristics;

(b) allowing a glycosylation reaction to proceed such that a glycosidic bond is formed, which links said 1G to the anomeric carbon of said 2G, to provide a disaccharide that is bound to the solid support and has a sequence of 1G-2G.

31. The method of claim 30 which is carried out in the presence of an effective amount of an acid scavenger or a sulfenic acid scavenger.

32. The method of claim 30 in which said 2G possesses a nucleophilic group blocked by a temporal protecting group (TPG).

33. The method of claim 32 which further comprises:

(c) removing said TPG to uncover the glycosyl accepting characteristics of said 2G;

(d) exposing the unprotected support-bound disaccharide to a third glycoside (3G) having an activated anomeric sulfoxide group, said 3G having glycosyl donating characteristics;

(e) allowing a glycosylation reaction to proceed such that a glycosidic bond is formed, which links said 2G to the anomeric carbon of said 3G, to provide an oligosaccharide that is bound to the solid support and has a sequence of 1G-2G-3G.

34. The method of claim 33 in which said 3G possesses a nucleophilic group blocked by a temporal protecting group (TPG) and the steps (c), (d), and (e)

are repeated, except that a fourth glycoside is introduced, to provide an oligosaccharide that is bound to the solid support and has a sequence of 1G-2G-3G-4G.

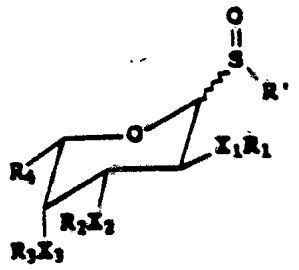
35. The method of claim 34 in which subsequent glycosides are introduced to the growing oligosaccharide chain.

36. The method of claim 33 which further comprises intermediate washing steps.

37. The method of claim 30, 33, 34, or 35 in which more than one type of 1G, 2G, 3G, 4G, or subsequent glycosides are used to provide a glycosidic library comprising a mixture of various oligosaccharides.

38. A chemical composition comprising:

(i) a glycoside of the formula (I), having potential glycosyl accepting and glycosyl donating characteristics,



(I)

in which X_1, X_2, or X_3 independently can be a hydrogen, nitrogen, oxygen, or sulfur atom but not all hydrogen; R' and R_4 can be an aromatic or aliphatic group comprising 1-25 carbon atoms; R_1, R_2, or R_3 independently can each be an H, aromatic or aliphatic group comprising 1-25 carbon atoms, except that when X_1, X_2, or X_3 is H, then the

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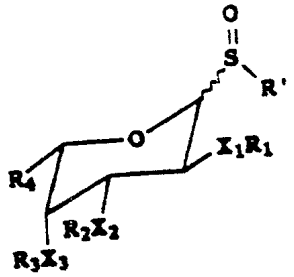
corresponding R_1, R_2, or R_3 is not present; said aromatic or aliphatic groups of R', R_1, R_2, R_3, or R_4, optionally, further comprising nitrogen, oxygen, phosphorus, silicon, or sulfur atoms; and

(ii) an activating agent capable of eliciting the manifestation of both the glycosyl accepting and glycosyl donating characteristics of said glycoside.

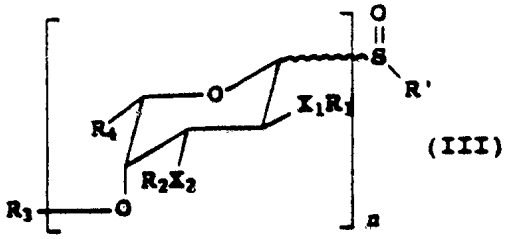
39. A glycoside of the formula (II), having potential glycosyl accepting and glycosyl donating characteristics,



(II)

in which X_1, X_2, or X_3 independently can be a hydrogen, nitrogen, oxygen, or sulfur atom but not all hydrogen; R' and R_4 can be an aromatic or aliphatic group comprising 1-25 carbon atoms; R_1, R_2, or R_3 independently can each be an H, aromatic or aliphatic group comprising 1-25 carbon atoms, except that when X_1, X_2, or X_3 is H, then the corresponding R_1, R_2, or R_3 is not present; said aromatic or aliphatic groups of R', R_1, R_2, R_3, or R_4, optionally, further comprising nitrogen, oxygen, phosphorus, silicon, or sulfur atoms; and provided that when R_3 is not H, the R_3-X_3 bond is susceptible to bond scission under the same conditions used to activate the anomeric sulfoxide group, thus allowing for the manifestation of both the glycosyl accepting and glycosyl donating characteristics of said glycoside.

40. A compound of the formula (III), having potential glycosyl accepting and glycosyl donating characteristics,



(III)

in which *n* can be an integer greater than zero; X_1 or X_2 independently can be a hydrogen, nitrogen, oxygen, or sulfur atom; R' and R_4 can be an aromatic or aliphatic group comprising 1-25 carbon atoms; R_1, R_2, or R_3 independently can each be an H, aromatic or aliphatic group comprising 1-25 carbon atoms, except that when X_1 or X_2 is H, then the corresponding R_1 or R_2 is not present; said aromatic or aliphatic groups of R', R_1, R_2, R_3, or R_4, optionally, further comprising nitrogen, oxygen, phosphorus, silicon, or sulfur atoms; and further provided that when R_3 is not H, the R_3-O bond is susceptible to bond scission under the same conditions used to activate the anomeric sulfoxide group, thus allowing for the manifestation of both the glycosyl accepting and glycosyl donating characteristics of said glycoside.

41. A glycosidic library comprising a mixture of oligosaccharides formed by the method of claim 1, 2, 5, 6, 13, 14, 26, or 35.

42. The glycosidic library of claim 41 in which said mixture of oligosaccharides includes polymers of varying lengths substantially comprised of the same glycoside units.

43. The glycosidic library of claim 41 in which said mixture of oligosaccharides includes a heteropolymer comprised of different glycoside units.

44. The glycosidic library of claim 43 in which said mixture further includes heteropolymers of varying lengths.

45. The method of claim 2 or 7 in which more than one type of SG or TG is present during the reaction.

46. The method of claim 30, 33, 34 or 35 in which said 1G, 2G, 3G, 4G, or subsequent glycosides are comprises of mono-, di-, or polysaccharide residues.

47. A catalytic method of forming a glycosidic linkage comprising:

(a) providing a mixture comprising a glycosyl donor (GD), bearing an anomeric sulfoxide group, and a glycosyl acceptor (GA), having glycosyl accepting characteristics, in an organic solvent under anhydrous conditions;

(b) cooling said mixture below ambient temperature;

(c) adding to said mixture a catalytic amount of an activating agent (AG) effective to activate said anomeric sulfoxide group of said GD;

(d) allowing a catalytic reaction to proceed under conditions effective to provide the formation of a glycosidic linkage between said GD and said GA.

48. The method of claim 47 in which said mixture further comprises a sulfenic acid scavenging agent (SASA), said SASA effective to sequester any sulfenic acid formed in the course of said reaction.

49. The method of claim 47 in which said cooling step includes cooling said mixture to a temperature below -30 °C.

50. The method of claim 47 in which said cooling step includes cooling said mixture to -78 °C.

51. The method of claim 50 in which the conditions of step (d) include allowing the reaction mixture to warm above -78 °C.

52. The method of claim 47 in which said mixture includes a GD that comprises a 2-deoxy GD.

53. The method of claim 47 in which said mixture includes a GA that bears a nucleophilic group.

54. The method of claim 53 in which said nucleophilic group comprises an unprotected hydroxyl group.

55. The method of claim 53 in which said nucleophilic group comprises a blocked hydroxyl group.

56. The method of claim 55 in which the blocked hydroxyl group is blocked by a silyl protecting group.

57. The method of claim 47 in which said AG comprises triflic acid.

58. The method of claim 47 in which said SASA comprises excess methyl propiolate.

59. A catalytic method of forming a glycosidic linkage comprising:

(a) providing a mixture comprising a glycosyl donor (GD), bearing an anomeric sulfoxide

group, and a glycosyl acceptor (GA), having glycosyl accepting characteristics, in an organic solvent under anhydrous conditions;

(b) cooling said mixture below -30 °C;

(c) adding to said mixture a catalytic amount of an activating agent (AG), comprising triflic acid, effective to activate said anomeric sulfoxide group of said GD;

(d) allowing a catalytic reaction to proceed under conditions effective to provide the formation of a glycosidic linkage between said GD and said GA.

60. The method of claim 59 in which said mixture further comprises excess sulfenic acid scavenging agent (SASA), said excess SASA effective to sequester any sulfenic acid formed in the course of said reaction.

61. The method of claim 59 in which said mixture includes a GA that bears a nucleophilic group.

62. A catalytic method of forming a glycosidic linkage comprising:

(a) providing a mixture comprising a glycosyl donor (GD), bearing an anomeric sulfoxide group, and a glycosyl acceptor (GA), bearing a nucleophilic group blocked by a silyl protecting group, in an organic solvent under anhydrous conditions;

(b) cooling said mixture to about -78 °C;

(c) adding to said mixture a catalytic amount of an activating agent (AG), comprising triflic acid, effective to activate said anomeric sulfoxide group of said GD;

(d) allowing a catalytic reaction to proceed under conditions effective to provide the formation of a glycosidic linkage between said GD and said GA.

63. The method of claim 47 in which said mixture includes a GA and a GD dissolved in the organic solvent.

64. The method of claim 47 in which said mixture includes a GA or GD bound to a solid resin.

65. The method of claim 47 in which said mixture includes a GD dissolved in the organic solvent and a GA bound to a solid resin.

66. The method of claim 47 in which said mixture includes a GD present in an equimolar amount relative to a GA.

67. The method of claim 47 in which said mixture includes a GD present in an amount that is about one-and-a-half molar equivalents relative to a GA.

68. The method of claim 47 in which said mixture includes an AG present in an amount that is about 0.05 molar equivalents relative to a GA.

69. The method of claim 47 in which said mixture, further includes a SASA present in an amount that is about twenty molar equivalents relative to a GA.

70. The method of claim 47 in which the organic solvent comprises methylene chloride.

71. The method of claim 59 in which said mixture includes a GD that comprises a 2-deoxy GD.

72. The method of claim 62 in which said mixture includes a GD that comprises a 2-deoxy GD.

73. A library of glycosidic compounds comprising a mixture of oligosaccharides of varying

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sequence or length, each oligosaccharide of said mixture having at least one glycosidic linkage that is obtained through the activation and condensation of a glycosyl donor bearing an anomeric sulfoxide group on a solid phase.

74. The library of claim 73 in which said mixture comprises oligosaccharides of the formula $A-(X)_n-B$, in which n is an integer ≥ 0, A represents a first terminal glycosidic residue, B represents a second terminal glycosidic residue, and X represents a glycosidic residue intermediate between A and B.

75. The library of claim 74 in which n is an integer ≥ 1.

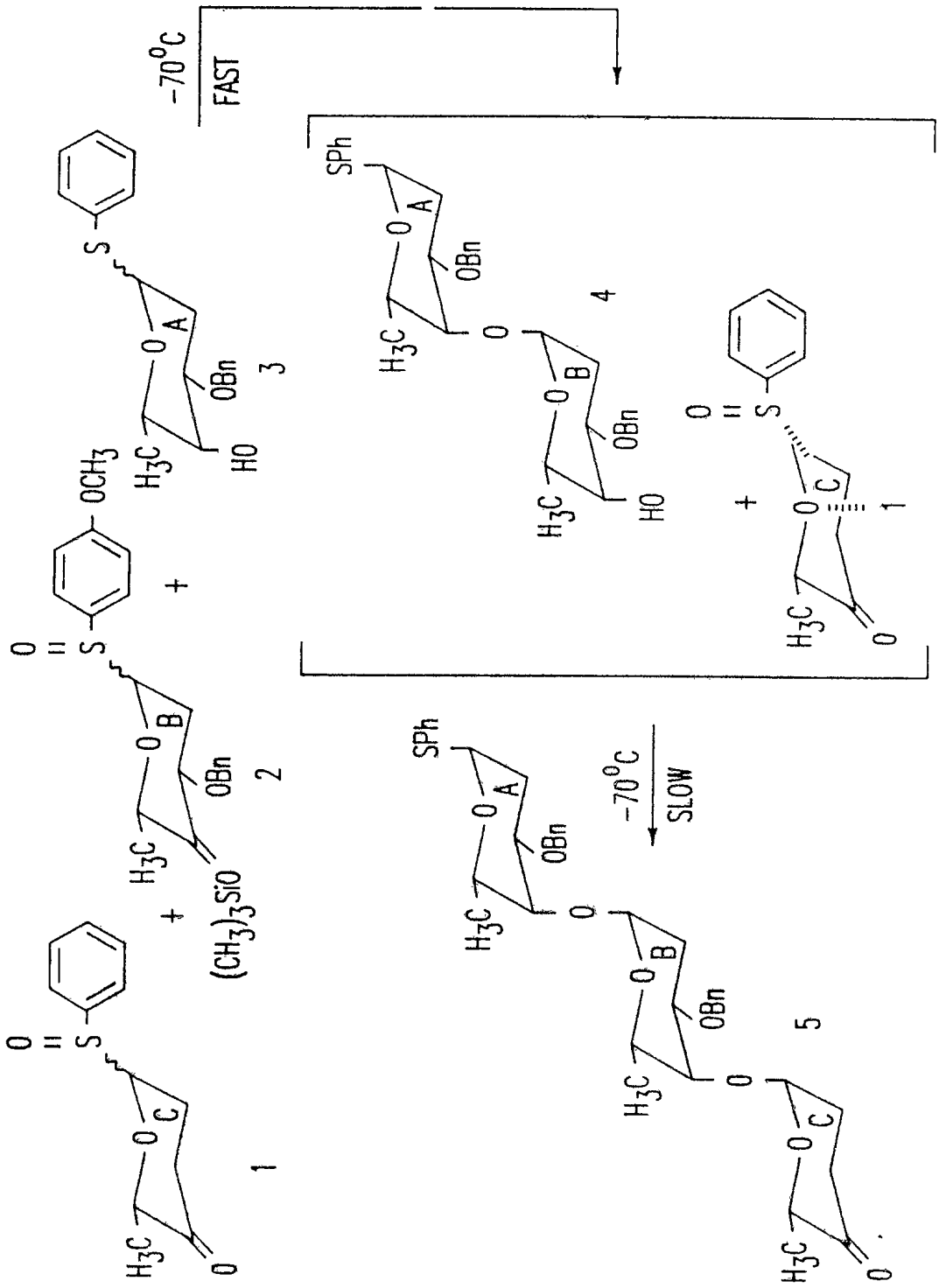
76. The library of claim 75 in which X represents different intermediate glycosidic residues.

77. The library of claim 73, 74, 75 or 76 in which said mixture comprises glycoconjugates of said oligosaccharides.

78. An oligosaccharide comprising three or more glycosidic residues prepared by the method of claim 1, 27, 28 or 30.

79. The oligosaccharide of claim 78 which is a Lewis blood group sugar.

80. The oligosaccharide of claim 79 which is selected from the group consisting of Lewis X, Lewis A or Lewis B.



SCHEME 1 PREMIXED 1(3.0eq), 2(2.0eq) AND 3(1.0eq), Et_2O–CH_2Cl_2(1:1), HC=CCOOCH$_3$(20eq), TfOH(0.05eq), -78°C TO -70°C, 45MIN, THEN QUENCHED WITH SAT. $NaHCO_3$ SOLUTION.

FIG. 1

FIG. 2

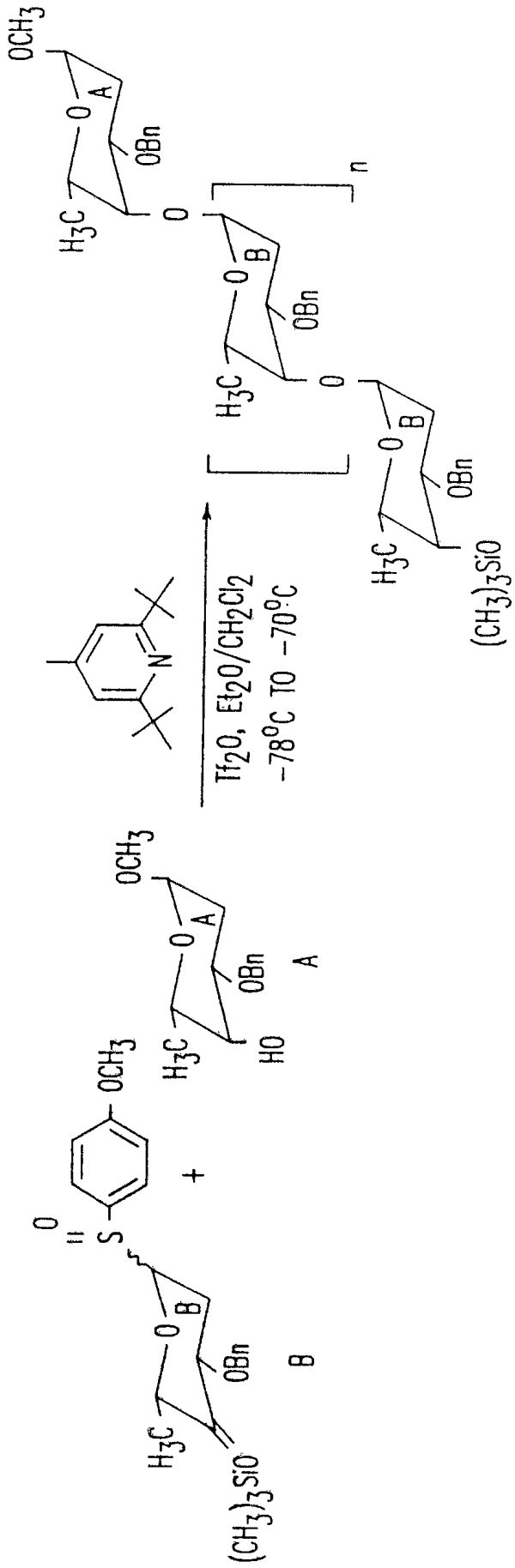
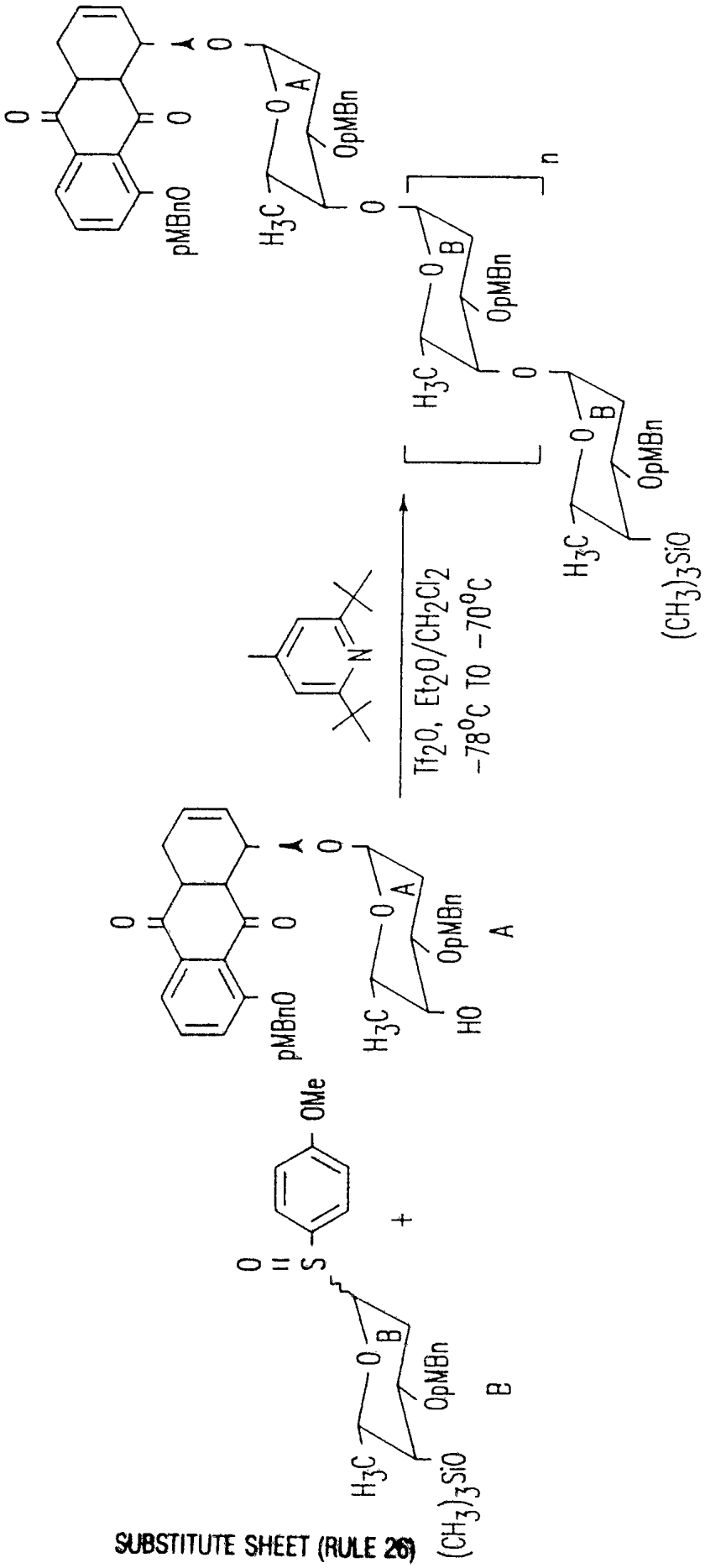


FIG. 3A


pMBnO
OMe
OpMBn
H_3C
$(CH_3)_3SiO$
HO
A
B
Tf_2O, Et_2O/CH_2Cl_2
-78^oC TO -70^oC
N
n

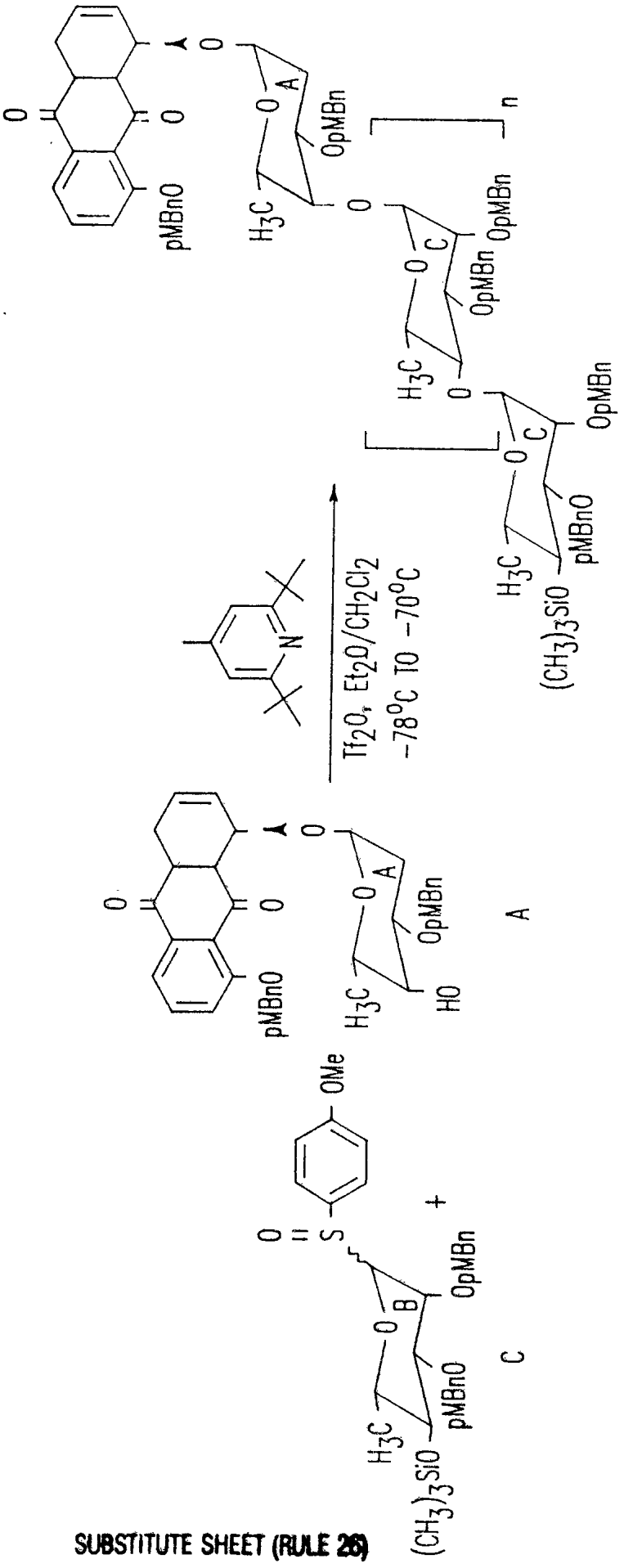

pMBnO
H3C
OpMBn
n
Tf2O, Et2O/CH2Cl2
-78°C TO -70°C
HO
A
OMe
S
B
(CH3)3SiO
C

FIG. 3B

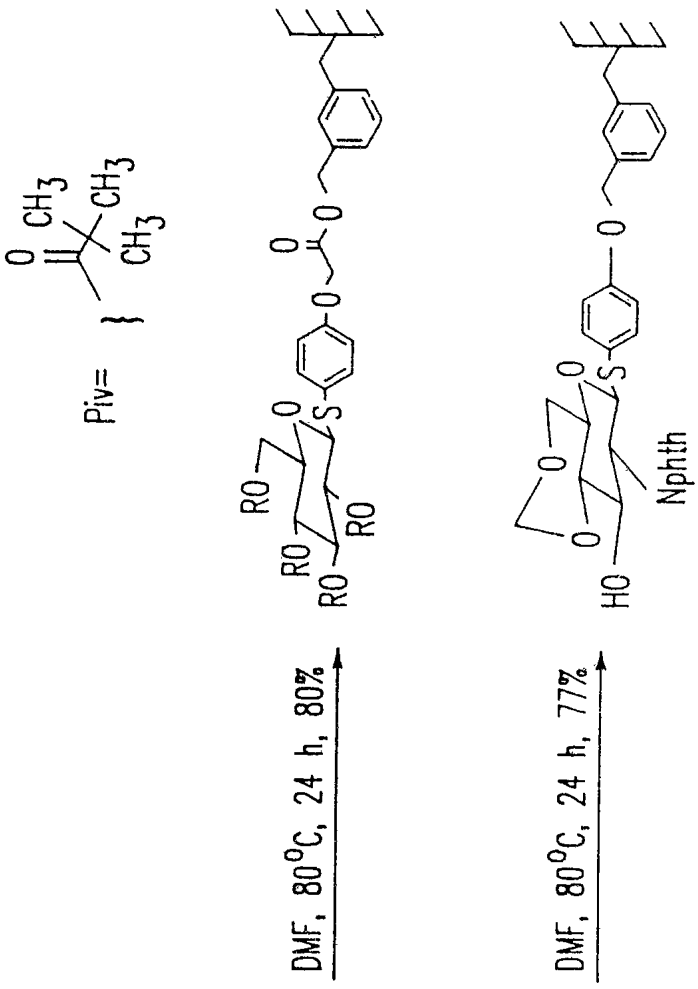
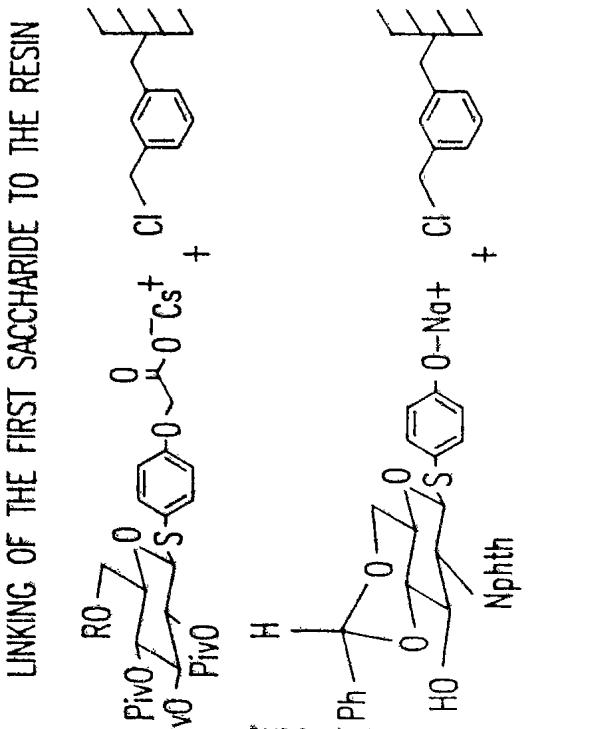

Piv=
DMF, 80°C, 24 h, 80%
DMF, 80°C, 24 h, 77%
Nphth
Nphth

FIG. 4A


LINKING OF THE FIRST SACCHARIDE TO THE RESIN
Nphth

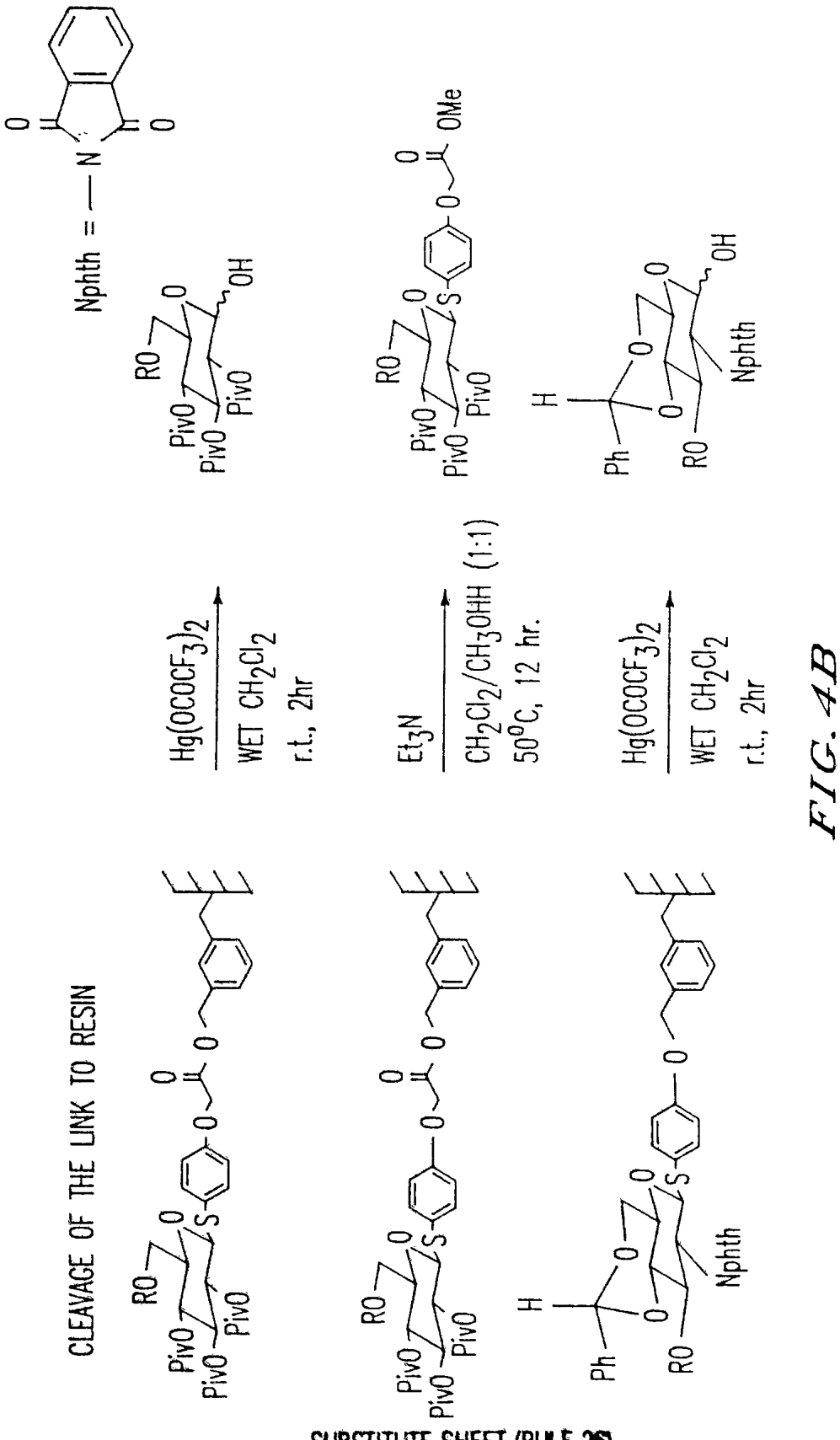

Nphth =
Hg(OCOCF3)2
WET CH2Cl2
r.t., 2hr
Et3N
CH2Cl2/CH3OHH (1:1)
50°C, 12 hr.
Hg(OCOCF3)2
WET CH2Cl2
r.t., 2hr
CLEAVAGE OF THE LINK TO RESIN

FIG. 4B

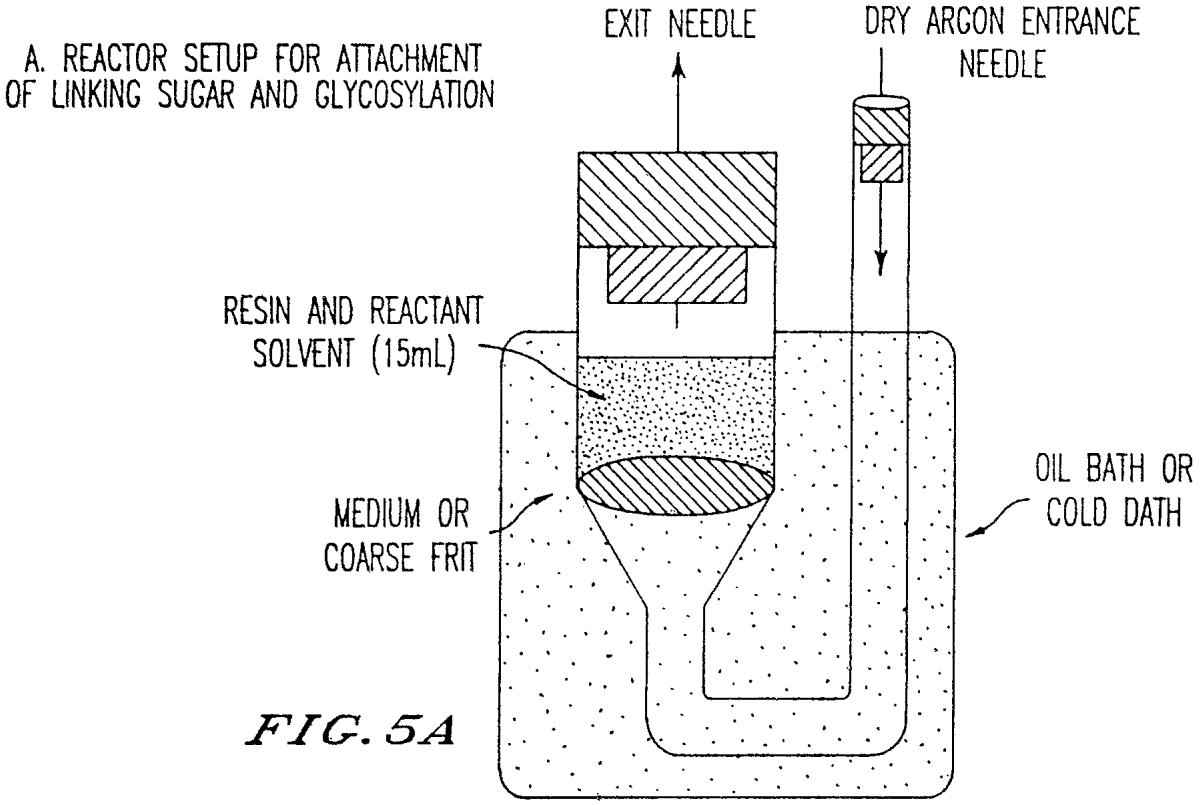

A. REACTOR SETUP FOR ATTACHMENT OF LINKING SUGAR AND GLYCOSYLATION
EXIT NEEDLE
DRY ARGON ENTRANCE NEEDLE
RESIN AND REACTANT SOLVENT (15mL)
MEDIUM OR COARSE FRIT
OIL BATH OR COLD DATH

FIG. 5A

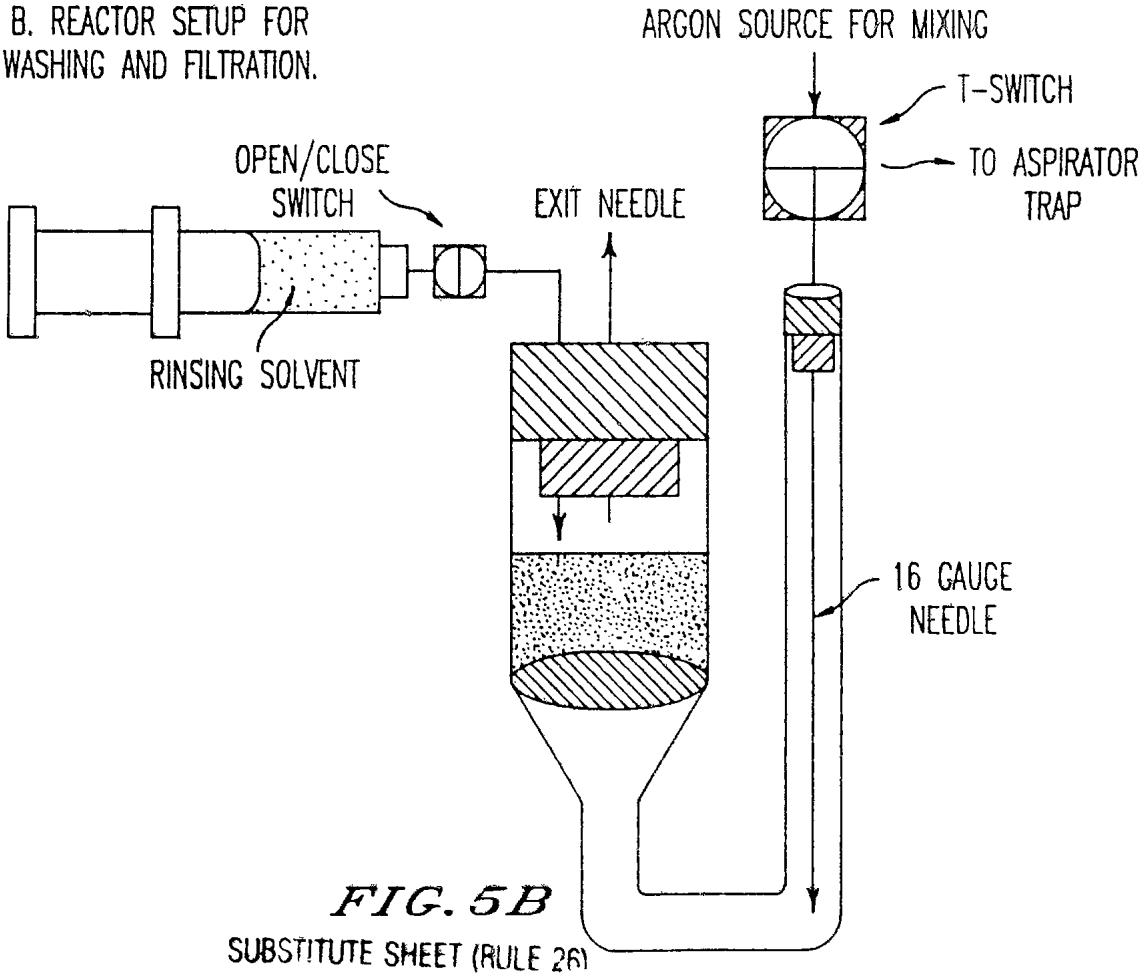

B. REACTOR SETUP FOR WASHING AND FILTRATION.
ARGON SOURCE FOR MIXING
T-SWITCH
TO ASPIRATOR TRAP
OPEN/CLOSE SWITCH
EXIT NEEDLE
RINSING SOLVENT
16 GAUGE NEEDLE

FIG. 5B

FIG. 6

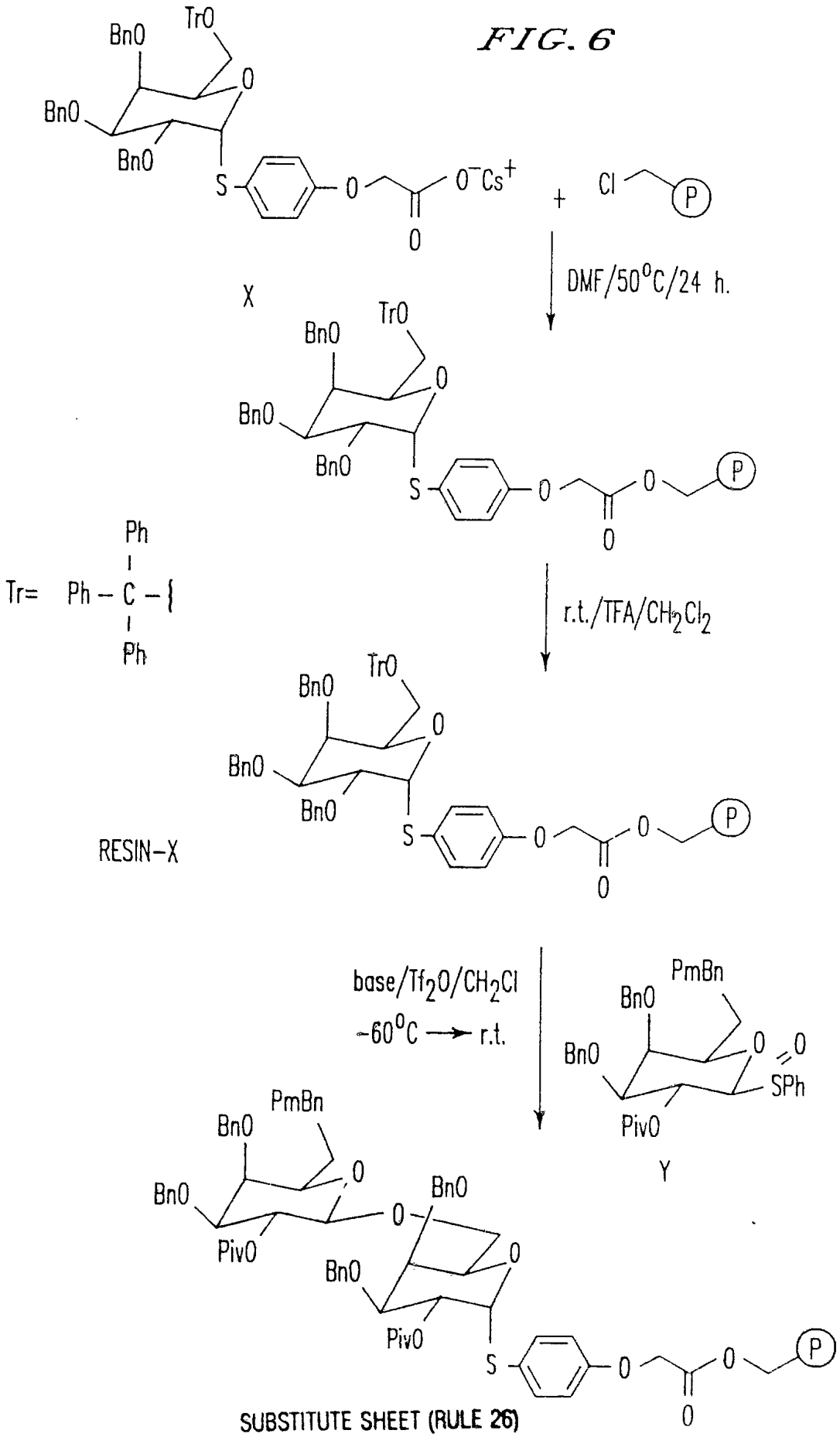
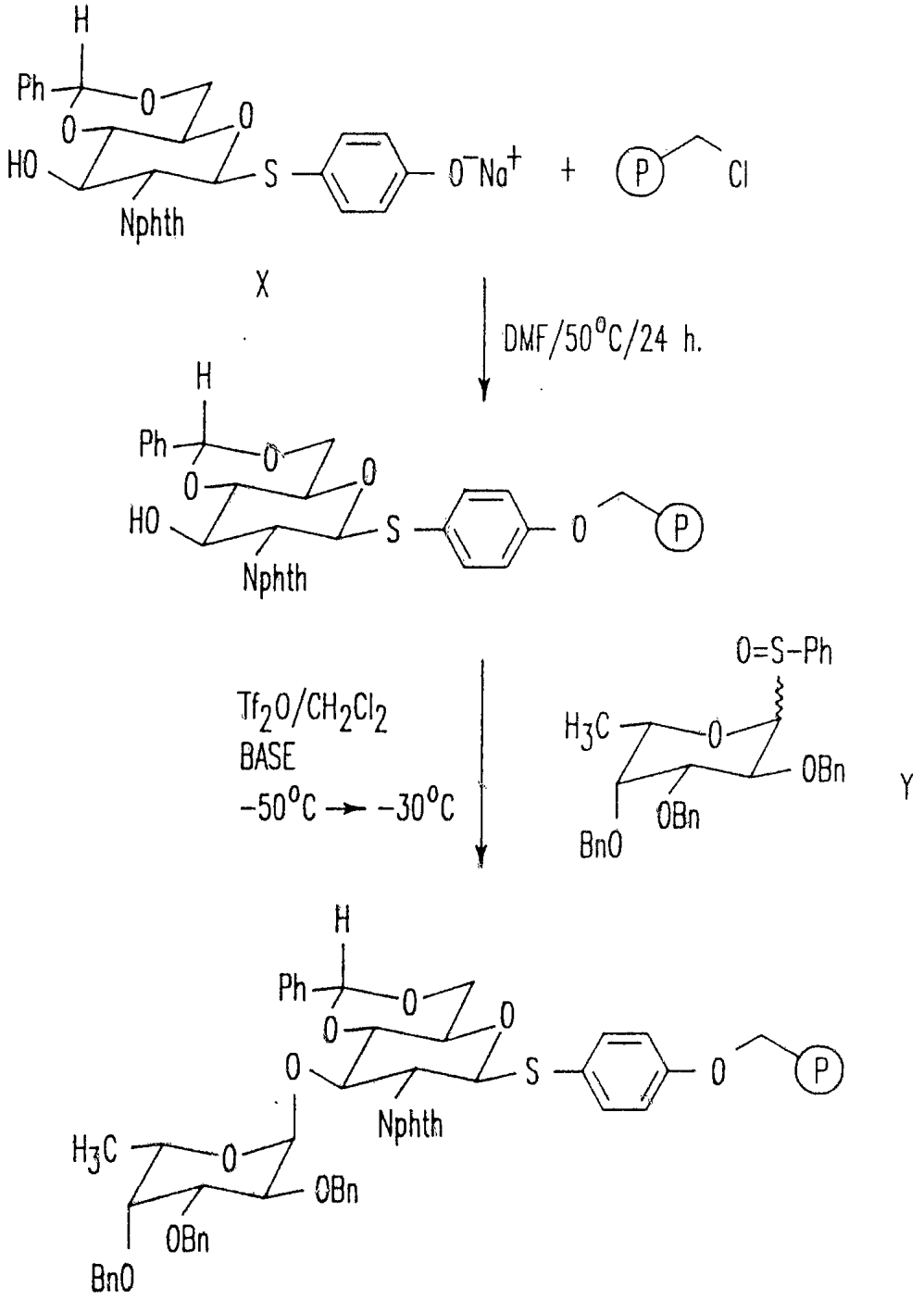

BnO
TrO
O
BnO
BnO
S
O
O⁻Cs⁺
O
+
Cl
P
DMF/50°C/24 h.
X
Tr=
Ph
Ph – C –
Ph
r.t./TFA/CH2Cl2
RESIN–X
base/Tf2O/CH2Cl
–60°C → r.t.
PmBn
SPh
PivO
Y

FIG. 7


H
Ph
O
O
O
HO
S
O⁻Na⁺
+
P
Cl
Nphth
X
DMF/50°C/24 h.
H
Ph
O
O
O
HO
S
O
P
Nphth
Tf2O/CH2Cl2
BASE
-50°C → -30°C
O=S-Ph
H3C
O
OBn
OBn
BnO
Y
H
Ph
O
O
O
O
S
O
P
Nphth
H3C
O
OBn
OBn
BnO

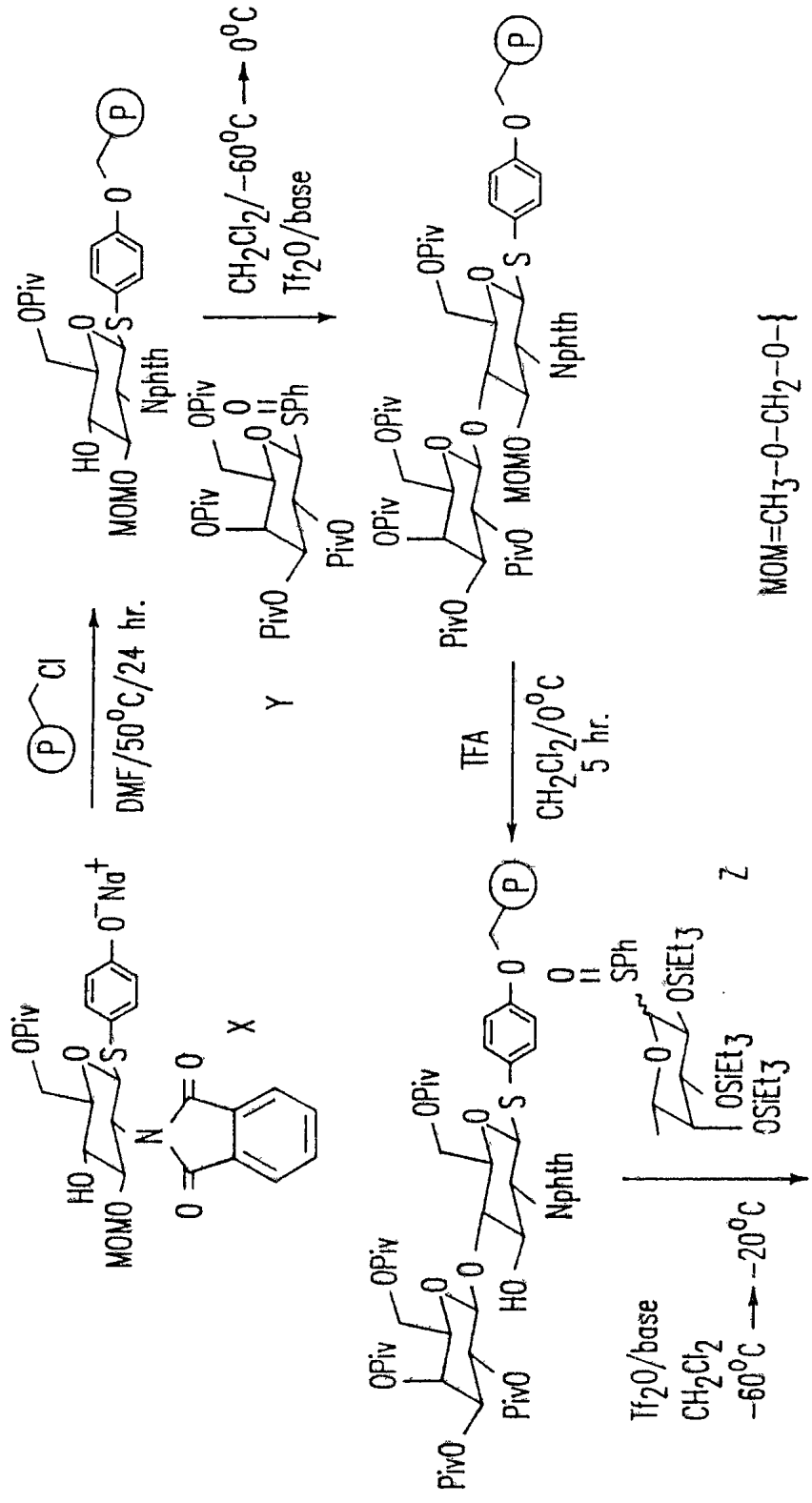


FIG. 8

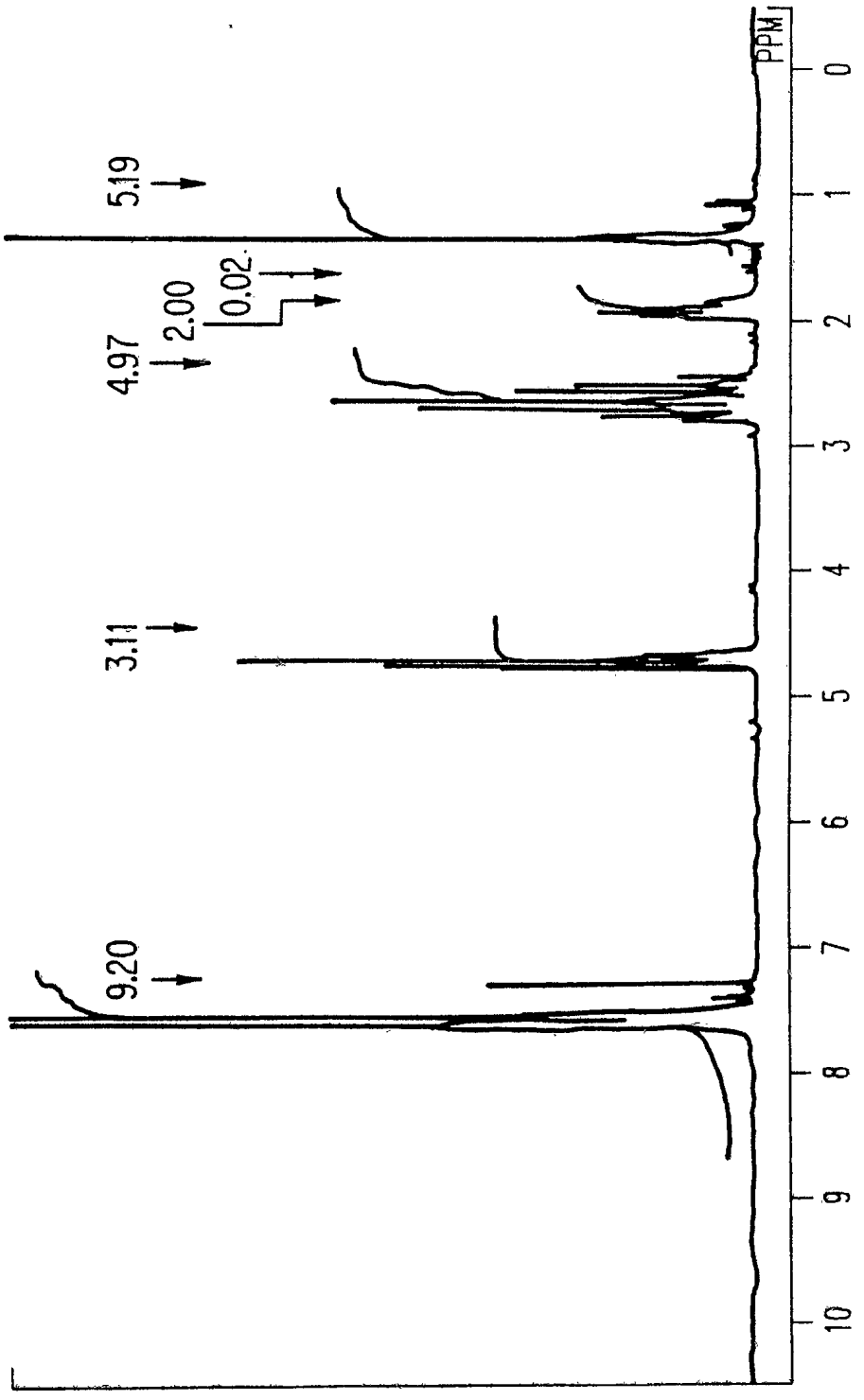
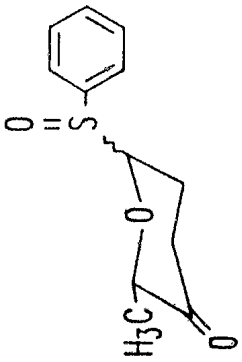


FIG. 9



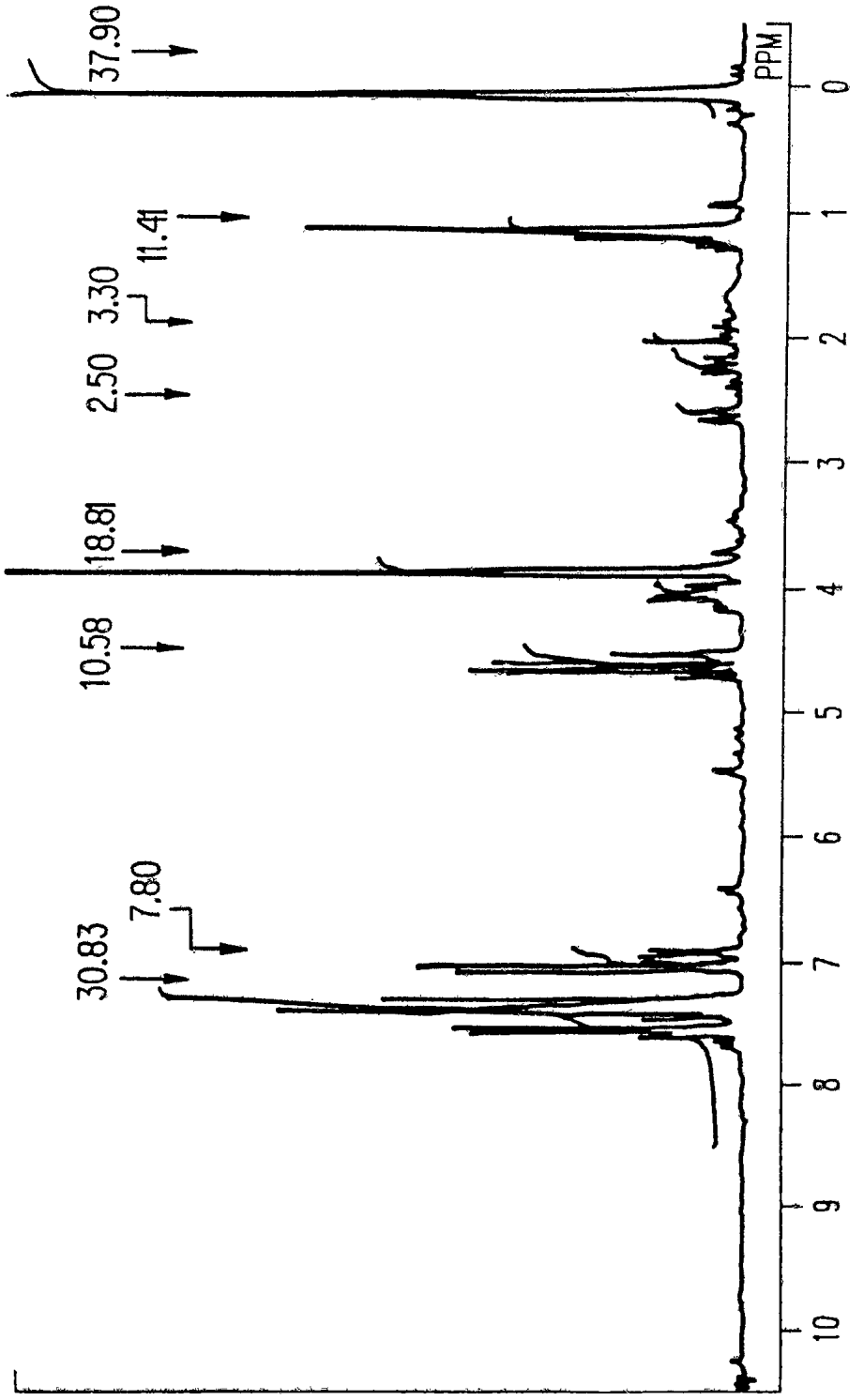

37.90
11.41
3.30
2.50
18.81
10.58
7.80
30.83
PPM
0
1
2
3
4
5
6
7
8
9
10
OCH3
O
S
O
H3C
OBn
(CH3)3SiO

FIG. 10

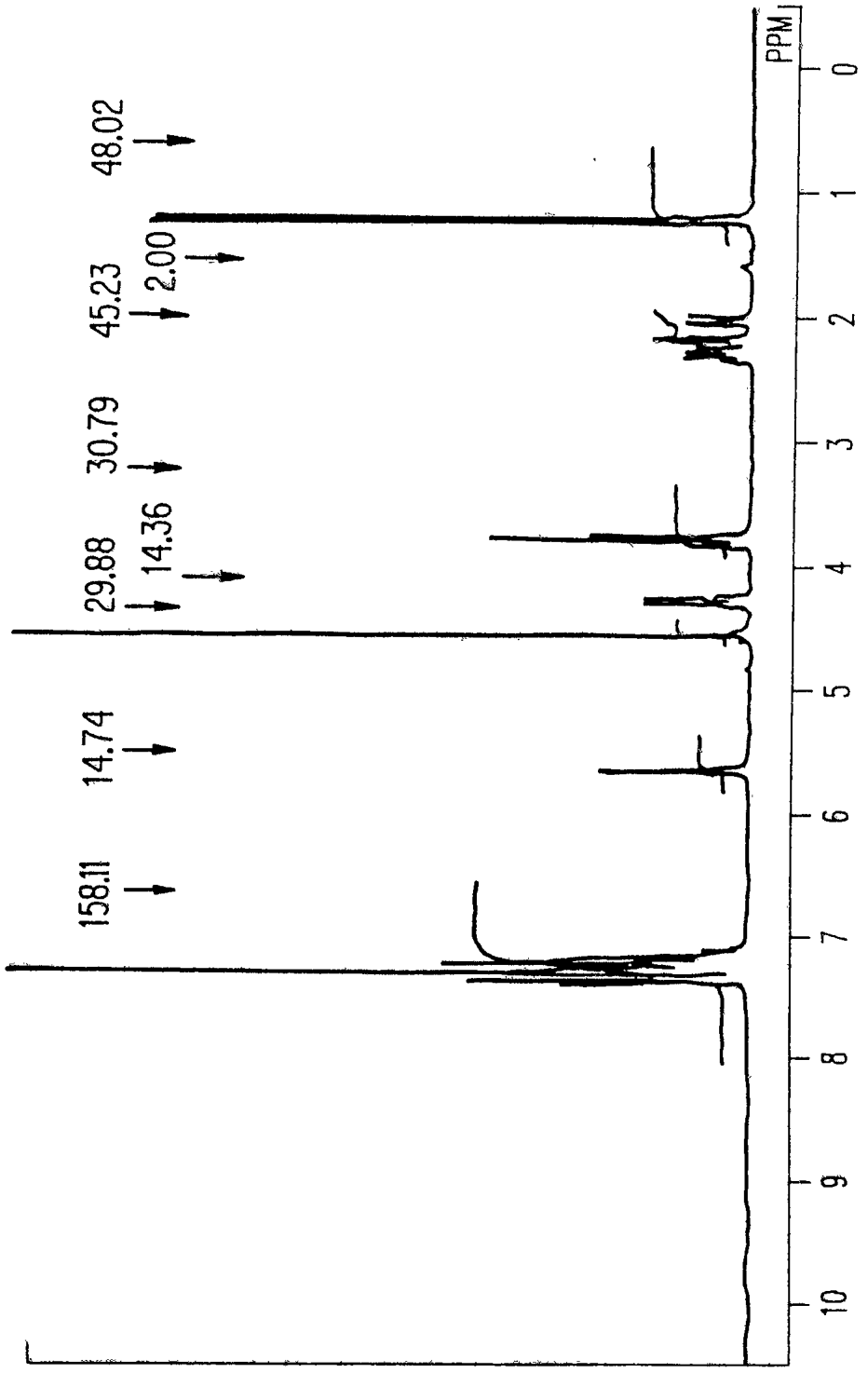
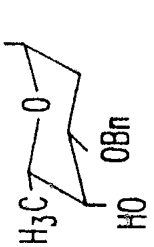

PPM
0
1
2
3
4
5
6
7
8
9
10
48.02
2.00
45.23
30.79
14.36
29.88
14.74
158.11

FIG. 11


SPh
O
H3C
OBn
HO

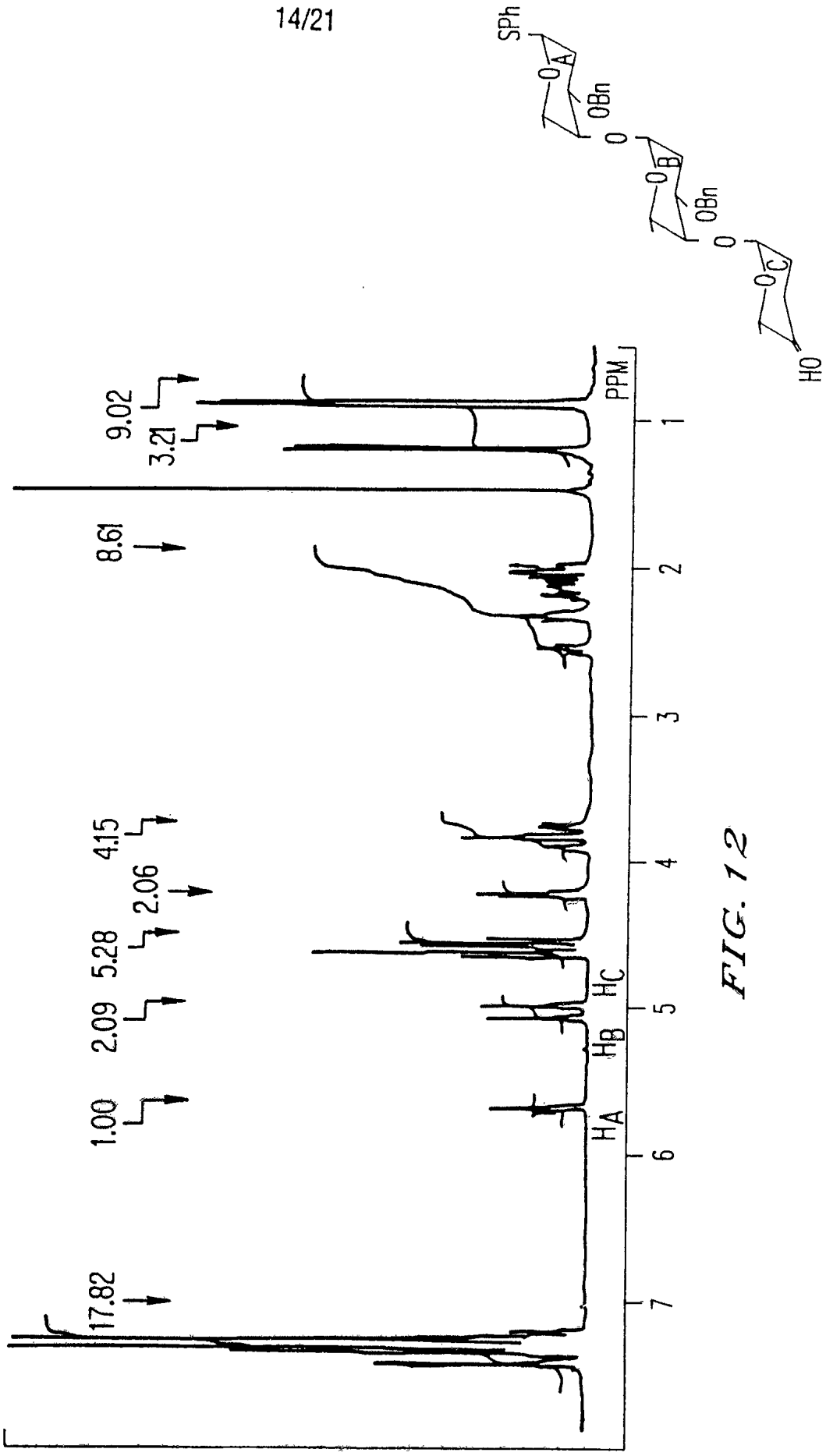

SPh
OA
OBn
O
OB
OBn
O
OC
HO
9.02
3.21
8.61
4.15
2.06
5.28
2.09
1.00
17.82
HA
HB
HC
PPM
1
2
3
4
5
6
7

FIG. 12

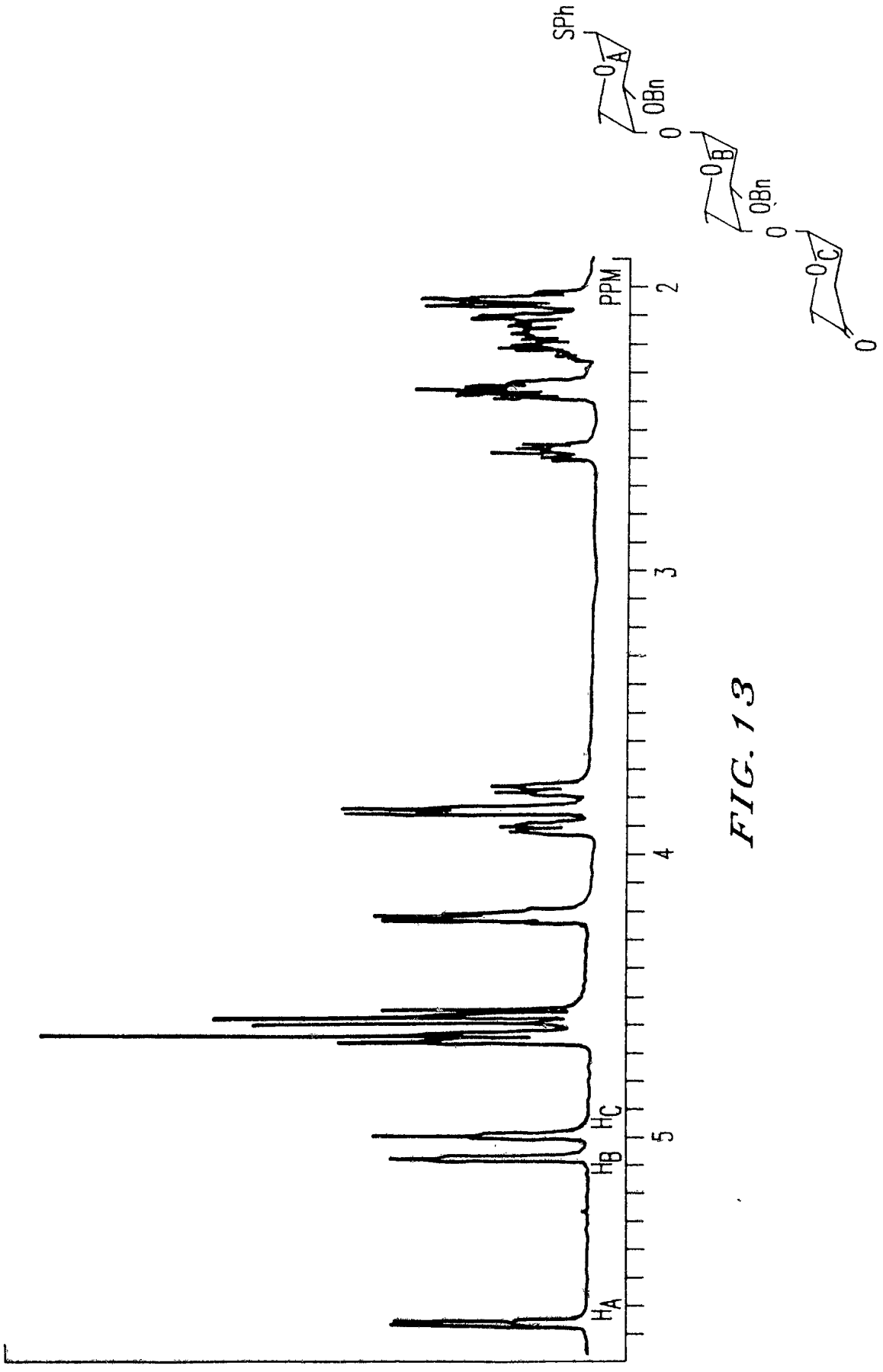


FIG. 13

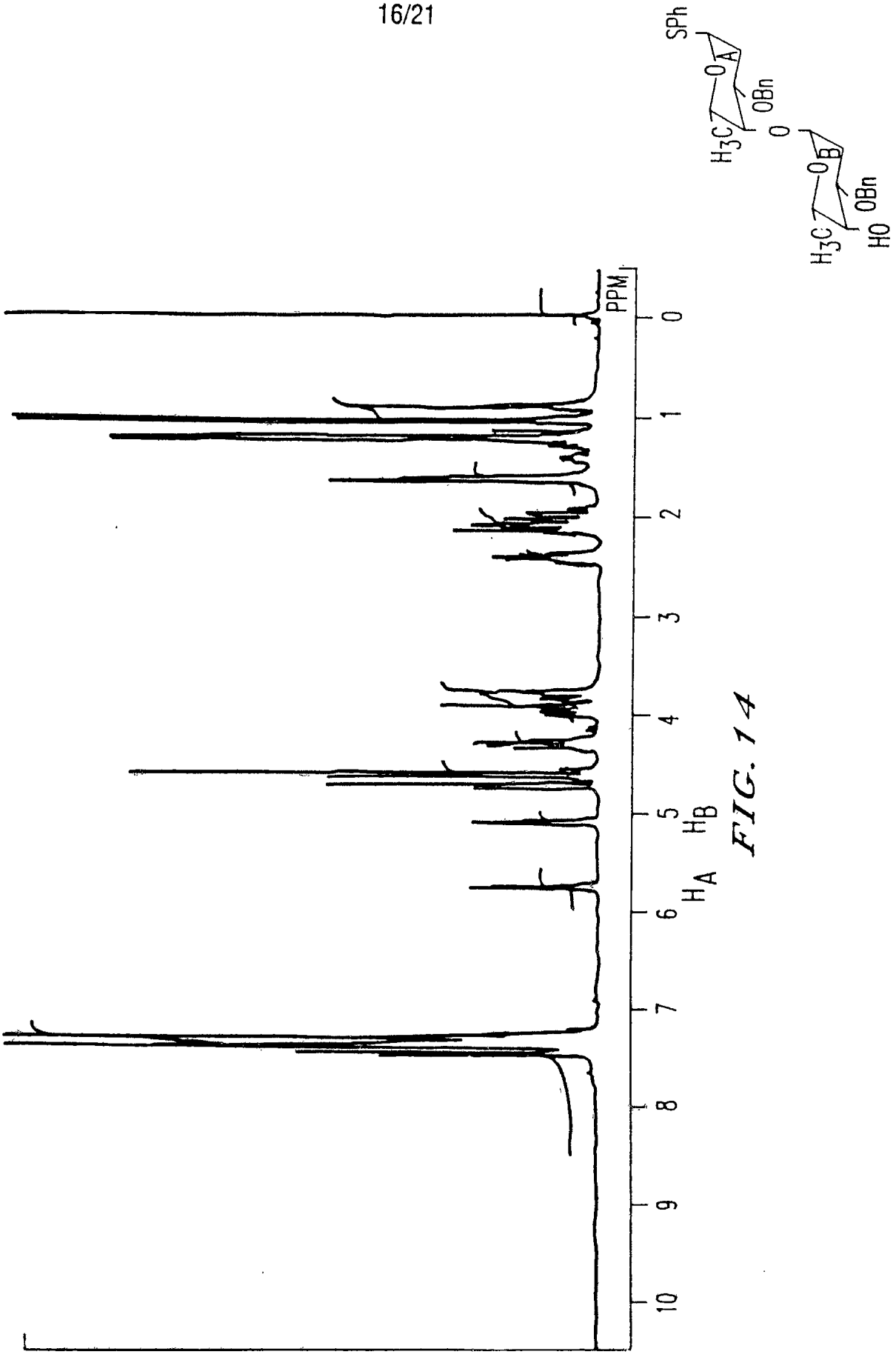


FIG. 14

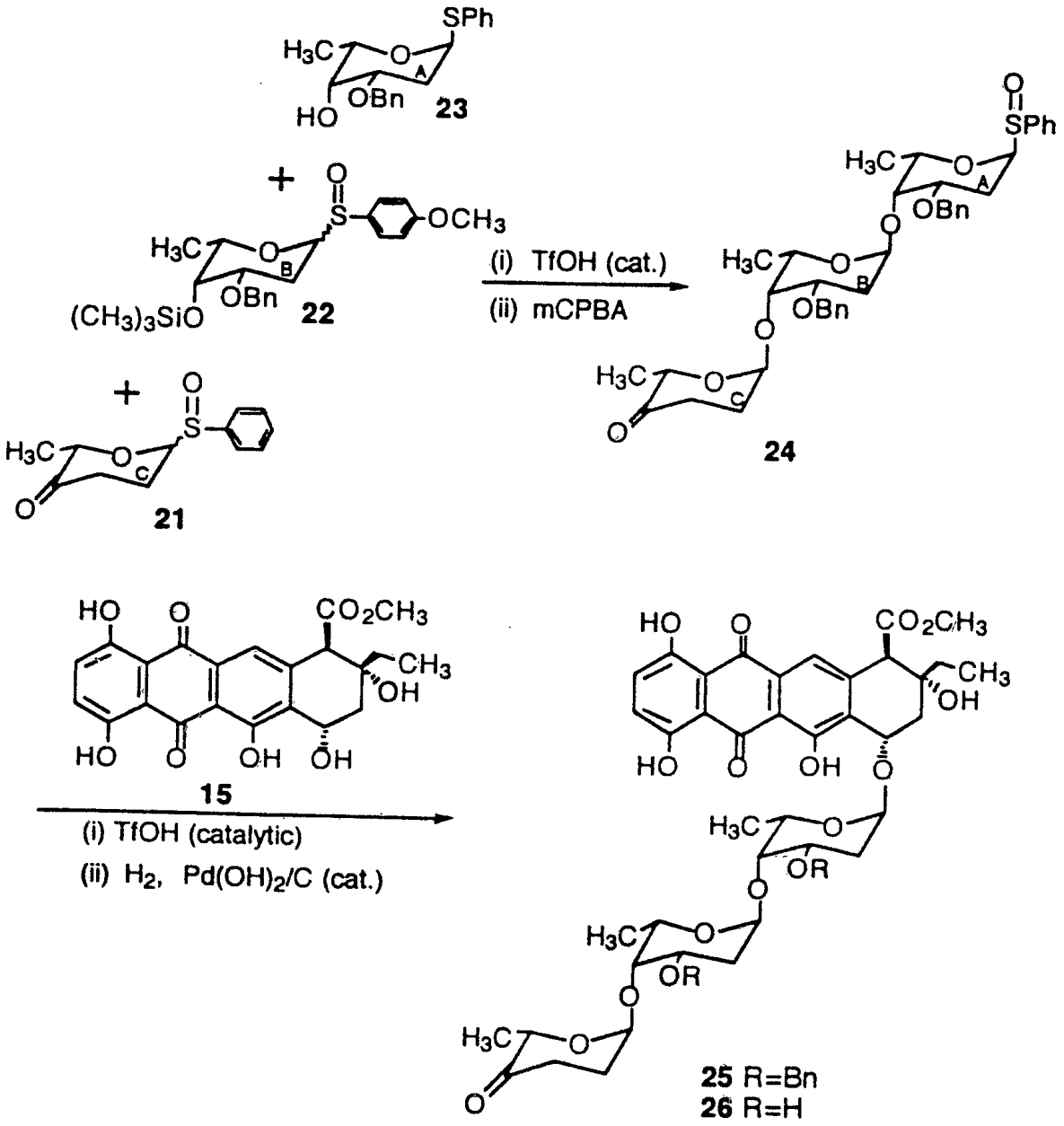

SPh
H3C
O
A
OBn
HO
23
+
O
S
OCH3
H3C
O
B
OBn
(CH3)3SiO
22
(i) TfOH (cat.)
(ii) mCPBA
O
SPh
H3C
O
A
OBn
O
H3C
O
B
OBn
O
H3C
O
C
O
24
+
O
S
H3C
O
C
O
21
HO
O
CO2CH3
CH3
OH
HO
O
OH
OH
15
(i) TfOH (catalytic)
(ii) H2, Pd(OH)2/C (cat.)
HO
O
CO2CH3
CH3
OH
HO
O
OH
O
H3C
O
OR
H3C
O
OR
O
H3C
O
O
25 R=Bn
26 R=H

FIGURE 15

SUBSTITUTE SHEET (RULE 26)

FIG. 16A

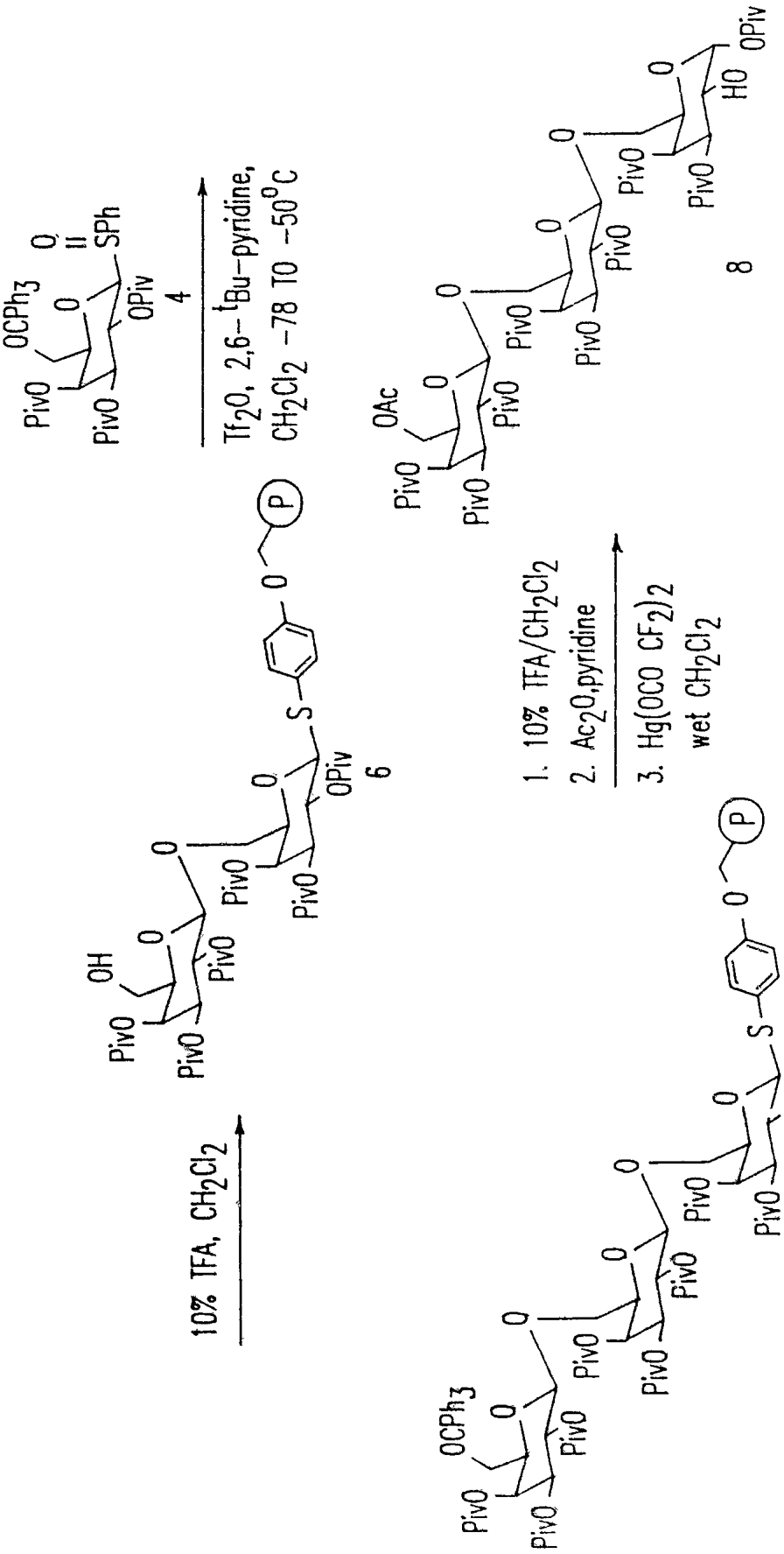
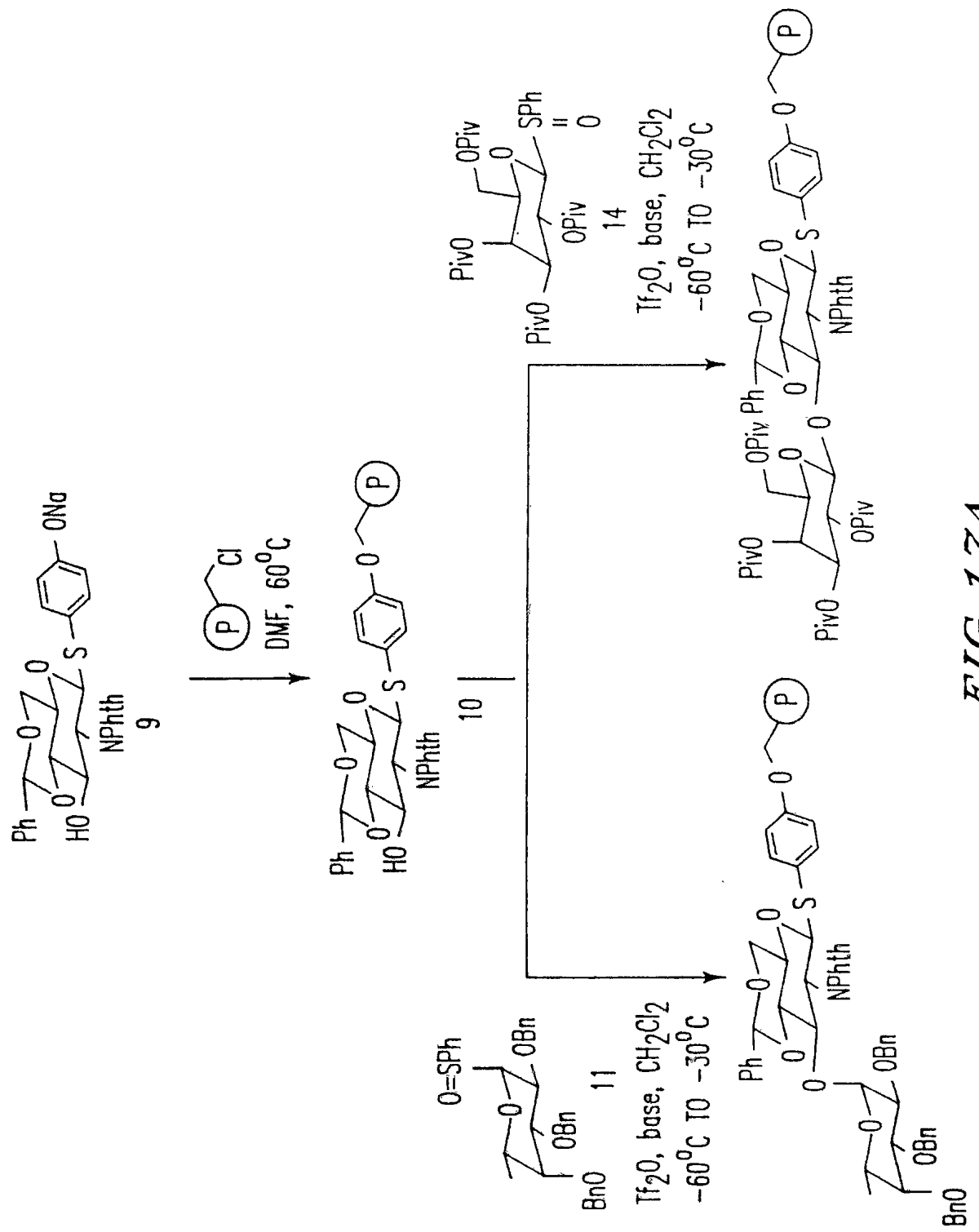

10% TFA, CH2Cl2
Tf2O, 2,6-tBu-pyridine,
CH2Cl2 -78 TO -50°C
1. 10% TFA/CH2Cl2
2. Ac2O,pyridine
3. Hg(OCO CF2)2
wet CH2Cl2

FIG. 16B



Ph
HO
O
NPhth
S
ONa
9
P
Cl
DMF, 60°C
10
O=SPh
OBn
BnO
11
Tf2O, base, CH2Cl2
-60°C TO -30°C
PivO
OPiv
SPh
14
Tf2O, base, CH2Cl2
-60°C TO -30°C

FIG. 17A

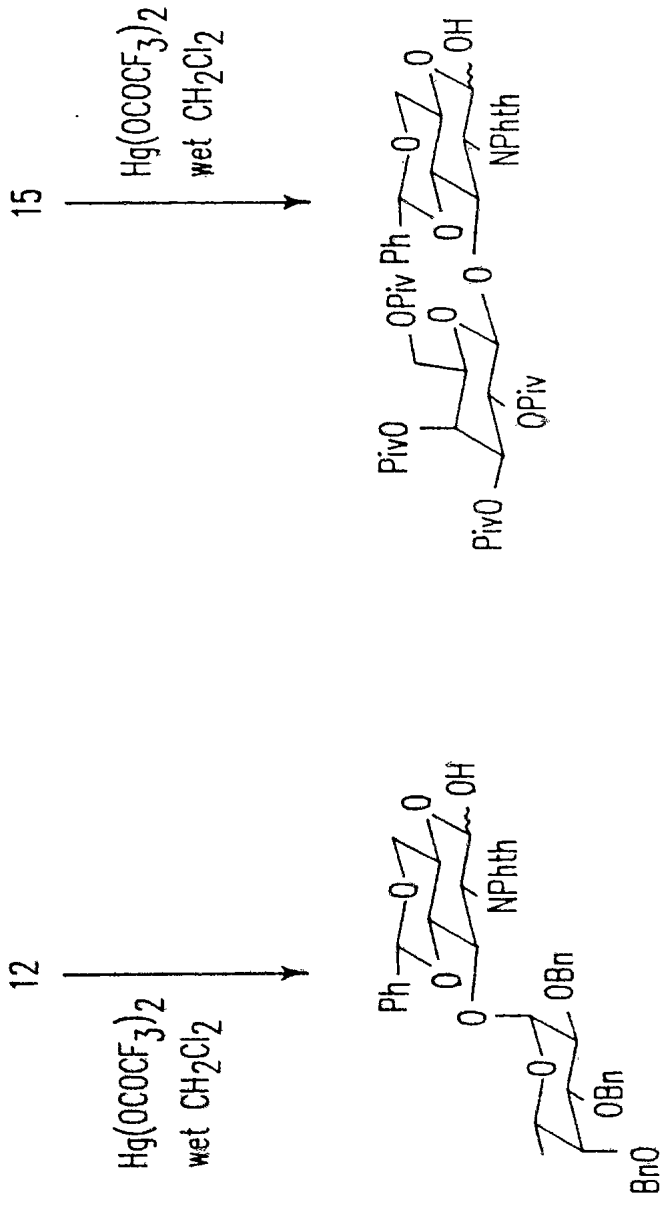

15
Hg(OCOCF3)2
wet CH2Cl2
PivO
OPiv
OPiv
Ph
O
OH
NPhth
12
Hg(OCOCF3)2
wet CH2Cl2
Ph
OH
NPhth
OBn
OBn
BnO

FIG. 17B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US94/01604

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :C07H 3/00; C07H 3/02; C07H 3/06
US CL :536/18.5, 18.6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S : 536/18.5, 18.6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Automated Patent System; CAS Online
Keywords: anomeric(W)sulfoxide, solid-phase, glycosidic linkage, glycosidic library; Kahne, Daniel E?/au

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,470,976 (MINER ET AL.) 11 September 1984, see entire document.	1 - 80
A	Ginsburg, Victor, ed., "Methods in Enzymology: Complex Carbohydrates", Volume 138, Part E, published 1987 by Academic Press (Boston), pages 359 - 404.	1 - 80
Y	Journal of the American Chemical Society, Volume 113, issued 1991, "Polymer-Supported Solution Synthesis of Oligosaccharides", pages 5095 - 5097, see entire article.	1 - 80

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 11 MAY 1994	Date of mailing of the international search report MAY 31 1994.
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No (703) 305-3230	Authorized officer GARY L. KUNZ Telephone No (703) 308-0196

Jill Warden for

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No
PCT/US94/01604

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Carbohydrate Research, Volume 233, issued 1992, "2,3,4,6-Tetra-O-benzyl-β-D-galacto-pyranosyl phenyl sulfoxide as a glycoyl donor. Synthesis of some oligosaccharide containing an alpha-D-galctopyranosyl group", pages 245 - 250, see entire document.	1 - 80
Y	Journal of the American Chemical Society, Volume 111, Number 17, issued 1989, "Glycosylation of unreactive substrates, see entire document, pages 6881 - 6882.	1 - 80