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(54) Title: SYNTHESIS OF ROTAVIRUS RECEPTOR SACCHARIDES (57) Abstract <p>Saccharides which are active as rotavirus receptor sites are disclosed. These saccharides are synthetically produced sugar derivatives recognized by the antireceptor of a rotavirus. The molecules are useful in studying the interaction between the rotavirus antireceptor and most receptor sites so as to develop drugs useful in blocking the connection between such viruses and the host cells. Further, the molecules can be modified in order to increase the degree to which they attract the antireceptor sites of the virus and/or modified by binding to them antiviral compounds which are effective in destroying the virus once the receptor molecule binds to the virus. Synthetic receptor molecules recognizable by a rotavirus are obtainable by the synthesis methods disclosed.</p>		

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5 SYNTHESIS OF ROTAVIRUS RECEPTOR SACCHARIDESCross-Reference

 This application is a continuation-in-part of
U.S. Patent Application Serial No. 07/450,026 filed 13
10 December 1989 which application is incorporated herein by
reference and to which application is claimed priority
under 35 USC Section 120.

Field of the Invention

15 This invention relates generally to the field
of rotavirus receptors. More particularly, this
invention relates to saccharide compounds and their
synthesis which compounds are recognized by the
antireceptor of a rotavirus which compounds can be
20 formulated into pharmaceutical compositions to treat
and/or prevent rotavirus infection.

Background of the Invention

 Rotaviruses are double stranded RNA viruses of
25 the family Reoviridae. These viruses replicate in the
intestinal epithelial cells of a wide range of animal
species including most mammalian and avian species and
are the major etiological agents of several
gastrointestinal disorders in humans and other animals.
30 For example, rotaviruses are responsible for infantile
diarrhea and enteritis, causing infant morbidity and
mortality. Rotaviruses also cause diarrheal illnesses in
calves and piglets, as well as other mammals. These
viruses are responsible for debilitating diarrhea in

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immune-compromised patients such as transplant recipients and AIDS sufferers and have been implicated as a significant cause of traveler's diarrhea. Currently, there is no effective prophylactic or therapeutic drug
5 available to combat rotaviral disorders and attempts to develop vaccines have been problematic.

In order to infect cells and replicate, viruses bind specific receptors on the target cell surface. After attachment, the virus fuses with the cell membrane
10 and is internalized where it uses the target cell's own metabolism to replicate. The initial attachment process is therefore essential to successful infection. For several viruses, a glycoprotein on the viral surface interacts with a specific target cell carbohydrate.

15 Like all viruses, a rotavirus has a virion which attaches to the surface of a host cell. The attachment is brought about by the specific binding of the virion protein (the antireceptor) to a constituent of the cell surface (the receptor). A classic example of an
20 antireceptor is the hemagglutinin of influenza virus (orthomyxovirus). The antireceptors are distributed throughout the surface of viruses which infect human cells. Complex viruses, such as vaccinia (a pox virus) and herpes simplex virus (a herpes virus), may have more
25 than one species of antireceptor molecules. Furthermore, antireceptor molecules may have several domains, each of which may react with a different receptor. Accordingly, complex structures and interactions are clearly involved with respect to receptors and antireceptors.

30 The details of the initial interaction between rotaviruses and the host cell surface have not been completely elucidated. However, sialic acid appears to be an important component of the rotavirus receptor, Yolken et al., J. Clin. Invest. 79: 148-154 (1987), and

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asialo GM1 binds rotavirus and inhibits viral replication in plaque reduction assays, Willoughby et al., abstract from Proceedings of U.S.-Japan International Rotavirus Meeting, Annapolis, MD, August 1989. Furthermore, bovine submaxillary mucin and chicken ovomucoid have been shown to prevent rotavirus gastroenteritis in mice. Volken et al., supra. Additionally, it has been shown that rotavirus strains isolated from one species cross-react with hosts of another species (see e.g. Leece et al., Infect. Immun. 14: 816-825 (1976); Mebus et al., Infect. Immun. 14: 471-474 (1976); Wyatt et al., Science 207: 189-191 (1980)), suggesting conservation of rotaviral receptors between species.

Viral binding molecules to which a rotavirus binds could be bound to antiviral drugs and rotaviral binding molecules by themselves could be used to hinder or prevent the subsequent infection of host cells. A distinct advantage of such an approach over traditional methods of preventing viral infections, e.g. vaccines, is that the portion of the viral protein normally binding to the specific cell surface carbohydrate does not mutate. Thus, antiviral agents which act by preventing viral binding to host cells are likely to remain effective in the face of mutations to other parts of the viral genome.

In U.S. patent application Serial No. 07/450,026 filed December 13, 1989, certain glycolipids were extracted from intestinal cells and found to bind to rotavirus. The present invention extends that of the 07/450,026 application by finding saccharides which bind to rotavirus and disclosing such saccharides, their method of synthesis and the formulation of such saccharides into pharmaceutical compositions useful in treating and/or preventing rotavirus infection.

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Summary of the Invention

Various saccharide compounds are disclosed which bind rotavirus. The compounds can be used to prevent interaction of the virus with a target cell and thus prevent viral infection. These compounds can be bound to antiviral drugs and advantageously employed to treat rotaviral infections. The discovery and chemical characterization of relatively small sugars able to bind rotavirus allows for the efficient production of pharmaceutical and/or food compositions useful in the prevention and treatment of rotaviral infections.

A primary object of the present invention is to provide a rotavirus receptor compound.

Another object of the subject invention is directed to a composition for preventing or treating rotaviral infection comprising a therapeutically effective amount of synthetic rotavirus receptor compound in combination with a pharmaceutically acceptable carrier.

Yet another object of the subject invention is directed to methods of producing molecules able to bind rotavirus.

Still other objects of the subject invention include methods for preventing or treating rotaviral induced disorders by administering to a subject a therapeutically effective amount of a synthetic rotavirus receptors.

A feature of the present invention is that the molecules are produced in an effective manner by synthetic means.

An advantage of the present invention is that the receptor molecules can be linked to antiviral drugs to provide effective antiviral compositions.

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Another advantage of the present invention is that the compounds can be linked to probes such as radioactive probes to form conjugates which can be used to test for the presence of rotavirus and/or the binding effectiveness of compound with respect to a rotavirus.

These and other objects, features and advantages of the present invention will become apparent to those persons skilled in the art upon reading the details of the structure, synthesis and usage as more fully set forth below, reference being made to the accompanying general structural formulae forming a part hereof.

Detailed Description of Preferred Embodiments

Before the present synthetic saccharide receptor molecules and processes for making and using such are described, it is to be understood that this invention is not limited to the particular sugar residues described. For example, derivatives of such saccharides can be used such as when such saccharides are bound to anti-viral compounds. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims.

It must be noted that as used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus for example, reference to "a synthetic receptor molecule" includes mixtures of such molecules, reference to "an antireceptor" includes mixtures of antireceptors of the type described and reference to "the process for synthesizing" includes similar processes which will

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become apparent to those skilled in the art upon reading this disclosure and so forth.

A. Definitions

5 In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

By "isolated rotavirus receptor glycolipid" is meant any sugar containing lipid that is capable of binding rotavirus. The glycolipid can be isolated from intestinal cells as described in U.S. patent application Serial No. 07/450,026, filed December 13, 1989, which is incorporated herein by reference in its entirety. The term also refers to those glycolipids which bind rotavirus which glycolipids which can be synthetically produced by applying the present disclosure in combination with methods well known in the art.

By "rotavirus receptor saccharide" is meant any synthetic saccharide of the invention such as the saccharide epitopes or analogs thereof which are capable of binding rotavirus as determined by standard viral binding assays including but not limited to overlay assays with thin layer chromatography plates and by probing receptor compounds adsorbed to PVC wells with labeled virus. A receptor compound of the invention will bind a rotavirus more avidly than asialo G_{M1}, as determined by orcinol staining in comparison with known amounts of asialo G_{M1}.

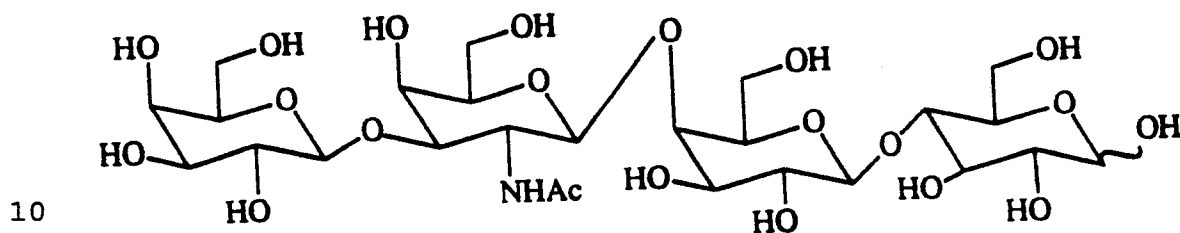
The glycolipid Ggose₄Cer (also known as G_{A1}) will run on thin layer chromatography (TLC) silica plates using chloroform:methanol:water (60:40:10) as solvent to provide a standard. The oligosaccharide moiety of the glycolipid G_{A1} is shown below as structural formula III and is Ggose₄ (also known as asialo G_{M1}). (Note that Ac

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is COR throughout and R is preferably CH₃ but may be any alkyl containing 1 to 30 carbons - in formulae I, II and III, R is CH₃.

III

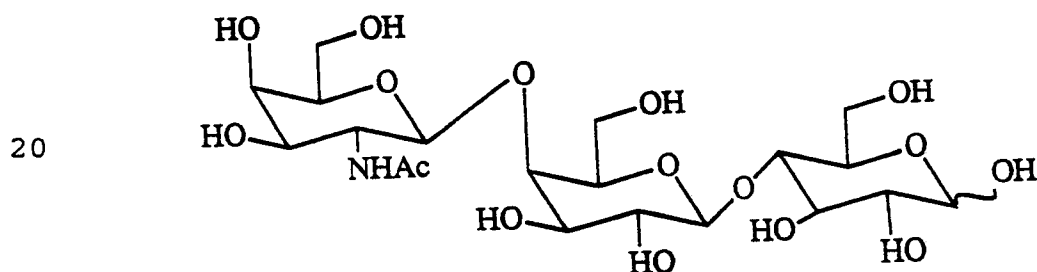
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The oligosaccharide Ggose₃ is the oligosaccharide moiety of the glycolipid Ggose₃Cer. The compound of structural formula II below is Ggose₃ (also known as asialo G_{M2}).

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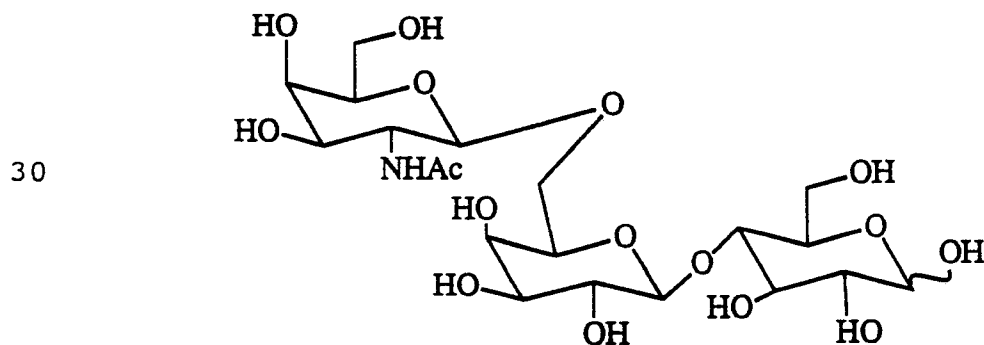
II



The beta-(1-6)-linked isomer of II is shown below as I:

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I



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The term "therapeutically effective amount" refers to the amount of a rotavirus receptor saccharide or conjugate of such a compound with an antiviral drug sufficient to prevent, (i.e., treat prophylactically), or
5 treat, (i.e., reduce or eliminate the symptoms of rotavirus) induced disorders in a recipient subject when administered.

B. Receptor Molecules for Rotavirus

10 Information regarding receptor sites for the antireceptor of a rotavirus are of particular interest to those involved in the research of the rotavirus and its infectious process. Since a rotavirus will readily attach to a receptor site, the production of receptor
15 sites other than those present on cell surfaces would provide false hosts to the viruses and aid in preventing infection of the host cells to be protected. Further, by providing such receptors, it is possible to link other compounds to the receptors, such as antiviral drugs which
20 can act to destroy the rotavirus after the rotavirus antireceptor has attached. A probe could be attached to or incorporated as part of the receptor so that the "tagged" receptor could be used to detect the presence of rotavirus. Saccharides presently disclosed can be
25 adapted for all or any of these uses.

This invention relates to: (1) synthetically produced saccharides such as saccharide epitopes or analogs thereof which are recognized by the antireceptor of a rotavirus; (2) pharmaceutical compositions
30 containing such saccharides in combination with a pharmaceutical acceptable excipient; and (3) the saccharides of (1) in combination with an antiviral compound or detectable probe. The rotavirus receptor molecules of the invention are structurally small, but

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functionally similar to the naturally occurring glycolipid receptor sites which are described further below.

5 Glycolipids can be isolated from cells receptive to rotavirus using any of several techniques for purifying glycolipids, well known in the art. A particularly useful technique is a modified Folch extraction as developed by Svennerholm and Fredman, Biochim. Biophys. Acta 617: 97-109 (1980), the disclosure of which
10 is incorporated by reference herein in its entirety to disclose such extraction techniques. Isolated glycolipids known to act as receptors can be used for comparison purposes with the synthetic saccharide receptors of the present invention.

15 Sugar residues and related compounds can also be synthesized using standard biochemical techniques, well known in the art. For example, the methods described by Tang et al., Biochem. Biophys. Res. Comm. 132: 474-480 (1985); Schnaar et al., J. Biol. Chem. 253:7940-7941 (1978); and Laine et al., J. Biol. Chem. 249:4460-4466 (1974), all incorporated herein by
20 reference in their entirety to disclose techniques which can be used in combination with the present disclosure to synthesize the saccharide compounds of the invention.

25 Once synthesized and purified the receptor saccharide compounds can be tested (in their neoglycolipid form as explained further below) for their ability to bind labeled rotavirus using thin layer chromatography plates, developed in a suitable solvent or
30 by adsorbing the saccharides to PVC wells and subsequently probing the immobilized receptor compounds with labeled virus. The efficacy of the saccharide binding receptor compounds can be directly tested in vitro in standard plaque reduction assays. Saccharides

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can also be directly tested for their ability to inhibit a rotavirus from binding to an immobilized glycolipid in PVC wells. These testing procedures are described in detail in the Examples.

5 Once receptor compounds such as natural glycolipids with the ability to bind rotavirus have been isolated, the sugar moieties can be cleaved therefrom using enzymes, including but not limited to neuraminidase, beta-glucuronidase, alpha-galactosidase, 10 beta-galactosidase, endogalactosidase, beta-hexosaminidase, ceramide glycanase, alpha-fucosidase, and the coenzymes sulfatase and phosphatase. The ability of these cleaved carbohydrates to bind to a rotavirus antireceptor can be tested and 15 compared with the binding of synthetic saccharide compounds of the invention to the same viral antireceptor.

 The cleavage products can be tested for their ability to bind rotavirus as described above and 20 compounds (such as the various saccharides of the invention) with binding capability can be further tested in standard rotavirus plaque reduction assays, such as described below. Receptor compounds with inhibitory activity can be used directly, or equivalent receptor 25 compounds can be synthesized by applying methods known in the art in combination with the present disclosure. For example, receptor oligosaccharides can be synthesized by the sequential addition of appropriate single sugar units to a growing chain covalently linked to an insoluble 30 solid support or by "block synthesis" where di- or trisaccharide blocks are synthesized which are then complexed to provide larger size oligosaccharides. The minimum binding and inhibitory carbohydrate epitope can be determined and used. Further, these sugars can be

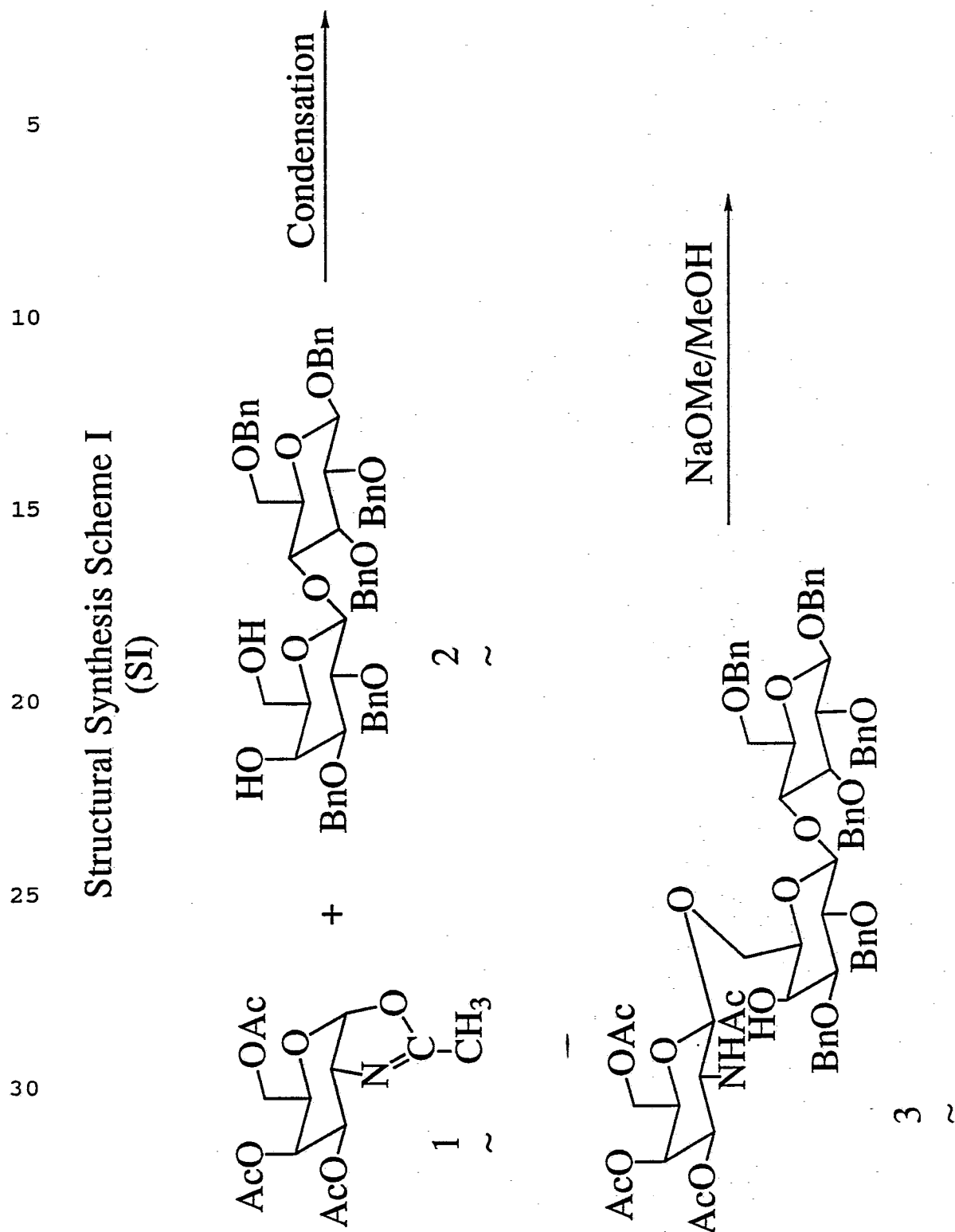
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structurally modified to create more avidly binding analogs and/or linked to antiviral drugs or detectable probes.

In order to determine receptor saccharides recognized by a rotavirus certain characteristics of such receptors were taken into consideration. Firstly, it was noted that the conversion of asialo G_{M1} into asialo G_{M2} did not cause a difference in the ability of a rotavirus to bind to these molecules as measured by the TLC assay. The microtiter well assay showed some decrease in binding, although G_{A2} still bound some virus, in comparison with other tested glycolipids which did not. G_{A2} does support viral binding but greater concentrations are needed when compared with G_{A1} in the PVC adsorption assay. This is in contrast to the TLC viral binding experiments in which G_{A1} and G_{A2} appear to be more equivalent. Further, it was noted that GalNAc by itself is of insufficient size to bind a rotavirus. Lastly, it was noted that the removal of the terminal Gal from $Ggose_4Cer$ (G_{A1}) generates $Ggose_3Cer$ (G_{A2}), a glycolipid which retains viral binding activity although at a reduced potency.

Based on the above discoveries, saccharide structures were deduced, synthesized and tested for rotavirus affinity. The following structural synthesis schemes SI, SII and SIII show how oligosaccharides I, II and III respectively are synthesized in accordance with the methods of synthesis of the present invention.

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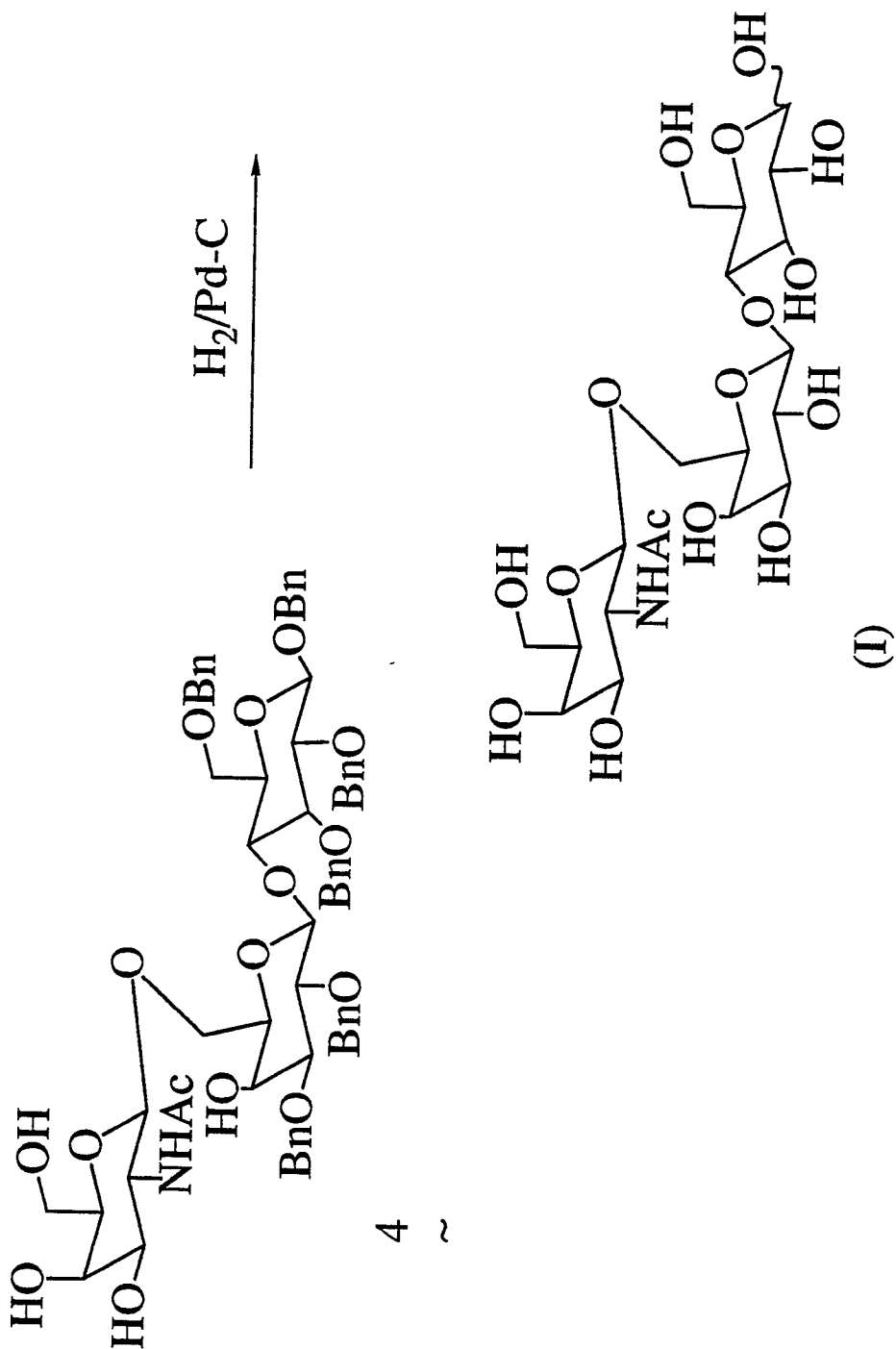
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(S I continued)



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Structural Synthesis Scheme II
(SII)

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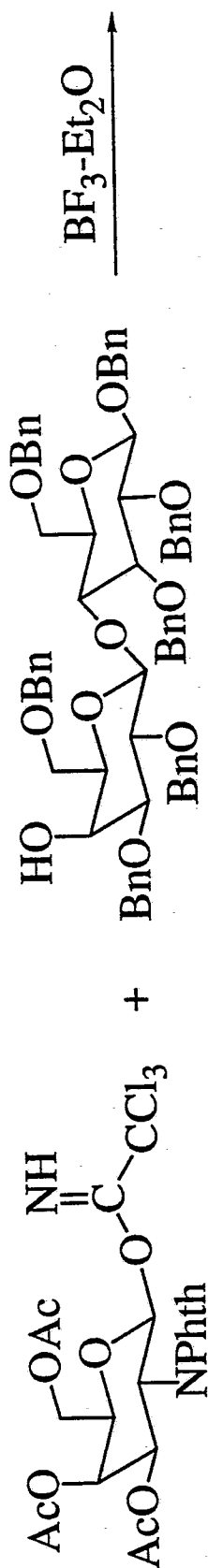
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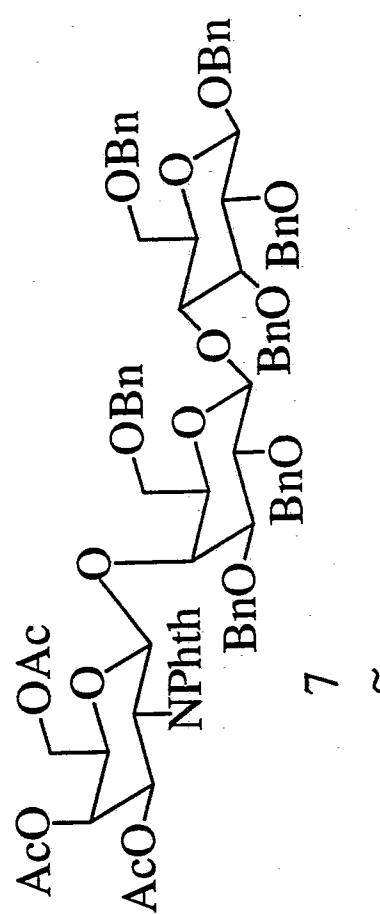
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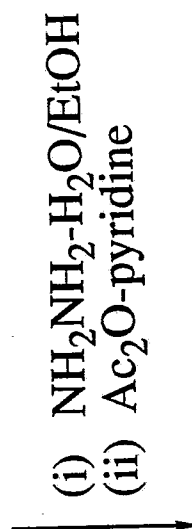
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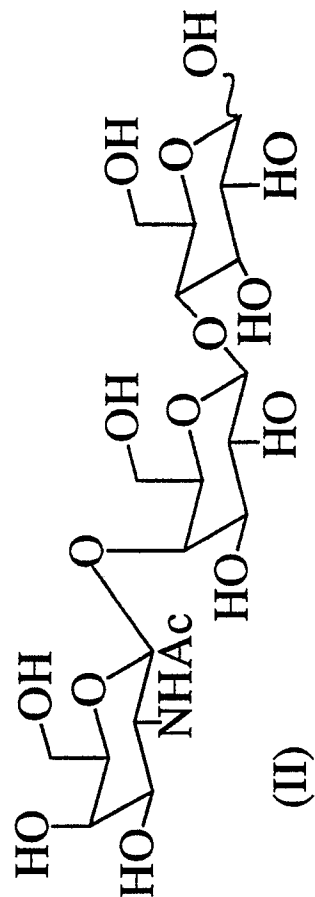
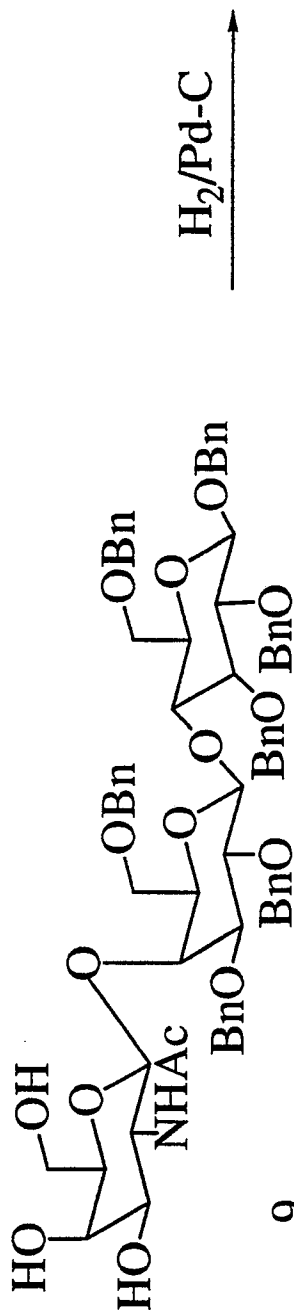
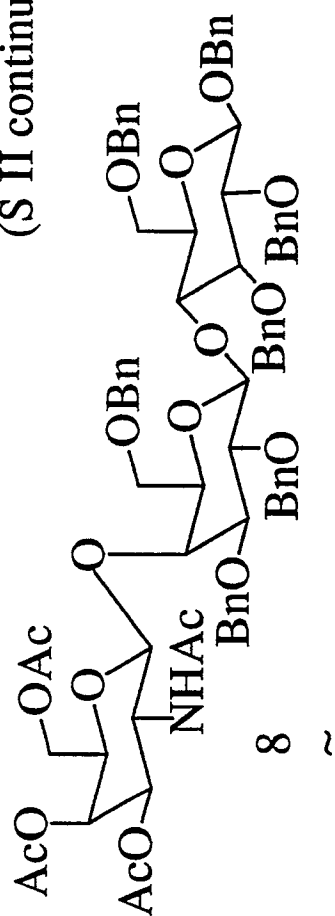
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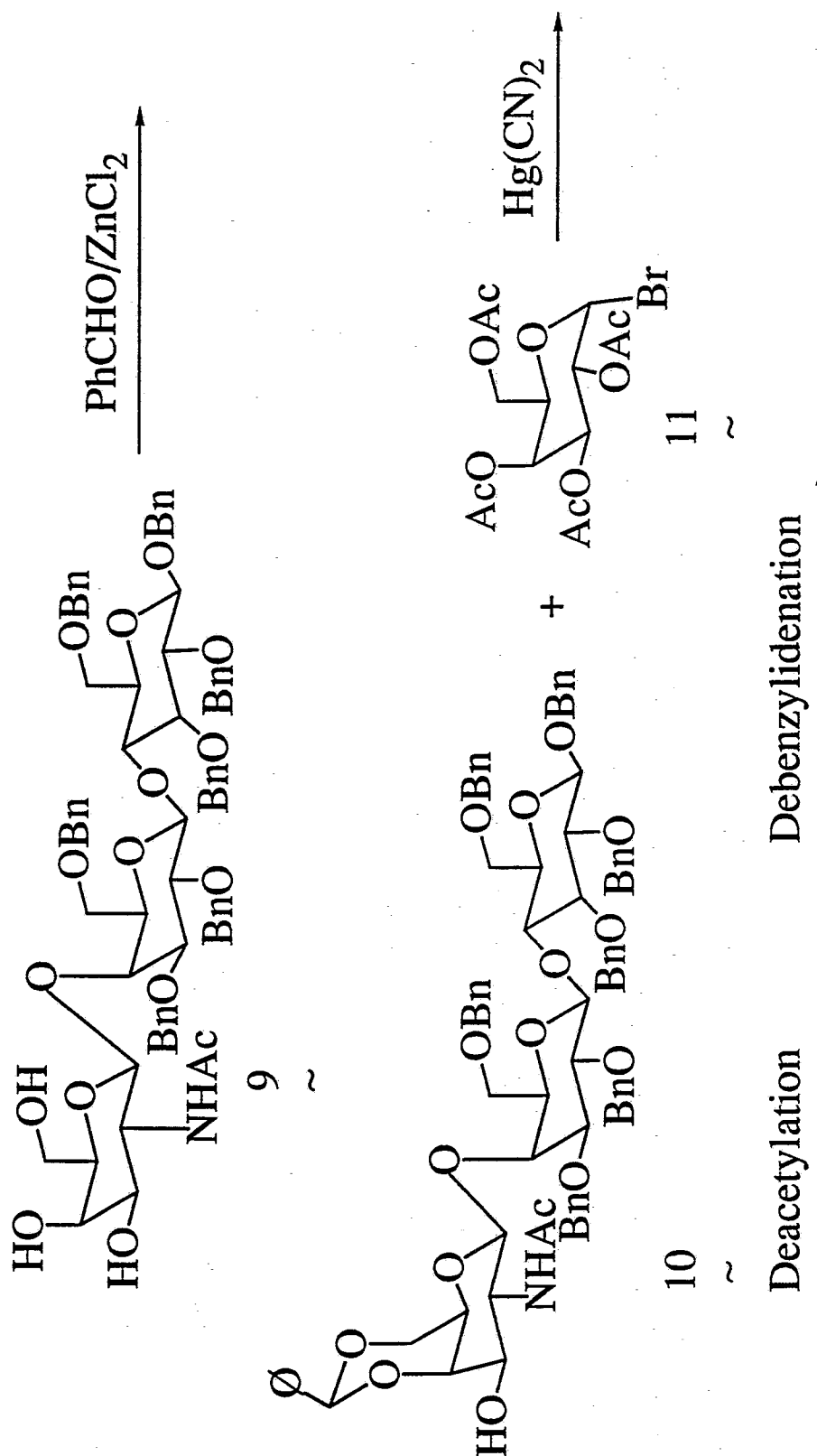
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(S II continued)



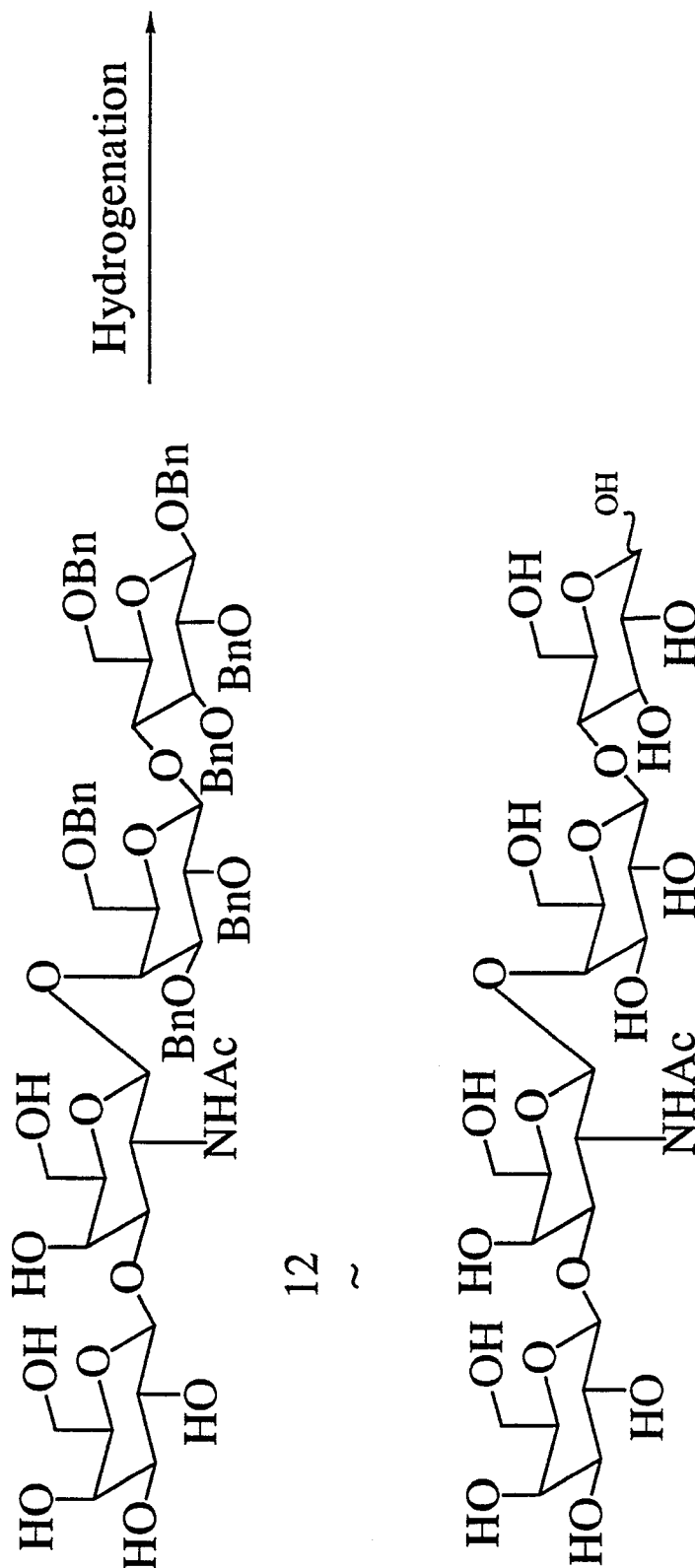
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Structural Synthesis Scheme III
(S III)



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Structural Synthesis Scheme III
(continued)



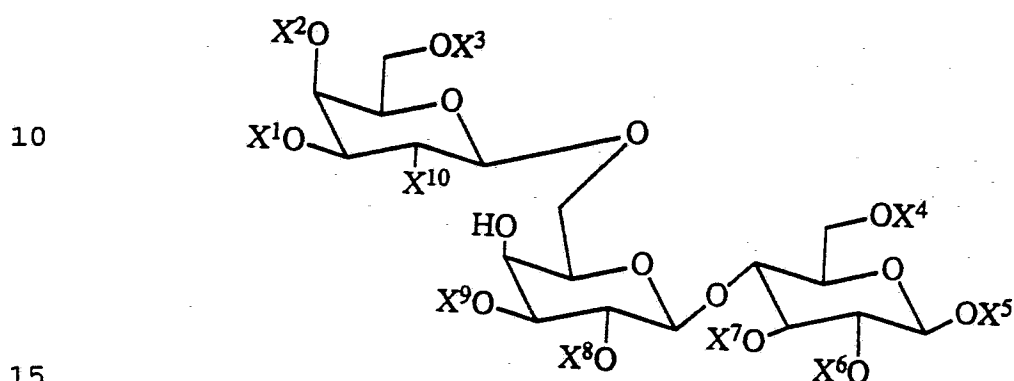
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General Structural Formulae

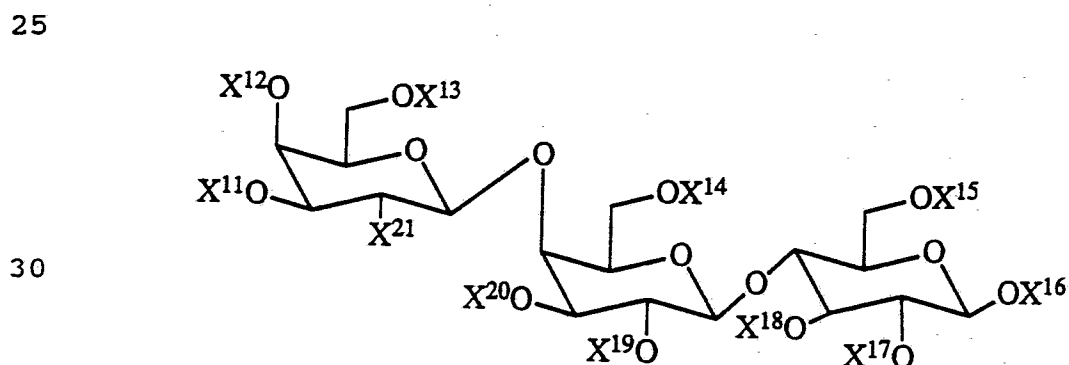
Based on the above Structural Synthesis Schemes SI, SII and SIII, the following general structural formulae for saccharide compounds useful in producing pharmaceutical formulations of the invention can be deduced.



IV

wherein each of X^1 to X^8 is independently H, an acyl, or a benzyl and X^{10} is an acylamine or OH wherein any acyl moiety may contain from 2 to 30 carbons. Preferably X^1 to X^9 are H and X^{10} is acylamine (most preferably $-\text{COCH}_3$) with the proviso that X^5 may be in the alpha or beta position.

Another general structure is as follows:

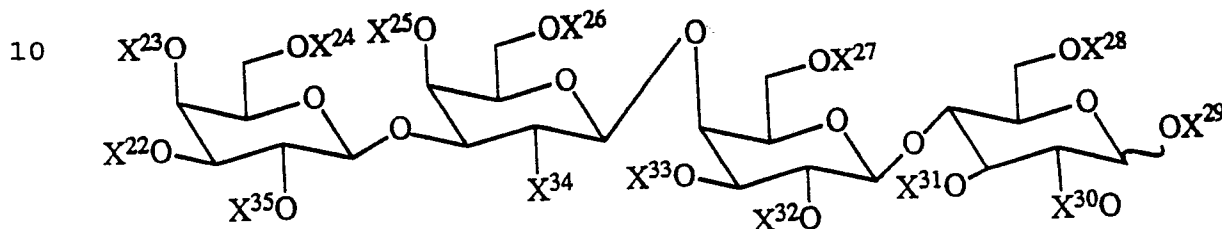


V

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wherein each of $X^{11} - X^{20}$ is independently H, acyl or benzyl and X^{21} is -OH or acylamine wherein any acyl can contain from 1 to 30 carbons. Preferably $X^{11} - X^{20}$ are each H and X^{21} is acylamine (most preferably -COCH₃) with the proviso that X^{16} may be in the alpha or beta position.

Yet another general structure is:



VI

wherein each of $X^{22} - X^{33}$ and X^{35} is independently H, an acyl, or a benzyl and X^{34} is an acylamine or OH. Preferably each of $X^{22} - X^{33}$ and X^{35} is H and X^{34} is acylamine (most preferably -COCH₃) with the proviso that any acyl moiety may contain from 2 to 30 carbon atoms and X^{29} may be in the alpha or beta position.

General Description of the Synthesis shown in
SI, SII and SIII

Description of Structural Reaction Scheme I - (SI)

Synthesis of β -D-GalNAc-(1-6)- β -D-Gal-(1-4)-D-Glc. I

Condensation of oxazoline 1 [K.L. Matta and O.P. Bahl, Carbohydr. Res., 21 (1972) 460-464] with the lactoside diol 2 [K. Koike et al. Carbohydr. Res., 163

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(1987) 189-208] according to established procedures, followed by column chromatographic purification on silica gel gave trisaccharide derivative 3 (72.5%). Compound 3 was deacetylated in methanolic sodium methoxide to give the partially protected trisaccharide 4 (83%). Hydrogenolysis of the benzyl groups of 4 ($H_2/Pd-C$ in glacial acetic acid at room temperature and ~ 345 kPa) followed by chromatographic purification afforded the title trisaccharide I (72%).

Description of Structural Reaction Scheme II - (SII)

Synthesis of β -D-GalNAc-(1-4)- β -D-Gal-(1-4)-D-Glc. II

Imidate 5 was prepared and reacted according to Schmidt's procedure [R.R. Schmidt and G. Grundler, *Angew. Chem. Int. Ed. Engl.*, **22** (1983) 776-777] with compound 6 [K. Koike *et al.* *Carbohydr. Res.*, **163** (1987) 189-208] to give trisaccharide 7 which was subjected to hydrazinolysis ($NH_2 \cdot NH_2 \cdot H_2O$ in ethanol) followed by acetylation (2:1 Pyridine-acetic anhydride) to give the fully protected trisaccharide 8 (65%, based on lactoside acceptor 6). Zemplen transesterification of 8 gave the partially protected derivative 9 (84%). Compound 9 was hydrogenolyzed in the usual manner to provide the title compound II (51.3%).

Description of Structural Reaction Scheme III - (SIII)

Synthesis of β -D-Gal-(1-3)- β -D-GalNAc-(1-4)- β -D-Gal-(1-4)-D-Glc. III

The benzylated trisaccharide 9 (scheme II) was converted into its 4",6"-O-benzylidene acetal 10 (84%) by reaction with benzaldehyde-zinc chloride complex. Compound 10 was condensed with acetobromogalactose 11

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under Helferich conditions to give a tetrasaccharide derivative which was O-deacetylated (NaOMe-MeOH) and then deacetalated (80% aqueous acetic acid, 90) to afford 12 (50%, based on trisaccharide 10). Hydrogenolysis of the
5 benzyl groups of 12 (50%, based on trisaccharide 10). Hydrogenolysis of the benzyl groups of 12 then afforded the desired tetrasaccharide III (44.5%).

DETAILED PROTOCOLS

10 Detailed Description of Reaction Scheme I-III

SI - SIII

The following detailed protocols are provided so as to provide those of ordinary skill in the art with a complete disclosure and description of how to carry out
15 Structural Reaction Schemes I, II and III and are not intended to limit the scope of what the inventors regard as their invention or as being the only methods of synthesizing such molecules. Efforts have been made to insure accuracy with respect to numbers used (e.g.,
20 amounts, temperature), but some experimental errors and deviation should be accounted for. Unless indicated otherwise, parts or parts by weight, temperature is in degrees centigrade, and pressure is at or near atmospheric. In these detailed synthesis, reference can
25 be made to SI-SIII where Ac is acetate, i.e., Ac is $-\text{COCH}_3$.

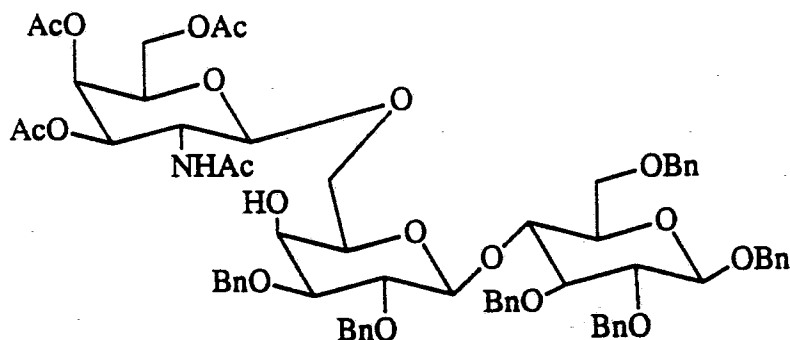
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Detailed Description of Structural Reaction Scheme I
Synthesis of β -GalNAc-(1-6)- β -Gal-(1-4)-D-Glc
 (SI)

5 Benzyl 0-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-galactopyranosyl)-(1-6)-0-(2,6-di-O-benzyl- β -D-galactopyranosyl)-(1-4)-2,3,6-tri-O-benzyl- β -D-glucopyranoside

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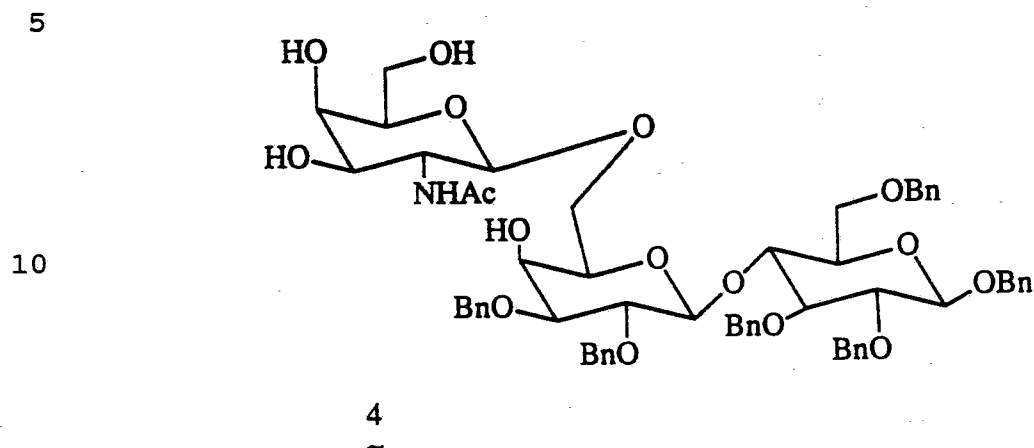
A mixture of benzyl 0-(2,6-di-O-benzyl- β -D-glucopyranoside 2 (0.88g, 1 mmol), 2-methyl (3,4,6-tri-O-acetyl-1,2-dideoxy- α -D-galactopyrano)-[2,1-d]-2-oxazoline 1 (0.66g, 2 mmol) and 4-toluenesulfonic acid (25 mg) in 1,2-dichloroethane (15 mL), protected from moisture, was heated (~72°) with stirring for 16h. T.L.C. (3:2 ethyl acetate-hexane) revealed the presence of a major product faster-migrating than 2; some unchanged 2, as well as some slower-migrating contaminants (presumably due to the decomposition of oxazoline 1) were also revealed by T.L.C. After cooling, a few drops of pyridine were added, the mixture evaporated to dryness, and the residue dissolved in a

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small volume of dichloromethane and applied to a column of silica gel (~100g). The column was eluted first with 1:2 and then 1:1 ethyl acetate-hexane. On evaporation, the first fractions gave unchanged 2 (0.1g). Fractions
5 corresponding to the product gave a residue (0.9 which was dissolved in a small volume of ethyl acetate). Addition of ether-hexane caused the precipitation of 3 0.87g (72.5%), $[\alpha]_D^{20} -3^\circ$ (≤ 0.43 , chloroform).

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Benzyl 0-(2-acetamido-2-deoxy-β-D-galactopyranosyl (1-6)-0-(2,6-di-0-benzyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-0-benzyl-β-D-glucopyranoside



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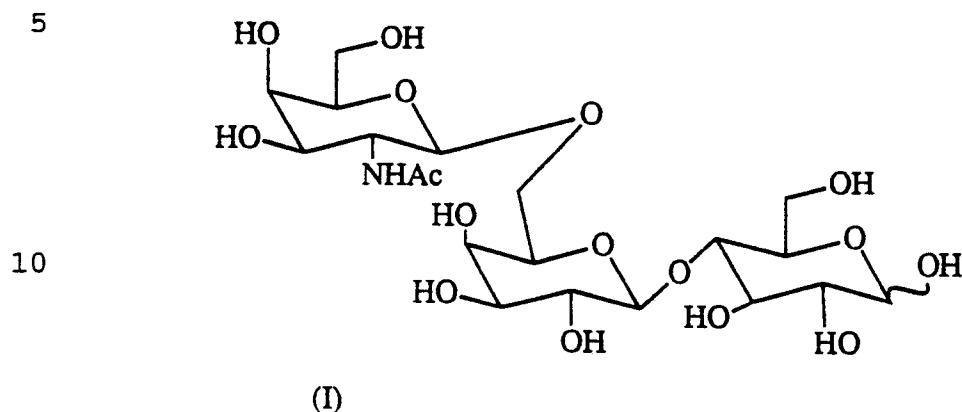
Trisaccharide derivative 3 (0.8g) was suspended in methanol (30mL) containing a catalytic anion of sodium methoxide and stirred at room temperature. The suspended 3 quickly dissolved, and, in a few minutes,

20 crystallization ensued. The stirring was continued overnight at room temperature, the base neutralized with a few drops of glacial acetic acid and the precipitation was filtered and thoroughly washed with cold methanol to give 4 (0.6g, 83%), $[\alpha]_D^{20} + 2.7^\circ$ (c 4.6, chloroform).

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-25-

O-(2-Acetamido-2-deoxy-β-D-galactopyranosyl) (1-6)-O-β-D-galactopyranosyl-(1-4)-D-glucopyranose



15

Compound 4 (0.42g) in glacial acetic (25 mL) was shaken under H₂ at ~345 kPa for 3 days at room temperature in the presence of 10% palladium-on-carbon (0.4 g). The suspension was filtered (a bed of Celite) and thoroughly washed with glacial acetic acid. The acetic acid was evaporated under diminished pressure and the residue was applied to a column of silica gel. Elution first with 13:6:1 and then 4:5:1 chloroform-methanol-water, and evaporation of the fractions corresponds to the product gave a residue (0.18g) which was dissolved in methanol, filtered, and concentrated. It was redissolved in water and lyophilized to give the desired trisaccharide (I) (0.152g, 72.4%) $[\alpha]_D^{20} + 23.4$ (c 0.32, methanol).

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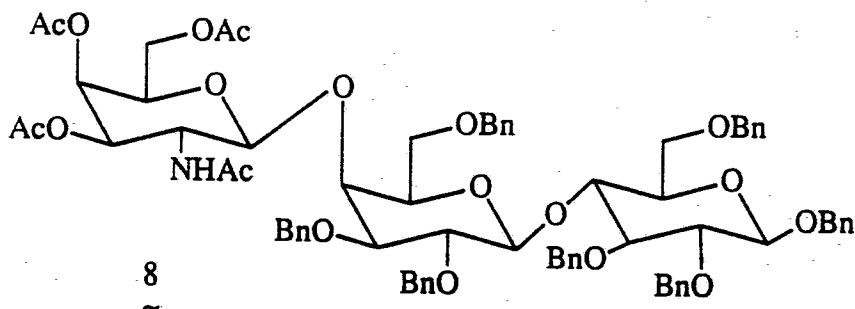
-26-

Detailed Description of Structural Reaction Scheme II
Synthesis of β -GalNAc-(1-4)- β -Gal-(1-4)-D-Glc
 (SII)

5 Benzyl 0-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-
galactopyranosyl)-(1-4)-0-(2,3,6-tri-O-benzyl- β -D-
galactopyranosyl)-(1-4)-2,3,6-tri-O-benzyl- β -D-
glucopyranoside

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A mixture of benzyl 0-(2,3,6-tri-O-benzyl- β -D-galactopyranosyl)-(1-4) 2,3,6-tri-O-benzyl- β -D-glucopyranoside 7 (0.64g, 0.66 mmol), ionidate 5 (0.77g, 1.3 mmol) and powdered molecular sieves (1-5g) in dry dichloromethane (15 mL), protected from moisture, was stirred for ~0.5h. at room temperature. It was then cooled (-20°, bath) and treated with boron trifluoride etherate (0.7 mL) and stirred for 0.5h. at -20°. It was then allowed to warm to room temperature and the stirring was continued for an additional 1h. T.L.C. (4:1 toluene-acetone) revealed the presence of a major product, slower-migrating than acceptor 6; a trace of 6 and some slower-migrating contaminants (presumably due to the decomposition of 5) were also revealed by T.L.C. The mixture was diluted with dichloromethane (100 mL) and

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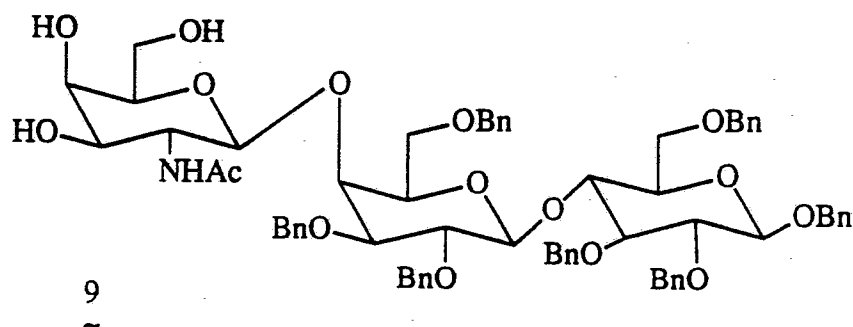
filtered (Celite) and the solution washed with water, dried, and concentrated. The residue was applied to a column of silica gel (~100g) and eluted with 10:1 toluene-acetone. On evaporation, fractions corresponding to the product gave 7 (0.75), 82%) which was slightly contaminated with a faster-migrating compound. This material was heated for 15 min. at ~70° in a mixture of ethanol (15 mL) and 85% hydrazine hydrate (7.5 mL). The mixture was evaporated, and several portions of ethanol were added to, and evaporated from the residue. The residue was thoroughly dried in vacuo and mixed with acetic anhydride (8 mL) and pyridine (16 mL) and heated for 20 min. at ~90°. The acetic anhydride and pyridine were removed under reduced pressure and the crude product was taken in chloroform, filtered and concentrated to a small volume. The concentrate was applied to a column of silica gel (~150g) and eluted with (10:) toluene-acetone (~1L). On evaporation, the fractions corresponding to the product gave 8 0.56g; 65% yield based on lactoside acceptor.

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Benzyl 0-(2-acetamido-2-deoxy-β-D-galactopyranosyl (1-4)-0-(2,3,6-tri-O-benzyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside

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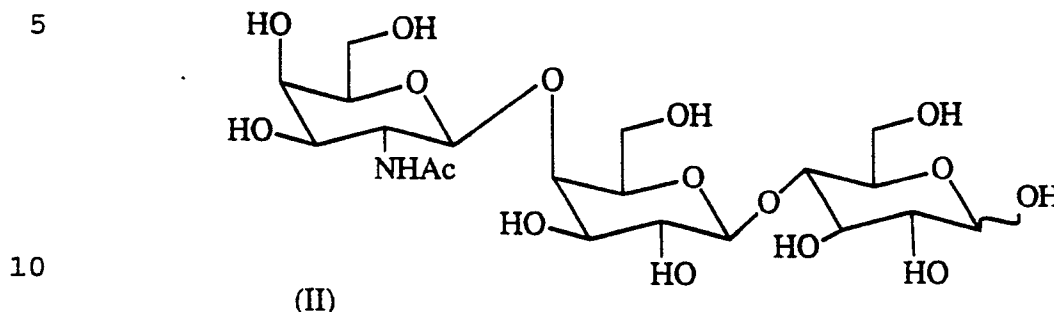
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Compound 8 (0.55g) was stirred overnight at room temperature in methanol (30 mL), containing a catalytic amount of sodium methoxide. The base was neutralized with a few drops of glacial acetic acid, the mixture concentrated, and taken into chloroform. The chloroform solution was washed twice with water, dried and concentrated to give a residue which was dissolved in a small volume of dichloromethane. Addition of ether-hexane caused the precipitation of 9 (0.42g, 84%).

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0-(2-Acetamido-2-deoxy-β-D-galactopyranosyl) (1-4)-0-β-D-galactopyranosyl-(1-4)-D-glucopyranose



The benzylated trisaccharide 9 (0.38g) in
 15 glacial acetic acid (30 mL) was shaken under H₂ at ~345
 kPa for 3 days at room temperature in the presence of 10%
 palladium-on-carbon (0.38g). T.L.C. (4:5:1 chloroform-
 methanol-water) indicated the presence of a major slower-
 migrating product as well as some faster-migrating
 20 contaminants which were faintly detectable in u.v. light.
 The latter contaminants are presumably due to incomplete
 debenzylation. The solids were removed by filtration and
 thoroughly washed with glacial acetic acid and then with
 1:2 water-methanol. After concentration, the residue was
 25 applied to a column of silica gel and eluted successfully
 with 13:6:1, 5:4:1 and 4:5:1 chloroform-methanol-water.
 On evaporation, the earlier fractions gave 100 mg. of the
 faintly u.v. visible contaminants. The fractions
 corresponding to the product yielded 95mg of material
 30 which was dissolved in methanol and filtered (Celite).
 It was concentrated, dissolved in water and lyophilized
 to give the desired trisaccharide (II) (87.9 mg, 51.3%),
 $[\alpha]_D^{20} + 21.5^\circ$ (c 0.26, methanol).

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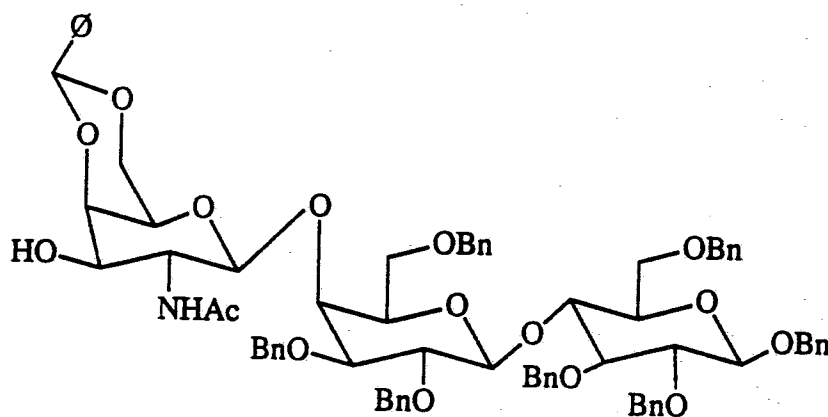
Detailed Description of Structural Reaction Scheme III
Synthesis of β -Gal-(1-3)- β -GalNac-(1-4)- β -Gal-(1-4)-
D-Glc

(SIII)

5

Benzyl 0-(2-acetamido-2-deoxy- β -D-galactopyranosyl 4,6-
0-benzylidene- β -D-galactopyranoside-(1-4)-0-(2,3,6-tri-
0-benzyl- β -D-galactopyranoside)-(1-4)-2,3,6-tri-0-benzyl-
 β -D-glucopyranoside

10



20

10

Zinc chloride (1g) was added quickly with
 25 stirring, to benzaldehyde (5 mL) and the mixture was
 stirred for ~20 min. Trisaccharide 9 (1g) was then added
 and the stirring continued for 4h. at room temperature.
 The mixture was taken in chloroform (150 mL) and washed
 with water, dried and concentrated to a small volume.
 30 The concentrate was applied to a column of silica gel and
 eluted first with 1:1 chloroform-hexane (150 mL) to
 remove excess benzaldehyde, and then with 4:1 chloroform-
 acetone to give 10 (0.9g, 84%), $[\alpha]_D^{20} + 20.8^\circ$ (c 0.53,
 chloroform).

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Condensation of Acetobrogalactose with the Trisaccharide
Acceptor 10

A mixture of trisaccharide 10 (0.6g, 0.5 mmol) and mercuric cyanide (0.18g, 0.75 mmol) in 1:1 benzene-nitro methane (50 mL) was boiled until ~25 mL of the solvent had distilled off. The temperature was then adjusted to 45° and treated with 2,3,4,6-tetra-O-acetyl α -D-galactopyranosyl bromide 11 (0.4g, 1 mmol) and the stirring was continued at 45-50° overnight. T.L.C. (20:1 chloroform-methanol) revealed the formation of a product, marginally faster-migrating than acceptor 10; some 10 and some faster-migrating contaminants (presumably due to decomposition of bromide 11) were also revealed by T.L.C. The mixture was concentrated and the residue taken in chloroform (~100 mL). The suspended material was filtered off and washed with chloroform. The chloroform solution was washed with 10% potassium iodide solution and water, dried and evaporated to dryness. The residue was taken in methanol (50 mL) containing a catalytic amount of sodium methoxide and stirred for 5h at room temperature. After deionization with Amberlite 1R-120 (H^+) the mixture was filtered and concentrated. T.L.C. 6:1 chloroform-methanol indicated the presence of a slower-migrating product; some unchanged trisaccharide as well as slower-migrating contaminants were also revealed by T.L.C. The mixture was taken in chloroform (~150 mL) and washed with water. T.L.C. then showed the disappearance of the slower-migrating contaminants. After concentration, the crude product mixture was applied to a column of silica gel (~130g) and eluted first with 20:1 and 6:1 chloroform-methanol. Evaporation of the earlier fraction gave unchanged trisaccharide 10 (0.2g). On evaporation, the fractions corresponding to

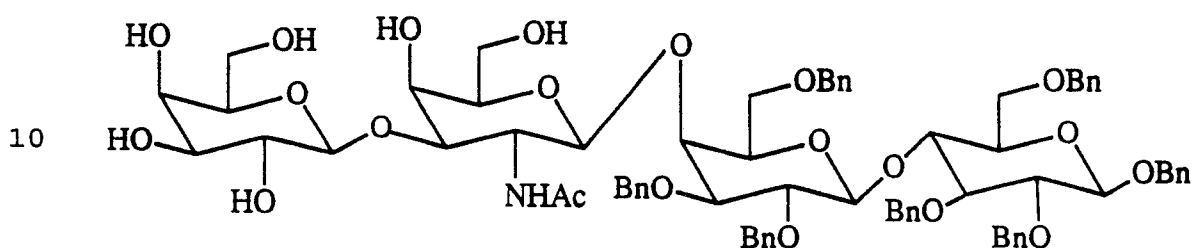
-32-

the product gave 0.37g of an amorphous white solid.
Yield 54.4% (86% based on reacted trisaccharide).

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Benzyl 0-β-D-galactopyranosyl-(1-3)-0-(2-acetamido-2-deoxy-β-D-galactopyranosyl-(1-4)-0-(2,3,6-tri-0-benzyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-0-benzyl-β-D-glucopyranoside

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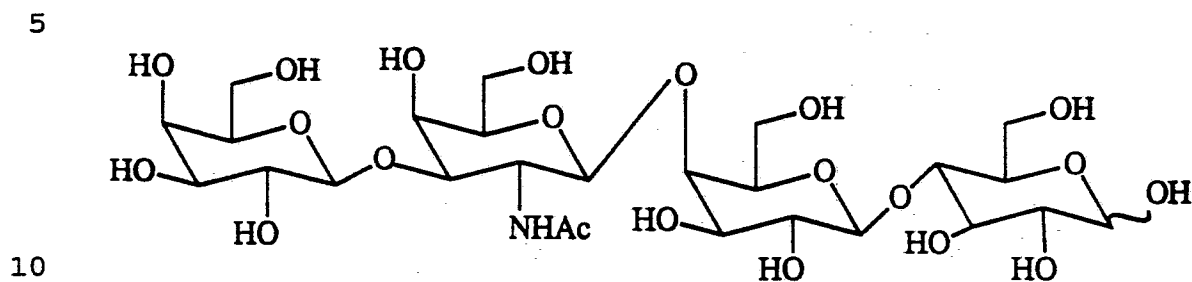
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12
~

The benzylidenated tetrasaccharide derivative 0.32g, in 80% aqueous acetic acid (25 mL) was stirred for 1h. at -90° . The acetic acid was evaporated and several portions of toluene were added to, and evaporated from the residue to give the compound 12 0.27g (90%), $[\alpha]_D^{20} + 18.8^{\circ}$ (c 0.43, CHCl_3).

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0- β -D-Galactopyranosyl-(1-3)-0-(2-acetamido-2-deoxy- β -D-galactopyranosyl-(1-4)-0- β -D-galactopyranosyl-(1-4)-D-glucopyranose



(III)

15

Compound 12 (0.21g) was hydrogenolyzed as described for 9 and then purified by column chromatography to give the desired tetrasaccharide in (44.5%), $[\alpha]_D^{20} + 16.7^\circ$ (c 0.24, methanol).

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USE and ADMINISTRATION

The above disclosed saccharide epitopes and analogs thereof can be administered to a subject either prophylactically or after rotaviral infection. The
5 saccharide receptor molecules are administered with a pharmaceutically acceptable carrier, the nature of the carrier differing with the mode of administration, for example, oral administration, usually using a solid carrier and I.V. administration a liquid salt solution
10 carrier. The method of choice, can be accomplished using a variety of excipients including, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium, stearate, sodium saccharin cellulose, magnesium carbonate, and the like. These oral composi-
15 tions may be taken in the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations, or powders. Particularly useful is the administration of the subject carbohydrate receptor molecules directly in infant nutrient formulae or
20 rehydration formulae, given to replenish electrolytes lost in severe bouts of diarrhea.

Alternatively, the receptor molecules of the invention can be administered orally, linked to supports such as beads, resins, or natural or synthetic polymers.
25 Methods for binding substances to such supports are well known in the art. The receptor saccharides may also be administered in small lipid particles comprising these receptor saccharides, such as in vesicles, micelles, or liposomes.

30 A sufficient amount of receptor saccharide molecules should be administered to bind to a substantial portion of the rotavirus expected to cause or actually causing infection so that infection can either be prevented or ameliorated. Typically, the oral composi-

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tions of the instant invention will contain from less than 1% to about 95% of the active ingredient, preferably about 10% to about 50%. Preferably, between about 12 μ g and 1.2 mg will be administered to a child and between
5 about 200 μ g and 10 mg will be administered to an adult. The frequency of administration will be determined by the care giver based on patient responsiveness. Other effective dosages can be readily determined by one of ordinary skill in the art through routine trials
10 establishing dose response curves.

Other modes of administration will also find use with the subject invention. For instance, the rotavirus receptor saccharides can be formulated in suppositories and, in some cases, aerosol and intranasal
15 compositions. For suppositories, the vehicle composition will include traditional binders and carriers such as, polyalkylene glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10%
20 (w/w), preferably about 1% to about 2%.

Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known
25 substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal
30 mucosa.

The receptor saccharides formulations of the instant invention may also be administered as injectables. Typically, injectable compositions are prepared as liquid solutions or suspensions; solid forms

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suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the active ingredient encapsulated in liposome vehicles. The
5 receptors saccharides can be mixed with compatible, pharmaceutically acceptable excipients.

Suitable vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the
10 vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences,
15 Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985. The composition or formulation to be administered will, in any event, contain a quantity of the receptor saccharides adequate to achieve the desired state in the subject being treated.

20 The various receptor saccharides of the present invention can be used by themselves or in combination with pharmaceutically exceptable excipient materials as described above. However, it is more preferable to use the receptor compounds of the invention as conjugates
25 wherein the compounds of the invention are linked in some manner to an antiviral drug. By forming such conjugates, the receptor saccharides of the invention act as biochemical delivery systems for the antiviral drugs. It is well known that many antiviral drugs are extremely
30 toxic. Accordingly, it is desirable to provide such drugs to a patient in extremely small amounts. The smaller the amount of the drug delivered, the less toxic affects. However, the decrease in toxic affects must be balanced against the need to provide sufficient amounts

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of the drug in order to sufficiently destroy the virus. The receptor saccharides of the invention aid in solving the problem by providing the biochemical delivery system. By forming conjugates of the receptor saccharides of the invention with antiviral drugs, the conjugates readily
5 attach to the viruses and destroy them. Thereby, smaller amounts of the antiviral drug need be delivered to a patient to obtain efficacious results.

The receptor saccharides of the invention could
10 also be used as laboratory probes to test for the presence of a rotavirus in a sample. Such probes are preferably labeled such as with a radioactive or fluorescent labels.

15 C. Experimental

The following examples are provided so as to provide those of ordinary skill in the art with a complete disclosure and description of how to test the receptor saccharides of the invention and are not
20 intended to limit the scope of what the inventors regard as their invention or as being the only methods of testing such molecules. Efforts have been made to insure accuracy with respect to numbers used (e.g., amounts, temperature), but some experimental errors and deviation
25 should be accounted for. Unless indicated otherwise, parts or parts by weight, temperature is in degrees centigrade, and pressure is at or near atmospheric.

Example 1

30 Rotavirus Thin Layer Overlay Binding Assay

The ability of any of the compounds of the invention to bind rotavirus can be tested in the following manner.

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Rotavirus SA11 for use in the following experiment can be grown in and isolated from MA104 cells, available from Whittaker Bioproducts, Walkersville MD., using previously described methods. See, e.g. Yolken et al., J. Clin. Invest. 79: 148-154 (1987) and Kabcenell et al., J. Virol. 62: 2929 (1988), the disclosures of which are incorporated herein by reference in their entirety. The virus can be iodinated using solid state iodobeads as reagent as described by Markwell, M.A., Analyt. Biochem. 125: 427-432 (1982), incorporated herein by reference. Approximately 25 ug of gradient purified virus (by protein assay) can be incubated with 2 mCi of Na ¹²⁵I and the virus subsequently purified by gel exclusion on a PD-10 column.

Silica 60 high performance thin layer chromatography plates (Merck) can be spotted with the isolated glycolipids and from 50 to 500 pmoles of asialo GM1 as standard. The plates can be run in chloroform:methanol:water 60:40:10 (v:v:v). The plates can be probed with 10⁷ dpm of ¹²⁵I-labeled rotavirus per 100 sq.cm. using a modification of the technique of Magnani et al., Anal. Biochem. 109: 399-402 (1980).

After probing, the plates can be treated with iodine and sprayed with orcinol (Alltech). Following autoradiography of the plates, it can then be determined if the molecules bind rotavirus, and whether they bind to a higher degree than asialo GM1 which normally binds virus if present in 50 pmoles in a 2 mm streak.

The above binding assay can be used for testing cleaved or synthesized sugar moieties and derivatives thereof after their attachment to lipids by means well known in the art of neoglycolipid synthesis. For example, the Ten Feizi method can be used whereby

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oligosaccharides are attached to phosphatidylethanolamine by reductive animation to make neoglycolipids.

Example 2

5 Rotavirus Binding to Adsorbed Glycolipids

 The ability of synthetic saccharides which are believed to be receptors to bind rotavirus can be tested by synthesizing the neoglycolipid form of the saccharide and using the following technique. Saccharides (in their
10 neoglycolipid form) to be tested are first adsorbed to PVC wells by evaporating the saccharide be tested out of chloroform:methanol:water (4:8:3, v:v:v) and resuspending by sonication in an appropriate volume of 2.6 μ M
phosphatidyl choline/2.6 μ M cholesterol in absolute
15 ethanol. The resuspended compounds being tested should then be diluted with an equal volume of water to give stock solutions between 0 and 20 μ M of test lipid. Prior to use, the PVC 96 well microtiter plates should be washed by immersion and agitation in n-butanol for 30
20 minutes, rinsed in absolute ethanol and air dried. 50 μ l of test solution should be added to the microtiter wells to yield between 0 and 1 nmole of test lipid with 130 pmoles each of phosphatidyl choline and cholesterol. The PVC plates should be allowed to stand at room temperature
25 for 80 minutes or just before the meniscus of the solution contacts the well bottom. Microtiter plates should then be washed three times with water and stored with water in each well until probed with labeled virus.

 The putative receptor saccharides (in their
30 neoglycolipid form) can be probed as follows:
¹²⁵I-Rotavirus is to be diluted in complete PBS supplemented with 10 mg/ml BSA (Fraction V), pH 7.4, to give approximately 20,000 cpm/100 μ l. This volume should be added to each microtiter well and the plates incubated at

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4⁰C. for 3 hours with gentle agitation. Following incubation, plates are to be washed three times with ice cold PBS to remove unbound virus. The bottom half of each well should then be clipped off and placed in vials
5 for measurement of bound radioactivity. This procedure will indicate if the tested compound demonstrates the ability to bind rotavirus.

Soluble rotavirus binding substances can also be tested (for their ability to inhibit binding to an
10 immobilized glycolipid) with this technique by first adding the putative rotavirus binders to microtiter wells in 50 ul volumes of PBS-BSA at twice the desired final concentration. Labeled rotavirus should then be added to the wells at approximately 20,000 cpm/50 ul and the
15 radioactivity in the wells determined as above.

Example 3

Rotavirus Plaque Reduction Assay

The ability of the above-described synthesized
20 sugar moieties to inhibit rotavirus infection can be tested in vitro using the following plaque reduction assay. SA11 rotavirus (at 100 pfu) is to be added to 6 well plates containing confluent MA104 cells. The cells are to be incubated for one hour at 37⁰C., the inoculum
25 removed and the cells washed once with EBSS medium. The cells are then to be overlaid with 3 mls of medium containing 0.7% agarose and the compounds to be tested. Known inhibitors, such as ovalbumin and bovine
submaxillary mucin and substances known not to inhibit
30 rotavirus, such as globoside and trihexosyl ceramide, can be added as positive and negative controls, respectively. After the overlay gels, the plates are to be incubated for 48 hours and the number of plaques counted.

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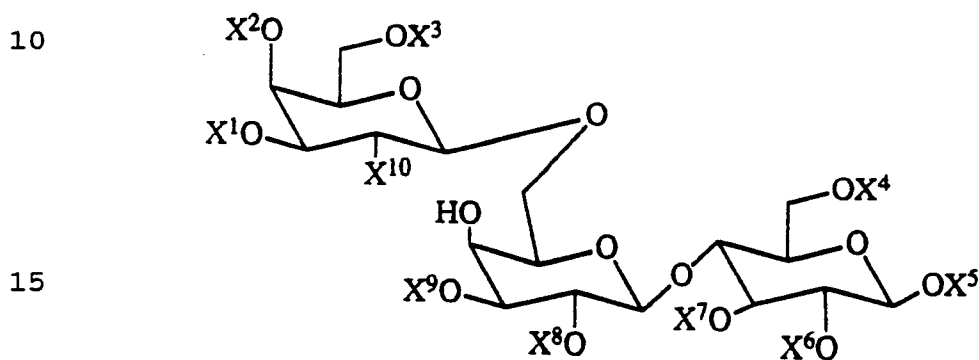
The instant invention is shown and described herein in what is considered to be the most practical, and preferred embodiments. It is recognized, however, that departures may be made therefrom which are within
5 the scope of the invention, and that obvious modifications will occur to one skilled in the art upon reading this disclosure.

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CLAIMS

1. A composition for preventing or treating rotaviral infection, comprising:

- 5 a pharmaceutically acceptable carrier; and
a therapeutically effective amount of a rotavirus receptor having the following structural formula III:



III

20 wherein each of X^1 to X^8 is independently H, an acyl, or a benzyl and X^{10} is acylamine or OH.

2. A composition for preventing or treating rotaviral infection as claimed in claim 1, wherein X^1 , X^2 and X^3 are acyl, X^4 , X^5 , X^6 , X^7 , X^8 and X^9 are benzyl and X^{10} is acylamine.

25

3. A composition for preventing or treating rotaviral infection as claimed in claim 1, wherein X^1 - X^3 are each H, X^4 - X^9 are each benzyl and X^{10} is $-NHCOR$ and R is an alkyl containing 1 to 30 carbon atoms.

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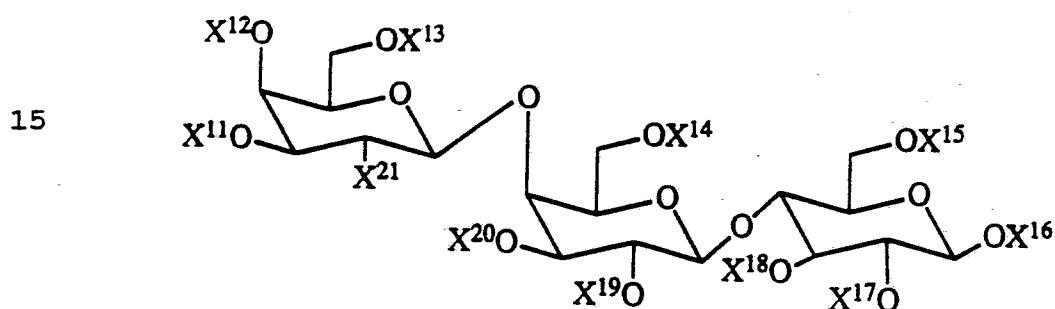
4. A composition for preventing or treating rotaviral infection as claimed in claim 1, wherein each of $X^1 - X^9$ is H, X^{10} is NHCOCH_3 with the proviso that X^5 may be in the alpha or beta positions.

5

5. A composition for preventing or treating rotaviral infection, comprising:

a pharmaceutically acceptable carrier; and

a therapeutically effective amount of a rotavirus receptor having the following structural formula IV:



20

IV

wherein each of $X^{11} - X^{20}$ are independently H, acyl or benzyl and X^{21} is -OH or acylamine.

25

6. A composition for preventing or treating rotaviral infection as claimed in claim 5, wherein each of $X^{11} - X^{13}$ is acyl, each of $X^{14} - X^{20}$ is benzyl and X^{21} is NHCOR and R is an alkyl containing 1 to 30 carbon atoms.

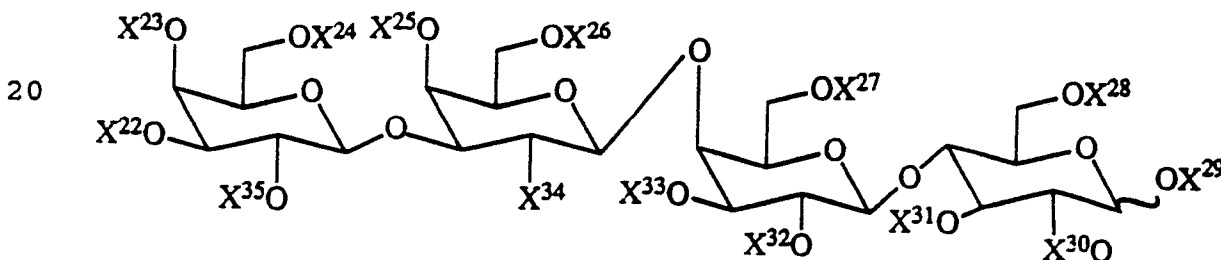
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-45-

7. A composition for preventing or treating rotaviral infection as claimed in claim 5, wherein each of X^{11} - X^{13} is OH, each of X^{14} - X^{20} is benzyl and X^{21} is NHCOR and R is an alkyl containing 1 to 30 carbon atoms.

8. A composition for preventing or treating rotaviral infection as claimed in claim 5, wherein each of X^{11} - X^{20} is H and X^{21} is NHCOCH_3 with the proviso that X^{16} may be in the alpha or beta position.

9. A composition for preventing or treating rotaviral infection, comprising:
a pharmaceutically acceptable carrier; and
a therapeutically effective amount of a rotavirus receptor having the following general structural formula V:



V

wherein each of X^{22} - X^{33} and X^{35} is independently H, an acyl, or a benzyl and X^{34} is OH or an acylamine.

10. The composition for preventing or treating rotaviral infections as claimed in claim 9, wherein each of X^{22} - X^{33} and X^{35} is H and X^{34} is $-\text{NHCOCH}_3$ with the proviso that X^{29} may be in the alpha or beta position.

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11. A composition for preventing or treating rotaviral infection as claimed in claim 1 wherein the pharmaceutically acceptable carrier is a carrier for oral delivery.

5

12. A composition for preventing or treating rotaviral infection as claimed in claim 11, wherein the pharmaceutically acceptable carrier is an infant nutrient formula.

10

13. A method for preventing or treating rotavirus induced disorders comprising administering to a subject a therapeutically effective amount of a composition according to claim 11.

15

14. A method for preventing or treating rotavirus induced disorders comprising administering to a subject a therapeutically effective amount of a composition according to claim 12.

20

15. A composition for preventing or treating rotaviral infection as claimed in claim 5 wherein the pharmaceutically acceptable carrier is a carrier for oral delivery.

25

16. A composition for preventing or treating rotaviral infection as claimed in claim 15 wherein the pharmaceutically acceptable carrier is an infant nutrient formula.

30

17. A method for preventing or treating rotavirus induced disorders comprising administering to a subject a therapeutically effective amount of a composition according to claim 15.

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18. A method for preventing or treating
rotavirus induced disorders comprising administering to a
subject a therapeutically effective amount of a
5 composition according to claim 16.

19. A composition for preventing or treating
rotaviral infection as claimed in claim 9 wherein the
pharmaceutically acceptable carrier is a carrier for oral
10 delivery.

20. A composition for preventing or treating
rotaviral infection as claimed in claim 19 wherein the
pharmaceutically acceptable carrier is an infant nutrient
15 formula.

21. A method for preventing or treating
rotavirus induced disorders comprising administering to a
subject a therapeutically effective amount of a composi-
20 tion according to claim 19.

22. A method for preventing or treating
rotavirus induced disorders comprising administering to a
subject a therapeutically effective amount of a composi-
25 tion according to claim 20.

23. A composition for preventing or treating
rotaviral infection as claimed in claim 10 wherein the
pharmaceutically acceptable carrier is a carrier for oral
30 delivery.

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24. A composition for preventing or treating rotaviral infection as claimed in claim 21 wherein the pharmaceutically acceptable carrier is an infant nutrient formula.

5

25. A method for preventing or treating rotavirus induced disorders comprising administering to a subject a therapeutically effective amount of a composition according to claim 21.

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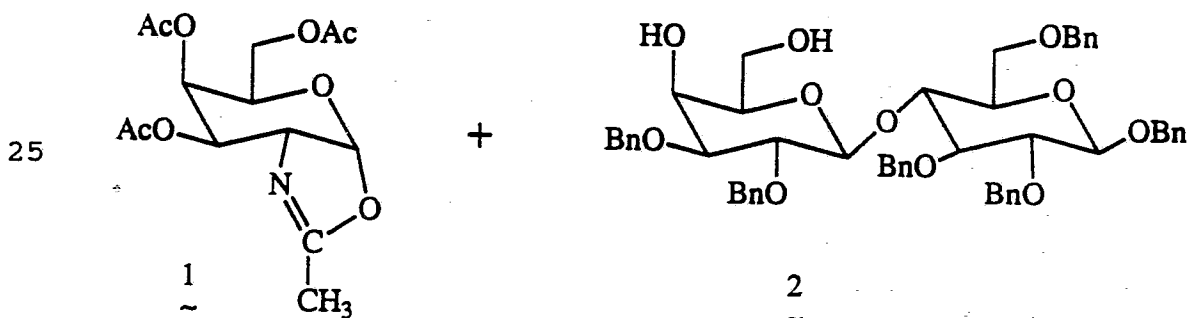
26. A method for preventing or treating rotavirus induced disorders comprising administering to a subject a therapeutically effective amount of a composition according to claim 22.

15

27. A method of synthesizing β -D-GalNAc-(1-6)- β -D-Gal-(1-4)-D-Glc comprising the steps of:

reacting the compound of formula 1 with the compound of formula 2

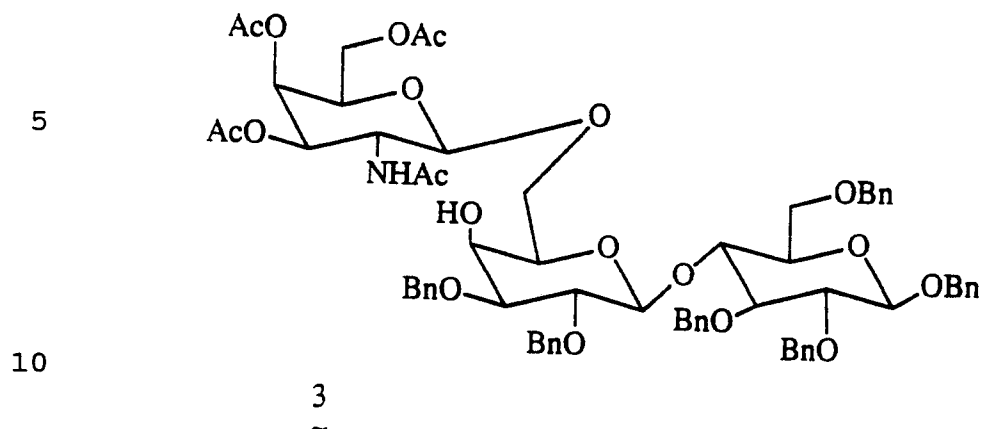
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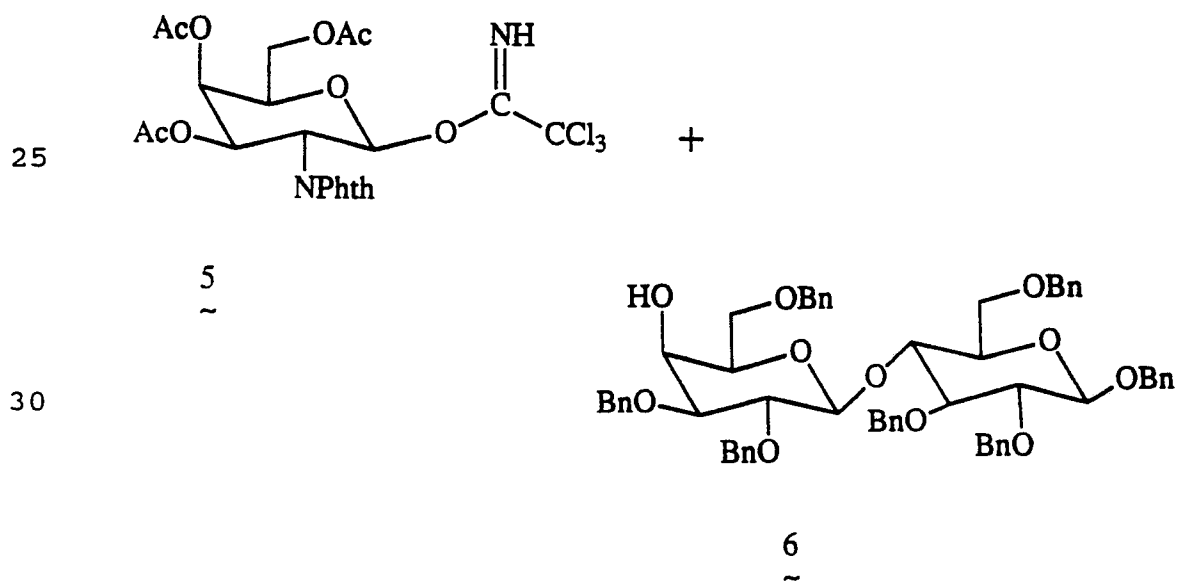
to obtain the condensation product of formula 3



deacetylating the compound of formula 3 and
 carrying out hydrogenolysis of the benzyl groups to
 15 obtain β -D-GalNAc-(1-6)- β -D-Gal-(1-4)-D-Glc.

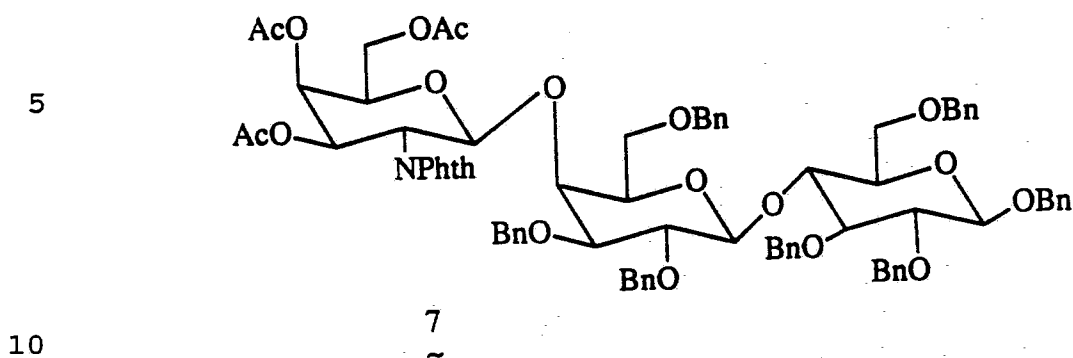
28. A method of synthesizing β -D-GalNAc-(1-4)- β -D-Gal-(1-4)-D-Glc, comprising the steps of:

reacting the compound of formula 5 with the
 20 compound of formula 6

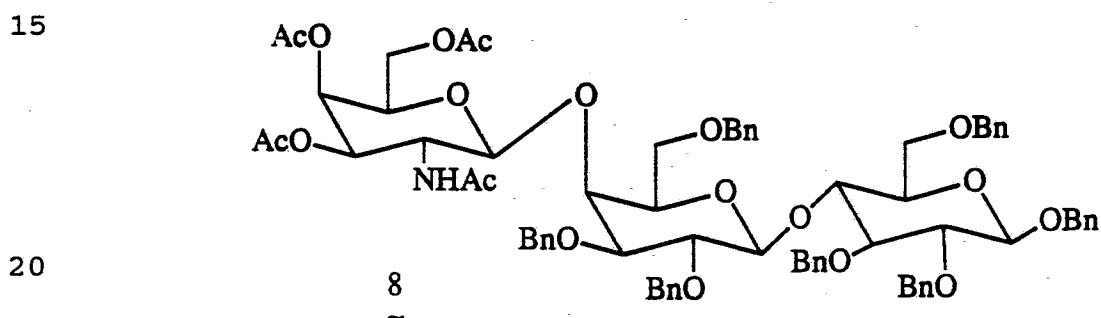


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to obtain the compound of formula 7



carrying out hydrazinolysis and acetylation of the compound of formula 7 to obtain the compound of formula 8

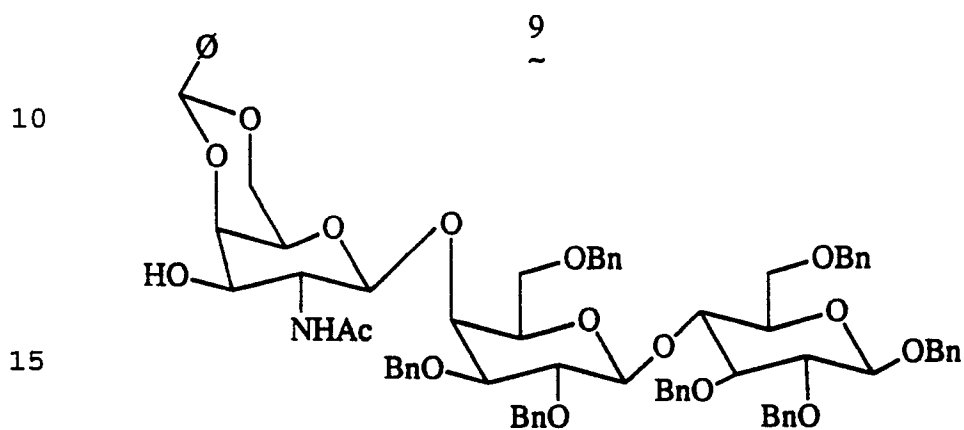
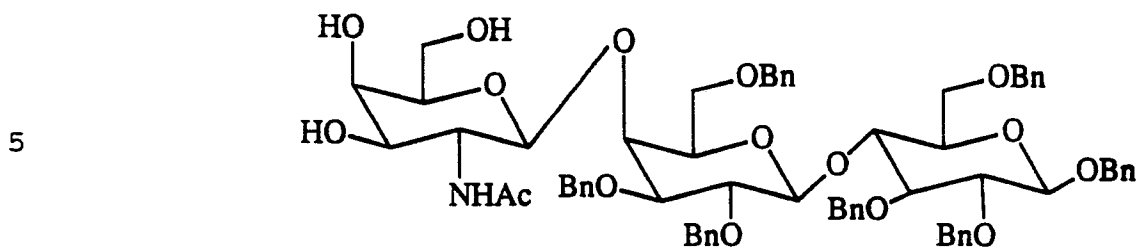


carrying out transesterification of the compound 8 followed by hydrogenolysis to obtain β -D-GalNac-(1-4)- β -D-Gal-(1-4)-D-Glc.

29. A method of synthesizing β -Gal-(1-3)- β -GalNac-(1-4)- β -Gal-(1-4)-D-Glc, comprising the steps of:

reacting the compound of formula 9 with a benzaldehyde-zinc chloride complex to obtain the compound of formula 10

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condensing the compound of formula 10 with
acctobromogalactose followed by deacetylation,
debenzylidenation and hydrogenation to obtain β -Gal-(1-
3)- β -GalNAc-(1-4)- β -Gal-(1-4)-D-Glc.

INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US90/07121**

I. CLASSIFICATION Or SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC(5):A61K 31/70, 39/12; C07H 3/06, 15/04; C12N 7/04		
U.S. CL.: 514/23.61; 435/236; 536/4.1, 53		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
U.S.	514/23, 61; 435/236; 536/4.1, 53	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
Chemical Abstracts Service: Searched carbohydrate structures as claimed: searched rotavirus or rotaviral and receptor(s) or epitope(s) and binding, bind(s), or bound.		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	US, A. 4,341,763 (ZYGRAICH) 27 July 1982, see entire document.	1-29
X	US, A. 4,464,360 (LEFFLER ET AL.) 07 August 1984, see entire document.	1-26
A	US, A. 4,657,849 (KALLENIOUS ET AL.) 14 April 1987, see entire document.	1-29
Y	US, A. 4,665,060 (MARDH ET AL.) 12 May 1987, see entire document.	1-29
X	US, A. 4,831,021 (TUBARO ET AL.) 16 May 1989, see entire document.	1-26
X	US, A. 4,859,769 (KARLSSON ET AL.) 22 August 1989, see entire document.	1-26
X,P	US, A. 4,918,009 (NILSSON) 17 April 1990, see entire document.	1-26
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>[*] Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date or another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"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</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
06 February 1991		28 MAR 1991
International Searching Authority		Signature of Authorized Officer
ISA/US		Nancy S. Carson <i>Nancy S. Carson</i>
		ebw

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	JP, A, 58-154513 (SENDAI BISEIBUTSU K) 14 September 1983, Abstract.	1-26
A	Infection and Immunity, volume 14, number 2, issued August 1976, C.A. Mebus et al, "Diarrhea in Gnotobiotic Calves Caused by the Reovirus-Like Agent of Human Infantile Gastroenteritis", pages 471-474.	1-26
A	Infection and Immunity, Volume 14, number 3, issued September 1976, J.G. LECCE et al., "Reovirus- Like Agent Associated with Fetal Diarrhea in Neonatal Pigs", pages 816-825.	1-26
A	SCIENCE, Volume 207, issued 11 January 1980, R.G. WYATT et al., "Human Rotavirus Type 2: Cultivation in vitro", pages 189-191.	1-26
Y	Infection and Immunity, Volume 29, Number 3, issued September 1980, J.W. Bastardo et al., "Attachment of SA-11 Rotavirus to Erythrocyte Receptors", pages 1134-1140, see entire document.	1-26
A	Journal of Virology, Volume 53, number 1, issued January 1985, M. Sabara et al., "Preliminary Characterization of an Epitope Involved in Neutralization and Cell Attachment That is Located on the Major Bovine Rotavirus Glycoprotein", pages 58-66.	1-26
A	Chemical Abstracts, Volume 104, issued 1986, Otnaess et al., "Studies on nonimmunoglobulin inhibitory activity in human milk against E. coli heat- labile enterotoxin, rotavirus, and respiratory syncytial virus", see page 520, column 2 to page 521, column 1, abstract no. 223817p, Nestle Nutr. Workshop' Ser., 5 (Hum. Milk Banking) 85-92.	1-26

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Journal of General Virology, Volume 68, issued 1987, M. Sabara et al., "Biochemical Evidence for the Oligomeric Arrangement of Bovine Rotavirus Nucleocapsid Protein and Its Possible Significance in the Immunogenicity of This Protein", pages 123-133.	1-26
X	Journal of Clinical Investigation, Volume 79, issued January 1987, R.H. YOLKEN et al., "Sialic Acid Glycoproteins Inhibit In Vitro and In vivo Replication of Rotaviruses", pages 148-154, see entire document.	1-26
A	Journal of Virology, Volume 63, Number 11, issued November 1989, K.S. Au et al. "Receptor Activity of Rotavirus Nonstructural Glycoprotein NS28", pages 4553-4562.	1-26
A	The EMBO Journal, Volume 8, Number 6, issued 1989, C.C. Bergmann et al., "Topology of the non-structural rotavirus receptor glycoprotein NS28 in the rough endoplasmic reticulum", pages 1695-1703.	1-26
X	Proceedings of U.S. Japan International Rotavirus Meeting, issued August 1989, (Annapolis MD), Willoughby et al, "Rotaviruses Bind Specifically to the Neutral Glycolipid Asialo-GM1", see entire abstract.	1-26
A	Virology, Volume 171, issued 1989, K. Nishikawa et al., "Rotavirus VP7 Neutralization Epitopes of Serotype 3 Strains", pages 503-515.	1-26
X,P	Journal of Virology, Volume 64, number 10, issued October 1990, R.E. Willoughby et al, "Rotaviruses Specifically Bind to the Neutral Glycosphingolipid Asialo-GM1", pages 4830-4835, see entire document.	1-26

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Carbohydrate Research, Volume 138, issued 1985, J. Dahmen et al., "Synthesis of Di-Tri-, and Tetra-saccharides corresponding to Receptor Structures Recognized by <u>Streptococcus pneumoniae</u> ", pages 17-28, see entire document.	27-29
Y	Carbohydrate Research, Volume 167, issued 1987, S. Sato et al., "Total Synthesis of X Hapten, III ³ Fuc-alpha-nLC ₄ Cer", pages 197-210, see entire document.	27-29