



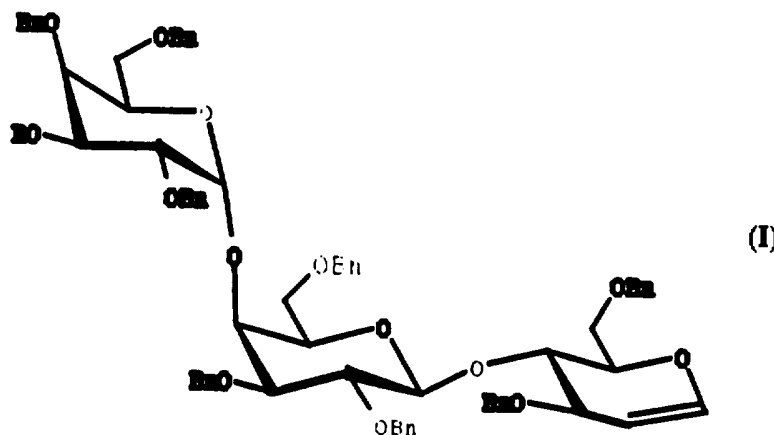
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<p>(21) International Application Number: PCT/US96/06109</p> <p>(22) International Filing Date: 26 April 1996 (26.04.96)</p> <p>(30) Priority Data: 08/430,355 28 April 1995 (28.04.95) US</p> <p>(71) Applicant: SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH [US/US]; 1275 York Avenue, New York, NY 10021 (US).</p> <p>(72) Inventors: DANISHEFSKY, Samuel, J.; 22 Brayton Street, Englewood, NJ 07631 (US). BILODEAU, Mark, T.; Apartment 21F, 303 East 63rd Street, New York, NY 10021 (US). HU, Shuang, Hua; Apartment 1C, 310 East 66th Street, New York, NY 10021 (US). PARK, Tae, Kyo; Apartment 2L, 504 East 81st Street, New York, NY 10028 (US). RANDOLPH, John, T.; Apartment 111, 30035 North Waukegan Road, Lake Bluff, IL 60044 (US). KIM, In, Jong; Apartment 6M, 504 East 81st Street, New York, NY 10028 (US). LIVINGSTON, Philip, O.; Apartment 6C, 156 East 79th Street, New York, NY 10021 (US).</p> <p>(74) Agent: WHITE, John, P.; Cooper & Dunham L.L.P., 1185 Avenue of the Americas, New York, NY 10036 (US).</p>	<p>(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report.</i></p>	

(54) Title: SYNTHESIS OF THE BREAST TUMOR-ASSOCIATED ANTIGEN DEFINED BY MONOCLONAL ANTIBODY MBR1 AND USES THEREOF

(57) Abstract

The present invention provides a process for synthesizing a compound having structure (I), wherein R is H, as well as related oligosaccharides useful as a vaccine for inducing antibodies to human breast cancer cells in an adjuvant therapy therefor.



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**SYNTHESIS OF THE BREAST TUMOR-ASSOCIATED ANTIGEN
DEFINED BY MONOCLONAL ANTIBODY MB1 AND USES THEREOF**

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This application is a continuation-in-part of U.S. Serial No. 08/213,053, filed March 15, 1994, the contents of which are hereby incorporated by reference into this application.

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This invention was made with government support under grants GM-15240-02, GM-16291-01, and AI-16943 from the National Institutes of Health. Accordingly, the U.S. Government has certain rights in the invention.

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Background of the Invention

Throughout this application, citations for various publications are provided within parentheses in the text. The disclosures of these publications are hereby incorporated in their entirety by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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The function of carbohydrates as structural materials and as energy storage units in biological systems is well recognized. By contrast, the role of carbohydrates as signaling molecules in the context of biological processes has only recently been appreciated. (M.L. Phillips, E. Nudelman, F.C.A. Gaeta, M. Perez, A.K. Singhal, S. Hakomori, J.C. Paulson, *Science*, 1990, 250, 1130; M.J. Polley, M.L. Phillips, E. Wagner, E. Nudelman, A.K. Singhal, S. Hakomori, J.C. Paulson, *Proc. Natl. Acad. Sci. USA*, 1991, 88, 6224; T. Taki, Y. Hirabayashi, H. Ishikawa, S. Kon, Y. Tanaka, M. Matsumoto, *J. Biol. Chem.*, 1986, 261, 3075; Y. Hirabayashi, A. Hyogo, T. Nakao, K. Tsuchiya, Y. Suzuki, M. Matsumoto, K. Kon, S. Ando, *ibid.*, 1990, 265, 8144; O. Hindsgaul, T. Norberg,

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J. Le Pendu, R. U. Lemieux, *Carbohydr. Res.*, **1982**, 109, 109; U. Spohr, R.U. Lemieux, *ibid.*, **1988**, 174, 211)

The elucidation of the scope of carbohydrate involvement in mediating cellular interaction is an important area of inquiry in contemporary biomedical research. The carbohydrate molecules, carrying detailed structural information, tend to exist as glycoconjugates (cf. glycoproteins and glycolipids) rather than as free entities. Given the complexities often associated with isolating the conjugates in homogeneous form and the difficulties in retrieving intact carbohydrates from these naturally occurring conjugates, the applicability of synthetic approaches is apparent. (For recent reviews of glycosylation see: Paulsen, H., *Agnew. Chemie Int. Ed. Engl.*, **1982**, 21, 155; Schmidt, R.R., *Angew. Chemie Int. Ed. Engl.*, **1986**, 25, 212; Schmidt, R.R., *Comprehensive Organic Synthesis*, Vol. 6, Chapter 1(2), Pergamon Press, Oxford, **1991**; Schmidt, R.R., *Carbohydrates, Synthetic Methods and Applications in Medicinal Chemistry*, Part I, Chapter 4, VCH Publishers, Weinheim, New York, **1992**. For the use of glycals as glycosyl donors in glycoside synthesis, see Lemieux, R.U., *Can. J. Chem.*, **1964**, 42, 1417; Lemieux, R.U., Faser-Reid, B., *Can. J. Chem.*, **1965**, 43, 1460; Lemieux, R.U., Morgan, A.R., *Can. J. Chem.*, **1965**, 43, 2190; Thiem, J., Karl, H., Schwentner, J., *Synthesis*, **1978**, 696; Thiem, J. Ossowski, P., *Carbohydr. Chem.*, **1984**, 3, 287; Thiem, J., Prahst, A., Wendt, T. *Liebigs Ann. Chem.*, **1986**, 1044; Thiem, J. in *Trends in Synthetic Carbohydrate Chemistry*, Horton, D., Hawkins, L.D., McGarvey, G.L., eds., ACS Symposium Series #386, American Chemical Society, Washington, D.C., **1989**, Chapter 8.)

The carbohydrate domains of the blood group substances contained in both glycoproteins and glycolipids are distributed in erythrocytes, epithelial cells and various secretions. The early focus on these systems centered on

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their central role in determining blood group specificities. (R.R. Race and R. Sanger, *Blood Groups in Man*, 6th ed., Blackwell, Oxford, 1975) However, it is recognized that such determinants are broadly implicated

5 in cell adhesion and binding phenomena. (For example, see M.L. Phillips, E. Nudelman, F.C.A. Gaeta, M. Perez, A.K. Singhal, S. Hakomori, J.C. Paulson, *Science*, 1990, 250, 1130.) Moreover, ensembles related to the blood group substances in conjugated form are encountered as

10 markers for the onset of various tumors. (K.O. Lloyd, *Am. J. Clinical Path.*, 1987, 87, 129; K.O. Lloyd, *Cancer Biol.*, 1991, 2, 421) Carbohydrate-based tumor antigenic factors might find applications at the diagnostic level, as resources in drug delivery or ideally in

15 immunotherapy. (Toyokuni, T., Dean, B., Cai, S., Boivin, D., Hakomori, S., and Singhal, A.K., *J. Am. Chem Soc.*, 1994, 116, 395; Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K., Jackson, V., Hamada, H., Paardoll, D., Mulligan, R., *Proc. Natl. Acad. Sci. USA*, 1993, 90, 3539; Tao, M-H., Levy, R., *Nature*, 1993, 362, 755; Boon, T., *Int. J. Cancer*, 1993, 54, 177; Livingston, P.O., *Curr. Opin. Immunol.*, 1992, 4, 624; Hakomori, S., *Annu. Rev. Immunol.*, 1984, 2, 103; K. Shigeta, et al., *J. Biol. Chem.*, 1987, 262, 1358)

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The present invention provides new strategies and protocols for oligosaccharide synthesis. The object is to simplify such constructions such that relatively complex domains can be assembled with high stereo-

30 specificity. Major advances in glycoconjugate synthesis require the attainment of a high degree of convergence and relief from the burdens associated with the manipulation of blocking groups. Another requirement is that of delivering the carbohydrate determinant with

35 appropriate provision for conjugation to carrier proteins or lipids. (Bernstein, M.A., and Hall, L.D., *Carbohydr. Res.*, 1980, 78, C1; Lemieux, R.U., *Chem. Soc. Rev.*, 1978,

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7, 423; R.U. Lemieux, et al., *J. Am. Chem. Soc.*, **1975**,
97, 4076) This is a critical condition if the
synthetically derived carbohydrates are to be
incorporated into carriers suitable for biological
5 application.

Antigens which are selective or ideally specific for
cancer cells could prove useful in fostering active
immunity. (Hakomori, S., *Cancer Res.*, **1985**, 45, 2405-
10 2414; Feizi, T., *Cancer Surveys*, **1985**, 4, 245-269) Novel
carbohydrate patterns are often presented by transformed
cells as either cell surface glycoproteins or as
membrane-anchored glycolipids. In principle, well chosen
synthetic glycoconjugates which stimulate antibody
15 production could confer active immunity against cancers
which present equivalent structure types on their cell
surfaces. (Dennis, J., *Oxford Glycosystems Glyconews*
Second, **1992**; Lloyd, K. O., in *Specific Immunotherapy of*
Cancer with Vaccines, **1993**, New York Academy of Sciences
20 pp. 50-58) Chances for successful therapy improve with
increasing restriction of the antigen to the target cell.
A glycosphingolipid was isolated by Hakomori and
collaborators from the breast cancer cell line MCF-7 and
immunocharacterized by monoclonal antibody MBrl.
25 (Bremer, E. G., et al., *J. Biol. Chem.*, **1984**, 259, 14773-
14777; Menard, S., et al., *Cancer Res.*, **1983**, 43, 1295-
1300) The novel glycosphingolipid structure **1b** (Figure
8) was proposed for this breast tumor-associated antigen
on the basis of methylation and enzymatic degradation
30 protocols. A ¹H NMR spectrum consistent with but not
definitive for the proposed structure was obtained from
trace amounts of isolated antigen. While individual
sectors of the proposed structure were not unknown, the
full structure was first described based on studies on
35 the breast cancer line. It should be noted that MBrl
also binds to normal human mammary gland tissue and
ovarian cancer cell lines. Therefore, **1b** as a total

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entity is likely not restricted to the transformed breast cells. Alternatively, smaller subsections of **1b** are adequate for antibody recognition and binding. (The synthesis of the DEF fragment of **1b** has been reported, and has been shown to bind to MB1: Lay, L.; Nicotra, F.; Panza, L.; Russo, G. *Helv. Chim. Acta*, 1994, 77, 509-514.)

The compounds prepared by processes described herein are antigens useful in adjuvant therapies as a vaccines capable of inducing MB1 antibodies immunoreactive with human breast tumor cells. Such adjuvant therapies have potential to reduce the rate of recurrence of breast cancer and increase survival rates after surgery. Clinical trials on 122 patents surgically treated for AJCC stage III melanoma who were trated with vaccines prepared from melanoma differentiation antigen GM2 (another tumor antigen which like MB1 is a cell surface carbohydrate) demonstrated in patients lacking the antibody prior to immunization, a highly significant increase in disease-free interval (P.O. Livingston, et al., J. Clin Oncol., 12, 1036 (1994)).

The present invention provides a method of synthesizing **1b** in quantity as well as artificial protein-conjugates of the oligosaccharide which might be more immunogenic than the smaller glycolipid. The antigen contains a novel array of features including the α -linkage between the B and the C entities, as well as the β -linked ring D gal-NAC residue. (For the synthesis of a related structure (SSEA-3) which lacks the fucose residue see: Nunomura, S.; Ogawa, T., *Tetrahedron Lett.*, 1988, 29, 5681-5684.) The present invention provides (i) a total synthesis of **1b**, (ii) rigorous proof that the Hakomori antigen does, in fact, correspond to **1b** and (iii) the synthesis of a bioconjugatable version of **1b**. The conciseness of the synthesis reflects the efficiency of

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glycal assembly methods augmented by a powerful method for sulfonamidoglycosylation (see, e.g., the transformation of 14b-15b, Figure 10).

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Brief Description of the Figures

- Figure 1** shows glycal assembly leading to neoglycoproteins.
- 5 **Figure 2** shows the synthesis of **4a**. **Reagents:** a) TBDPSCl, imidazole/DMF 84%; b) carbonyldiimidazole, cat. imidazole, THF (65%) c) **5a**, di-tert-butylpyridine, AgClO₄, SnCl₂, ether (51%); PhSO₂NH₂, I(sym-coll)₂ClO₄ (94%).
- 10 **Figure 3** shows the synthesis of **8a**. **Reagents:** a) **9a**, AgBF₄, 4A mol. sieves, THF (75%); b) i. TBAF, THF; ii. Na/NH₃; iii Ac₂O, pyr. c) i. 3,3-dimethioxirane; allyl alcohol, ZnCl₂ (72%); ii. NaOMe, MeOH (quant.).
- 15 **Figure 4** shows a strategy for the solid-phase of oligosaccharides using the glycal assembly method.
- Figure 5** shows the application of the solid-support method to the assembly of 1,2-branching patterns of complex carbohydrates.
- 20 **Figure 6** shows the synthesis of a tetrasaccharide having H-type 2 blood group specificity. **Reagents:** (a) 1. 3,3-dimethyldioxirane, CH₂Cl₂; 2. **8**, ZnCl₂, THF; (b) **10**, Sn(OTf)₂, di-tert-butylpyridine, THF; (c) TBAF, AcOH, THF; (d) TIPSCl, imidazole, DMF; (e) I(coll)₂ClO₄, PhSO₂NH₂, CH₂Cl₂; (f) **15**, AgBF₄, 4A M.S., THF; (g) 1. TBAF, AcOH, THF; 2. Na/NH₃; 3. Ac₂O, pyridine.
- 25 **Figure 7a and 7b** show the synthesis of a Le^b hexasaccharide in bioconjugatable form. **Reagents:** (a) 1. 3,3-dimethyldioxirane, CH₂Cl₂; 2. **19**, ZnCl₂, THF; (b) **10**, Sn(OTf)₂ di-tert-butylpyridine, THF; (c) TBAF, AcOH, THF; (d) TIPSCl, imidazole, DMF; (e) I(coll)₂ClO₄, PhSO₂NH₂, CH₂Cl₂; (f) **24**, AgBF₄, 4A M.S., THF; (g) 1. TBAF, AcOH, THF; 2. Na/NH₃; 3. Ac₂O, pyridine; (h) 1. 3,3-dimethyl-
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dioxirane, CH_2Cl_2 ; 2. allyl alcohol, ZnCl_2 ; 3. NaOMe, MeOH.

Figure 8 shows the structure of the MBr1 antigen and a reaction pathway to a trisaccharide intermediate.

5 Reagents: a. $n\text{-Bu}_2\text{SnO}$, PMBCl , TBABr , PhH , 70%; b. NaH, BnBr , DMF, 95%; c. (i) 3,3-dimethyldioxirane, CH_2Cl_2 ; (ii) TBAF, THF; (iii) NaH, BnBr , DMF, 40% (three steps); d. NaH, BnBr , DMF, 80%; e. (i) TBAF, THF; (ii) NaOMe, MeOH, 93% (two steps); f. $(n\text{-Bu}_3\text{Sn})_2\text{O}$, BnBr , TBABr , PhH , 90%; g.
10 SnCl_2 , AgClO_4 , 2,6-di-butylpyridine, 4 Å mol. sieves, Et_2O , 40% & (4.5:1 &:B); h. DDQ, CH_2Cl_2 , H_2O , 84%.

Figure 9 shows a reaction pathway to a trisaccharide intermediate.

15 Reagents: a. (i) 3,3-dimethyldioxirane, CH_2Cl_2 ; (ii) **10a**, ZnCl_2 , THF, 87%; b. SnCl_2 , AgClO_4 , Et_2O , 47%; c. $\text{I}(\text{coll})_2\text{ClO}_4$, PhSO_2NH_2 , 4 Å mol. sieves, 47%.

Figure 10(a) shows a reaction pathway to the
20 hexasaccharide MBr1 antigen.

Reagents: a. EtSH, LiHMDS, DMF, 75%. B. **8b** (0.5 equiv), MeOTf , 4 Å Mol. sieves, 70-85% B, (10:1 B &); c. (i) 3,3-dimethyldioxirane, CH_2Cl_2 (ii) **17b** (5 equiv), $\text{Zn}(\text{OTf})_2$, 20%; d. Ac_2O , Et_3N , DMAP, CH_2Cl_2 95%; e. Lindlar's cat.,
25 H_2 palmitic anhydride, EtOAc, 90%; f. (i) TBAF, THF; (ii) NaOMe, MeOH, 94%; g. (i) Na, NH_3 , THF; (ii) Ac_2O , Et_3N , DMAP, CH_2Cl_2 , 80% h. NaOMe, MeOH, quant.

Figure 10(b) shows a reaction pathway to the allyl
30 glycoside.

Reagents: a. TBAF, THF, 94%; b. (i) Na, NH_3 , THF; (ii) Ac_2O , Et_3N , DMAP, THF, DMF, 85%; c. (i) 3,3-dimethyldioxirane, CH_2Cl_2 , (ii) allyl alcohol, 65% (+ 29% of &-manno isomer); d. NaOMe, MeOH, quant.

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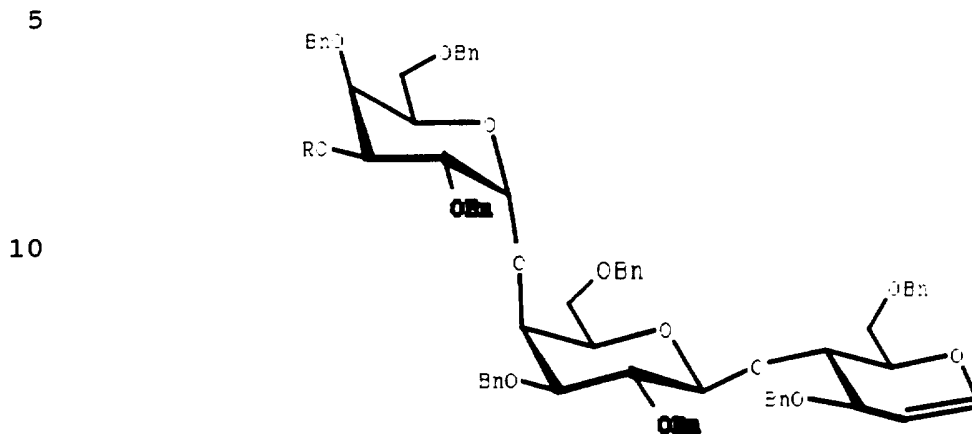
Figure 11 shows a reaction pathway to intermediates for preparing the hexasaccharide antigen MBr1.

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Figure 12 shows a reaction pathway to the hexasaccharide antigen MBr1 by a 4+2 synthetic approach.

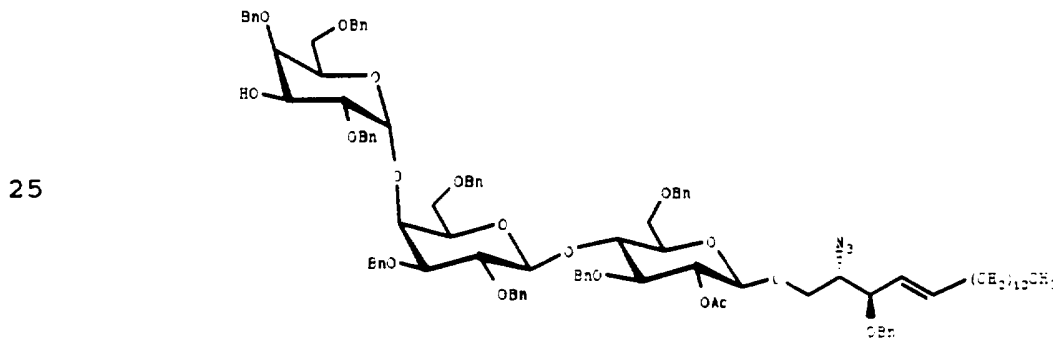
Summary of the Invention

The present invention provides a process for synthesizing a compound having the structure:

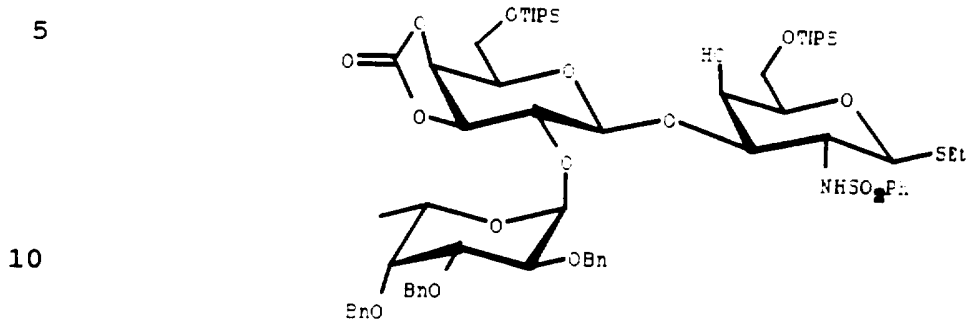


wherein R is H.

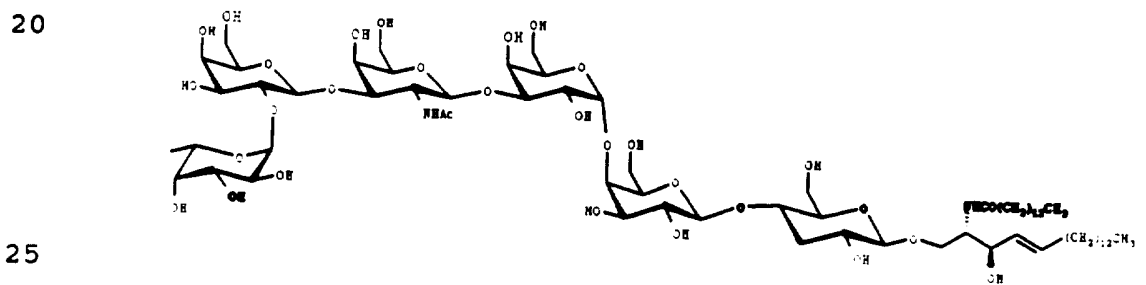
The present invention also provides a process for synthesizing a trisaccharide ceramide having the structure:



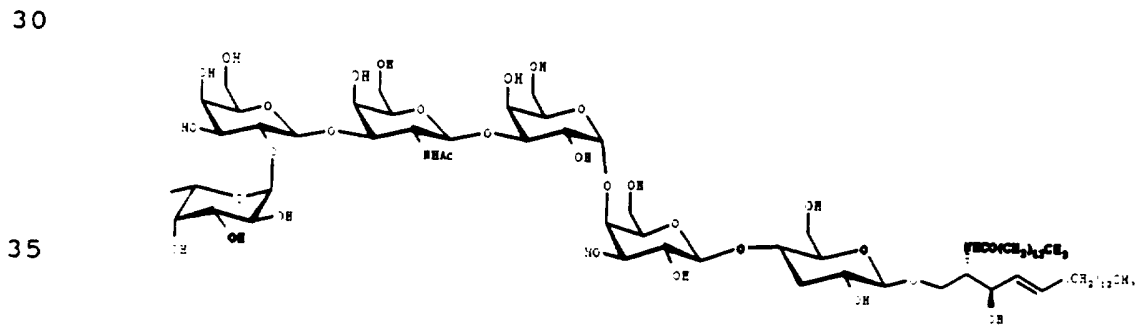
The present invention further provides a process for synthesizing a mercaptotrisaccharide having the structure:



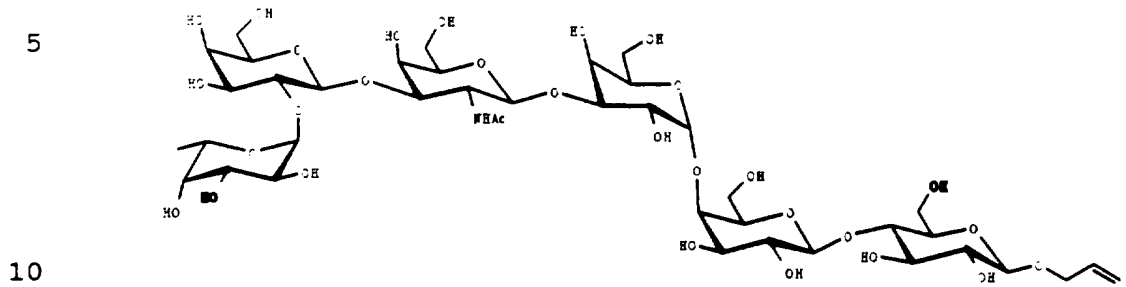
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The present invention also provides a process of synthesizing a hexasaccharide ceramide having the structure:



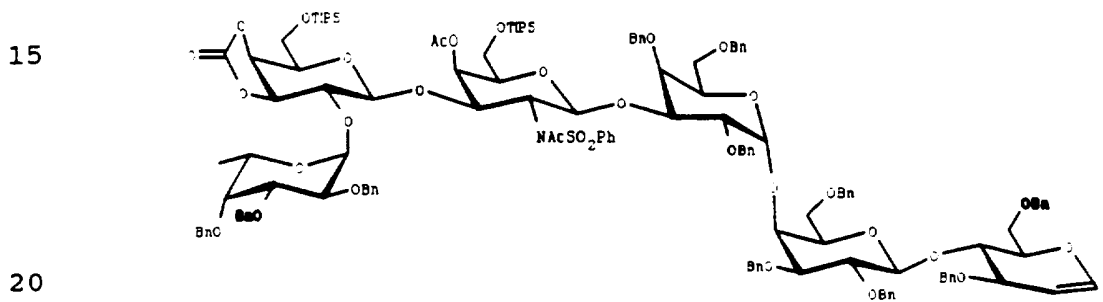
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The present invention also provides a process of synthesizing a hexasaccharide ceramide having the structure:



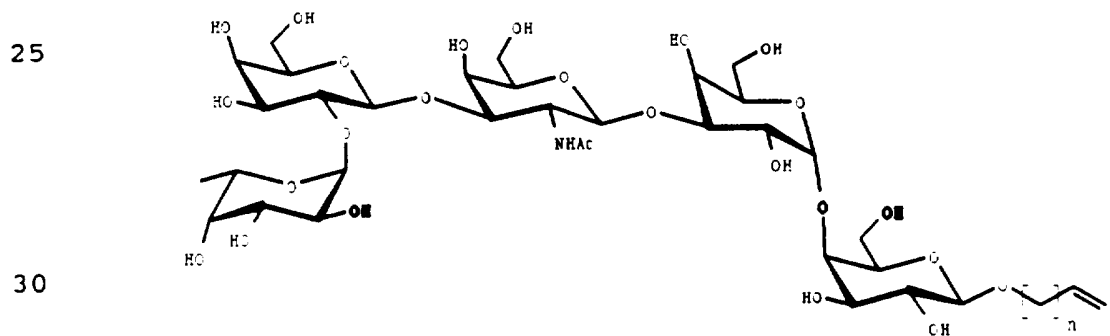
The present invention also provides a process of synthesizing an allyl hexasaccharide having the structure:



The present invention provides a process of synthesizing a hexasaccharide having the structure:



The present invention further provides a compound having the structure:

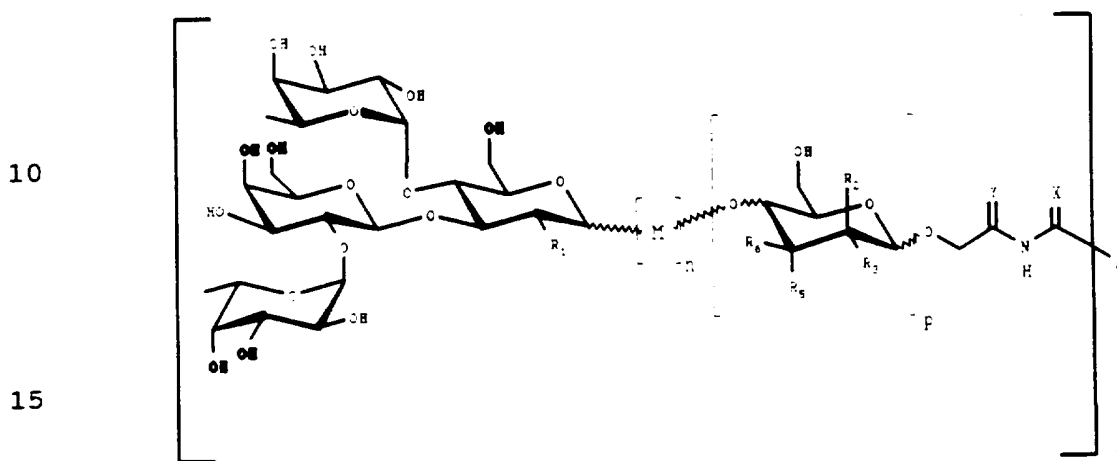


wherein n is an integer between about 0 and about 9.

Detailed Description of the Invention

The present invention provides a compound having the structure:

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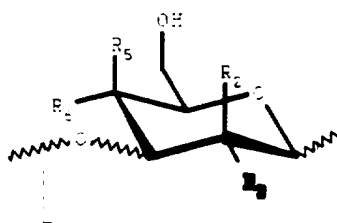
20 wherein A is selected from the group consisting of (i) an amino acid bearing an ω -amino group or an ω -(C=O)- group, (ii) an amino acid residue of a peptide, which residue bears an ω -amino group or an ω -(C=O)- group, and (iii) an amino acid residue of a protein, which residue bears an

25 ω -amino group or an ω -(C=O)- group; wherein R_1 is H, OH, NH_2 or NHR_4 , where R_4 is SO_2Ph , a linear or branched chain

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alkyl or acyl group, or an aryl group; wherein M has the structure:

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wherein n is an integer from 0 to 18, and where n is greater than 1, each M is independently the same or different; wherein p is either 0 or 1; wherein R₂, R₃, R₅ and R₆ are independently the same or different and are H or OH, with the proviso that geminal R₂ and R₃ are not both OH, and geminal R₅ and R₆ are not both OH; wherein each wavy line between a carbon atom and an oxygen atom denotes an R or S configuration at the carbon atom; wherein X and Y are independently the same or different and are H₂ or O; and wherein k is an integer greater than or equal to 1, with the proviso that when A is an amino acid bearing an ω-amino group or an ω-(C=O)- group, k is equal to 1.

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In one embodiment, the present invention provides the compound disclosed hereinabove wherein A is lysine or a lysine residue.

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In another embodiment, the present invention provides the compound disclosed hereinabove wherein A is glutamic acid or a glutamic acid residue.

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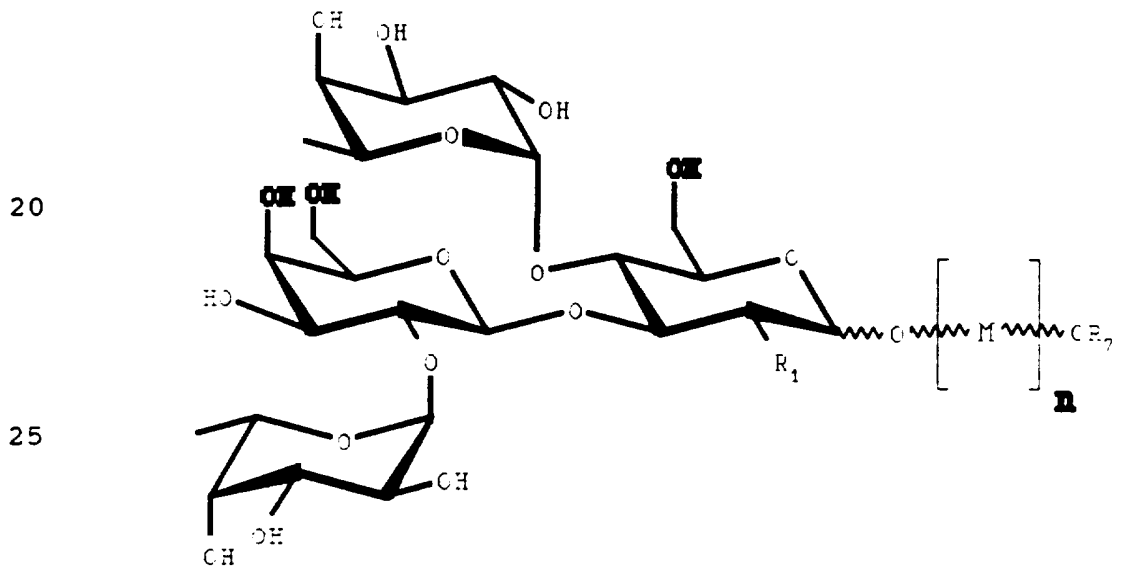
In another embodiment, the present invention provides the compound disclosed hereinabove wherein A is aspartic acid or an aspartic acid residue.

The invention also provides the compound disclosed hereinabove wherein A is an amino acid residue of a globular protein. In one embodiment, the invention provides the compound wherein the globular protein is selected from the group consisting of bovine serum albumin and human serum albumin.

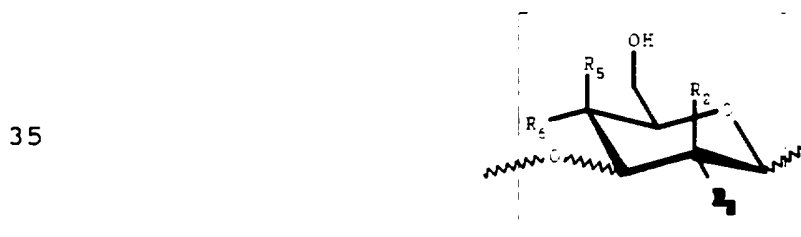
In one embodiment, the invention provides the compound disclosed hereinabove wherein k is 1.

In another embodiment, the invention provides the compound disclosed hereinabove wherein n and p are both equal to 0.

The invention provides a compound having the structure:



wherein R_1 is H, OH, NH_2 or NHR_4 , where R_4 is SO_2Ph , a linear or branched chain alkyl or acyl group, or an aryl group; wherein M has the structure:



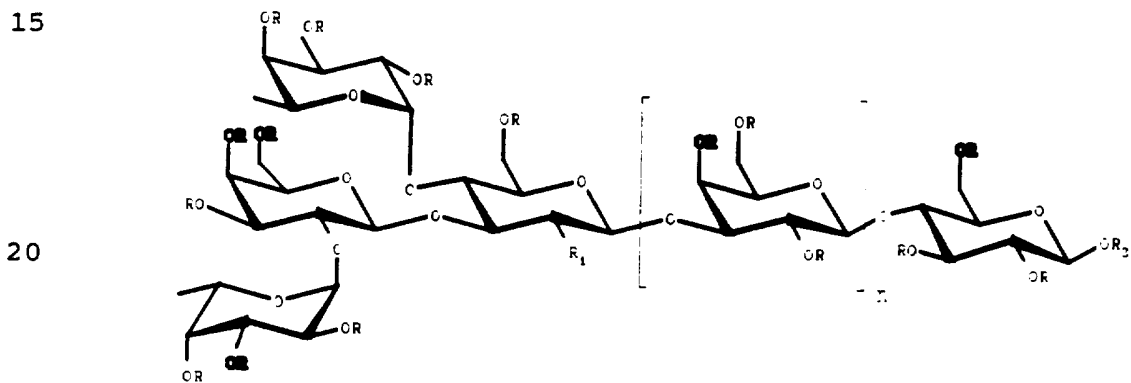
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wherein n is an integer from 0 to 18, and where n is greater than 1, each M is independently the same or different; wherein R_2 , R_3 , R_5 and R_6 are independently the same or different and are H or OH, with the proviso that
 5 geminal R_2 and R_3 are not both OH, and geminal R_5 and R_6 are not both OH; wherein each wavy line between a carbon atom and an oxygen atom denotes an R or S configuration at the carbon atom; and wherein R_7 is a substituted or unsubstituted allyl group.

10

The invention also provides a compound having the structure:

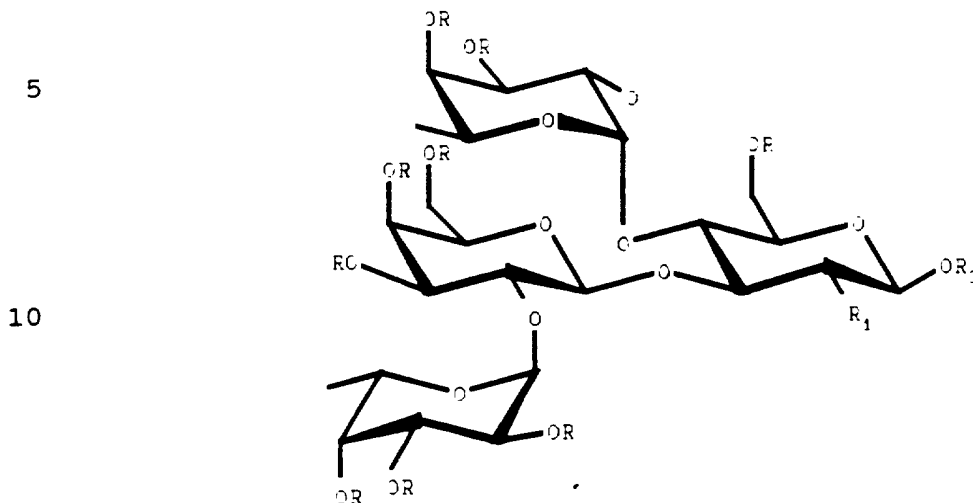
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wherein n is an integer from 1 to 18; wherein R is H or
 25 a linear or branched chain acyl group; wherein R_1 is H, OH, NH_2 or NHR_4 , where R_4 is SO_2Ph , a linear or branched chain alkyl or acyl group, or an aryl group; and wherein R_2 is a substituted or unsubstituted allyl group. In one embodiment, the invention provides the compound wherein
 30 n is 1.

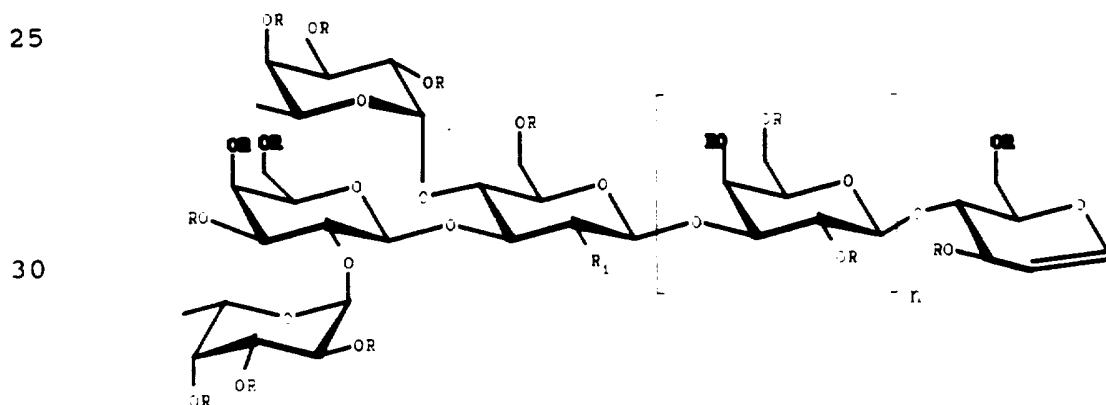
The invention further provides a compound having the structure:



15 wherein R is H or a linear or branched chain acyl group; wherein R_1 is H, OH, NH_2 or NHR_4 , where R_4 is SO_2Ph , a linear or branched chain alkyl or acyl group, or an aryl group; and wherein R_2 is a substituted or unsubstituted allyl group.

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The invention also provides a compound having the structure:



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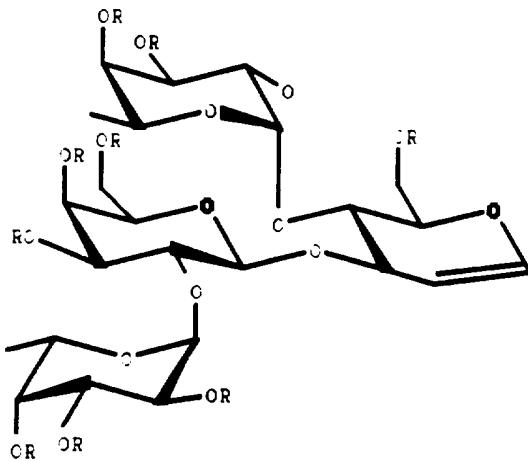
wherein R is H or a linear or branched chain acyl group; wherein R_1 is H, OH, NH_2 or NHR_4 , where R_4 is SO_2Ph , a linear or branched chain alkyl or acyl group, or an aryl

group; wherein R_2 is a substituted or unsubstituted allyl group; and wherein n is an integer from 1 to 18. In one embodiment, the invention provides the compound wherein n is 1.

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The invention also provides a compound having the structure:

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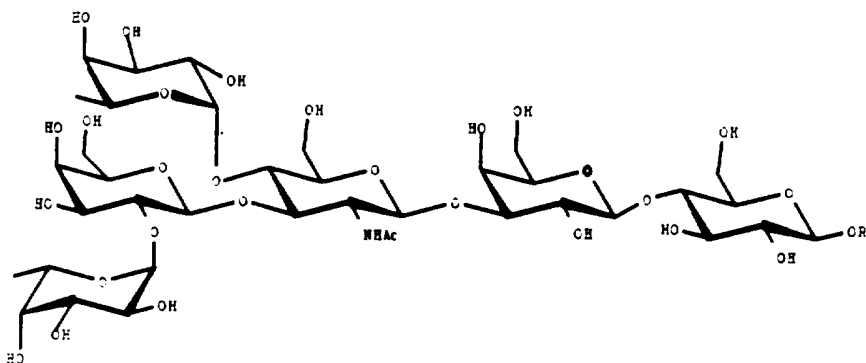
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wherein R is H or a linear or branched chain acyl group.

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The invention also provides a process for synthesizing a compound having the structure:

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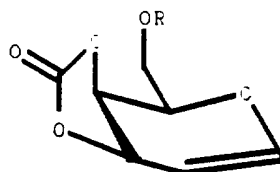


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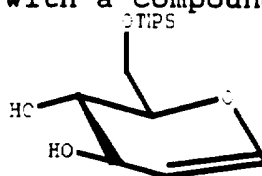
wherein R is a substituted or substituted allyl group, which comprises the steps of (a) synthesizing a compound having the structure:

5



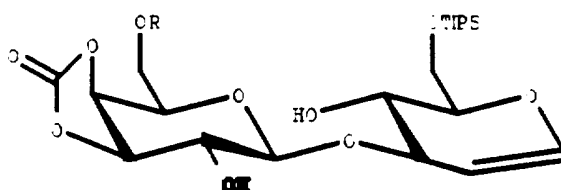
10 wherein R is a trialkylsilyl, aryldialkylsilyl, alkyldiarylsilyl or triarylsilyl group; (b) reacting the compound of step (a) with a compound having structure:

15



under suitable conditions to form a compound having the structure:

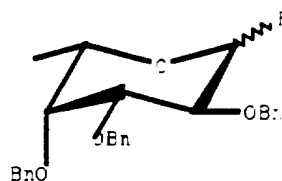
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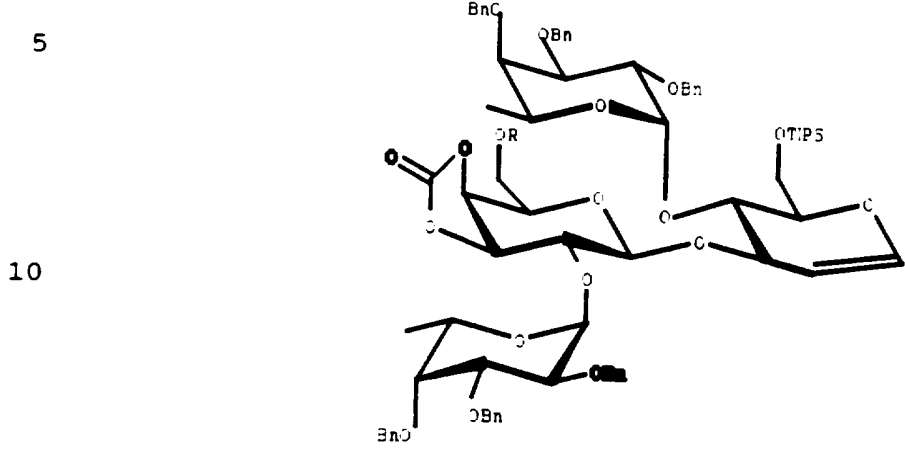
wherein R is a trialkylsilyl, aryldialkylsilyl, alkyldiarylsilyl or triarylsilyl group; (c) reacting the compound formed in step (b) with a compound having the structure:

30

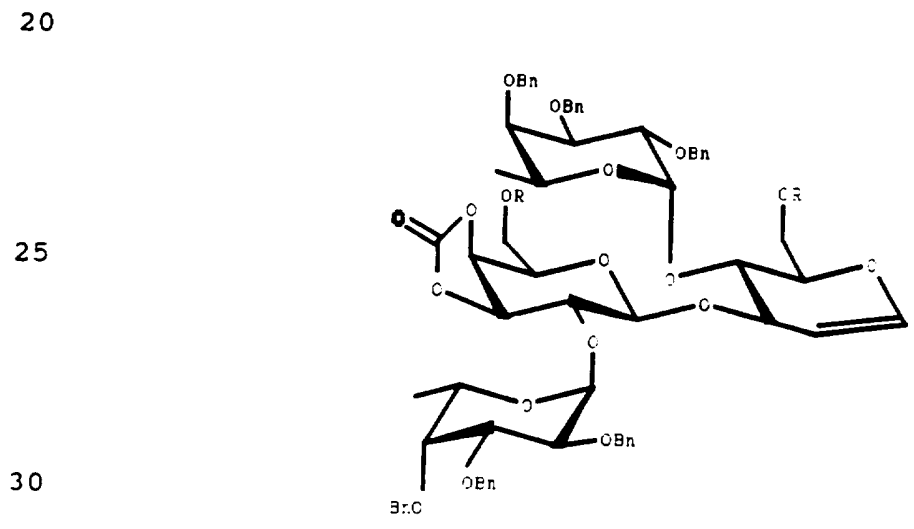


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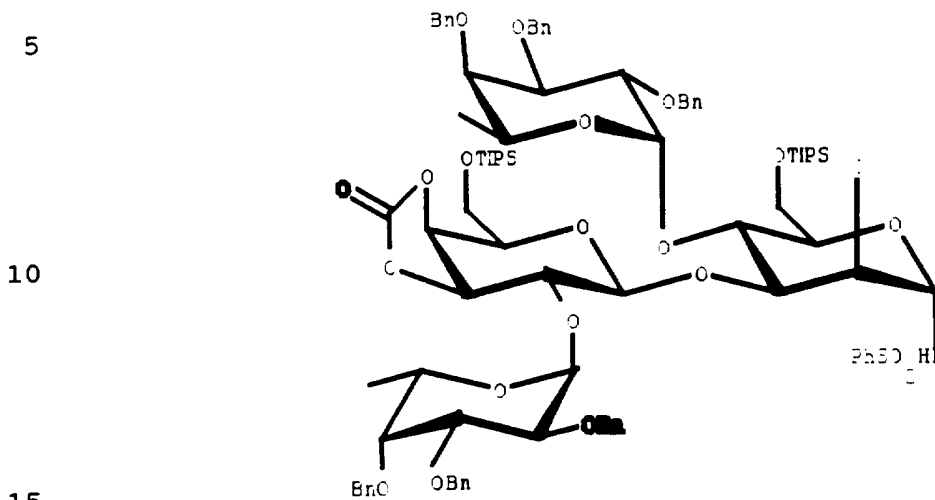
under suitable conditions to form a compound having the structure:



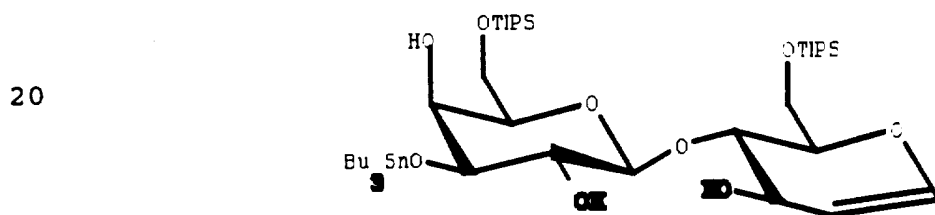
15 wherein R is a trialkylsilyl, aryldialkylsilyl, alkyldiarylsilyl or triarylsilyl group; (d) deprotecting and re-protecting the compound formed in step (c) under suitable conditions to form a compound having the structure:



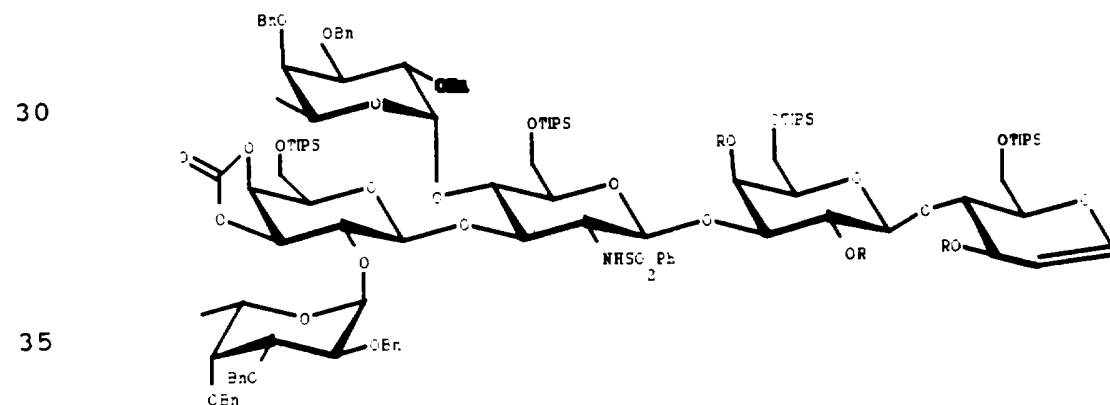
wherein R is TIPS; (e) iodosulfonamidating the compound formed in step (d) under suitable conditions to form a compound having the structure:



(f) reacting the compound formed in step (e) with a compound having the structure:

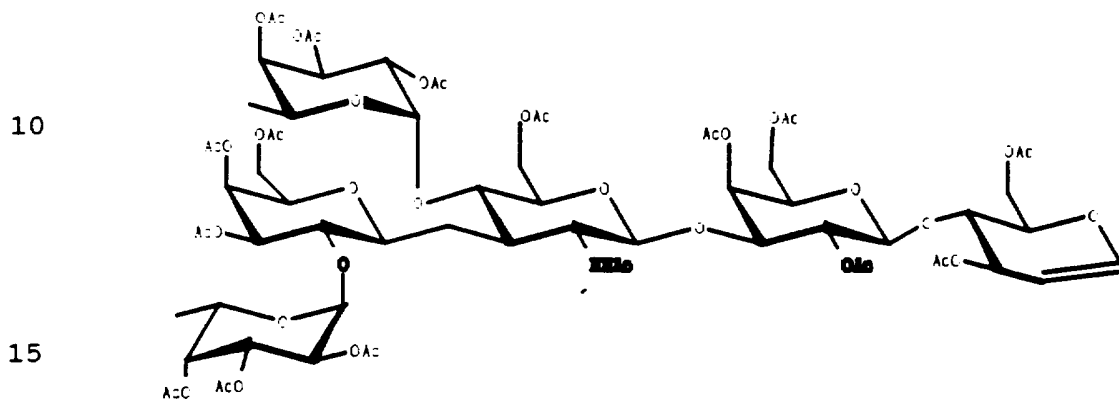


under suitable conditions to form a compound having the structure:



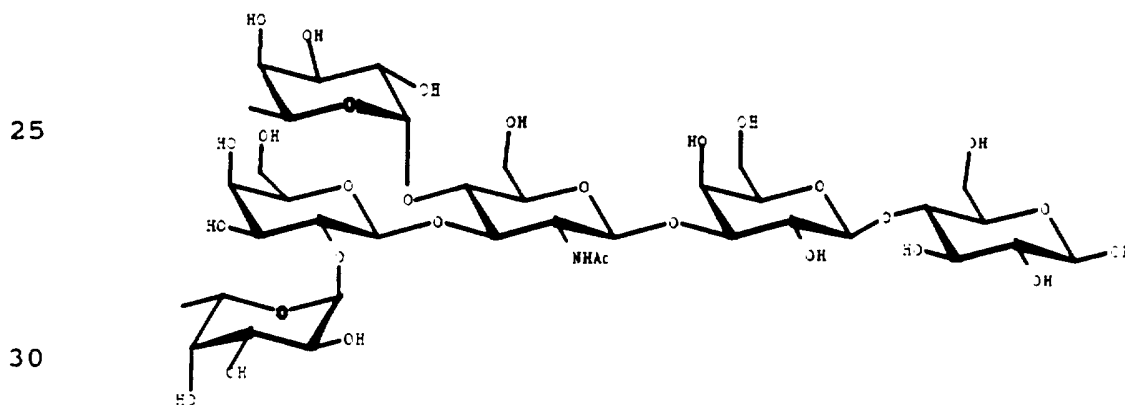
wherein R is H; (g) deprotecting and peracetylating the compound formed in step (f) under suitable conditions to form a compound having the structure:

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(h) epoxidizing the compound formed in step (g) under suitable conditions to form an epoxide thereof and reacting the epoxide under suitable conditions to form a compound having the structure:

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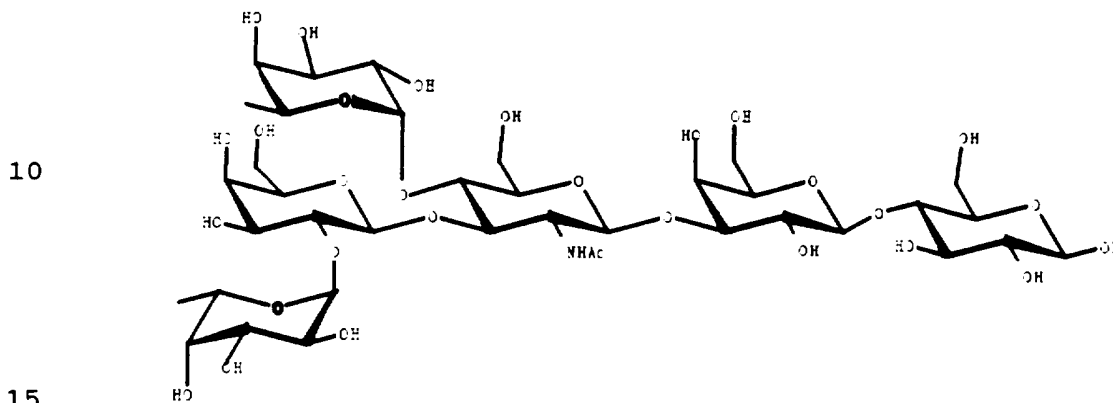


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-24-

wherein R is a substituted or unsubstituted allyl group;
and (i) treating the compound formed in step (h) under
suitable conditions to form a compound having the
structure:

5



20 wherein R is a substituted or unsubstituted allyl group.
In the above process the suitable conditions necessary
for the various reactions and treatments may be found in
the Experimental Details section which follows
hereinafter. However, it is within the confines of the
25 present invention that the specific reagents and solvents
provided as well as the specific conditions necessary for
reaction or treatment may be substituted with other
suitable reactants, solvents and conditions well known to
those skilled in the art.

30

The allyl compound may be conjugated to a peptide or
protein via amine or carboxylic acid side chain. In
practicing the invention, a bioconjugate is prepared
35 according to the protocol of Bernstein and Hall
(Carbohydr. Res. 1980, 78, C1). The allyl group is
ozonolyzed to form either an aldehyde or carboxylic acid,

-25-

which is condensed to a terminal amine to form, respectively, an imine or an amide. The imine is reduced with sodium borohydride to the amine. Alternatively, the aldehyde is reductively aminated using procedures known in the art to form an amine which is reacted with a side-chain terminal carboxylic acid to form an amide conjugate.

The invention provides a pharmaceutical composition which comprises a therapeutically effective amount of the compound disclosed hereinabove and a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

The invention further provides a method for treating a subject afflicted with a disorder caused by Helicobacter pylori which comprises administering to the subject a therapeutically effective amount of the pharmaceutical

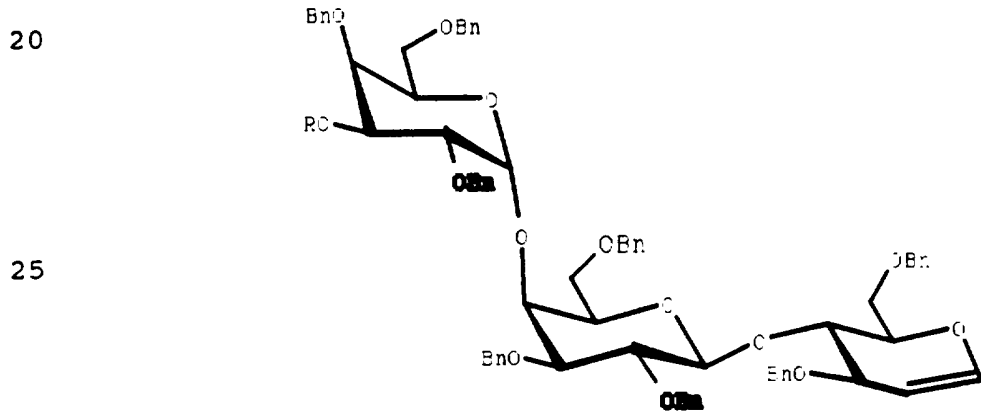
composition disclosed hereinabove so as to treat the subject afflicted with the disorder.

In one embodiment, the invention provides a method of treating a subject afflicted with gastric or duodenal ulcer.

In another embodiment, the invention provides a method of treating a subject afflicted with gastric adenocarcinoma.

In addition, the invention provides a method for inhibiting the adhesion of Helicobacter pylori to gastric epithelium in a subject which comprises administering to the subject an amount of the compound disclosed hereinabove effective to inhibit the adhesion of Helicobacter pylori to gastric epithelium in the subject.

The present invention also provides a process for synthesizing a compound having the structure:

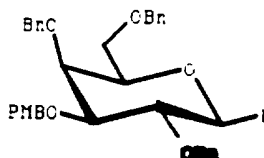


30 wherein R is H which comprises: (a) (i) reacting a compound having the structure:

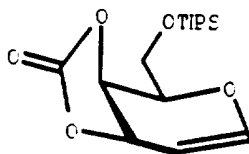


-27-

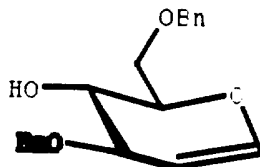
with an epoxidizing agent under suitable conditions to form an epoxide; (ii) cleaving the epoxide formed in step (a)(i) under suitable conditions with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group to form a fluoroalcohol; and (iii) alkylating the fluoroalcohol formed in step (b)(ii) under suitable conditions with a non-nucleophilic base and an organic halide having the formula $C_6H_5CH_2X$ wherein X is Br, Cl, I or F to form a compound having the structure:



(b) (i) synthesizing a compound having the structure:



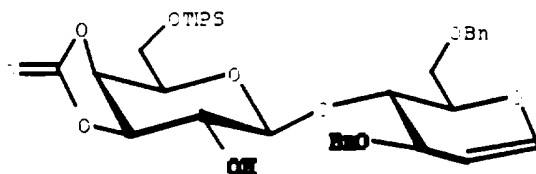
(c) (i) treating the compound formed in step (b) with an epoxidizing agent under suitable conditions to form an epoxide; and (ii) coupling the epoxide formed in step (c)(i) with a compound having the structure:



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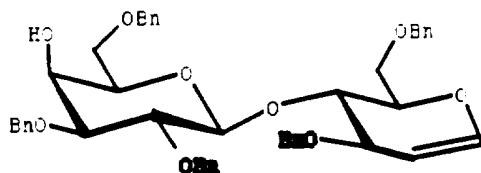
under suitable conditions to form a compound having the structure:

5



(d) (i) alkylating the compound formed in step (c)(ii) under suitable conditions with a non-nucleophilic base and an organic halide having the formula $C_6H_5CH_2X$ wherein X is Br, Cl, I or F; and (ii) de-silylating the compound formed in step (d)(i) under suitable conditions with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group; (iii) treating the compound formed in step (d)(ii) under suitable conditions with a metal alkoxide to form a deprotected disaccharide; and (iv) alkylating the disaccharide formed in step (d)(iii) under suitable conditions to form a selectively deprotected disaccharide having the structure:

25

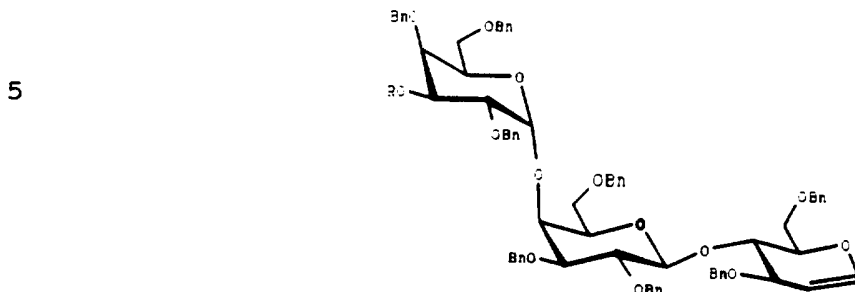


(e) (i) coupling the selectively deprotected disaccharide formed in step (d)(iv) with the compound formed in step (a)(iii) under suitable conditions to form a protected trisaccharide; and (ii) de-protecting the protected

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trisaccharide formed in step (e)(i) under suitable conditions to form a trisaccharide having the structure:



10 wherein R is H. In step (a) reaction (i) may be carried out using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide. A preferred agent is 3,3-dimethyldioxirane. Non-nucleophilic, inert solvents may be used, such as dichloromethane. Reaction (a)(ii) may be performed using organic ammonium fluoride salts, including tetrabutylammonium fluoride, in a range of solvents, including ethereal solvents, preferably in tetrahydrofuran. Step (iii) may be performed using a non-nucleophilic base such as sodium hydride in a non-nucleophilic solvent such as DMF. In step (b) the compound shown may be prepared as described herein. Step (c)(i) may be carried out using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane. Coupling step (c)(ii) may be carried out using a metal catalyst, such as zinc chloride, in an inert solvent, such as THF. Step (d)(i) is carried out using a non-nucleophilic base such as sodium hydride in a non-nucleophilic solvent such as DMF. In step (d)(ii) de-silylation is effected using an organic ammonium fluoride salt, including tetrabutylammonium fluoride, in a range of solvents, including ethereal solvents, preferably in tetrahydrofuran. The carbonate ester is cleaved using a metal alkoxide, such as sodium methoxide, in an alcoholic

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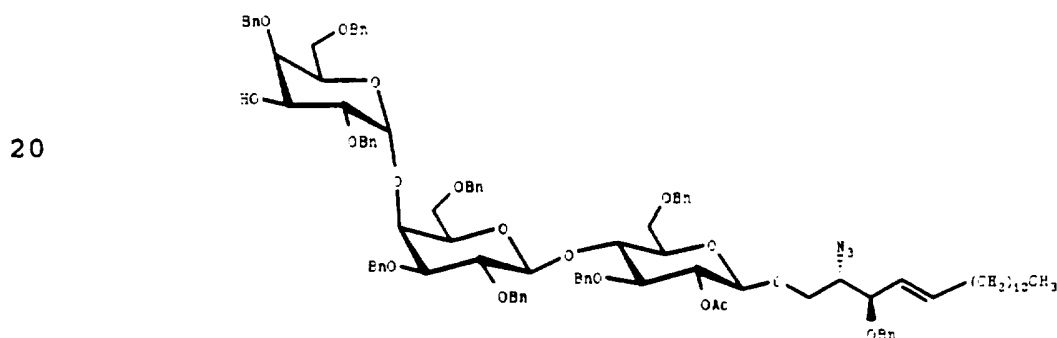
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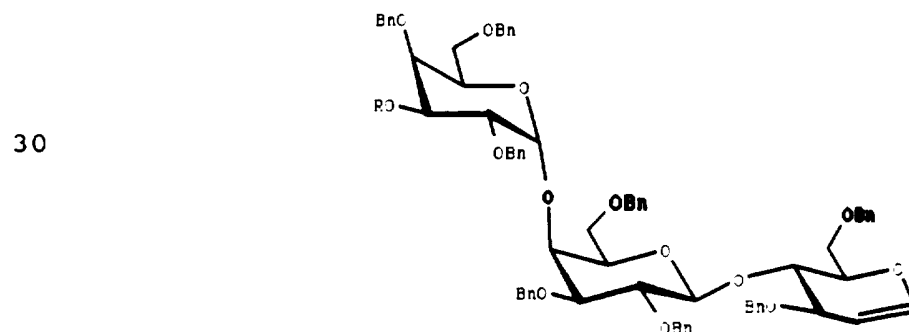
-30-

medium such as methanol. Step (d)(iv) is selectively performed using a metal oxide, such as $(n\text{-Bu}_3\text{Sn})_2\text{O}$, in the presence of an organic ammonium bromide, such as tetra-*n*-butylammonium bromide, in an inert solvent such as benzene. Step (e) is a coupling performed in the presence of a metal halide salt, such as SnCl_2 , in the presence of silver perchlorate and 2,6-di-*t*-butylpyridine, in a solvent, such as ether, containing molecular sieves. Oxidative removal of PMB is performed with an oxidizing agent such as DDQ in an inert solvent system, which may preferably be heterogeneous, for example, using water/dichloromethane.

The present invention also provides a process for synthesizing a trisaccharide ceramide having the structure:



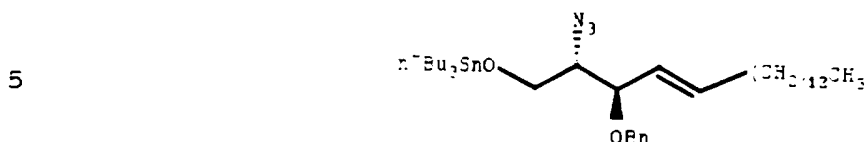
25 which comprises: (a) synthesizing a trisaccharide having the structure:



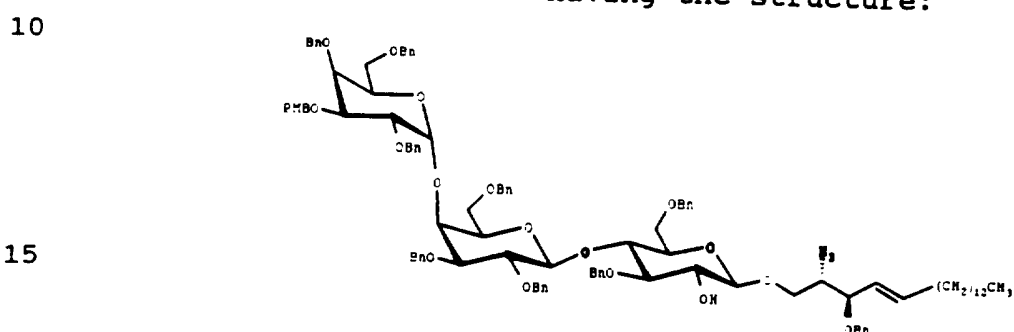
35 wherein R is PMB; (b) (i) reacting the trisaccharide formed in step (a) with an epoxidizing agent under suitable conditions to form a trisaccharide epoxide; and

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(ii) reacting the trisaccharide epoxide formed in step (b)(i) with a compound having the structure:



under suitable conditions to form a protected trisaccharide ceramide having the structure:



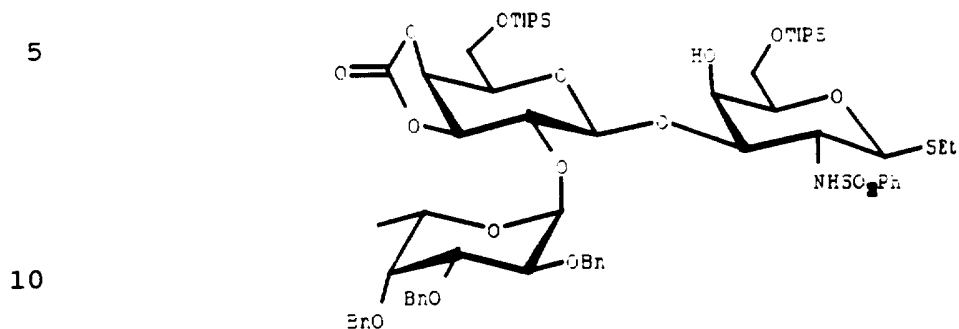
(c) (i) acylating the ceramide formed in step (b)(ii) under suitable conditions; and (ii) selectively de-protecting the compound formed in step (c)(i) under suitable conditions to form the trisaccharide ceramide.

20

In step (a) the trisaccharide may be synthesized as described herein. Step (b)(i) is performed using using a variety of epoxidizing agents including peracetic acid, 25 m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane. Coupling step (b)(ii) may be carried out using a tributyltin ether of the ceramide precursor and a metal catalyst, such as zinc chloride, in an inert solvent, such as THF. In step (c)(i) acylation is performed using a linear or branched chain alkyl anhydride preferably acetic anhydride or halide in the presence of 30 triethylamine and DMAP in an inert organic solvent such as dichloromethane. The PMB protecting group is removed oxidatively, preferably as described above.

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The present invention further provides a process for synthesizing a mercaptotrisaccharide having the structure:



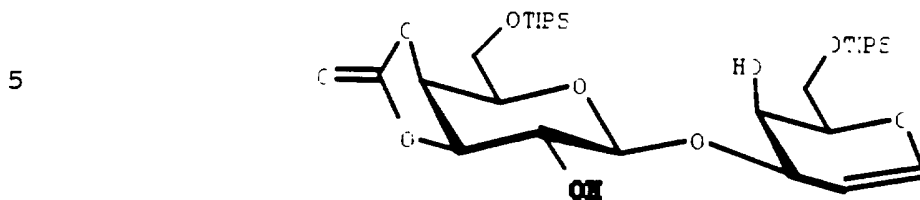
15 which comprises: (a) (i) synthesizing a compound having the structure:



(ii) coupling the compound of step (a) (i) with a compound having structure:



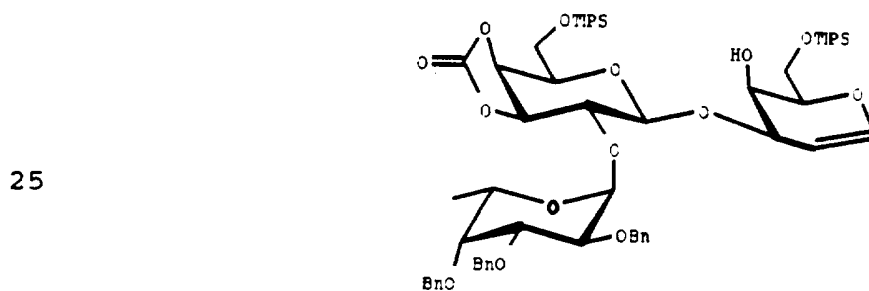
under suitable conditions to form a disaccharide having the structure:



10 (b) coupling the disaccharide formed in step (a) (ii) with a compound having the structure:

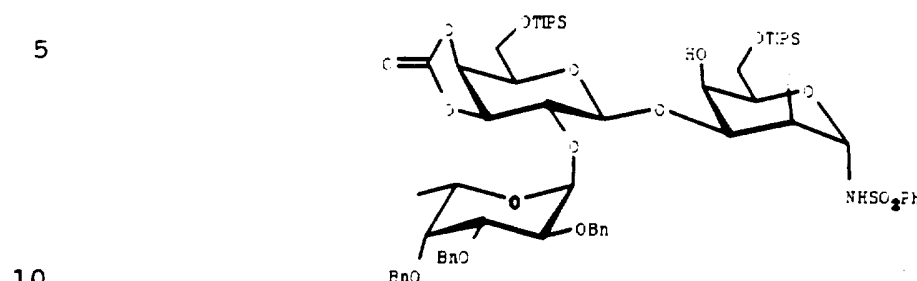


20 under suitable conditions to form a trisaccharide having the structure:



-34-

(c) iodosulfonamidating the trisaccharide formed in step (b) under suitable conditions to form a iodosulfonamide having the structure:



and (d) reacting the iodosulfonamide formed in step (c) under suitable conditions with a thiolate to form the mercaptotrisaccharide.

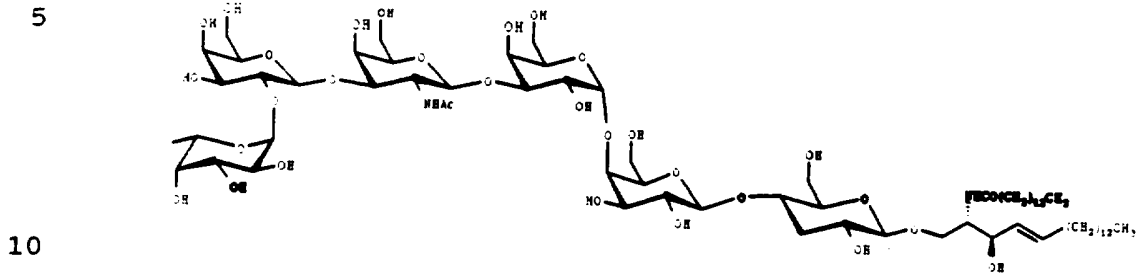
20 Step (a)(ii) is performed by reacting the compound of step (a)(i), which may be obtained as described herein or otherwise, with a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane, followed by coupling with the diol monosaccharide of step (a)(ii) which may be carried out using a metal catalyst, such as zinc chloride, in an inert solvent, such as THF. Coupling with the

25 fluorosugar is carried out in step (b) in the presence of a metal halide salt, such as SnCl_2 , in the presence of silver perchlorate and 2,6-di-t-butylpyridine, in a solvent, such as ether, containing molecular sieves. Step (c) is performed using $\text{I}(\text{coll})_2$ perchlorate and

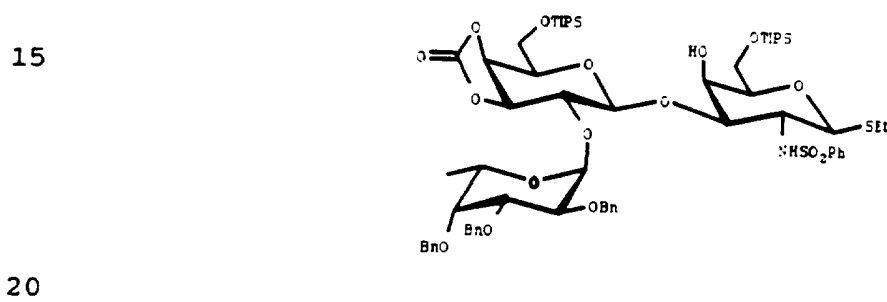
30 PhSO_2NH_2 in the presence of molecular sieves. Step (d) is carried out using alkyl thiol and a base such as LiHMDS in an inert solvent as DMF.

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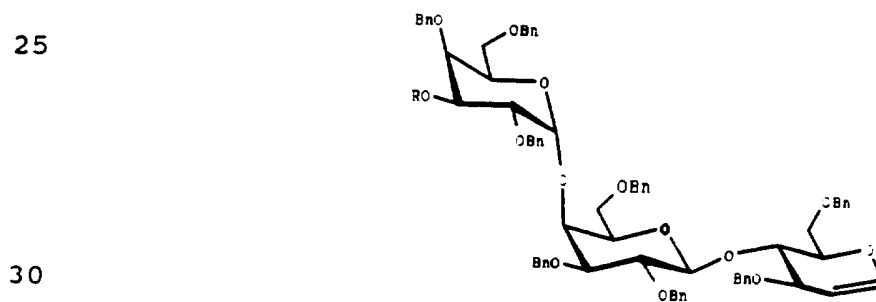
The present invention also provides a process of synthesizing a hexasaccharide ceramide having the structure:



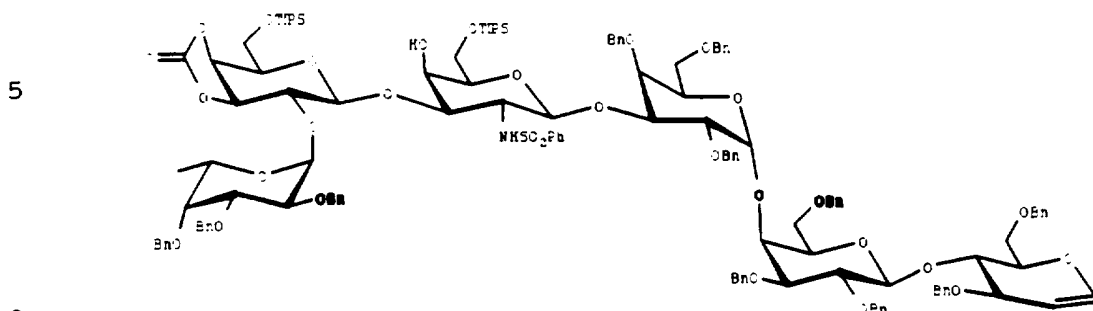
which comprises: (a) coupling a compound having the structure:



with a compound having the structure:

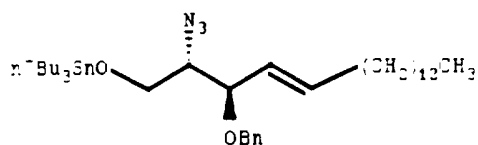


under suitable conditions to form a compound having the structure:



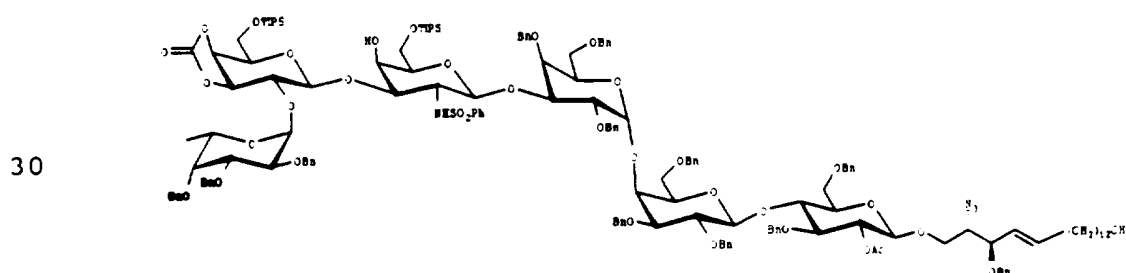
(b) (i) reacting the compound formed in step (a) with an epoxidizing agent under suitable conditions to form a hexasaccharide epoxide; and (ii) reacting the hexasaccharide epoxide with a stannyl ether having the structure:

15



under suitable conditions to form a hexasaccharide alcohol; (c) acylating the hexasaccharide alcohol formed in step (b)(ii) under suitable conditions to form a hexasaccharide acetate having the structure:

25



(d) reductively acylating the hexasaccharide acetate formed in step (c) under suitable conditions in the

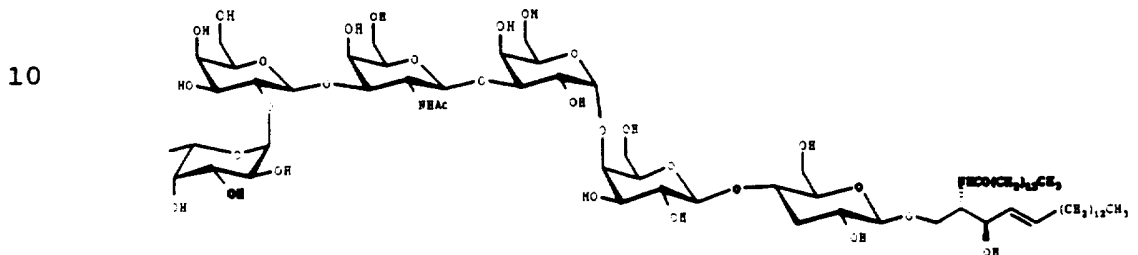
-37-

presence of palmitic anhydride to form a hexasaccharide ceramide; (e) desilylating and partially deprotecting the hexasacchararide ceramide under suitable conditions to form a partially deprotected hexasaccharide ceramide; (f) 5 (i) reducing the partially deprotected hexasaccharide ceramide under suitable conditions to form a deprotected hexasaccharide ceramide acetate; and (ii) acylating the deprotected hexasaccharide ceramide acetate under suitable conditions to form a hexasaccharide ceramide peracetate; and (g) saponifying the hexasaccharide ceramide peracetate under suitable conditions to form the hexasaccharide ceramide. 10

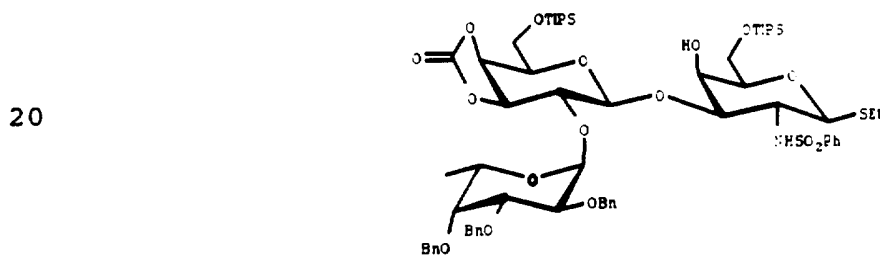
Step (a) is performed using triflate esters, such as 15 methyl triflate, in the presence of molecular sieves in an inert solvent. Step (b)(i) is carried out using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane. 20 Step (b)(ii) is performed using a stannyl ether of the ceramide precursor, preferably the tri-n-butyl stannylether, in the presence of a metal salt, such as Zn triflate, in an inert solvent, such as THF. Step (c) is carried out using acetic anhydride in the presence of a 25 base such as triethylamine and DMAP. Step (d) is carried out using a noble metal catalyst such as Lindlar's catalyst and hydrogen gas in the presence of palmitic anhydride in an inert solvent such as ethyl acetate. 30 Desilylation step (e) is effected using organic ammonium fluoride salts, such as tetra-n-butylammonium fluoride in THF. The carbonate ester is cleaved using a metal alkoxide such as NaOMe in an alcohol such as methanol. In step (f)(i) reduction is performed using a metal such as 35 lithium or sodium in liquid ammonia and an inert solvent such as THF. Step (f)(ii) is carried out using acetic anhydride in the presence of a base such as Et₃N and DMAP

in an inert solvent such as dichloromethane. The peracetate is saponified using a metal alkoxide such as sodium methoxide in an alcohol such as methanol.

5 The present invention also provides a process of synthesizing a hexasaccharide ceramide having the structure:

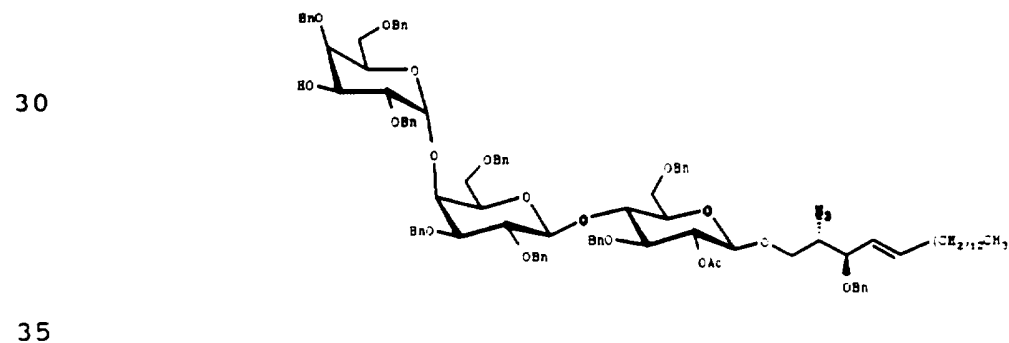


15 which comprises: (a) coupling a compound having the structure:



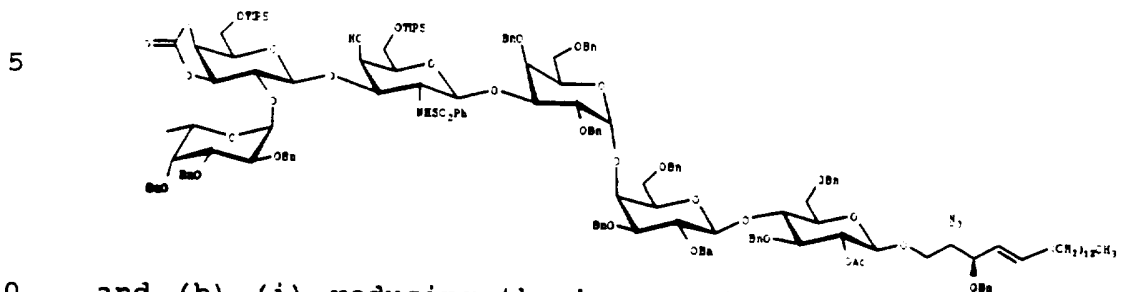
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with a compound having the structure:



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under suitable conditions to form a hexasaccharide having the structure:

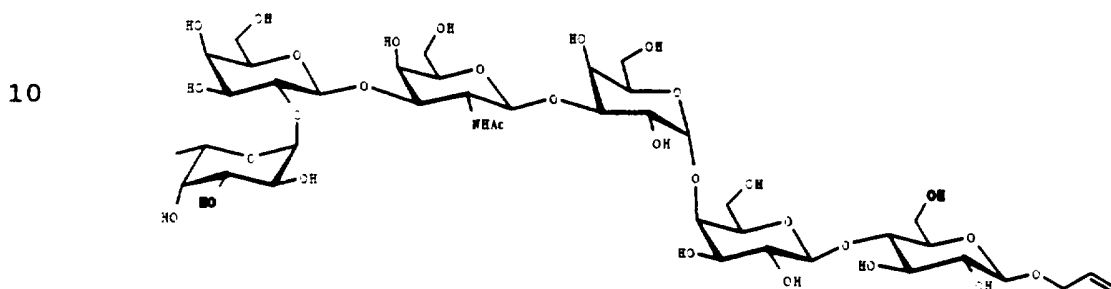


10 and (b) (i) reducing the hexasaccharide formed in step (a) under suitable conditions in the presence of palmitic anhydride to form a palmitoyl amide; (ii) desilylating the palmitoyl amide with R_4NF wherein each R is independently the same or different and is a linear or
15 branched chain alkyl, aralkyl or aryl group under suitable conditions to form a partially deprotected hexasaccharide; (iii) de-protecting the hexasaccharide formed in step (b)(ii) under suitable conditions to form a deprotected hexasaccharide; (iv) acylating the
20 hexasaccharide formed in step (b)(iii) under suitable conditions to form a hexasaccharide ceramide peracetate; and (v) saponifying the hexasaccharide ceramide peracetate under suitable conditions to form the hexasaccharide ceramide.

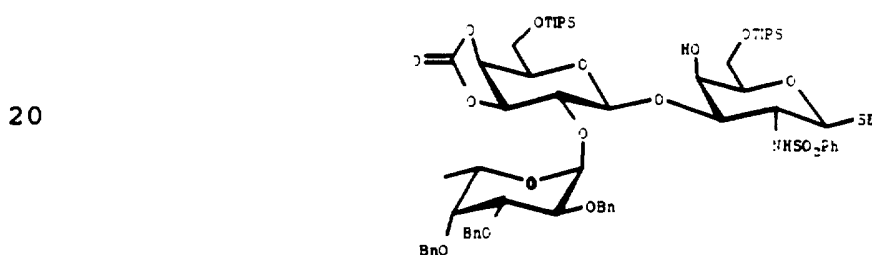
25 Step (a) is performed using triflate esters, such as methyl triflate, in the presence of molecular sieves in an inert solvent. Step (b)(i) is carried out using using a noble metal catalyst such as Lindlar's catalyst and
30 hydrogen gas in the presence of palmitic anhydride in an inert solvent such as ethyl acetate. Step (b)(ii) is performed using organic ammonium fluoride salts, such as tetra-n-butylammonium fluoride in THF. In step (b)(iii) reduction is performed using a metal such as lithium or
35 sodium in liquid ammonia and an inert solvent such as THF. Step (b)(iv) is carried out using acetic anhydride in the presence of a base such as Et_3N and DMAP in an

inert solvent such as dichloromethane. In step (v) the peracetate carbonate is saponified using a metal alkoxide such as sodium methoxide in an alcohol such as methanol.

5 The present invention also provides a process of synthesizing an allyl hexasaccharide having the structure:

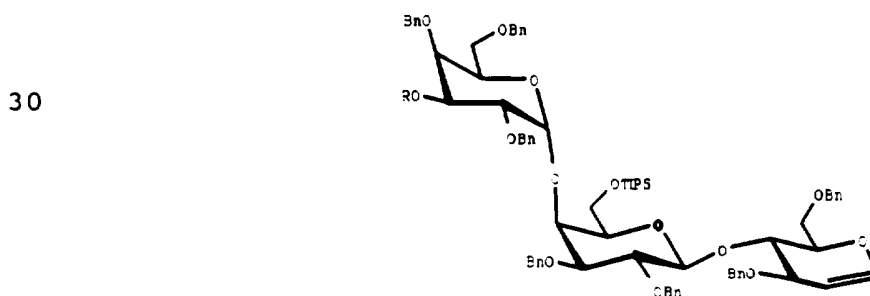


15 which comprises: (a) coupling a compound having the structure:



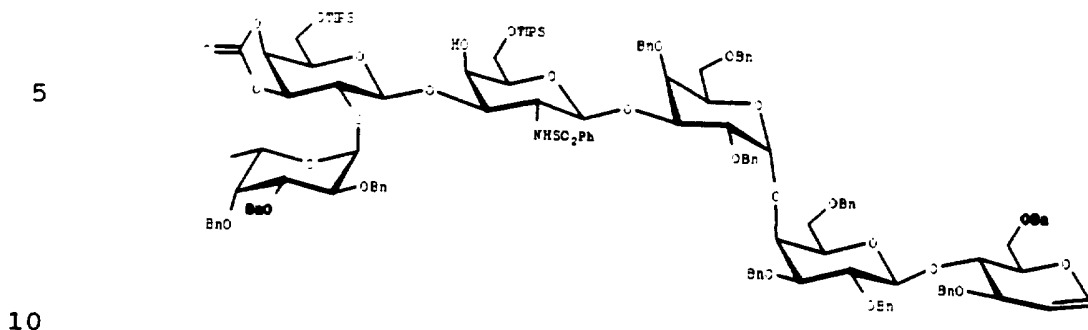
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with a compound having the structure:



-41-

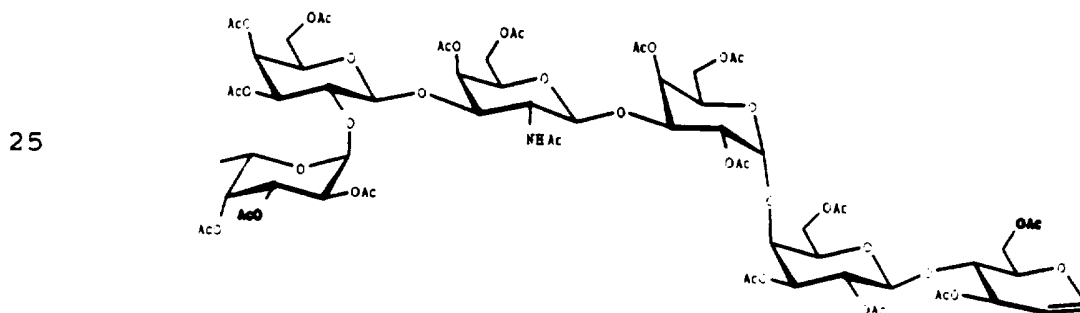
wherein R is H under suitable conditions to form a hexasaccharide having the structure:



(b) (i) desilylating the compound formed in step (a) with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group under suitable conditions to form a partially deprotected hexasaccharide; (ii) de-protecting the hexasaccharide formed in step (b)(i) under suitable conditions to form a deprotected hexasaccharide; and (iii) peracetylating the compound formed in step (b)(ii) under suitable conditions to form a hexasaccharide peracetate having the structure:

15

20



(c) (i) reacting the hexasaccharide peracetate formed in step (b)(iii) with an epoxidizing agent under suitable conditions to form an hexasaccharide epoxide peracetate; (ii) treating the hexasaccharide epoxide peracetate formed in step (c)(i) with allyl alcohol under suitable conditions to form an allyl hexasaccharide peracetate; and (iii) saponifying the allyl hexasaccharide peracetate

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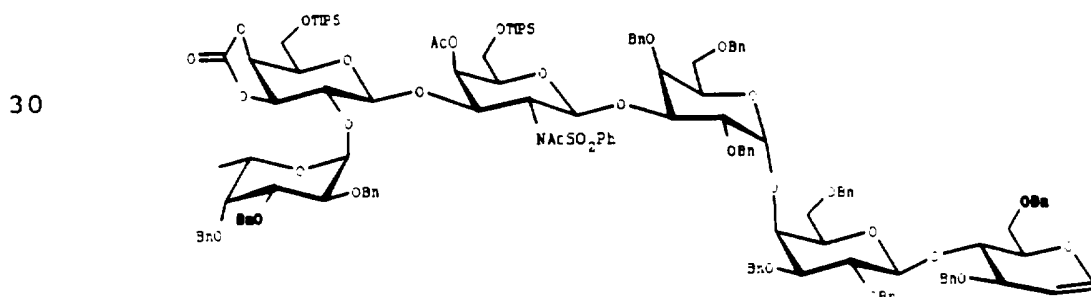
-42-

under suitable conditions to form the allyl hexasaccharide.

Step (a) is performed using triflate esters, such as methyl triflate, in the presence of molecular sieves in an inert solvent. Step (b)(i) is carried out using organic ammonium fluoride salts, such as tetra-n-butylammonium fluoride in THF. Step (b)(ii) is performed using a metal alkoxide such as sodium methoxide in an alcohol such as methanol, followed by reduction performed using a metal such as lithium or preferably sodium in liquid ammonia and an inert solvent such as THF. Step (b)(iii) is carried out using acetic anhydride in the presence of a base such as Et₃N and DMAP in an inert solvent such as dichloromethane. In step (c)(i) is carried out using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane. Step (c)(ii) is carried out using allyl alcohol in an inert solvent. Step (c)(iii) the peracetate carbonate is saponified using a metal alkoxide such as sodium methoxide in an alcohol such as methanol.

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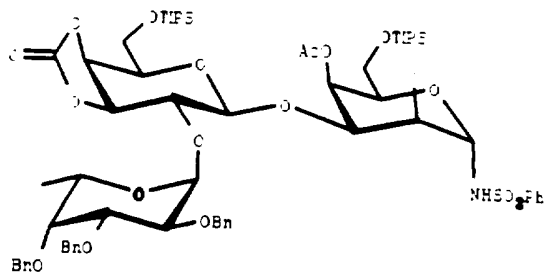
The present invention provides a process of synthesizing a hexasaccharide having the structure:



which comprises: (a) coupling a compound having the

structure:

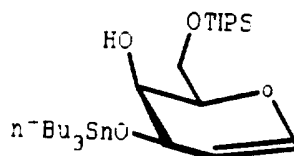
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with a compound having the structure:

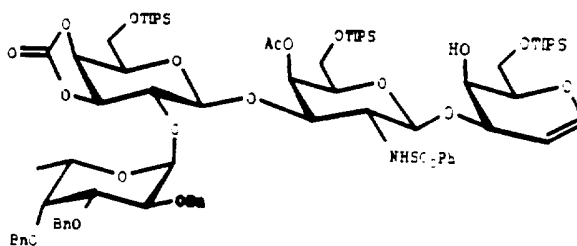
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under suitable conditions to form a compound having the structure:

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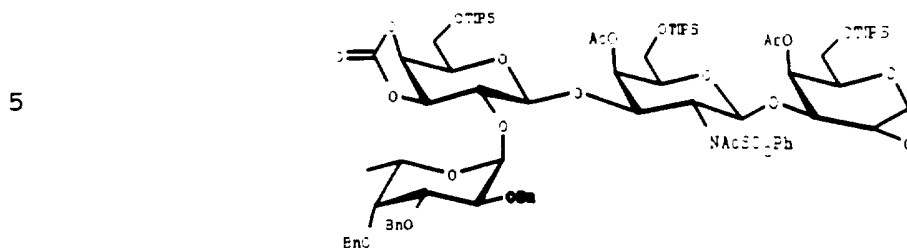


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(b) (i) acylating the compound formed in step (a) under suitable conditions; and (ii) reacting the compound formed in step (b)(i) with an epoxidizing agent under

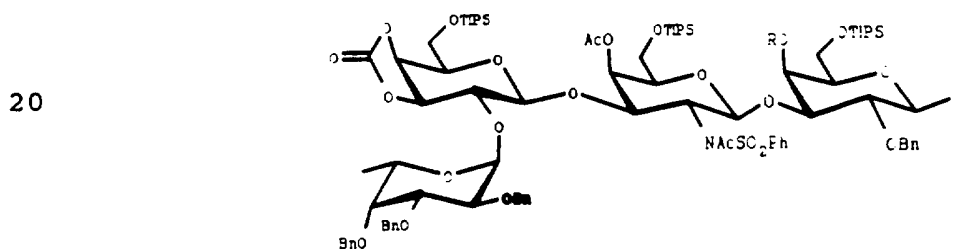
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suitable conditions to form an epoxide having the structure:

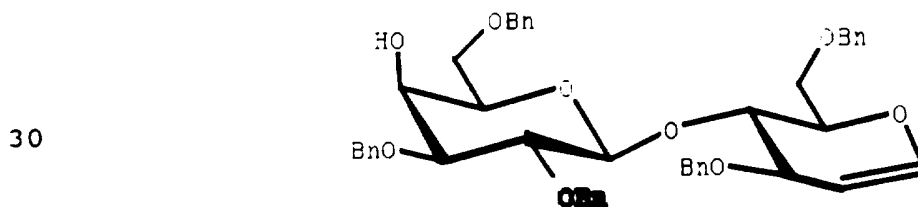


10

(c) (i) treating the epoxide with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group under suitable conditions; and (ii) alkylating the compound formed in step (c)(i) under suitable conditions to form a compound having the structure:



25 wherein R is H or acyl; (d) coupling the compound formed in step (c)(ii) with a compound having the structure:

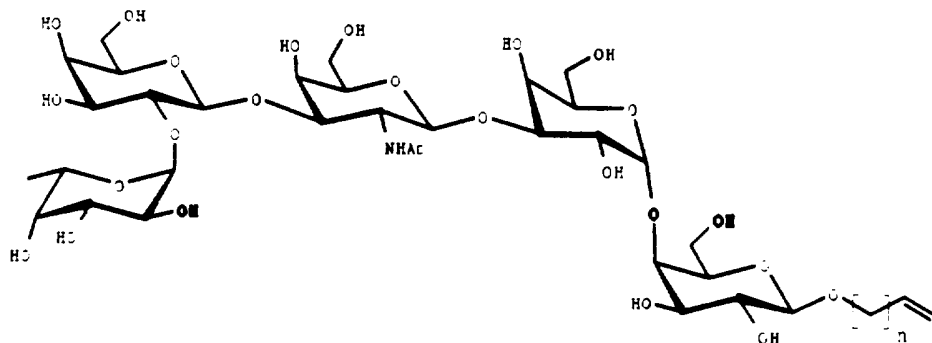


35 under suitable conditions to form the hexasaccharide.

-45-

Step (a) is performed using a metal catalyst such as silver tetrafluoroborate in an inert solvent. Step (b)(i) is carried out using acetic anhydride in the presence of a base such as Et₃N and DMAP in an inert solvent such as dichloromethane. Step (b)(ii) is carried out using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane. Step (c)(i) is effected with organic ammonium fluoride salts, such as tetra-n-butylammonium fluoride in THF. Step (c)(ii) is performed using a non-nucleophilic base such as sodium hydride in an inert solvent. Step (d) is performed using a metal salt catalyst such as tin dichloride in the presence of silver perchlorate in an inert solvent such as di-*t*-butylpyridine. Further transformations provide deprotected products or conjugates with proteins or other carriers.

The present invention further provides a compound having the structure:



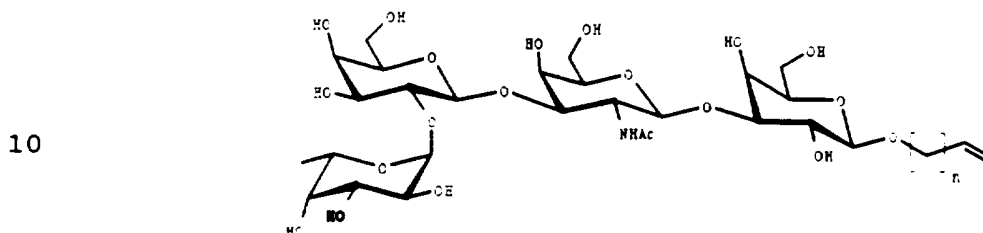
wherein *n* is an integer between about 0 and about 9.

The allyl glycoside shown is prepared using the glycal coupling methods taught herein, and may be bound to protein carriers using general reactions described herein or by standard methods in the art. For example, the allyl glycoside may be prepared by coupling compound 9b

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disclosed herein with a suitably protected 8b, followed by coupling with 12b, then coupling with allyl alcohol and an appropriate deprotection sequence.

5 The present invention also provides a compound having the structure:

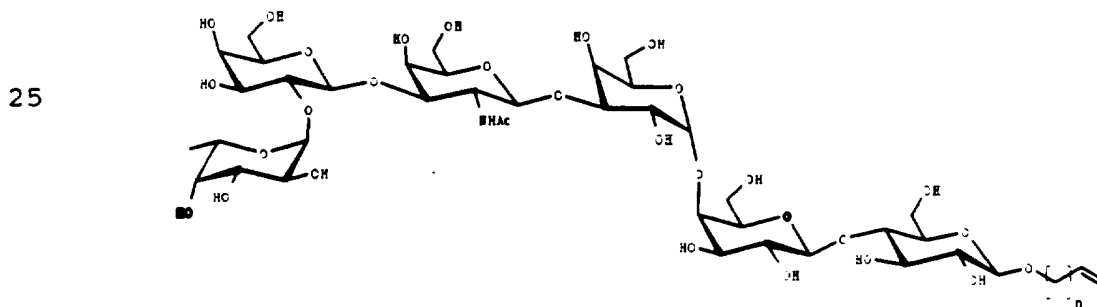


wherein n is an integer between about 0 and about 9.

15 The allyl glycoside shown is prepared using the glycal coupling methods, allylation and a deprotection sequence as taught herein (see Fig. 12), and may be bound to protein carriers using general reactions described herein or by standard methods in the art.

20

The present invention also provides a compound having the structure:



30 wherein n is an integer between about 0 and about 9.

The allyl glycosides shown are prepared using the glycal coupling methods taught herein, and may be bound to protein carriers using general reactions described herein or by standard methods in the art.

35

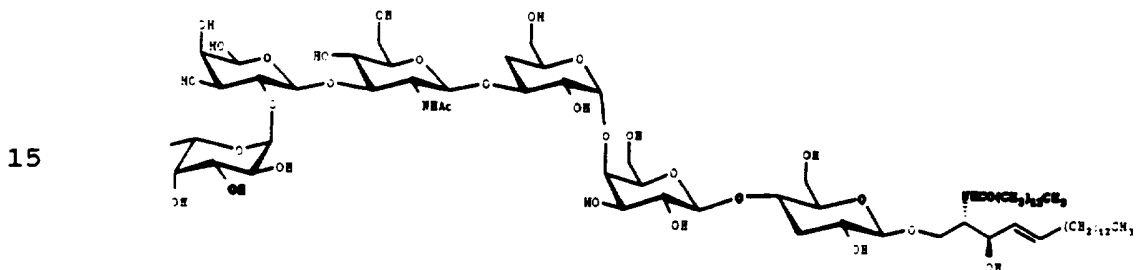
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It is within the scope of the present invention to vary the combination of protecting groups for the various sugar hydroxyl groups in accord with ordinary skill in the art.

5

The present invention provides a method of inducing antibodies in a human subject, wherein the antibodies are immunoreactive with human breast tumor cells, which comprises administering to the subject an amount of a compound having the structure:

10



alone or bound to a suitable immunological adjuvant effective to induce the antibodies. In one embodiment, the present invention provides a method wherein the antibodies induced are MB1 antibodies. In another embodiment, the present invention provides a method wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease. In another embodiment, the present invention provides a method wherein the adjuvant is a protein carrier, bacteria or liposomes. In yet another embodiment, the present invention provides wherein the adjuvant is bacille Calmette-Guerin (BCG).

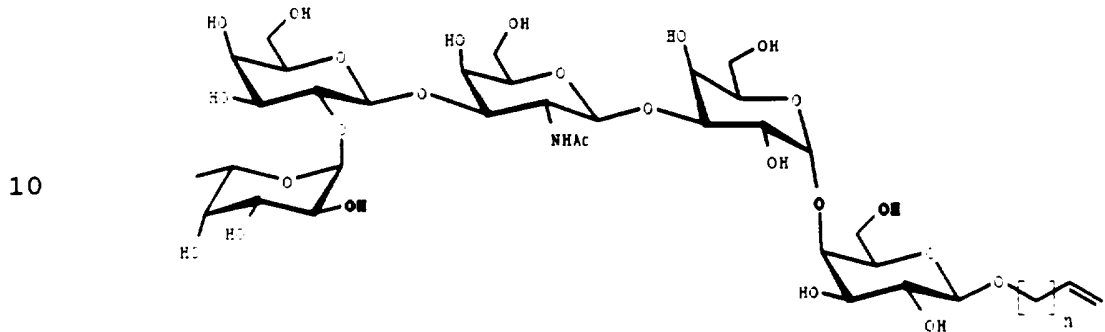
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The present invention provides a method of preventing recurrence of breast cancer in a subject which comprises vaccinating the subject with the compound shown hereinabove either alone or bound to a suitable immunological carrier, adjuvant or vehicle.

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The present invention also provides a method of inducing antibodies in a subject, wherein the antibodies are immunoreactive with human breast tumor cells, which comprises administering to the subject an amount of the compound having the structure:

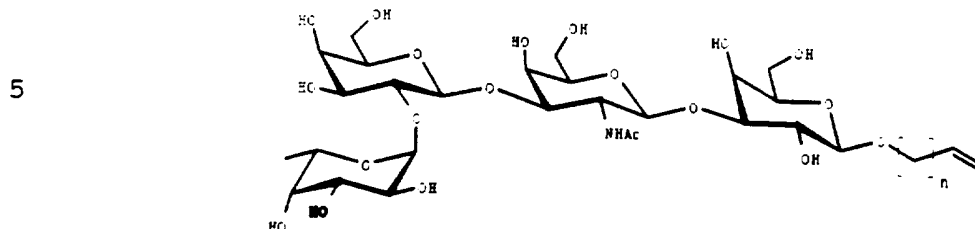


15 wherein n is an integer between about 0 and about 9 either alone or bound to a suitable immunological adjuvant effective to induce the antibodies. In one embodiment, the present invention provides a method wherein the antibodies induced are MBrl antibodies. In another embodiment, the present invention provides a method wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease. In another embodiment, the present invention provides a method wherein the adjuvant is a protein carrier, bacteria or liposomes. In yet another embodiment, the present invention provides wherein the adjuvant is bacille Calmette-Guerin.

30 The present invention provides a method of preventing recurrence of breast cancer in a subject which comprises vaccinating the subject with the compound shown hereinabove either alone or bound to a suitable immunological carrier, adjuvant or vehicle.

35 The present invention also provides a method of inducing antibodies in a subject, wherein the antibodies are immunoreactive with human breast tumor cells, which

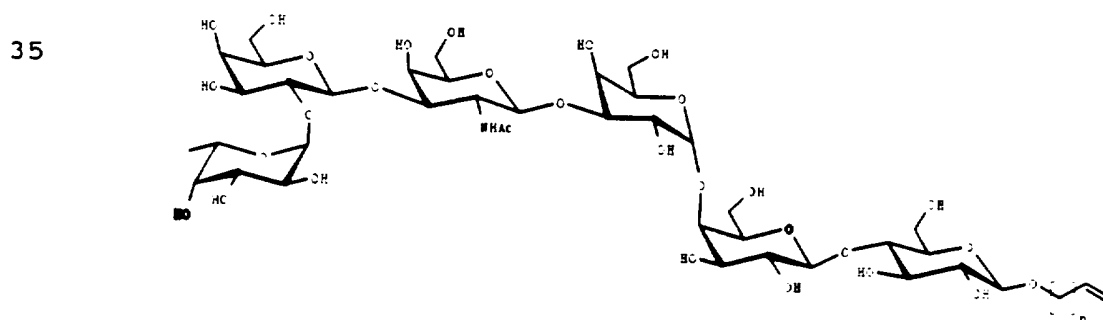
comprises administering to the subject an amount of the compound having the structure:



10 wherein n is an integer between about 0 and about 9 either alone or bound to a suitable immunological adjuvant effective to induce the antibodies. In one embodiment, the present invention provides a method wherein the antibodies induced are MBrl antibodies. In another embodiment, the present invention provides a method wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease. In another embodiment, the present invention provides a method wherein the adjuvant is a protein carrier, bacteria or liposomes. In yet another embodiment, the present invention provides wherein the adjuvant is bacille Calmette-Guerin.

25 The present invention also provides a method of preventing recurrence of breast cancer in a subject which comprises vaccinating the subject with the compound shown hereinabove either alone or bound to a suitable immunological carrier, adjuvant or vehicle.

30 The present invention additionally provides a method of inducing antibodies in a subject, wherein the antibodies are immunoreactive with human breast tumor cells, which comprises administering to the subject an amount of the compound having the structure:



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wherein n is an integer between about 0 and about 9
either alone or bound to a suitable immunological
adjuvant effective to induce the antibodies. In one
5 embodiment, the present invention provides a method
wherein the antibodies induced are MBrl antibodies. In
another embodiment, the present invention provides a
method wherein the subject is in clinical remission or,
where the subject has been treated by surgery, has
10 limited unresected disease. In another embodiment, the
present invention provides a method wherein the adjuvant
is a protein carrier, bacteria or liposomes. In yet
another embodiment, the present invention provides
wherein the adjuvant is bacille Calmette-Guerin.

15

The present invention also provides a method of
preventing recurrence of breast cancer in a subject which
comprises vaccinating the subject with the compound shown
hereinabove either alone or bound to a suitable
20 immunological carrier, adjuvant or vehicle.

Experimental DetailsGeneral Procedures

5 All air- and moisture-sensitive reactions were performed
in a flame-dried apparatus under an argon atmosphere
unless otherwise noted. Air-sensitive liquids and
solutions were transferred via syringe or canula.
10 Wherever possible, reactions were monitored by thin-layer
chromatography (TLC). Gross solvent removal was
performed in vacuum under aspirator vacuum on a Buchi
rotary evaporator, and trace solvent was removed on a
high vacuum pump at 0.1-0.5 mmHg.
Melting points (mp) were uncorrected and performed in
15 soft glass capillary tubes using an Electrothermal series
IA9100 digital melting point apparatus.

Infrared spectra (IR) were recorded using a Perkin-Elmer
1600 series Fourier-Transform instrument. Samples were
20 prepared as neat films on NaCl plates unless otherwise
noted. Absorption bands are reported in wavenumbers (cm^{-1}).

Only relevant, assignable bands are reported.

25 Proton nuclear magnetic resonance (^1H NMR) spectra were
determined using a Bruker AMX-400 spectrometer at 400
MHz. Chemical shifts are reported in parts per million
(ppm) downfield from tetramethylsilane (TMS; $\delta=0$ ppm)
using residual CHCl_3 as a lock reference ($\delta=7.25$ ppm).
30 Multiplicities are abbreviated in the usual fashion:
s=singlet; d=doublet; t=triplet; q=quartet; m=multiplet;
br=broad.

35 Carbon nuclear magnetic resonance (^{13}C NMR) spectra were
performed on a Bruker AMX-400 spectrometer at 100 MHz
with composite pulse decoupling. Samples were prepared
as with ^1H NMR spectra, and chemical shifts are reported

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relative to TMS (0 ppm); residual CHCl_3 was used as an internal reference ($\delta=77.0$ ppm).

5 All high resolution mass spectral (HRMS) analyses were determined by electron impact ionization (EI) on a JEOL JMS-DX 303HF mass spectrometer with perfluorokerosene (PFK) as an internal standard. Low resolution mass spectra (MS) were determined by either electron impact ionization (EI) or chemical ionization (CI) using the
10 indicated carrier gas (ammonia or methane) on a Delsi-Nermag R-10-10 mass spectrometer. For gas chromatography/mass spectra (GCMS), a DB-5 fused capillary column (30 m, 0.25mm thickness) was used with helium as the carrier gas. Typical conditions used a
15 temperature program from 60-250°C at 40°C/min.

Thin layer chromatography (TLC) was performed using precoated glass plates (silica gel 60, 0.25 mm thickness). Visualization was done by illumination with
20 a 254 nm UV lamp, or by immersion in anisaldehyde stain (9.2 mL p-anisaldehyde in 3.5 mL acetic acid, 12.5 mL conc. sulfuric acid and 338 mL 95% ethanol (EtOH)) and heating to colorization.

25 Flash silica gel chromatography was carried out according to the standard protocol.

Unless otherwise noted, all solvents and reagents were commercial grade and were used as received, except as
30 indicated hereinbelow, where solvents were distilled under argon using the drying methods listed in parentheses: CH_2Cl_2 (CaH_2); benzene (CaH_2); THF (Na/ketyl); Et_2O (Na/ketyl); diisopropylamine (CaH_2).

35 Abbreviations

OTf triflate

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	TLC	thin layer chromatography
	EtOAc	ethyl acetate
	TIPS	triisopropylsilyl
	PMB	p-methoxybenzyl
5	Bn	benzyl
	Ac	acetate
	hex	hexane
	THF	tetrahydrofuran
	coll	collidine
10	LiHMDS	lithium hexamethyldisilazide
	DMF	N,N-dimethylformamide
	DMAP	2-dimethylaminopyridine
	DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
	TBAF	tetra-n-butylammonium fluoride
15	M.S.	molecular sieves
	r.t.	room temperature
	r.b.	round bottom flask

EXAMPLE 1

20

Preparation of Polymer-Bound Glucal 18:

Polymer-bound galactal 7 (500 mg; S.J. Danishefsky, et al., *J. Am. Chem. Soc.* **1992**, 8331) was placed in a 100 mL polymer flask and dried in vacuo. On cooling to 0°C under N₂, dry CH₂Cl₂ (20 mL) and freshly prepared Murray solution (30 mL; R.W. Murray and R. Jeyaraman, *J. Org Chem.* **1985**, 2847) was added. After stirring at 0°C for ~90 min., solubles were filtered using N₂ pressure. The oxidation procedure was repeated. The resulting epoxide of 7 kept on a vacuum line for ~3 h to dry. A solution of glucal 19 (1.0 g in 8 mL dry THF) was added, and the mixture was cooled to -23°C (dry ice-CCl₄). A solution of ZnCl₂ in THF (0.8 mL 1.0 M) was added. The mixture was slowly allowed to warm to r.t. (over ~2 h), and then stirred at r.t. overnight. The polymer-bound glucal 18

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was rinsed with 3 x 20 mL THF, and dried on a vacuum line.

Preparation of Polymer-Bound Tetrasaccharide 20:

5
Polymer-bound glucal 18 and Sn(OTf)₂ (0.80 g, 1.92 mmol) were combined and dried in vacuo. On cooling to 0°C under N₂, a solution of fucosyl donor 10 (1.8 g, 4.1 mmol) in 20 mL dry THF with di-t-butylpyridine (1.7 mL, 7.57
10 mmol) was added. The mixture was allowed to warm slowly to r.t., and stirred overnight. The polymer was washed with 2 x 20 mL dry THF, 2 x 20 mL dry dioxane, 20 mL DMSO, and 2 x 20 mL THF. The resulting polymer-bound tetrasaccharide 20 was kept on a vacuum line to dry.

15

Preparation of Tetrasaccharide Glycal 21:

The polymer-bound tetrasaccharide 20 (50 mg) was stirred in 2 mL THF, and treated with 0.2 mL each of 1.0 M
20 solutions of TBAF and AcOH in THF. The mixture was stirred at 40°C overnight. The polymer was washed with 3 x 5 mL THF. The combined rinsings were concentrated and column-chromatographed on silica (2:1 EtOAc:hex), providing tetrasaccharide glycal 21 as a colorless gum.
25 Yield: 9.0 mg.

EXAMPLE 2

Preparation of Diol 18':

30 Galactal 7' (0.100 g, 0.304 mmol) in 5 mL dry CH₂Cl₂ at 0°C under a N₂ atmosphere was treated with 10 mL Murray solution (freshly prepared) and stirred at 0°C for 40 min. TLC (1:1 EtOAc:hex) showed no trace of 7'.
35 Solvents were evaporated using a dry N₂ stream. The residual epoxide of 7' was kept on a vac. line ~2h. To the epoxide under a N₂ atmosphere was added a solution of

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glucal derivative 3' (0.150 g, 0.496 mmol) in 3 mL dry THF. On cooling to -78°C , 1.0 M ZnCl_2 in Et_2O (0.50 mL, 0.50 mmol) was added. The mixture was allowed to slowly warm to r.t. (over ~2 h) and stirred overnight. TLC (1:1 EtOAc:hex) showed that the reaction was complete. Saturated aq. NaHCO_3 (20 mL) was added, and the mixture was then extracted with EtOAc (3 x 20 mL). The organic layer was dried over MgSO_4 . Column chromatography on silica (1:3 EtOAc:hex) afforded diol 18' as a colorless solid. Yield: 173 mg (89%). $[\alpha]_D^{23} - 9.8^{\circ}$ (c 1.0, CH_2Cl_2).

Preparation of Tetrasaccharide 22:

Diol 18' (86 mg, 0.133 mmol) and fucosyl donor 10 (0.290 g, 0.665 mmol) were azeotropically dried using benzene. The mixture was dissolved in 3 mL dry THF together with 0.65 mL di-*t*-butylpyridine and then added via canula to a flask containing $\text{Sn}(\text{OTf})_2$ (0.30 g, 0.72 mmol) and 4 Å MS (500 mg) at 0°C under N_2 atm. The mixture was stirred at 0°C ~7 h. TLC (1:3 EtOAc:hex) shows no trace of diol 18'. The mixture was partitioned between saturated aq. NaHCO_3 (100 mL) and EtOAc (2 x 100 mL). The organic layer was dried over MgSO_4 . The organic layer was filtered through silica using EtOAc to obtain crude material, which was then purified by chromatography on silica (1:9 EtOAc:hex) affording tetrasaccharide 22. Yield: 170 mg (86%).

Preparation of Iodosulfonamide 23:

Procedure 1.

Tetrasaccharide glycal 22 (120 mg, 81.1 μmol) and PhSO_2NH_2 (20 mg, 0.13 mmol) were azeotropically dried using benzene. Added (glove bag) 4 Å MS (0.2 g). After cooling to 0°C under N_2 , dry CH_2Cl_2 (1.0 mL) was added. The mixture was treated with a solution of $\text{I}(\text{coll})_2\text{ClO}_4$

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(prepared from 100 mg $\text{Ag}(\text{coll})_2\text{ClO}_4$, 5 mL collidine, and 60 mg I_2 in 1 mL dry CH_2Cl_2) via canula through a plug of flame-dried celite and 4 Å MS. The mixture was stirred at 0°C for 40 min. TLC (1:4 EtOAc:hex) showed
5 iodosulfonamide 23 as the major component. The mixture was filtered through celite, which was rinsed with Et_2O . The organic layer was extracted with saturated aq. $\text{Na}_2\text{S}_2\text{O}_3$, saturated aq. CuSO_4 , brine, and then dried over MgSO_4 . Column chromatography on silica (1:4 EtOAc:hex)
10 gave iodosulfonamide 23 as a colorless solid.
Yield: 115 mg (80%).

Procedure 2.

Tetrasaccharide glycal 22 (200 mg, 0.135 mmol), PhSO_2NH_2
15 (42 mg, 0.27 mmol), and 200 mg powdered 4 Å MS in 2.0 mL dry CH_2Cl_2 at 0°C under a N_2 atmosphere was treated with $\text{I}(\text{coll})_2\text{ClO}_4$ (prepared from 120 mg $\text{Ag}(\text{coll})_2\text{ClO}_4$ and 67 mg I_2 in 1 mL dry CH_2Cl_2). The mixture was stirred at 0°C (protected from light using foil) for 30 min. TLC (1:2
20 EtOAc:hex) showed mainly iodosulfonamide with some glycal.

After ~1 h more at 0°C, TLC showed no noticeable improvement. The mixture was filtered through celite, which was washed with Et_2O . After extracting with saturated aq.
25 $\text{Na}_2\text{S}_2\text{O}_3$, saturated aq. CuSO_4 , brine, the organics were dried over MgSO_4 . Column chromatography on silica (1:3 EtOAc:hex) gave 23 as a colorless solid.
Yield: 165 mg (69%). $[\alpha]_D^{23} = -85.7^\circ$ (c 1.0, CH_2Cl_2).

30

Preparation of Hexasaccharide 25:

Iodosulfonamide 23 (60 mg, 34 mmol) in a 35 mL r.b. was treated with 200 mg powdered 4 Å MS (glove bag). To this
35 flask under N_2 was added a solution of protected lactal 24 in THF (1.5 mL). On cooling the mixture to -78°C, a solution of AgBF_4 (40 mg, 0.206 mmol) was added in 0.25

-57-

mL dry THF. The mixture was stirred and slowly warmed to r.t. overnight. The mixture was warmed to 45°C and stirred ~36 h. TLC showed only a trace of iodosulfonamide. Saturated aq. NH₄Cl (5 mL) was added, and the mixture was extracted with 3 x 10 mL EtOAc. The organic layer was dried over MgSO₄. Column chromatography on silica (1:3 EtOAc:hex) afforded 25 as a colorless oil. Yield: 42 mg (55%).

$[\alpha]_D^{23} = -33.8^\circ$ (c 2.0, CH₂Cl₂)

Preparation of Hexasaccharide 25a:

Hexasaccharide 25 (55 mg, 24.4 mmol) in ~1.5 mL THF was treated at 0°C with TBAF (0.25 mL, 1.0 M solution in THF, 0.25 mmol), and stirred at r.t. overnight. TLC (1:9 MeOH:CH₂Cl₂) showed a 3:1 mixture of 25a vs. a less polar substance. Additional 1.0 M TBAF (0.10 mL) was added, and the mixture was stirred overnight at r.t. TLC showed that the reaction was complete. Solvents were removed using a N₂ stream. Column chromatography on silica (1:19 MeOH:CH₂Cl₂) afforded a ~1:2 mixture corresponding to two compounds which differ only in the presence or absence of a 3,4-cyclic carbonate group. Crude yield: 35 mg total weight for two products. The crude mixture was used as such for the next reaction.

Preparation of Peracetylated Hexasaccharide 26:

Hexasaccharide 25a (36 mg) in 0.25 mL dry THF was added via canula to ~8 mL bright blue Na/NH₃ solution at -78°C (dry ice bath) under N₂ atm. After removing the dry ice bath, the mixture was stirred in refluxing NH₃ (dry ice condenser) for 15 min. After adding 2 mL dry MeOH (slowly!), the resulting mixture was stirred while blowing off NH₃ with a N₂ stream. The MeOH solution was treated with Dowex 50 x 8 [H⁺] until pH ~8-9, and then

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filtered. The resin was washed with MeOH. The residue was concentrated and kept on a vacuum line to dry. Under a N₂ atmosphere, the residue was treated with 1 mL dry pyridine and 0.5 mL Ac₂O, and stirred at r.t. overnight. TLC (EtOAc) showed that hexasaccharide 26 is major component. Upon concentration, the residue was purified by column chromatography on silica (1:4 hex:EtOAc).

Preparation of Hexasaccharide 17:

Hexasaccharide 26 (10.0 mg, 6.3 mmol) under N₂ at 0°C was treated with 0.5 mL dry CH₂Cl₂. Dioxirane solution (0.20 mL) was added, and the mixture was stirred at 0°C -40 min. TLC (EtOAc) showed no trace of 26. Solvents were evaporated with a N₂ stream. The epoxide was dried on a vacuum line for -2 h. The epoxide was treated under a N₂ atmosphere with 0.5 mL allyl alcohol (passed through basic alumina to dry) and 0.5 mL dry THF. On cooling to -78°C, 1.0 M ZnCl₂ (10 mL) in dry Et₂O was added. After warming slowly to r.t., the mixture was stirred overnight. Saturated aq. NaHCO₃ (5 mL) was added, and the mixture was extracted with 3 x 5 mL EtOAc. The combined organic layers were dried over MgSO₄, filtered and concentrated to an oil, which was dried on a vacuum line for -2 h. The residue was treated to pyridine:Ac₂O (2:1, 1.5 mL) while stirring overnight. Solvents were removed, and the residue was purified by column chromatography on silica (1:4 hex:EtOAc), affording hexasaccharide 17 as a colorless solid. Yield: 5.5 mg.

Results and Discussion

A Highly Convergent Synthesis of the Lewis Y Blood Group Determinant in Conjugatable Form

Construction of the Le^Y determinant commences with lactal (1a) (W.N. Haworth, E.L. Hirst, M.M.T. Plant, R.J.W.

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Reynolds, *J. Chem. Soc.* **1930**, 2644) as shown in Figure 2. Capping both primary hydroxyl groups as their TBDPS ethers under standard conditions was followed by simple engagement of the 3' and 4' hydroxyl functions as a cyclic carbonate **2a**. The stereospecific introduction of two α -linked fucose residues gave tetrasaccharide glycal **3a** in 51% yield in a single step. The donor used was the known fluorosugar **5a** (S.J. Danishefsky, J. Gervay, J.M. Peterson, F.E. McDonald, K. Koseki, T. Oriyama, D.A. Griffith, C-H. Wong, D.P. Dumas, *J. Am. Chem. Soc.* **1992**, 114, 8329) following a modification of the original Mukaiyama conditions. (T. Mukaiyama, Y. Murai, S. Shoda, *Chem. Lett.* **1981**, 431) Glycal **3a** corresponds to the Le^Y hapten, lacking the N-acetyl function in the glucose residue. The problem was then to introduce this group as well as a galactose spacer module.

Methodology developed previously (D.A. Griffith, S.J. Danishefsky, "On the Sulfonamidoglycosylation of Glycals. A Route to Oligosaccharides With 2-Aminohexose Subunits+", *J. Am. Chem. Soc.* **1990** 112, 5811) proved appropriate to attain these goals. Glycal **3a** was treated with iodonium dicollidine perchlorate and benzenesulfonamide to afford iodosulfonamide **4a**. Azaglycosylation using the 3-stannyl ether of galactal (**9a**) (S.J. Danishefsky, K. Koseki, D.A. Griffith, J. Gervay, J. M. Peterson, F.E. McDonald, T. Oriyama, *J. Am. Chem. Soc.* **1992**, 114, 8331) in the presence of silver tetrafluoroborate gave pentasaccharide glycal **6a** in 75% yield as shown in Figure 3. Having **6a** in hand, one can iterate the azaglycosylation sequence or activate the glycal as its epoxide and continue with further glycosylations. To demonstrate the ability to fashion a conjugatable form of Le^Y hapten, formation of the allyl glycoside was important. The feasibility of converting the sulfonamido group into the target acetamide was demonstrated. Glycal **6a** was deprotected in two steps as

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shown. Peracetylation afforded acetamido glycal **7a**. Activation of the glycal as its epoxide with dimethyldioxirane (R.L. Halcomb, S.J. Danishefsky, *J. Am. Chem. Soc.* **1989**, 111, 6661), followed by epoxide opening with allyl alcohol in the presence of zinc chloride gave the desired peracetylated β -allyl pentasaccharide which was deacetylated by action of methoxide to provide the target Le^Y hapten as its β -allyl glycoside **8a**. (**8a** $[\alpha]_D - 72.7^\circ$ (c. 1 MeOH); IR (thin film) 3350, 2940, 2900, 2830, 1650, 1550, 1365, 1300, 1155, 1070, 1030; ¹H NMR (400 MHz, CD₃OD) δ 5.95 (m, 1H), 5.32 (d, $J=17.25$ Hz, 1H), 5.14-5.19 (m, 2H), 5.04 (d, $J= 3.83$ Hz, 1H), 5.02 (d, $J=3.50$ Hz, 1H). 4.68 (d, $J= 8.15$ Hz, 2H), 4.51 (d, $J= 5.70$ Hz, 1H) 3.40-4.38 (m, 27H). 1.96 (s, 3H), 1.23 (m, 6H); HRMS (FAB) calcd for C₃₅H₅₆NO₂₄Na 900.3325 found 900.3310) The aldehyde, derived by ozonolysis of **8a**, could be conjugated to a carrier protein by the method of Bernstein and Hall.

This synthesis is the most direct route to the Le^Y determinant known. (O. Hindsgaul, T. Norberg, J. Le Pendu, R. U. Lemieux, *Carbohydr Res.* **1982**, 109, 109; U. Spohr, R.U. Lemieux *ibid*, **1988**, 174, 211; for previous syntheses, see: J.C. Jacquinet, P. Sinay, *J. Org. Chem.* **1977**, 42, 720; S. Nilsson, H. Lohn, T. Norberg, *Glycoconjugate J.* **1989**, 6, 21; R.R. Schmidt, A. Topfer, *Tetrahedron Lett.* **1991**, 32, 3353; W. Kinzy, A. Low, *Carbohydrate. Res.* **1993**, 245, 193) The method is stereospecific at each step, and it illustrates the versatility of glycals both as donors and acceptors and takes advantage of 1,2-glycal epoxides and their presumed N-sulfonylaziridine counterparts. The method also makes possible extensive analog preparation and variation of conjugation strategies.

35

The synthesis of **3a** and **6a** are shown below:

3a: To 2.00g (2.47 mmol) of lactal carbonate **2a** was added 4.44g (9.86 mmol) of fucosyl fluoride **5a**. The mixture was azeotroped 5 times with benzene and placed under high vacuum for two hours. Under an argon atmosphere 2.77 ml (12.33 mmol) of di-tert-butyl pyridine and 16ml of dry ether were added. 2.0 g of freshly activated 4A molecular sieves were added and the mixture stirred one hour at room temperature. In an argon glove bag, 2.34g (12.33 mmol) of stannous chloride (SnCl_2) and 2.56g (12.33 mmol) of silver perchlorate (AgClO_4) were added. The flask was equipped with a reflux condenser and the reaction brought to reflux for 72 hours. The reaction was quenched with 5ml of saturated bicarbonate and filtered through a pad of celite. Diluted with 50ml ethyl acetate and washed 2 times with sat. bicarbonate, 2 times with sat. copper sulfate and 2 times with sat. brine. The organics were dried over MgSO_4 and concentrated. Flash chromatography in 20% ethyl acetate/hexanes afforded 2.10g (51%) of a white foam **3a**: $[\alpha]_D -78.9$ (c.555, CHCl_3); IR (thin film) 3040, 3000, 2905, 2860, 2830, 1820, 1800, 1710, 1635, 1585, 1570, 1480, 1460, 1440, 1415, 1370, 1350, 1300, 1260, 1205, 1145, 1100, 950, 735, 695, ^1H NMR (400MHz, CDCl_3) δ 8.09 (d, $J= 8.12$ Hz, 2H), 8.00 (d, $J= 8.26$ Hz, 2H) 7.66 (m, 4H), 7.59 (d, $J= 6.74$ Hz, 4H), 7.56 (t, $J= 7.27$ Hz, 1H), 7.30-7.50 (m, 22H) 7.16-7.26 (m, 10H) 7.09 (m, 2H), 6.99 (t, $J= 7.59$ Hz, 2H) 6.89 (t, $J= 7.97$ Hz, 1H), 6.43 (d, $J= 6.08$ Hz, 1H), 5.46 (bs, 1H), 5.38 (bs, 1H), 5.35 (d, $J= 3.42$ Hz, 1H), 4.89 (d, $J= 11.35$ Hz, 1H), 4.75-4.80 (m, 4H), 4.72 (d, $J= 5.88$ Hz, 2H), 4.69 (d, $J= 4.27$ Hz, 2H), 4.36-4.55 (m, 5H), 4.28 (q, $J= 6.51$ Hz, 1H), 4.17 (bd, $J= 5.46$ Hz, 1H), 3.90-4.00 (m, 6H), 3.85 (d, $J= 2.99$ Hz, 1H), 3.82 (d, $J= 2.89$ Hz, 1H), 3.56-3.78 (m, 4H), 1.07 (m, 24H); HRMS (FAB): calcd for $\text{C}_{99}\text{H}_{106}\text{O}_{20}\text{Si}_2\text{Na}$ 1694.6740 found 1694.6787.

6a: 230 mg (0.12mmol) of iodosulfonamide **4a** was azeotroped 5 times with dry benzene and placed under high

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vacuum for two hours. Added 2.4ml of THF solution of
15eq. of tin ether **9a** (generated by azeotropic removal
of water overnight with a Dean-Stark trap equipped with
freshly activated 4A mol. sieves from 561 mg (1.80mmol)
5 of **6a**-TIPS-galactal and 673 μ l (1.32mmol) bis(tributyltin)
oxide in 80 ml of benzene). To this solution stirring
under an argon atmosphere was added 200 mg of freshly
activated 4A powdered molecular sieves. Stirred one hour
at room temperature. Cooled solution to -78°C and added,
10 via cannula, a solution of 187 mg (.96mmol) of silver
tetrafluoroborate in 2.4 ml of THF. Warmed to room
temperature over 15 hours and quenched the reaction,
which had turned bright yellow, with 2ml. of sat.
bicarbonate. The reaction mixture was filtered through
15 a pad of celite into a separatory funnel. The celite pad
was washed thoroughly with ethyl acetate. The organics
were washed twice with sat. bicarbonate and twice with
sat. brine. The organics were dried over MgSO₄.
Concentration and chromatography in 25% ethyl
20 acetate/hexanes gave 193 mg (75%) as a white foam **6a**:
[α]_D-126.4°(c, 505, CHCl₃), IR (thin film) 3500, 3040, 3000,
2905, 2840, 1820, 1800, 1705, 1635, 1590, 1440, 1410,
1255, 1195, 1100, 1080, 1035, 815, 730, 695; ¹H NMR (400
MHz, CDCl₃) δ 8.09 (app t, 4H), 7.08-7.65 (m, 46H), 6.90
25 (t, J=7.65 Hz, 3H), 6.76 (d, J=6.91 Hz, 2H), 6.12 (d,
J=6.59 Hz, 1H), 5.50 (bs 1H), 5.45 (bs 1H), 5.28 (app t,
2H), 3.03-4.91 (m, 36H), 1.09 (m, 45H); LRMS (FAB): calcd
for C₁₂₀H₁₄₁NO₂₆SSi₃Na 2153 found 2153.

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**A Strategy for the Assembly of Complex, Branched
Oligosaccharide Domains on a Solid Support: An
Application to a Concise Synthesis of the Lewis^b Domain
in Bioconjugatable Form.**

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The assembly of the Le^b (type 1) domain is a relatively
more difficult undertaking than was the Le^y (type 2)

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target, wherein lactal was used as a convenient starting material. In the case of the type 1 determinant, lactal is not a useful starting material. The synthesis of the Le^b system offered an opportunity to apply the polymer-based oligosaccharide construction method. (S.J. Danishefsky, K.F. McClure, J.T. Randolph, R.B. Ruggeri, *Science* 1993, 260, 1307) The strategy is summarized in Figure 4, wherein polymer-bound glycal 1 is activated for glycosyl donation via direct formation of a 1,2-anhydro derivative 2. Reaction of 2 with acceptor glycal 3 furnishes 4. Reiteration is achieved by means of direct epoxidation and reaction with acceptor 3. The self-policing nature of the method and the simple "one time" purification at the end of the synthesis are useful features.

The present invention discloses an important additional dimension of the polymer-bound method. The logic is captured by inspection of Figure 5. Each glycosylation event generates a unique C₂ hydroxyl. In principle (and in fact, see *infra*) this hydroxyl can function as a glycosyl acceptor upon reaction with a solution based donor. The glycal linkage of 5, still housed on the support, can be further elongated. In this way, branching at C₂ is accomplished while minimizing the requirement for protecting group machinations. (For an application of this strategy in the synthesis of a complex saponin, see: J.T. Randolph, S.J. Danishefsky, *J. Am Chem Soc.* 1993, 115, 8473)

In principle, this branching can be implemented at any site in a growing chain. For such an extension, it would be necessary to cap all previously generated hydroxyl groups generated on the "polymer side" (non-reducing end) of the growing domain. Thus, the polymer-bound oligosaccharide can serve as either donor or acceptor, wherever appropriate.

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Initial efforts at reduction to practice identified tetrasaccharide glycal **6**, bearing H-type 2 blood group specificity, as a goal. Polymer-supported galactal **7** (using as polymer support polystyrene crosslinked with 1% divinylbenzene functionalized using published procedures: T-H. Chan, W.-Q. Huang, *J. Chem. Soc., Chem. Commun.* **1985**, 909; M.J. Farrall, J.M.J. Frechet, *J. Org. Chem.* **1976**, 41, 3877) reacted with a solution of 3,3-dimethyldioxirane (R.W. Murray, R. Jeyaraman, *J. Org. Chem.* **1985**, 50, 2847), to provide the corresponding 1,2-anhydrosugar glycosyl donor, which was treated with a solution of glucal derivative **8** in the presence of $ZnCl_2$ to provide **9** (R.L. Halcomb, S.J. Danishefsky, *J. Am. Chem Soc.* **1989**, 111, 6661) This polymer-bound disaccharide acted as a glycosyl acceptor upon treatment with a solution of fucosyl fluoride **10** (K.C. Nicolau, C.W. Hummel, Y. Iwabuchi, *J. Am. Chem. Soc.* **1992**, 114, 3126) in the presence of $Sn(OTf)_2$ thereby giving **11**. Retrieval of the trisaccharide glycal from the support was accomplished using tetrabutylammonium fluoride (TBAF) to afford **12** in 50% overall yield from **7**.

The trisaccharide, retrieved from the polymer, could then be further elaborated. Toward this end, compound **12** was converted to silyl ether **13** by reaction with TIPSCl. The latter was converted to the iodosulfonamide derivative **14** by the action of $I(coll)_2ClO_4$ in the presence of $PhSO_2NH_2$. Reaction of **14** with galactal stannyl ether derivative **15** in the presence of $AgBF_4$ gave **16** 77% yield. (D.A. Griffith, S.J. Danishefsky, *J. Am. Chem Soc.* **1990**, 112, 5811) Tetrasaccharide glycal **16** was deprotected and peracetylated to afford **6**. (S.J. Danishefsky, K. Koseki, D.A. Griffith, J. Gervay, J.M. Peterson, F.E. McDonald, T. Oriyama, *J. Am. Chem Soc.* **1992**, 114, 8331)

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Thus, the synthesis of the full H-type determinant was achieved by sequential polymer- and solution-based

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maneuvers. The next target was the more complex Le^b hexasaccharide 17. The campaign proceeded as shown in Figure 6. Polymer-bound galactal 7 was converted to 18 upon epoxidation with 3,3-dimethyldioxirane followed by
5 reaction with glucal derivative 19. This disaccharide diol was then bisfucosylated using fucosyl donor 10 in the presence of Sn(OTf)₂ to afford 20. Retrieval from the support with TBAF provided 21, which was obtained in 40% overall yield from 7. Compound 21 reacted with TIPSCl to
10 give 22.

Iodosulfonamide 23, obtained from 22 using I(coll)₂ClO₄ and PhSO₂NH₂, reacted with lactal derivative 24 in the presence of AgBF₄ to provide hexasaccharide glycal 25 in
15 55% yield. Deprotection of 25 was accomplished in two stages (TBAF to remove the silyl ethers, followed by Na/NH₃ reduction to remove the aromatic protecting groups), and the crude product was peracetylated to give 26 in a 51% overall yield. Compound 26 was converted,
20 via the 1,2-anhydrosugar derivative, to allyl glycoside 17, which can be activated by ozonolysis to the aldehyde (R = CH₂CHO) for subsequent coupling to a protein by the method of Bernstein and Hall.

25 In sum, the present invention extends the solid-support glycal assembly method for complex carbohydrate domain synthesis to include the branching patterns critical for biorecognition. Specifically, the determinant for the binding of *H. pylori* to human gastric epithelium has been
30 stereospecifically fashioned, with simplicity, in a way which provides significant relief from some of the complexities of protecting group manipulations.

35 *Experimental Procedure:*

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6: ^1H NMR (400 MHz, CDCl_3); δ 6.39 (d, 1H, $J=6.2$ Hz, H_1 galactal), 5.65 (d, 1H, $J=8.9$ Hz, NHAc), 5.35 (d, 1H, $J=3.8$ Hz), 5.33 (m, 1H), 5.29 (d, 1H, $J=2.6$ Hz), 5.27 (d, 1H, $J=3.1$ Hz), 5.17-5.09 (m, 2H), 4.97-4.90 (m, 2H), 4.81 (dd, 1H, $J=3$ Hz, $J=6.1$ Hz, H_2 galactal), 4.75 (d, 1H, $J=8.0$ Hz), 4.52 (m, 1H), 4.48 (dd, 1H, $J=12.0$ Hz), 4.44-4.06 (m, 8H), 3.88-3.77 (m, 4H), 3.61 (m, 1H), 2.18-1.97 (m, 33 H, COCH_3), 1.18 (d, 3H, $J=6.5$ Hz, CH_3 fucose); ^{13}C NMR (CDCl_3): δ 170.80, 170.77, 170.72, 170.67, 170.62, 170.34, 170.21, 170.09, 170.01, 169.99, 169.65, 144.92 (C_1 galactal), 100.22, 98.83, 98.58, 95.55, 74.48, 73.38, 73.13, 73.06, 71.48, 71.01, 70.68, 67.97, 67.42, 67.18, 67.05, 65.94, 64.83, 62.35, 62.22, 60.88, 60.37, 54.21, 23.23, 22.15, 20.85, 20.82, 20.79, 20.76, 20.65, 20.61, 20.57, 15.51, (C_6 fucose); IR (thin film): 3368.7 (NH), 2965.6, 2934.6, 1746.5 ($\text{C}=\text{O}$), 1537.5, 1435.9, 1371.3, 1228.5, 1065.0, 1046.0; $[\alpha]_{\text{D}}^{23} = -51.1^\circ$ (c 1.8, CH_2Cl_2); HRMS (FAB); calcd. for $\text{C}_{46}\text{H}_{63}\text{NNaO}_{28}$: $m/z = 1100.3434$, found 1100.3436.

20

21: Polymer-bound galactal 7 (loading = 0.85 mmol glycal/g), which had been placed in a round-bottom flask equipped with a fritted outlet, was suspended in CH_2Cl_2 under N_2 , cooled to 0°C , and then treated with a solution of 3,3-dimethyldioxirane. The mixture was stirred (teflon-coated magnetic stir bar) for 40 min. at 0°C , after which time solubles were removed by filtration through the fritted outlet (N_2 pressure). The polymer bound 1,2 anhydrosugar was evacuated (ca. 0.1 torr) for several hours in order to dry the material for the next step. This material was once again placed under N_2 before being treated with 19 (~10 molar equivalents as a 0.5 M solution in THF). The suspension was cooled to -40°C , and treated with ZnCl_2 (~2 molar equivalents as a 1.0 M solution in THF). The reaction mixture was allowed to slowly warm to rt (over ca. 2 h), and then stirred an additional 3-4 h. Solubles were removed by filtration,

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and polymer 18 was washed several times with THF and then dried in vacuo. To compound 18 was added, in a glove bag, solid Sn(OTf)₂ (~ molar equivalents), and the mixture was placed under N₂ and cooled to 0 °C before being treated with 10 (~5 molar equivalents as a 0.2 M solution in THF and di-tert-butylpyridine (~8 molar equivalents). The suspension was allowed to warm to rt and stirred 8-10 h. The mixture was rinsed with anhydrous THF (2 times), 1,4-dioxane (2 times), again with THF, and then dried in vacuo. Compound 20 (100 mg) was suspended in THF, treated with a 1:3 mixture of AcOH and TBAF (~0.2 M in TBAF, ~10 molar equivalents), and the mixture was stirred for 18 h at 40 °C. The polymer was rinsed with THF (3 times), and the combined rinsings were concentrated and purified by column chromatography on silica gel (1:1 EtOAc: hexanes). Compound 21 (18 mg) was obtained as a colorless solid (40% overall yield from 7): ¹H NMR (400 MHz, CDCl₃): δ 7.40-7.25 (m, 30H, Ar H), 6.18 (d, 1H, J=6.0 Hz, H₁ glucal), 5.26 (d, 1H, J=3.5 Hz, H₁ fucose), 5.09 (d, 1 H, J= 3.7 Hz, H₁ fucose), 4.96 (t, 2 H, J= 10.8 Hz, PhCH₂), (4.90-4.56 (m, 13 H), 4.43 (m, 1H), 4.15-4.06 (m, 4 H), 3.97 (dt, 1 H, J=8.3 Hz, J=2.4 Hz), 3.87-3.65 (m, 10H), 3.64 (d, 1 H), 3.57 (d, 1 H), 2.69 (br, 1 H, OH), 2.52 (br, 1 H, OH), 1.11 (d, 3 H, J= 7.0 Hz, CH₃ fucose), 1.09 (d, 3H, J= 7.0 Hz, CH₃ fucose); ¹³C NMR (CDCl₃): δ 153.37 (C=O), 145.75 (C₁ glucal), 138.60, 138.52, 138.19, 137.61, 128.55, 128.52, 128.44, 128.24, 128.16, 128.07, 127.62, 127.56, 127.45, 98.71, 98.38, 97.65, 97.34, 79.26, 78.87, 78.67, 78.01, 77.79, 77.65, 76.37, 76.10, 74.92, 74.40, 74.16, 73.95, 72.86, 72.64, 72.53, 67.43, 67.29, 61.31, 60.90, 16.65 (C₆ fucose), 16.53 (C₆ fucose); IR (thin film): 3467.0 (OH), 3029.6, 2923.6, 1807.2 (C=O), 1647.3, 1496.0, 1453.5, 1358.1, 1240.2, 1095.6, 1049.2, 738.5, 697.2; [α]_{D23} = -82.5° (c 0.4, CH₂Cl₂); HRMS (FAB); calcd. for C₆₇H₇₄NaO₁₈: m/z = 1189.4772, found 1189.4757.

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25: To a mixture of **23** (60 mg, 34 μ mol) and powdered 4A molecular sieves (200 mg) under N_2 was added, via canula, a solution of **24** (0.21 mmol) in anhydrous THF (1.5 mL). The stirred suspension was cooled to $-78^\circ C$ before being
5 treated with a solution of $AgBF_4$ (0.21 mmol) in 0.25 mL of anhydrous THF. The mixture was stirred and allowed to slowly warm to rt overnight. The suspension, which had developed a bright-yellow color, was heated, with stirring, at $45^\circ C$ for an additional 36 h, until the TLC
10 (2.5 EtOAc:hexanes) showed no trace of **23**. The mixture was treated with saturated aqueous NH_4Cl (5 mL) and then extracted with EtOAc (3 x 10 mL), and the organics were dried over $MgSO_4$. The crude product was purified by silica gel chromatography (1:3 EtOAc:hexanes) to give **25**
15 as a colorless oil (42 mg, 55%): 1H NMR (400 MHz, acetone- d_6): δ 8.17(d, 2 H, $J=7.3$ Hz, $PhSO_2$), 7.50-7.20 (m, 33H, ArH), 6.52 (d, 1 H, $J=10.5$ Hz, NH), 6.30 (dd, 1 H, $J=6.0$ Hz, H_1 glucal), 5.35-5.32 (m, 2H), 5.25 (d, 1H, $J=7.9$ Hz), 5.15 (m, 2 H), 4.99-4.92 (m, 3H), 4.86-4.52
20 (m, 14 H), 4.45 (dd, 1H, $J=7.91$ Hz, $J=2.4$ Hz), 4.32-4.23 (m, 3H), 4.22 (dd, 1 H), 4.17 (d, 1 H, $J=10.1$ Hz), 4.08-3.84 (m, 18 H), 3.79-3.73 (m, 2H), 3.66 (m, 1H), 3.55 (t, 1 H, $J=6$ Hz), 3.50 (dd, 1 H, $J=9.7$ Hz), 1.33 (d, 3 H, $J=6.5$ Hz, CH_3 fucose), 1.31 (d, 3H, $J=6.4$ Hz, CH_3
25 fucose), 1.20-0.98 (m, 84 H, 3 x $Si(i-Pr)_3$); ^{13}C NMR (acetone- d_6): 145.66 (C=O), 132.72, 131.48, 131.45, 131.28, 131.16, 130.77, 130.48, 121.31, 120.11, 119.86, 119.78, 119.25, 95.63, 94.70, 91.37, 89.64, 89.31, 86.52, 73.38, 72.24, 71.00, 70.71, 70.37, 69.80, 69.59, 69.06,
30 68.23, 67.92, 67.38, 67.10, 66.49, 65.67, 65.33, 64.60, 64.34, 64.03, 63.45, 63.30, 59.46, 58.83, 58.37, 54.45, 53.32, 49.86, 19.67 (C_6 fucose), 18.42 (C_6 fucose), 9.55, 9.48, 9.45, 9.31, 9.23, 3.82, 3.70, 3.64; IR (thin film): 3491.9 (OH), 3030.1, 2941.2, 2865.5, 1835.8, 1819.5,
35 1649.8, 1496.2, 1462.3, 1349.9, 1245.5, 1155.2, 1095.1, 1049.4, 882.2, 734.8, 692.0; $[\alpha]_{D23} = -33.8^\circ$ (c 2.0,

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CH₂Cl₂); HRMS (FAB): calcd for ¹²C₁₂₀¹³CH₁₇₉N₂NaO₂₉SSi₄: m/z = 2278.1292, found 2278.1296.

17: ¹H NMR (400 MHz, CD₃OD): δ 6.00 (m, 1H, J = 5.6 Hz, CH₂CH=CH₂), 5.37 (dd, 1 H, J = 1.6 Hz, J = 7.3 Hz, CH₂CH=CH₂), 5.20 (dd, 1 H, J = 1.6 Hz, J = 9.5 Hz, CH₂CH=CH₂), 5.18 (d, 1 H, J = 3.9 Hz, H₁ fucose), 5.10 (d, 1H, J = 3.8 Hz, H₁ fucose), 4.64 (d, 1 H, J = 6.9 Hz), 4.45 (d, 1H, J = 7.4 Hz), 4.43-4.23 (m, 2H), 4.27 (dd, 1H, J = 9.3 Hz, J = 10.6 Hz), 4.23-4.11 (m, 2H), 4.02-3.29 (m, 31 H), 2.06 (s, 3H, NAc), 1.31 (d, 3H, J = 6.6 Hz, CH₃ fucose), 1.29 (d, 3 H, J = 6.6 Hz, CH₃ fucose); ¹³C NMR (CD₃OD): δ 173.20 (C=O), 135.73 (CH₂CH=CH₂), 105.13, 103.30, 102.49, 101.62, 99.63, 96.86, 80.79, 80.67, 78.44, 76.67, 76.49, 75.89, 74.80, 74.59, 73.94, 73.61, 73.40, 71.55, 71.38, 71.16, 70.42, 70.26, 70.14, 67.77, 67.30, 67.21, 62.79, 62.34, 61.99, 55.54, 22.97 (NAC), 16.65 (2 C's, C₆ fucose); IR (thin film): 3376.6 (OH), 2924.2, 1652.5 (C=O), 1383.1, 1032.4; [α]_{D23} = -12.8° (c 0.25, MeOH); HRMS (FAB): calcd. for C₄₁H₆₉N₂NaO₂₉: m/z = 1062.3853, found 1062.3837

Glycal Assembly Method Applied to the Synthesis of Human Breast Tumor-Associated Antigen

The present invention provides a convergent synthesis of the hexasaccharide wherein the two trisaccharide domains have been efficiently assembled in forms readily amenable for coupling. The synthesis of the ABC trisaccharide is presented in Figure 8. The α-linkage of this trisaccharide might be formed by employing a fluorosugar donor **4b**, using established conditions. (Gordon, D. M.; Danishefsky, S. J., *Carbohydr. Res.*, **1990**, 206, 361-366.) Preparation of the appropriate disaccharide acceptor commenced with **5b** (Danishefsky, S. J.; Behar, V.; Randolph, J. T.; Lloyd, K. O., *J. Am. Chem. Soc.*, **1995**, 0000), itself obtained from a glycal coupling.

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Benzylation followed by desilylation, carbonate removal and selective dibenylation afforded the disaccharide **6b**. The acceptor thus obtained was reacted with the fluorosugar **4b** using modified Mukaiyama conditions (Mukaiyama, T.; Murai, Y.; Shoda, S., *Chem. Lett.*, **1981**, 431-433) to provide the trisaccharide glycal **7b**. Deprotection of the PMB ether provided the ABC trisaccharide **8b**, which was poised for coupling with a suitable DEF trisaccharide donor.

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The synthesis of the DEF trisaccharide is described in Figure 9. Epoxidation of the galactal **9b** and standard coupling (Halcomb, R.L.; Danishefsky, S.J., *J. Am. Chem. Soc.*, **1989**, 111, 6661-6666.) with acceptor **10b** afforded, regioselectively, the disaccharide **11b**. Fucosylation employing the fluoro-fucose **12b** (Dejter-Juszynski, M.; Flowers, H.M., *Carbohydr. Res.*, **1973**, 28, 61) provided a 5:1 ratio of monoglycosylation regioisomers, the major isomer being the desired trisaccharide **13b**. This material was treated under standard conditions to afford the trans-diaxial iodosulfonamide **14b**.

Direct coupling reactions (Griffith, D.A.; Danishefsky, S.J., *J. Am. Chem. Soc.*, **1990**, 112, 5811-5819; Danishefsky, S.J.; Koseki, K.; Griffith, D.A.; Gervay, J.; Peterson, J.M.; McDonald, F.E.; Oriyama, T., *J. Am. Chem. Soc.*, **1992**, 114, 8331-8333) employing iodosulfonamides such as **14b** with ABC trisaccharide acceptors failed, leading to a different donor functionality in the trisaccharide. In practice, the iodosulfonamide **14b** was treated with excess lithium ethanethiolate to afford the ethyl thioglycoside **15b** (Figure 10). Precedent established by the present inventors lead to the prediction of sulfonamide participation to provide the desired β -linked product from **15b**. (Griffith, D.A., Ph.D. Thesis, Yale University, 1992) When donor **15b** was treated with MeOTf in the

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presence of acceptor **8b**, a 10:1 mixture of hexasaccharide isomers was obtained. The major product **16b** was obtained in 70-85% yield.

5 Ceramide attachment and elaboration commenced with
epoxidation of **16b**, followed by reaction with the stannyl
ether **17b** promoted by $Zn(OTf)_2$. (Liu, K.K.-C.;
Danishefsky, S.J., *J. Am. Chem. Soc.*, 1993, 115, 4933-
10 4934) Although the yield of this ceramide coupling is
low, when this reaction was performed on trisaccharide
7b, the corresponding product was obtained in 66% yield.
This material can then be used to obtain **18b**. Following
acetylation, the ceramide side-chain was elaborated by
15 reduction of the azide functionality using Lindlar's
catalyst under an atmosphere of H_2 in the presence of
palmitic anhydride to provide **18b**. Desilylation and
saponification was followed by dissolving metal
deprotection and MeOH quench. Peracetylation of the
20 crude mixture, followed by saponification provided the
glycosphingolipid **1b**. Only the chemical shifts and
coupling constants of the anomeric protons have been
reported for the natural material. The spectrum of
synthetic **1b** is in complete agreement with this data.
Furthermore, the product was characterized by exact mass,
25 and 1H and ^{13}C NMR. The synthetic material has also been
shown to bind to the monoclonal antibody MBrl.

In addition, the present invention provides the
30 corresponding allyl glycoside (Figure 11). Deprotection
of **16b**, as above, and acetylation afforded the peracetate
of the hexasaccharide glycal. Epoxidation, reaction with
allyl alcohol, and saponification provided the allyl
glycoside **19b**.

35 As in the case of the Le determinant, ozonolysis of the
allyl group of **19b** will set the stage for reductive
coupling to lysine residues of proteins.

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Synthesis of 3b:**3-O-(4-Methoxybenzyl)-D-galactal**

A suspension of D-galactal (**2b**) (3.70g, 25.3 mmol) and dibutyltin oxide (6.30g, 1.0 equiv) in dry benzene (150 mL) was heated to reflux for 2h with azeotropic removal of water. The reaction was cooled and treated with PMBCl (3.80 mL, 1.1 equiv) and tetrabutylammonium bromide (9.10g, 1.1 equiv) and refluxed for 4h. The reaction was filtered through silica column and eluted with EtOAc/hexanes (4:1). Fractions containing product were concentrated and the residue triturated in hexanes to give 4.50g (67%) of product as white crystalline solid.

mp (hexanes) 117-118 °C; $(\alpha)^{23}_D = -23.0^{\circ}$ (CHCl₃, c = 1.1); IR (KBr) 3313 (br), 1645, 1513, 1228, 1082, 821 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.28 (2H, d, J = 8.4 Hz), 6.89 (2H, d, J = 8.4 Hz), 6.44 (1H, dd, J = 6.4 Hz), 4.70 (1H, dt, J = 6.3, 1.9 Hz), 4.59-4.52 (2H, ABq, J = 11.4 Hz), 4.20-4.18 (1H, m), 4.04-3.97 (1H, m), 3.90-3.82 (2H, m), 3.81 (3H, s), 2.73 (1H, d, J = 3.1 Hz, C4-OH), 2.54 (1H, dd, J = 8.2, 4.2 Hz, C6-OH); ¹³C-NMR (100 MHz, CDCl₃) δ 159.46, 145.02, 142.05, 129.46, 113.95, 99.36, 76.12, 70.17, 70.14, 63.65, 62.74, 55.26; LRMS(NH₃) 284 (M+NH₄)⁺, 266 (M)⁺, 249.

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4,6-di-O-benzyl-3-O-(4-methoxybenzyl)-D-galactal (3b).

A solution of 3-O-(4-methoxybenzyl)-D-galactal (2.28 g, 8.56 mmol) and benzyl bromide (3.75 mL, 3.68 mol equiv; freshly passed through basic alumina) in DMF (30 mL) under N₂ at 0 °C was treated with NaH (1.37 g, 4.0 mol equiv) in two portions. The reaction was stirred 0.5h at 0 °C and 1h at rt. The reaction was carefully poured into 50 g of crushed ice, diluted to 100 mL with water, then extracted with EtOAc-hexanes (1:1, 100 mL x 3). Organic extracts were washed with water (100mL x 2), dried (Na₂SO₄) and concentrated. Flash chromatography with 15%

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EtOAc-hexanes gave 3.58 g (94%) of the title compound as a clear liquid.

$[\alpha]_D^{23} = -48.2^{\circ}$ (CHCl_3 , $c = 0.85$); IR (neat) 3030, 2867, 1645, 1613, 1513 1247, 1092, 821, 736 cm^{-1} ; $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.34-7.23(12H, m), 4.62 (1H, d, $J = 12.0$ Hz), 4.59-4.51 (2H, ABq, $J = 11.7$ Hz), 4.50-4.39 (2H, ABq, $J = 11.9$ Hz) $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 159.04, 143.99, 138.30, 137.90, 130.43, 128.26, 128.20, 128.03, 127.77, 127.57, 127.56, 113.67, 100.00, 75.58, 73.28, 73.17, 71.13, 70.42, 70.28, 68.35, 55.15; LRMS (NH_3) 464 ($\text{M} + \text{NH}_4^+$, 100), 326 (18), 309 (48), 253 (17).

Synthesis of 4b:

A solution of galactal 3b (3.20g, 7.17 mmol) in dry CH_2Cl_2 under N_2 at 0°C was treated with dimethyldioxirane (0.09M, 80 mL) and stirred until all of the glycol was consumed (0.5-1h; TLC 30% EtOAc in hexanes). Volatiles were removed at 0°C with stream of dry N_2 . The residue was dissolved in 30 mL of dry THF under N_2 at 0°C and treated TBAF (36 mL, stored over molecular sieves) then stirred at ambient temperature for 20h. The dark brown solution was filtered through a pad of silica (~4cm depth) and washed with EtOAc (200 mL). The filtrate was washed with water (200 mL x 3) and dried (MgSO_4) and concentrated. The residue was redissolved in 30% EtOAc-hexanes (50 mL) and filtered through short silica column (10 cm d x 4 cm h) and washed with the same solvent system (1L). The filtrate was concentrated to give 2.59g of fluorohydrin with >90% purity. The residue was dissolved in dry DMF (30 mL) under N_2 at 0°C and treated with benzyl bromide (958 μL , 1.5 equiv, freshly filtered through basic alumina), finally with NaH (322 mg, 60% dispersion, 1.5 equiv) and stirred for 30 min at 0°C and 30 min at rt. The reaction was quenched by pouring into 100g of ice, and extracted with 1:1 EtOAc-hexanes (150 mL x 2). The organic extracts were washed with water (150 mL x 2),

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dried (MgSO₄) and concentrated in vacuo. Flash chromatography with 10% EtOAc-hexanes gave 2.00g (49%) of the title compound as a yellowish liquid.

5 $[\alpha]_D^{23} = +15.3^0$ (CHCl₃, c = 0.85); IR (CHCl₃ film) 2916, 1612, 1513, 1248, 1103, 1056, 734 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.35-7.24 (17H, m), 6.84 (2H, d, J = 8.4 Hz), 5.15 (1H, dd, J = 53.2, 7.0 Hz), 4.92 (1H, d, J = 11.6 Hz), 4.48-4.74 (2H, ABq, J = 11.8 Hz), 3.96-3.89 (1H, m),
10 3.86 (1H, br s), (3H, s), 3.65-3.56 (3H, m), 3.51 (1H, dd, J = 9.8, 2.8 Hz); ¹³C-NMR (100 MHz, CDCl₃) δ 159.22, 138.33, 138.11, 137.62, 130.16, 129.19, 128.40, 128.29, 128.21, 128.04 (2C), 127.90, 127.81, 127.69, 127.59, 113.77, 110.20 (d, J = 214 Hz), 80.60 (d, J = 11.3 Hz),
15 79.00 (d, J = 20.5 Hz), 74.92, 74.52, 73.59 (d, J = 5.0 Hz), 73.54, 72.99, 72.70, 68.34, 55.20; LRMS (NH₃) 454 (M + NH₄⁺, 100).

Synthesis of 6b:

20 A solution of TIPS-carbonate galactal 5b (Danishefsky, S.J.; Behar, V.; Randolph, J.T.; Lloyd, K., *J. Am. Chem. Soc.*, 1995, 0000) (4.28g, 5.62 mmol) in THF (25mL)-MeOH (5 mL) was treated with TBAF solution (1.0M, 6.75 mL, 1.2 equiv). After 6h, additional TBAF (4mL) was added and
25 stirred additional 3h. The reaction was concentrated and directly chromatographed with 4:1 EtOAc-hexanes to obtain 2.20g of the triol. Remaining mixtures of cyclic carbonate and mixed carbonate was hydrolysed in MeOH with MeONa (1.0 mL, 25 wt%) and purified chromatographically.
30 Total yield was 3.02g (93%). This material was directly used for the dibenylation step.

¹H-NMR (400 MHz, CDCl₃) δ 7.35-7.24 (15H, m), 6.43 (1H, d, J = 6.3 Hz), 4.87 (1H, dd, J = 6.3, 3.4 Hz), 4.84 (1H, d, J = 11.4 Hz), 4.63 (2H, apparent s), 4.61 (1H, d, J = 11.4 Hz), 4.53-4.47 (3H, m), 4.19-4.16 (3H, m), 3.87-3.84 (2H, m), 3.78-3.66 (3H, m), 3.46 (2H, apparent d, J = 4.6

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Hz), 3.29 (1H, t, J = 5.5 Hz), 3.08 (1H, br), 2.73 (2H, br); ¹³C-NMR (100 MHz, CDCl₃) δ 144.70, 138.41, 138.22, 137.83, 128.45, 128.33 (2C), 128.12, 127.84, 127.73, 127.64, 127.57, 102.28, 99.74, 78.99, 76.03, 74.64, 74.07, 73.24 (2C), 73.17, 72.64, 70.20, 69.10, 67.79, 62.15.

A mixture of triol glycal from above (2.95g, 5.1 mmol), dibutyltin oxide (1.33g, 1.05 equiv) and bistrabutyltin oxide (1.69 mL, 0.65 equiv) in dry benzene (50 mL) under N₂ was refluxed for 5h with azeotropic removal of water. The reaction was cooled below boiling and treated with benzyl bromide (2.43 mL, 4.0 mol equiv) and tetrabutylammonium bromide (3.29g, 2.0 equiv). 10 mL of benzene was distilled off and the reaction refluxed for 16h. The reaction was directly loaded on silica column and eluted with 15-20% EtOAc-hexanes to give 3.48g (90%) of product **6b** as a clear oil.

[α]_D²³ = -3.3⁰ (CHCl₃, c = 0.87); IR (CHCl₃ film) 2867, 1652 1454, 1364, 1097, 736 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.35-7.21 (25H, m), 6.45 (1H, d, J = 6.2 Hz), 4.88 (1H, dd, J = 6.2, 3.9Hz), 4.83 (1H, d, J = 10.9 Hz), 4.69 (2H, apparent s), 4.68 (1H, d, J=10.9Hz), 4.59 (2H, apparent s), 4.55 (1H, d, J = 7.8 Hz), 4.49 (2H, apparent s), 4.47 (2H, apparent s), 4.29 (1H, dd, J = 9.6, 5.8 Hz), 4.18 (1H, t, J = 4.4 Hz), 4.13 (1H, m), 3.99 (1H, br s), 3.85 (1H, dd, J = 10.6, 6.4 Hz), 3.75-3.60 (4H, m), 3.47-3.41 (2H, m); ¹³C-NMR (100 MHz, CDCl₃) δ 144.43, 138.64, 138.42, 137.99, 137.84, 137.80, 128.40, 128.34, 128.26, 128.23, 128.18, 128.15, 127.82, 127.75, 127.69, 127.67, 127.65, 127.55, 127.51, 127.46, 127.31102.56, 99.56, 80.57, 78.69, 75.72, 75.10, 73.57, 73.32, 72.94, 72.28, 71.94, 70.12, 68.90, 67.85, 66.62; LRMS (NH₃) 776 (M + NH₄⁺, 100).

Synthesis of 7b:

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Lactal **6b** (1.32g, 1.74 mmol, 1.0 equiv) and fluoro sugar **4b** (1.49g, 2.60 mmol, 1.5 equiv) were combined in ether and concentrated. The mixture were dried by evaporation in dry benzene (25 mL x 2), in vacuum for 2h then treated with di-*t*-butylpyridine (389 μ L, 1.0 equiv) in glove bag and dissolved in dry ether (18 mL) under nitrogen atmosphere. In a separate 50 mL flask was placed 4A M.S. (4.0g) then flame-dried under vacuum, cooled to room temperature. Anhydrous silver perchlorate (360 mg, 1.0 equiv) and SnCl₂ (329 mg, 1.0 equiv) were added in glove bag and flushed with nitrogen. The salt mixture was placed in water bath and sugar solution was introduced via double tipped needle and the mixture sonicated for 2 min. The reaction was wrapped with aluminum foil and stirred for 45h at rt. The filtrate (200 mL) was washed with dil NaHCO₃ (100 mL x 2), dried (MgSO₄) and concentrated. Flash chromatography with 15-20 % EtOAc/hexanes yielded trisaccharides (1.107g, 49%) and impure lactal. The trisaccharide portion was rechromatographed with 2% ether-methylene chloride to give 879 mg (39%) of the desired α -product and 195 mg (8.6%) of β -product. The impure lactal fraction was rechromatographed with 3-4% ether-methylene chloride to give 479 mg (36%) of clean lactal. 77% of coupling (61% α -product) yield based on recovered starting material.

$[\alpha]_D^{23} = +41.8^0$ (CHCl₃, c = 1.8); IR (CHCl₃ film) 2867, 1648, 1513, 1496, 1453, 1364, 1248, 1097, 735 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.33-7.12 (42H, m) 6.83 (2H, d, J = 8.4 Hz), 6.45 (1H, d, J = 6.0 Hz), 5.03 (1H, d, J = 2.3 Hz), 4.91-4.76 (6H, m), 4.68-4.40 (12H, m), 4.23-3.97 (11H, m), 3.86-3.82 (1H, dd, J = 2.3 Hz), 3.76 (3H, s), 3.69-3.64 (2H, m), 3.53 (1H, t, J = 8.7 Hz), 3.47-3.43 (1H, m), 3.40-3.36 (1H, m), 3.34-3.31 (1H, dd, J = 9.9, 2.8 Hz), 3.22 (1H, dd, J = 8.3, 4.8 Hz); ¹³C-NMR (100 MHz, CDCl₃) δ 158.93, 144.59, 138.98, 138.84, 138.78, 138.64, 138.58, 138.06, 138.02 (2C), 130.82, 129.04, 128.33,

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128.24, 128.21, 128.15, 128.08, 128.05, 127.83, 127.81,
127.72, 127.64, 127.58, 127.55, 127.50, 127.44, 127.41,
127.36, 127.33, 127.31, 113.65, 103.02, 100.39, 100.01,
80.93, 78.93, 78.70, 76.53, 76.11, 75.14, 74.84, 74.79,
5 74.35, 73.91, 73.59, 73.36, 73.15, 73.10, 72.98, 72.15,
72.10, 71.99, 70.55, 69.25, 67.92 (2C), 67.69, 55.19.

Synthesis of 8b:

A solution of PMB-trisaccharide (37 mg, 0.028 mmol) in
10 CH_2Cl_2 (1 mL) at 0°C . The reaction was directly loaded on
silica column and eluted with 20% EtOAc-hexanes to give
28 mg (84%) of desired product.

$[\alpha]^{23}_{\text{D}} = +45.6^\circ$ (CHCl_3 , $c = 1.78$); IR (CHCl_3 film) 2866,
15 1648, 1496, 1453, 1248, 1097, 735 cm^{-1} ; $^1\text{H-NMR}$ (400 MHz,
 CDCl_3) δ 7.36-7.15 (40H, M), 6.43 (1H, d, $J = 6.2$ Hz),
5.09 (1H, d, $J = 3.3$ Hz), 4.85 (1H, dd, $J = 6.2, 3.6$ Hz),
4.83-4.65 (5H, m), 4.61-4.41 (9H, m), 4.29-4.08 (8H, m),
4.02 (1H, d, $J = 2.6$ Hz), 3.97 (1H, d, $J = 2.2$ Hz), 3.93
20 (1H, t, $J = 8.4$ Hz), 3.86-3.78 (2H, m), 3.67-3.61 (2H,
m), 3.53 (1H, dd, $J = 8.5, 4.8$ Hz); $^{13}\text{C-NMR}$ (100 MHz,
 CDCl_3) δ 144.38, 138.78, 138.62, 138.47, (2C), 138.20,
138.00, 137.88, (2C, 128.31, 128.29, 128.23, 128.19,
128.16, 128.05, 127.88, 127.83, 127.62, 127.57, 127.49,
25 127.45, 127.43, 127.41, 127.37, 127.32, 127.23, 102.68,
99.89, 99.34, 80.82, 78.72, 77.49, 77.10, 75.88, 75.13,
75.03, 74.23, 73.62, 73.05, 73.01, (3C), 72.62, 72.19
(2C), 70.46, 69.66, 68.92, 67.85, 67.74, 67.54.

Synthesis of 11b:

Glycol **9b** (4.32 g, 3.14 mmol) was dissolved in CH_2Cl_2 (20
ml) and cooled to 0°C . It was then treated with
dimethyldioxarane (219 ml, 3.14 mmol) at 0°C . The
epoxidation finished within 1 h and then the reaction
35 mixture was concentrated to dryness using dry N_2 stream.
The residue was further azeotroped once with benzene (20
ml) and put on a vacuum line for 30 min at 0°C before

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being dissolved in THF (60 ml) and cooled to -78°C . Into the above solution was added, via canula, azeotropically dried galactal **10b** (3.32 g, 10.95 mmol, 20 ml THF) and followed by ZnCl_2 (26.3 ml, 1.0 M in ether). The reaction mixture was warmed up to room temperature and stirred overnight. After treatment with sat'd aq. Na_2CO_3 (40 ml), the reaction mixture was concentrated and extracted with ether (500 ml). The organic phase was washed with sat'd aq. NaCl , dried (MgSO_4) and concentrated. The crude product was purified by silica gel chromatography (1:4 EtOAc-hexanes) to give 6.20 g of **11b** as a white foam (87.4%).

IR (CHCl_3 film) ν_{max} cm^{-1} ; $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 6.45 (1H, dd, $J = 6.4, 1.6$ Hz), 4.85 (1H, dd, $J = 6.4, 2.0$ Hz), 4.72-4.68 (2H, m), 4.65 (1H, d, $J = 7.2$ Hz), 4.55 (1H, m), 4.21 (1H, m), 4.08 (1H, dd, $J = 9.6, 5.6$ Hz), 3.96-3.82 (6H, m), 3.33 (1H, d, $J = 3.2$ Hz, OH), 3.27 (1H, d, $J = 2.8$ Hz, OH), 1.16-1.04 (42H, m); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 154.45, 145.75, 99.14, 98.27, 77.83, 76.59, 74.27, 72.04, 71.62, 70.86, 64.52, 62.57, 61.60, 17.84, 11.78, 11.77. ; LRMS (NH_3) 664 ($\text{M} + \text{NH}_4^+$, 100), 647 ($\text{M} + 1^+$, 5), 422 (21), 380 (25).

25 **Synthesis of 13b:**

Disaccharide **11b** (2.64 g, 4.08 mmol) was azeotropically dried three times (3 x 10 ml) together with fluoro-fucose **12b** (1.64 g, 3.77 mmol) and molecular sieves (4 A, 4.0 g) in THF (20 ml) with 2,6-di-tert-butypyridine. The solution was added via canula to a flask containing AgClO_4 (1.56 g, 7.54 mmol), SnCl_2 (1.43 g, 7.54 mmol) and molecular sieves (4 A, 4.0 g) in THF (15 ml) at -40°C . The reaction mixture was stirred 30 min at -40°C and then 34 h at 5°C until the disappearance of fluoro-fucose. After treatment with sat'd aq. NaHCO_3 (40 ml) at 5°C , the mixture was extracted with EtOAc (700 ml). The organic phase was washed with sat'd NaCl , dried (MgSO_4) and

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concentrated. The crude product was purified by silica gel chromatography to give 1.93 g of the desired trisaccharide glycal 13b (48%, based on fluoro-fucose used) and 500 mg of the recovered disaccharide with only a trace of the other monofucosyl product.

Synthesis of 15b:

An azeotropically dried mixture of the trisaccharide glycal 13b (1.11 g, 1.05 mmol) and benzenesulphonamide (0.82 g, 5.24 mmol) was dissolved in the THF (20 ml) together with molecular sieves (4 A, 2.6 g). The mixture was cooled to -40°C and then was added, via canula, a solution of $\text{I}(\text{sym-coll})_2\text{COI}_4$ prepared *in situ* by stirring I_2 (0.54 g, 2.09 mmol) with $\text{Ag}(\text{sym-coll})_2\text{COI}_4$ (0.986 g, 2.29 mmol) in THF (20 ml) at room temperature for about 30 min until the disappearance of the brown color of I_2 . The mixture was warmed up to 0°C within 1 h and stirred for another 1 h. After quenching with sat'd aq. $\text{Na}_2\text{S}_2\text{O}_3$, the mixture was filtrate and extracted with EtOAc (3 x 100 ml). The combined organic phase was washed with sat'd aq. CuSO_4 (100 ml), sat'd NaCl (100 ml x 2) and dried (Na_2SO_4). After concentration, the crude product was purified by silica gel chromatography (1:4 EtOAc-hexanes) to give 981 mg of a colorless oil as a 21 mixture of the desired α -trans-diaxial iodosulphonamide and its *cis* isomer. The iodosulphonamide mixture was then added with stirring into a flask containing ethanthiol (226.3 mg, 3.64 mmol) and lithium hexamethydisilylazide (1.46 ml, 1.46 mmol) in DMF (10 ml) at -40°C . The reaction mixture was stirred at -40°C overnight, and then quenched with sat'd aq. NaHCO_3 and extracted with ether (3 x 100 ml). The combined organic phase was washed with sat'd aq. NaCl and dried (Na_2SO_4). After concentration, the crude product was purified by silica gel chromatography (3:97 EtOAc- CHCl_2) to yield 438 mg of 15b (33%) and 333 mg of the intact *cis* iodosulphonamide.

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Synthesis of 16b:

A mixture of acceptor trisaccharide **8b** (92 mg, 0.077 mmol, 1.0 equiv), thioglycoside **15b** (198 mg, 2.0 equiv) and freshly activated 4Å-MS (560 mg) under N₂ at rt was suspended in CH₂Cl₂-Et₂O (1:2, 3.9 mL) and stirred for 10 min. The reaction was cooled to 0°C, then treated with methyl triflate (52.4 uL, 6.0 equiv). The reaction was stirred for 4.5h at 0°C and 1.5h while warming to 15°C. The reaction was quenched with TEA(1.0 mL), filtered through a pad of silica and rinsed with Et₂O. The filtrate (70 mL) was washed with sat'd NaHCO₃ (50 mL x 2), dried (Na₂SO₄) and concentrated. The crude product was purified by HPLC (17% EtOAc in hexanes, 15 mL/min, 260 nm UV dection) to give 158 mg (85%) of the desired product and 27.7 mg of α-linked byproduct (ca 55% purity).

Retention time=22 min; $[\alpha]^{23}_D = -13.3^0$ (CHCl₃, c = 1.4); IR (CHCl₃ film) 2940, 2865, 1792, 1652, 1454, 1161, 1101, 734 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.8 (2H, m), 7.38-7.06 (58H, m), 6.43 (1H, d, J = 6.1 Hz), 5.15 (1H, br s), 5.07 (1H, d, J = 3.6 Hz), 5.03 (1H, d, J = 3.6 Hz), 4.99 (1H, d, J = 11.6 Hz), 4.89-4.61 (12H, m), 4.54-4.46 (4H, m), 4.42 (2H, app s), 4.38 (1H, d, J = 11.9 Hz), 4.34-4.26 (3H, m), 4.21-4.18 (4H, m), 4.13-4.03 (7H, m), 3.98-3.76 (14H, m), 3.70-3.61 (4H, m), 3.46-3.27 (7H, m), 2.84 (1H, OH), 1.16 (3H, d, J = 6.4 Hz), 1.13-1.02 (42H, m); ¹³C-NMR (100 MHz, CDCl₃) δ 155.35, 144.55, 140.78, 138.99, 138.75, 138.68, xxx, 138.54, 138.43, 138.13, 138.03, 137.94, 137.82, 132.31, 128.81, 128.52, xxx, 128.38, 128.36, 128.27, 128.24, 128.20, 128.16, 128.02, 127.93, 127.72, 127.66, 127.58, 127.48, 127.43, 127.37, 127.20, 103.41, 102.75, 99.79, 99.55, 98.29, 97.76, 80.49, 80.39, 79.09, 78.91, 78.25, 77.68, xxx, 76.51, 75.88, 75.09, 74.99, 74.91, 74.73, 74.15, 74.02, 73.92, 73.52, 73.19, 73.10, 72.94, 72.67, 72.25, 72.07, 71.76, 71.56, 71.33, 70.33, 69.45, 69.32, 68.48, 68.08, 67.86, 67.75, 61.97, 61.60, 56.14, 17.99, 17.96, 17.95, 17.92, 16.75, 11.86; HRMS

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(FAB) calcd for $C_{138}H_{169}NO_{30}SSi_2Na$ (M + Na) 2432.0920, found 2432.0970.

Synthesis of 19b:

5 A solution of hexasaccharide glycal **16b** (85 mg, 0.035 mmol) in THF (6mL) under N_2 at rt was treated with TBAF (1.0 M, 353 uL, 10 equiv). After 38h at rt, the reaction was concentrated to ca 1 mL, then dissolved in EtOAc (60 mL), washed with water (30 mL x 2), dried (Na_2SO_4) and
10 concentrated. Flash chromatography with 4% MeOH in CH_2Cl_2 gave 70.0 mg (98%) of the desilyl-decarbonated product.

$[\alpha]_D^{23} = 1.8^0$ ($CHCl_3$ film) 2868, 1652, 1455, 1157, 1094, 735 cm^{-1} ; 1H -NMR (400 MHz, $CDCl_3$) δ 7.80 (2H, d, J = 7.4 Hz), 7.47 (2H, d, J = 7.2 Hz), 7.37-6.95 (56H, m), 6.45 (1H, d, J = 6.3 Hz), 5.86 (1H, br s), 5.35 (1H, d, J = 11.6Hz), 5.30 (1H, D, J = 2.8 Hz), 4.95 (1H, d, J = 11.3 Hz), 4.89 (1H, d, J = 3.5 Hz), 4.8644.67 (9H, m), 4.54-4.39 (9H, m), 4.34 (1H, dd, J = 10.4, 2.8 Hz), 4.26-4.06 (9H, m), 3.98-3.45 (23H, m), 3.41 (1H, d, J = 10.0 Hz), 3.29-3.20 (5H, m), 0.73 (3H, d, J = 6.3 Hz); ^{13}C -NMR (100 MHz, $CDCl_3$) δ 144.87, 142.49, 139.49, 139.11, 138.87, 138.63, 138.54, 138.37, 138.00, 137.98, 137.97, 137.18, 131.64, 128.74, 128.52, 128.43, 128.33, 128.28, 128.25, 25 128.21, 128.02, 127.99, 127.97, 127.80, 127.074, 127.67, 127.63, 127.61, 127.54, 127.53, 127.50, 127.44, 127.33, 127.31, 127.02, 126.86, 103.39, 102.78, 100.75, 100.09, 99.80, 99.75, 81.42, 80.64, 78.98, 78.86, 77.82, 77.40, 77.26, 76.26, 75.16, 75.09, 75.07, 74.95, 74.69, 74.30, 30 73.58, 73.17, 73.11, 72.71, 72.67, 72.65, 72.55, 72.36, 72.18, 69.65, 69.53, 68.54, 68.18, 68.08, 67.85, 67.79, 67.21, 54.95, 16.60.

To liquid ammonia (ca 8 mL) under N_2 at -78^0C was added
35 metallic sodium (95 mg) and stirred for 2 min. To the blue solution was added a solution of the hexasaccharide glycal above (70 gm, 33.8 umol) in dry THF(2mL). After

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45 min at 78⁰C, the reaction was quenched with absolute methanol (4 mL). Most of ammonia was removed with stream of nitrogen (final volume was ca 4 mL) and the reaction diluted with methanol to ca 10 mL. To the solution was added Dowex 50-X8 (890 mg, washed and dried) and stirred for 5 min. The solution was filterate and rinsed with methanol, finally with ammoniacal methanol (5 mL), and the filterate was concentrated in vacuo. The residue and DMAP (2.4 mg) were placed under N₂ and suspended in DMF (1.0 mL), THF (1.0 mL) and TEA (1.0 mL), then treated with Ac₂O (0.3 mL). After 20h (TLC analysis with EtOAc), the reaction was poured into water (40 mL), and extracted with EtOAc (40 mL x 2), washed with dil NaHCO₃ (30 mL), with water (30 mL), dried (Na₂SO₄) and concentrated. Flash chromatography with 80% EtOAc in CH₂Cl₂ gave 52.0 mg (93%) of product as white foam.

mp 132-134⁰C; $[\alpha]_D^{23} = +4.7^0$ (CHCl₃, c = 1.4); IR (CHCl₃ film) 1742, 1652, 1371, 1227, 1069 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 6.68 (1H, d, J = 6.8 Hz), 6.42 (1H, d, J = 6.0 Hz), 5.58 (1H, d, J = 3.2 Hz), 5.47 (1H, d, J = 3.4 Hz), 5.40-5.37 (2H, m), 5.29 (1h, dd, J = 10.9, 3.1 Hz), 5.25-5.15 (5H, m) 5.06 (1H, dd, J = 11.2, 3.3 Hz), 5.02 (1H, d, J = 3.6 Hz), 4.99-4.92 (2H, m), 4.84-4.81 (2H, m), 4.67 (1H, d, J = 7.8 Hz), 4.56-4.51 (2H, m), 4.45-4.38 (3H,m), 4.29 (1H, dd, J = 10.6, 3.4 Hz), 4.22-3.95 (13H, m), 3.90-3.77 (3H, m), 2.19-1.92 (51H, m), 1.15 (3H, d, J = 6.4 Hz); ¹³C-NMR (100 MHz, CDCl₃) δ 172.40, 171.45, 170.84, 170.54, 170.52, 170.48, 170.45, 170.40, 170.39, 170.34, 170.23, 169.99, 169.82, 169.74, 169.36, 169.00, 145.43, 102.01, 101.17, 98.83, (2C), 98.45, 94.24, 75.65, 74.95, 73.98, 73.64, 73.49, 72.32, 71.84, 71.53, 71.44, 70.81, 70.74, 70.66, 70.12, 69.77, 68.97, 68.71, 68.67, 68.02, 67.97, 67.88, 67.60, 67.35, 64.43, 61.88, 61.81, 61.42, 61.29, 61.04, 56.18, 23.06, 21.02, 20.81, 20.76, 20.68, 20.64, 20.62, 20.58, 20.57, 20.55, 20.49, 20.43, 15.88.

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Peracetyl hexasaccharide glycal above (52 mg) was divided into two portions (22 mg and 30 mg). A solution of hexasaccharide glycal (22.0 mg, 13.4 μmol) in dry CH_2Cl_2 (2 mL) under N_2 at 0°C , then treated with allyl alcohol (5 mL). The mixture was stirred for 15h at room temperature. Excess allyl alcohol was removed in vacuo. The other batch (30 mg) was treated similarly. The crude products were combined and chromatographed with 85% EtOAc- CH_2Cl_2 to give 35.8 mg (66%) of less polar product and 15.7 mg (29%) of more polar product. A 33.2 mg (19 μmol) of the less polar material under N_2 was dissolved in absolute MeOH (14 mL) and treated with MeONa solution in methanol (165 μL , 25% by weight). After 6h, the reaction was neutralized with Dowex 50-X8 (200 mg, washed and dried), filtered and concentrated to give quantitative yield of the title compound 19b.

mp $204-206^\circ(\text{dec})$; $[\alpha]_D^{23} = +5.5^\circ$ (MeOH, $c = 0.67$); IR (MeOH film) 3356 (br), 2923, 1658, 1374, 1071 cm^{-1} ; $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ 5.99-5.93 (1H, m), 5.24 (1H, d, $J = 3.8$ Hz), 5.18-5.14 (1H, m), 4.93 (1H, d, $J = 3.9$ Hz), 4.56-4.54 (2H, m), 4.42-4.06 (10H, m), 3.99 (1H, s), 3.91-3.47 (26H, m), 3.41-3.37 (1H, m), 3.27 (1H, t, $J = 8.8$ Hz), 2.01 (3H, s), 1.24 (3H, d, $J = 6.5$ Hz); $^{13}\text{C-NMR}$ (100 MHz, CD_3OD , ref = $\delta 49.05$) δ 174.55, 135.73, 117.57, 105.48, 105.42, 103.94, 103.26, 102.79, 101.08, 81.21, 80.67, 80.05, 79.20, 78.09, 76.79, 76.56, 76.48, 76.44, 76.41, 75.54, 74.86, 74.68, 73.57, 72.63, 72.50, 71.57, 71.16, 70.64, 70.41, 69.68, 68.16, 62.67, 62.64, 62.57, 61.96, 61.63, 53.11, 23.58, 16.78.

For the purposes of the preparative synthesis of structure 1b a ceramide precursor was attached to the ABC trisaccharide (Scheme 5). Epoxidation of 7b, followed by reaction with the ceramide precursor 17b (as its tributylstannyl ether) promoted by $\text{Zn}(\text{OTf})_2$ provided 20b. Acetylation and PMB removal proceeded smoothly to furnish

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21b which is poised for coupling with a suitable DEF trisaccharide donor.

5 When trisaccharide 15b was treated with MeOTf in the presence of acceptor 21b, a 4:1 mixture of hexasaccharide isomers was obtained. The major product 22b was obtained in 50% yield.

10 The ceramide side-chain was elaborated by reduction of the azide functionality using Lindlar's catalyst under an atmosphere H₂ in the presence of palmitic anhydride to provide 18b directly. Desilylation was followed by dissolving metal deprotection of the sulfonamide and benzyl groups and MeOH quench to remove the carbonate and
15 acetate groups. Peracetylation of the crude mixture afforded a 78% yield of peracetylated hexasaccharide. Saponification of this material using NaOMe provided the natural product 1b in 96% yield. The coupling constants and chemical shifts of the anomeric protons of 1b matched
20 reported data. In addition, the product was characterized by exact mass, and ¹H and ¹³C NMR.

Synthesis of 20b:

25 The benzylated ceramide precursor (475 mg, 1.14 mmol) was dissolved in 4 mL PhH. Bis(tribuyltin) ether (0.29 mL, 0.34 g, 0.57 mmol) was added and the reaction vessel (equipped with a Dean-Stark trap) was heated to reflux. After 3 h the reaction was allowed to cool and was concentrated under a flow of N₂. In a separate flask, the
30 glycal 7b was dissolved in 1 mL anhydrous CH₂Cl₂ and the resulting solution was cooled to 0°C and a solution of 3,3-dimethyldioxirane (2.8 mL, 0.25 mmol, 0.09 M in acetone) was added. After 45 min the solution was concentrated under a flow of N₂, then under vacuum. The
35 tin ether was dissolved in 1 mL anhydrous THF and added via cannula to a mixture of Zn(OTf)₂ (170 mg, 0.468 mmol) in 1 mL THF at -78°C (wash 1 x 0.5 mL THF). The reaction

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was allowed to warm to room temperature over 12 h and then was quenched with distilled water. The aqueous phase was extracted 3 x with EtOAc. The combined organic phases were dried over anhydrous MgSO₄. Flash column chromatography (3:1 hexane/EtOAc, 3 x 16 cm silica gel) afforded 265 mg (66%) of the target compound 20b.

¹H NMR (CDCl₃) δ 7.43-7.15 (m, 45H), 7.03 (d, J= 8.6 Hz, 2H), 6.79 (d, J= 8.6 Hz, 2H), 5.76 (dt, J= 6.7, 15.4 Hz, 1H), 5.43 (dd, J= 8.5, 15.4 Hz, 1H), 5.07 (d, J= 3.5 Hz, 1H), 5.05 (d, J= 12.0 Hz, 1H), 4.90 (d, J= 12.9 Hz, 2H), 4.83-4.77 (m, 3H), 4.69 (d, J= 12.0 Hz, 1H), 4.61 (d, J= 11.9 Hz, 1H), 4.54-4.45 (m, 3H), 4.42-4.25 (m, 7H), 4.18-4.05 (m, 6H), 4.01-3.91 (m, 4H), 3.83 (dd, J= 4.4, 10.6 Hz, 1H), 3.79 (s, 3H), 3.71-3.65 (m, 4H), 3.57-3.32 (m, 7H), 3.20 (m, 1H), 2.29 (bs, 1H), 2.11 (bq, J= 6.7 Hz, 2H), 1.42-1.29 (m, 22H), 0.91 (t, J= 6.6 Hz, 3H); ¹³C NMR (CDCl₃) δ 158.8, 139.1, 139.0, 138.7, 138.6, 138.34, 138.29, 138.2, 138.1, 130.8, 128.7, 128.55, 128.50, 128.4, 128.33, 128.28, 128.26, 128.12, 128.06, 127.84, 127.76, 127.7, 127.64, 127.60, 127.5, 127.45, 127.36, 125.8, 113.5, 102.7, 100.6, 81.9, 81.5, 79.4, 77.4, 77.0, 76.7, 76.6, 76.4, 75.5, 74.9, 74.7, 74.4, 73.9, 73.3, 73.2, 73.11, 73.06, 72.3, 72.1, 70.0, 69.4, 68.7, 68.1, 67.9, 67.7, 64.2, 55.2, 32.4, 31.9, 29.70, 29.65, 29.5, 29.4, 29.2, 29.0, 22.7, 14.2; IR (thin film) 3447, 3062, 3029, 2923, 2853, 2099, 1612, 1586, 1514, 1496, 1454, 1364 cm⁻¹; [α]_D²³ +25.0 (c 0.70).

30 **Synthesis of 21b:**

The above trisaccharide (256 mg, 0.147 mmol) was dissolved in 2 mL anhydrous CH₂Cl₂. Triethylamine (0.105 mL, 76 mg, 0.753 mmol), DMAP (2 mg, 0.02 mmol) and acetic anhydride (0.042 mL, 45 mg, 0.445 mmol) were added sequentially. The reaction was stirred for 1 h then quenched with saturated aqueous NaHCO₃. The extracts were

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dried with anhydrous MgSO_4 , filtered and concentrated. Purification by flash column chromatography (4:1 hexane/EtOAc, 2 x 16 cm silica gel) afforded 235 mg (90%) of the desired compound.

5

^1H NMR (CDCl_3) δ 7.42-7.17 (m, 45H), 7.03 (d, $J=8.6$ Hz, 2H), 6.81 (d, $J=8.6$ Hz, 2H), 5.75 (dt, $J=6.7, 15.4$ Hz, 1H), 5.43 (dd, $J=8.6, 15.4$ Hz, 1H), 5.07 (d, $J=3.4$, 1H), 4.99-4.90 (m, 4H), 4.85 (d, $J=11.3$ Hz, 2H), 4.77 (d, $J=11.9$ Hz, 1H), 4.76 (d, $J=12.4$ Hz, 1H), 4.70 (d, $J=12.0$ Hz, 1H), 4.62 (d, $J=11.7$ Hz, 1H), 4.57-4.52 (m, 3H), 4.49-4.34 (m, 7H), 4.30 (d, $J=11.8$ Hz, 1H), 4.25 (d, $J=11.8$ Hz, 1H), 4.14-4.06 (m, 7H), 4.01-3.95 (m, 2H), 3.91 (dd, $J=5.6, 8.6$ Hz, 1H), 3.85 (dd, $J=4.3, 11.1$, Hz, 1H), 3.80 (s, 3H), 3.74 (d, $J=9.8$ Hz, 1H), 3.69 (dd, 7.7, 9.9 Hz, 1H), 3.63-3.51 (m, 5H), 3.43-3.34 (m, 3H), 3.22 (dd, $J=4.6, 8.2$ Hz, 1H), 2.12 (dd, $J=6.8, 13.6$, 2H), 1.87 (s, 3H), 1.43-1.30 (m, 22H), 0.93, (t, $J=6.6$ Hz, 3H); ^{13}C NMR (CDCl_3) δ 169.3, 158.8, 139.1, 139.0, 138.69, 138.65, 138.6, 138.31, 138.26, 138.2, 138.1, 138.0, 130.8, 128.8, 128.6, 128.41, 128.35, 128.30, 128.28, 128.14, 128.0, 127.9, 127.8, 127.64, 127.60, 127.58, 127.51, 127.47, 127.38, 126.0, 113.5, 102.7, 100.8, 100.6, 81.5, 79.9, 79.5, 79.4, 79.3, 77.4, 77.1, 76.8, 75.5, 75.3, 74.9, 74.5, 74.2, 73.9, 73.2, 73.1, 73.0, 72.4, 72.2, 72.1, 70.2, 69.4, 68.1, 68.0, 67.9, 67.5, 63.8, 55.2, 32.4, 32.0, 29.72, 29.67, 29.5, 29.4, 29.2, 29.1, 22.7, 20.9, 14.2; IR (thin film) 3028, 2923, 2852, 2098, 1751, 1611, 1513, 1496, 1453, 1365, 1232 cm^{-1} ; $[\alpha]_D^{23} +20.3$ (c 0.45).

35

The trisaccharide from above (230 mg, 0.129 mmol) was dissolved in 4 mL CH_2Cl_2 . Distilled water (1 mL) was added and the mixture was cooled to 0°C . DDQ (35 mg, 0.15 mmol) was added and the reaction was stirred for 1 h. The reaction was quenched with saturated aqueous NaHCO_3 . The aqueous phase was extracted 3 x with CH_2Cl_2 . The

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combined organic phases were washed dried over anhydrous MgSO_4 . Flash column chromatography (4:1 hexane/EtOAc, 2 x 16 cm silica) afforded 182 mg (85%) of the target compound **21b**.

5

^1H NMR (CDCl_3) δ 7.38-7.13 (m, 45H), 5.73 (dt, $J=6.7$, 15.4 Hz, 1H), 5.41 (dd, $J=8.6$, 15.4 Hz, 1H), 5.09 (d, $J=3.2$ Hz, 1H), 4.98 (d, $J=12.5$ Hz, 1H), 4.95 (dd, $J=8.0$, 9.2 Hz, 1H), 4.87 (d, $J=11.2$ Hz, 1H), 4.80 (d, $J=11.3$ Hz, 1H), 4.77 (d, $J=10.9$ Hz, 1H), 4.70 (d, $J=11.4$ Hz, 1H), 4.65-4.50 (m, 6H), 4.45-4.42 (m, 3H), 4.38-4.34 (m, 3H), 4.28 (bs, 2H), 4.15 (d, $J=11.7$ Hz, 1H), 4.11 (d, $J=11.8$ Hz, 1H), 4.08-4.01 (m, 3H), 3.98-3.94 (m, 3H), 3.88 (dd, $J=5.5$, 8.5 Hz, 1H), 3.82 (dd, $J=4.3$, 7.0 Hz, 1H), 3.77 (dd, $J=3.1$, 10.1 Hz, 1H), 3.70 (d, $J=9.8$ Hz, 1H), 3.64-3.51 (m, 5H), 3.46 (dd, $J=5.4$, 9.4 Hz, 1H), 3.39 (m, 1H), 3.34-3.30 (m, 2H), 3.21 (dd, $J=4.7$, 8.4 Hz, 1H), 2.09 (m, 2H), 1.90 (s, 3H), 1.84 (d, $J=5.1$ Hz, 1H), 1.41-1.27 (m, 22H), 0.90 (t, $J=6.5$ Hz, 3H); ^{13}C NMR (CDCl_3) δ 169.3, 165.9, 139.3, 138.7, 138.6, 138.5, 138.3, 138.2, 138.1, 138.0, 128.5, 128.4, 128.32, 128.27, 128.25, 128.17, 128.00, 127.94, 127.91, 127.8, 127.75, 127.70, 127.67, 127.61, 127.55, 127.49, 127.45, 127.21, 125.9, 107.8, 102.6, 100.8, 99.4, 81.4, 80.6, 79.3, 77.5, 77.3, 77.0, 76.9, 76.7, 75.5, 75.3, 75.2, 74.3, 73.2, 73.1, 73.0, 72.9, 72.3, 72.1, 70.1, 70.0, 69.1, 68.1, 68.0, 67.8, 67.4, 63.8, 32.4, 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 22.7, 20.9, 14.1; IR (thin film) 3570, 3087, 3062, 3029, 2924, 2853 2099, 1950, 1873, 1752, 1496, 1453, 1366, 1231 cm^{-1} ; $[\alpha]_D^{23} +17.6$ (c 1.40).

30

Synthesis of 22b:

Thioglycoside **15b** (188 mg, 0.151 mmol) and the acceptor **21b** (125 mg, 0.0751 mmol) were azeotropically dried with benzene twice. The mixture was then dissolved in 2.6 mL anhydrous Et_2O and 1.3 mL CH_2Cl_2 and to this solution was added 500 mg of 4 Å mol. sieves. This mixture was

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stirred for 1 h and then was cooled to 0 °C and MeOTf (0.051 mL, 74 mg, 0.45 mmol) was added. The reaction was stirred at 0°C for 9 h. Triethylamine (1 mL) was then added and reaction was filtered through a plug of silica
5 and washed with Et₂O. The filtrate was washed with saturated aqueous NaHCO₃ and dried over anhydrous MgSO₄. Purification by preparative HPLC (85:15 hexane/EtOAc) afforded 108 mg (50%) of the target compound **22b**. The b/a ratio of the reaction was 4:1.

10

¹H NMR (CDCl₃) δ 7.75 (d, J= 7.2 Hz, 2H), 7.46-7.05 (m, 63H), 5.75 (dt, J= 6.8, 15.2 Hz, 1H), 5.43 (dd, J= 8.6, 15.5 Hz, 1H), 5.13 (m, 2H), 5.09 (d, 3.6 Hz, 1H), 5.05 (d, J= 11.6 Hz, 1H), 5.00 (d, J= 11.5 Hz, 1H), 4.94-4.86
15 (m, 5H), 4.83-4.65 (m, 14H), 4.59 (d, 11.7 Hz, 2H), 4.53-4.43 (m, 6H), 4.39-4.31 (m, 4H), 4.23 (d, J= 11.9 Hz, 1H), 4.18 (d, J= 11.9 Hz, 1H), 4.15-4.08 (m, 2H), 4.05-3.57 (m, 31H), 3.54 (d, J= 9.1 Hz, 1H), 3.49-3.45 (m, 2H), 3.38 (m, 1H), 3.31-3.23 (m, 3H), 2.92-2.89 (m, 2H),
20 2.75 (bt, 6.0 H, H), 2.12 (bq, J= 6.9 Hz, 2H), 1.85 (s, 3H), 1.20-1.09 (m, 42H), 0.92 (t, J= 6.6 Hz, 3H); ¹³C NMR (CDCl₃) δ 169.1, 165.9, 155.5, 140.9, 139.2, 139.0, 138.8, 138.64, 138.59, 138.47, 138.43, 138.3, 138.2, 138.10, 138.07, 138.0, 132.1, 129.1, 128.69, 128.65,
25 128.56, 128.43, 128.40, 128.36, 128.35, 128.26, 128.17, 128.12, 128.08, 127.97, 127.77, 127.66, 127.64, 127.60, 127.54, 127.49, 127.45, 127.41, 127.3, 126.0, 103.0, 102.7, 100.8, 99.7, 99.2, 98.0, 81.2, 80.6, 79.5, 79.2, 79.0, 78.3, 77.7, 76.8, 76.5, 75.5, 75.3, 75.1, 75.03,
30 74.97, 74.91, 74.87, 74.0, 73.2, 73.10, 73.07, 72.98, 72.93, 72.6, 72.3, 72.1, 72.0, 71.32, 71.25, 70.2, 69.4, 69.32, 69.25, 68.1, 67.9, 67.5, 68.3, 62.1, 62.0, 56.1, 32.4, 31.9, 29.71, 29.68, 29.66, 29.48, 29.38, 29.2, 29.1, 22.7, 20.7, 18.13, 18.11, 18.01, 17.98, 16.9, 14.2,
35 11.9; IR (thin film) 3344, 3030, 2924, 2864, 2101, 1789, 1754, 1496, 1453, 1366, 1232 cm⁻¹.

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Synthesis of 18b:

The hexasaccharide **22b** (66 mg, 0.023 mmol) was dissolved in 1 mL EtOAc. Lindlar's catalyst (66 mg) was added followed by the addition of palmitic anhydride (23 mg, 0.046 mmol). The system was purged under vacuum and then put under 1 atm of H₂. After 24 h the reaction was filtered through a plug of silica gel, washed with EtOAc, and concentrated. Purification by preparative HPLC (4:1 hexane/EtOAc) afforded 64 mg (90%) of the desired product **18b**.

¹H NMR (CDCl₃) δ 7.72 (d, J= 7.2 Hz, 2H), 7.42-7.02 (m, 63H), 5.65 (d, J= 9.1 Hz, 1H), 5.62 (dt, J= 6.6, 15.3 Hz, 1H), 5.31 (dd, J= 8.6, 15.3 Hz, 1H), 5.10 (m, 2H), 5.05 (d, J= 3.6 Hz, 1H), 5.02 (d, J=11.5 Hz, 1H), 4.96 (d, J= 11.4 Hz, 1H), 4.90-4.62 (m, 13H), 4.57-4.38 (m, 8H), 4.32-4.26 (m, 3H), 4.21-4.07 (m, 9H), 4.01-3.41 (m, 31H), 3.30 (m, 1H), 3.23 (m, 3H), 2.20 (m, 4H), 1.82 (s, 3H), 1.52 (bm, 2H), 1.32-1.19 (m, 53H), 1.15-1.08 (m, 42H), 0.88 (t, J= 6.8 Hz, 6H); IR (thin film) 3531, 3346, 3063, 3030, 2924, 2854, 1790, 1748, 1674, 1496, 1454, 1365, 1236 cm⁻¹; [α]²³_D -17.9 (c 0.65).

Synthesis of 1b:

The hexasaccharide from above (20 mg, 0.0065 mmol) was dissolved in 0.5 mL THF. A solution of tetrabutylammonium fluoride (1.0 M in THF, 0.050 mL, 0.050 mmol) was added and the reaction was stirred for 2 h. The solution was filtered through a plug of silica, washed with EtOAc and concentrated. The residue was dissolved in 1 mL of anhydrous MeOH and NaOMe (10 mg, 0.19 mmol) was added. The reaction was stirred for 3 h, neutralized with 40 mg of Dowex-50 resin, filtered and concentrated. Purification by flash column chromatography (1.5 x 4 cm 10-40 μ silica gel, 95:5 CH₂Cl₂/MeOH) afforded 16.5 mg (94%) of the desired compound.

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¹H NMR (CDCl₃) δ 7.78 (d, J= 7.6 Hz, 2H), 7.46 (d, J= 7.4 Hz, 2H), 7.41-6.97 (m, 61H), 6.02 (d, J= 9.1 Hz, 1H), 5.76 (bs, 1H), 5.67 (dt, J= 6.6, 15.3 Hz, 1H), 5.37-5.30 (m, 2H), 5.19 (d, J= 2.6 Hz, 1H), 4.96 (d, J= 11.3 Hz, 1H), 4.93 (d, J= 3.4 Hz, 1H), 4.90-4.83 (m, 3H), 4.78-4.66 (m, 7H), 4.56 (d, J= 11.1 Hz, 1H), 4.53 (d, J= 10.2 Hz, 1H), 4.47-4.32 (m, 5H), 4.28-4.06 (m, 14H), 4.01-3.13 (m, 36H), 2.73 (bt, 1H), 2.61 (bs, 1H), 2.54 (bs, 1H), (2.05 (m, 4H), 1.50 (m, 2H), 1.38-1.23 (m, 46H), 0.88 (t, J= 6.6 Hz, 6H), 0.78 (d, 6.3 Hz, 3H); ¹³C NMR (CDCl₃) δ 173.4, 142.4, 139.5, 139.0, 138.7, 138.5, 138.33, 138.26, 138.14, 138.09, 137.9, 137.2, 137.1, 131.6, 129.0, 128.8, 128.54, 128.47, 128.37, 128.32, 128.27, 128.22, 128.17, 128.14, 128.05, 127.99, 127.79, 127.73, 127.68, 127.63, 127.59, 127.49, 127.46, 127.37, 127.32, 126.98, 126.58, 104.1, 102.83, 102.76, 100.3, 100.2, 82.1, 81.5, 81.2, 79.6, 79.2, 79.0, 78.0, 77.3, 77.0, 76.7, 75.6, 75.3, 75.1, 75.0, 74.8, 74.6, 73.5, 73.4, 73.2, 73.0, 72.7, 72.6, 71.9, 70.1, 69.6, 68.5, 68.2, 68.0, 67.5, 62.4, 61.9, 54.8, 52.3, 36.9, 32.3, 31.9, 29.71, 29.67, 29.54, 29.50, 29.43, 29.37, 29.28, 29.20, 25.7, 22.7, 16.7, 14.1; IR (thin film) 3424, 3062, 3023, 2923, 2852, 1641, 1530, 1496, 1453, 1362, 1325 cm⁻¹; [α]_D²³ -3.2 (c 0.83).

A flask was equipped with a dry ice condenser and was charged with 4 mL NH₃. Sodium (18 mg, 0.78 mol) was added and to the resulting blue solution was added 29 mg of the above hexasaccharide (0.010 mmol). The reaction was stirred at -78°C for 45 min. Quench by the addition of MeOH (3 mL). Nitrogen was blown over the solution to evaporate the NH₃. The reaction was neutralized with 170 mg of Dowex-50 resin, filtered and concentrated. The resulting residue was dissolved in 1 mL of 4:1 THF/DMF. Triethylamine (0.5 mL) was added followed by the addition of DMAP (3 mg) and acetic anhydride (0.200 mL). After 2 h the reaction was concentrated in vacuo. Purification by flash column (1.5 x 5 cm 10-40 m silica, 9:1

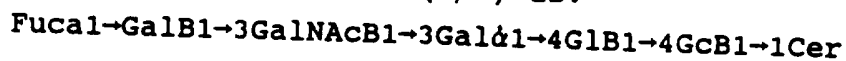
-91-

EtOAc/hexane) afforded 18 mg (78%) of the peracetate. A sample of this hexasaccharide (15 mg, 0.0065 mmol) was dissolved in 0.5 mL of anhydrous MeOH and a NaOMe solution (30% in MeOH, 0.010 mL, 0.05 mmol) was added. The solution was stirred for 3 h, neutralized with 9 mg Dowex-50 resin, filtered and concentrated. The residue was purified by flash column chromatography (1.5 x 4 cm C-18 reverse phase silica, MeOH) to afford 9.6 mg of the natural product 1. Spectral data agree with those reported by Hakomori, et al.

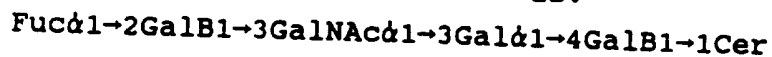
Biological Results

The MBR1 hexasaccharide has been prepared in two forms, the natural "B" form and the unnatural "A" form as shown below.

The natural structure (" β ") is:



The unnatural structure " α " is:



Both have been linked to ceramide to facilitate testing for immunological reactivity with monoclonal antibody (mAb) MBR1.

By Thin Layer Chromatography (TLC) the 2 preparations migrate as similar single bands. Immune TLC (see Ritter, G., et al., Cancer Res. 50, 1403-10 (1990)) demonstrates that both forms react with the MBR1 monoclonal antibody specifically but that the β -form reacts 10 times more strongly (comparable staining is seen with 1/10 the amount of antigen). The high level of reactivity of the β structure with mAb MBR1 was confirmed using flow cytometry inhibition assays. Reactivity of MAb MBR1 with breast cancer cell lines such as MCF-7 was 98% inhibited

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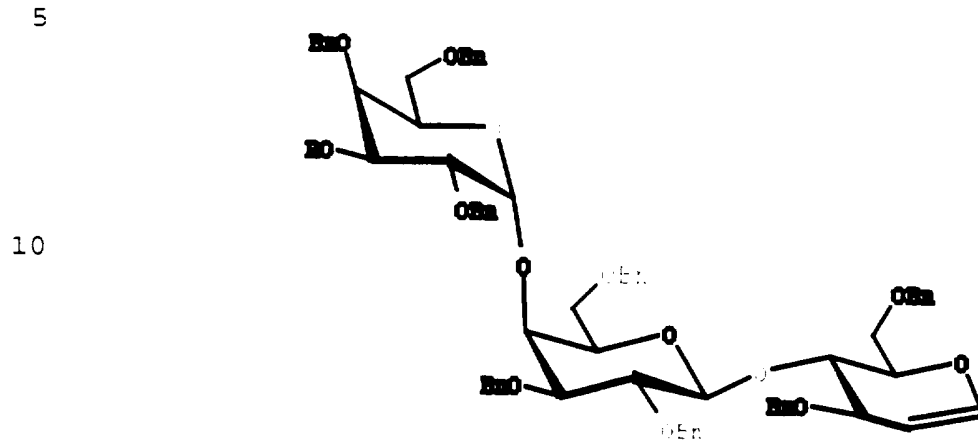
by 8 $\mu\text{g/ml}$ of the β linkage preparation but was only 6% inhibited by 8 μg of the α -linkage preparation. GD3 ganglioside (negative control) showed no inhibition at all.

5

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What is claimed is:

1. A process for synthesizing a compound having the structure:



wherein R is H which comprises:

- (a) (i) reacting a compound having the structure:



with an epoxidizing agent under suitable conditions to form an epoxide;

- (ii) cleaving the epoxide formed in step (a) (i) under suitable conditions with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group to form a fluoroalcohol; and

35

(iii) alkylating the fluoroalcohol formed in step (b) (ii) under suitable conditions with a

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non-nucleophilic base and an organic halide having the formula $C_6H_5CH_2X$ wherein X is Br, Cl, I or F to form a compound having the structure:

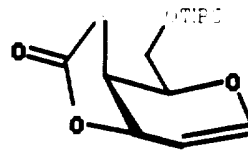
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(b) treating a compound having the structure:

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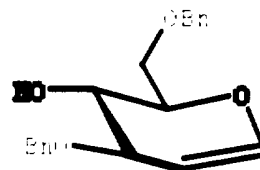


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with an epoxidizing agent under suitable conditions to form an epoxide; and

(c) coupling the epoxide formed in step (b) with a compound having the structure:

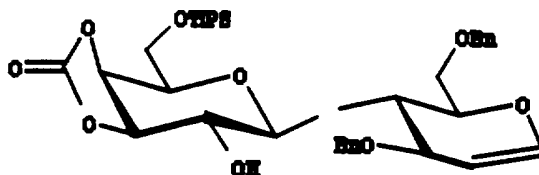
25



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under suitable conditions to form a compound having the structure:

5



10

- (d) (i) alkylating the compound formed in step (c)(ii) under suitable conditions with a non-nucleophilic base and an organic halide having the formula $C_6H_5CH_2X$ wherein X is Br, Cl, I or F; and

15

- (ii) de-silylating the compound formed in step (d)(i) under suitable conditions with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group;

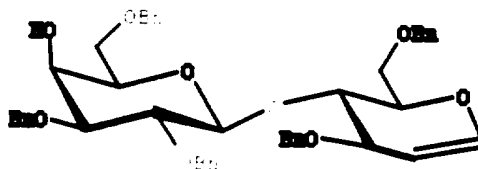
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- (iii) treating the compound formed in step (d)(ii) under suitable conditions with a metal alkoxide to form a deprotected disaccharide; and

25

- (iv) alkylating the disaccharide formed in step (d)(iii) under suitable conditions to form a selectively deprotected disaccharide having the structure:

30

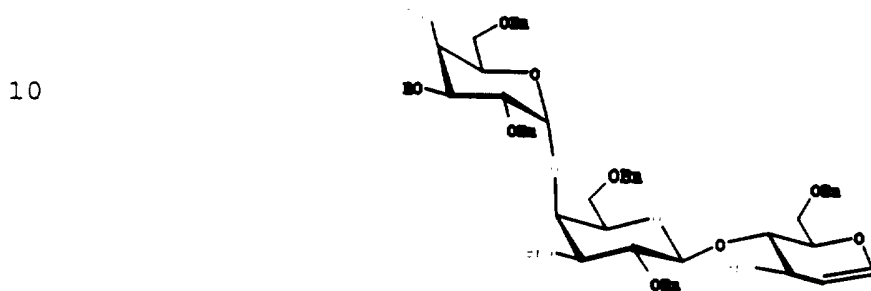


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- (e) (i) coupling the selectively deprotected disaccharide formed in step (d)(iv) with the

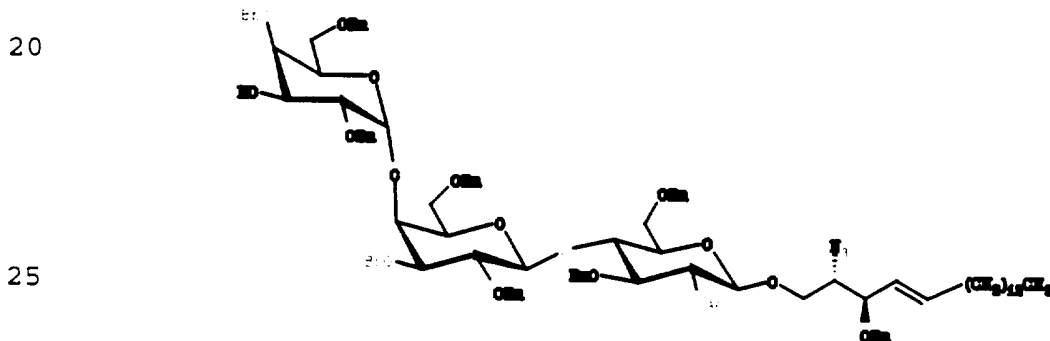
compound formed in step (a) (iii) under suitable conditions to form a protected trisaccharide; and

- 5 (ii) de-protecting the protected trisaccharide formed in step (e) (i) under suitable conditions to form a trisaccharide having the structure:



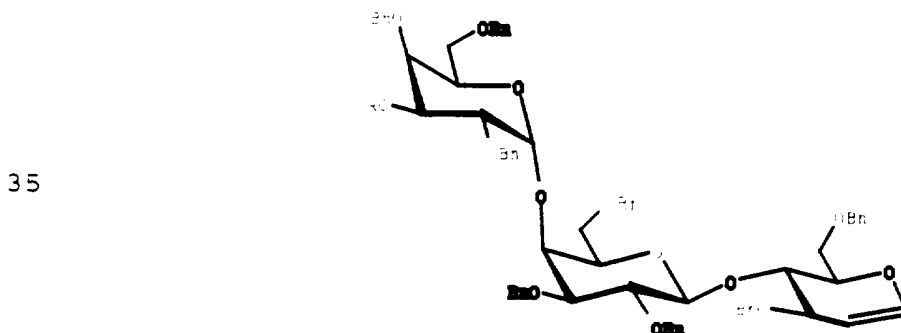
15 wherein R is H.

2. A process for synthesizing a trisaccharide ceramide having the structure:



25 which comprises:

- 30 (a) synthesizing a trisaccharide having the structure

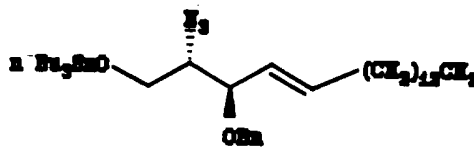


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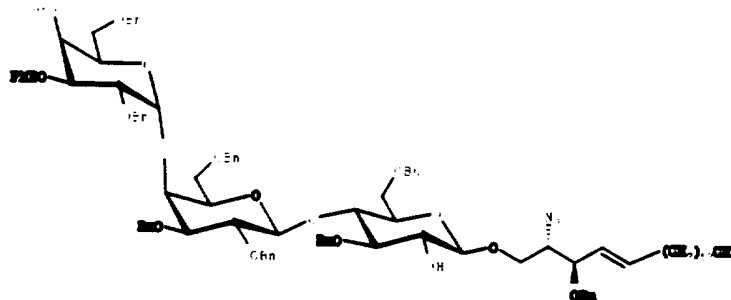
wherein R is PMB;

5 (b) (i) reacting the trisaccharide formed in step (a) with an epoxidizing agent under suitable conditions to form a trisaccharide epoxide; and

(ii) reacting the trisaccharide epoxide formed in step (b)(i) with a compound having the structure:



15 under suitable conditions to form a protected trisaccharide ceramide having the structure:

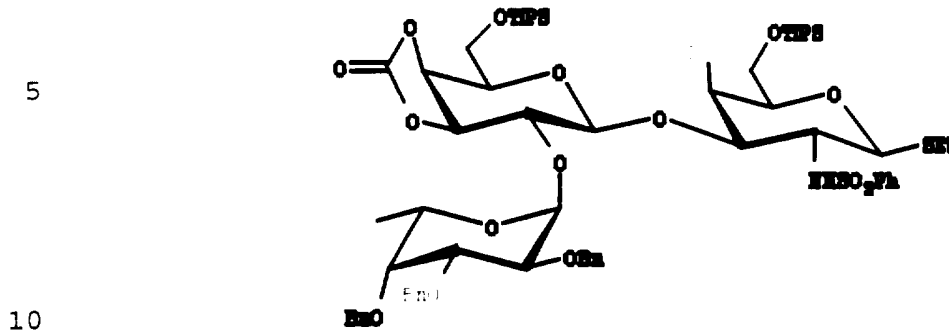


25 (c) (i) acylating the ceramide formed in step (b)(ii) under suitable conditions; and

(ii) selectively de-protecting the compound formed in step (c)(i) under suitable conditions to form the trisaccharide ceramide.

30

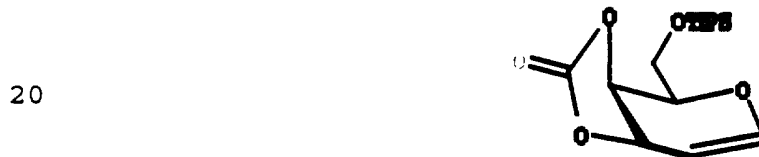
3. A process for synthesizing a mercaptotrisaccharide having the structure:



which comprises:

15

(a) coupling a compound having the structure:

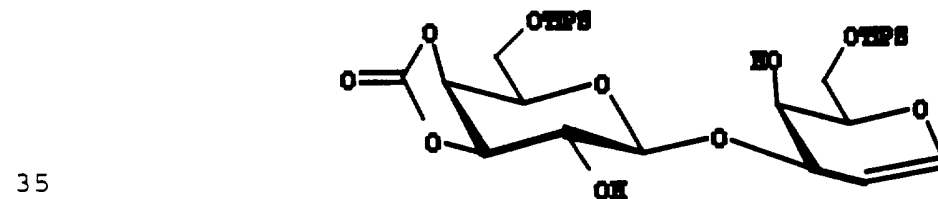


with a compound having structure:



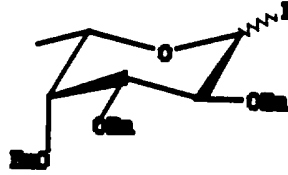
under suitable conditions to form a disaccharide having the structure:

30



(b) coupling the disaccharide formed in step (a) (ii) with a compound having the structure:

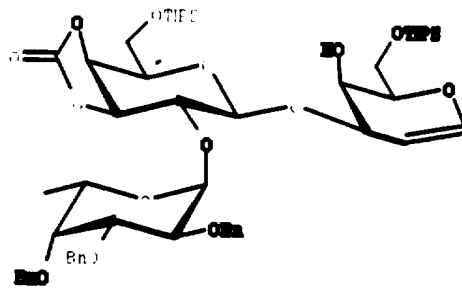
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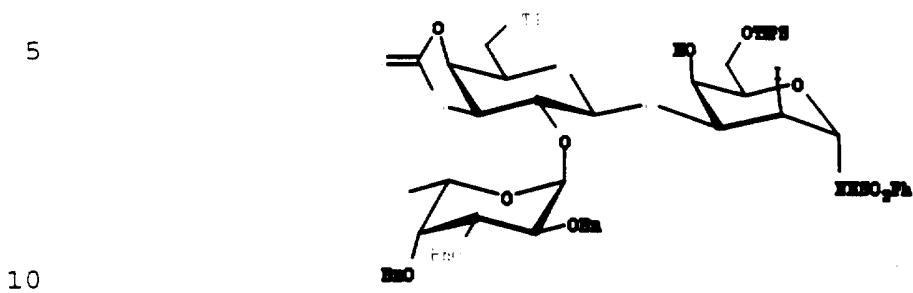
under suitable conditions to form a trisaccharide having the structure:

15



20

(c) iododisulfonamidating the trisaccharide formed in step (b) under suitable conditions to form a iododisulfonamide having the structure:



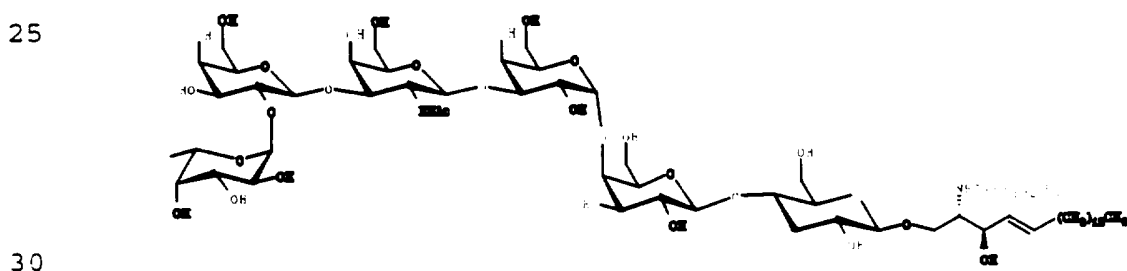
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and

(d) reacting the iododisulfonamide formed in step (c) under suitable conditions with a thiolate to form the mercaptotrisaccharide.

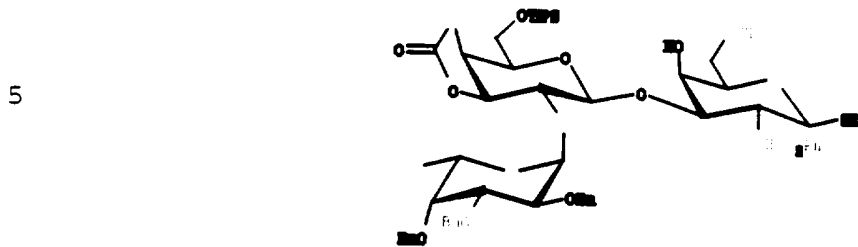
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4. A process of synthesizing a hexasaccharide ceramide having the structure:



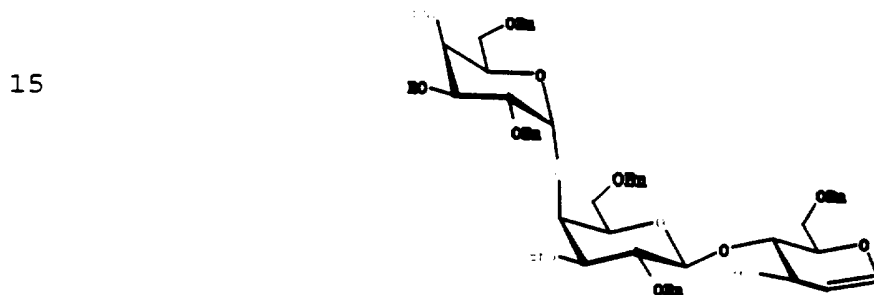
which comprises:

(a) coupling a compound having the structure:

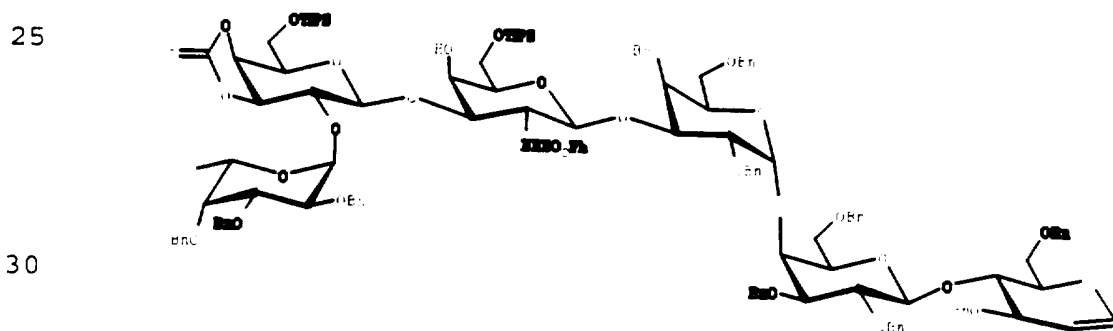


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with a compound having the structure:



under suitable conditions to form a compound having the structure:



(b) (i) reacting the compound formed in step (a) with an epoxidizing agent under suitable conditions to form a hexasaccharide epoxide; and

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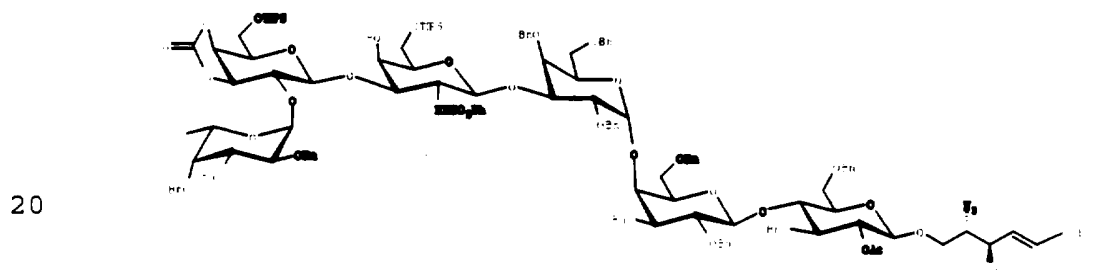
(ii) reacting the hexasaccharide epoxide with a stannyl ether having the structure:



10 under suitable conditions to form a hexasaccharide alcohol;

(c) acylating the hexasaccharide alcohol formed in step (b) (ii) under suitable conditions to form a hexasaccharide acetate having the structure:

15



25 (d) reductively acylating the hexasaccharide acetate formed in step (c) under suitable conditions in the presence of palmitic anhydride to form a hexasaccharide ceramide;

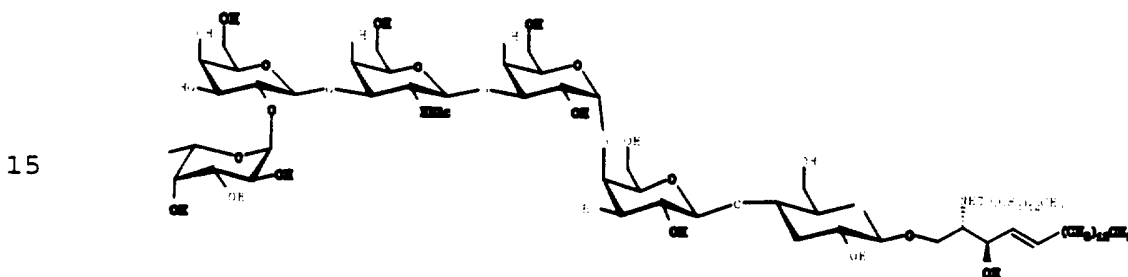
30 (e) desilylating the hexasaccharide ceramide under suitable conditions to form a partially deprotected hexasaccharide ceramide;

35 (f) (i) reducing the partially deprotected hexasaccharide ceramide under suitable conditions to form a deprotected hexasaccharide ceramide acetate; and

(ii) acylating the deprotected hexasaccharide ceramide acetate under suitable conditions to form a hexasaccharide ceramide peracetate; and

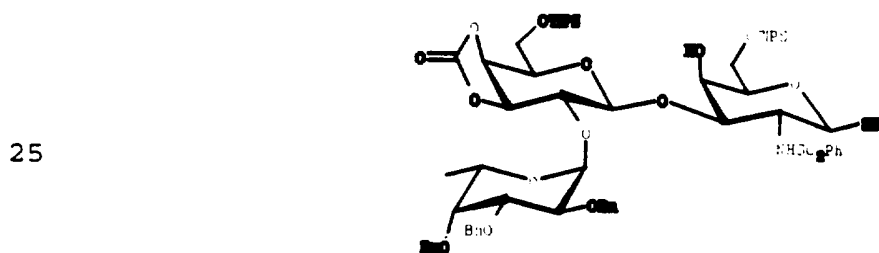
5 (g) saponifying the hexasaccharide ceramide peracetate under suitable conditions to form the hexasaccharide ceramide.

10 5. A process of synthesizing a hexasaccharide ceramide having the structure:



which comprises:

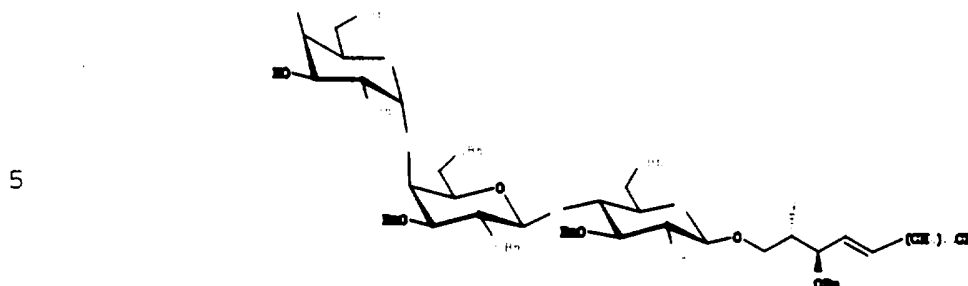
20 (a) coupling a compound having the structure:



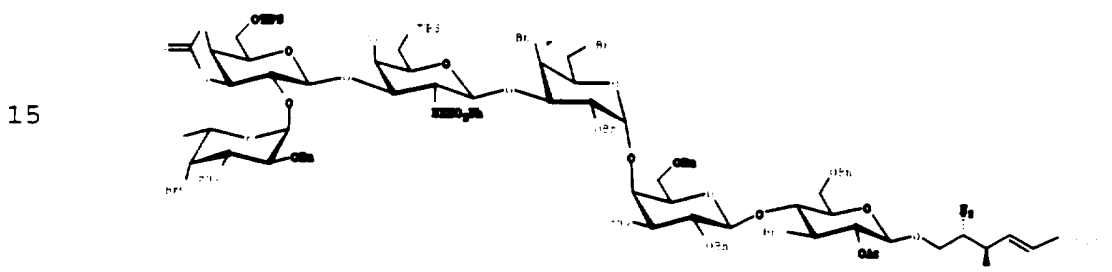
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with a compound having the structure:

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10 under suitable conditions to form a hexasaccharide having the structure:



and

20

(b) (i) reducing the hexasaccharide formed in step (a) under suitable conditions in the presence of palmitic anhydride to form a palmitoyl amide;

25

(ii) desilylating the palmitoyl amide with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group under suitable conditions to form a partially deprotected hexasaccharide;

30

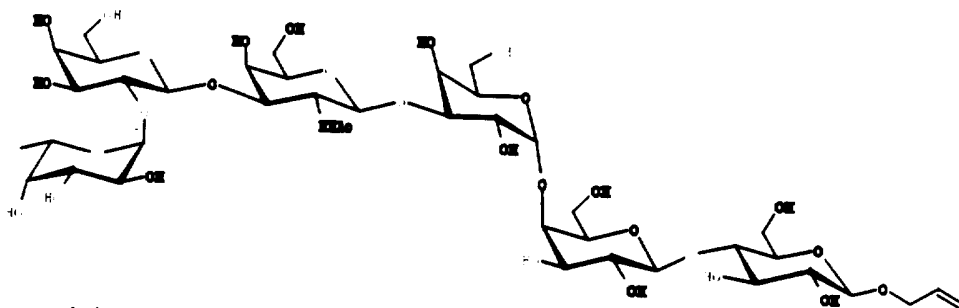
(iii) de-protecting the hexasaccharide formed in step (b)(ii) under suitable conditions to form a deprotected hexasaccharide;

35

(iv) acylating the hexasaccharide formed in step (b)(iii) under suitable conditions to form a hexasaccharide ceramide peracetate; and

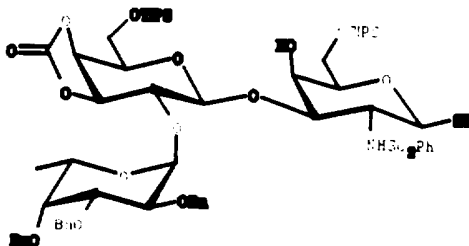
5 (iv) saponifying the hexasaccharide ceramide peracetate under suitable conditions to form the hexasaccharide ceramide.

10 6. A process of synthesizing an allyl hexasaccharide having the structure:



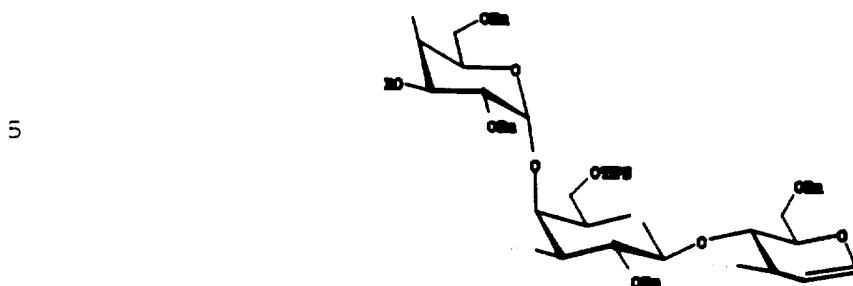
which comprises:

20 (a) coupling a compound having the structure:



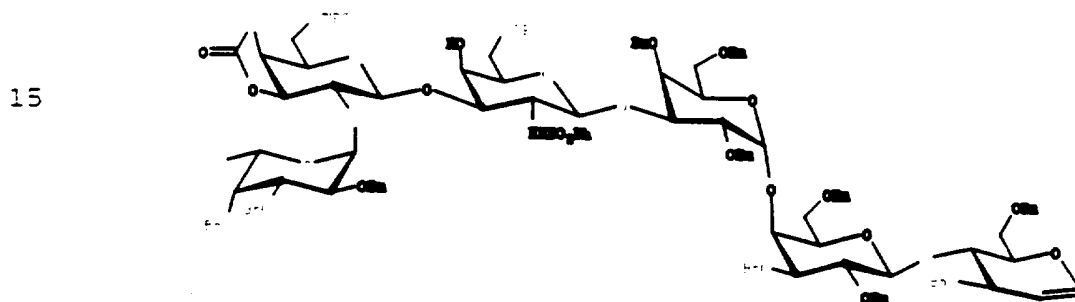
-106-

with a compound having the structure:



10

wherein R is H under suitable conditions to form a hexasaccharide having the structure:



20

(b) (i) desilylating the compound formed in step (a) with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group under suitable conditions to form a partially deprotected hexasaccharide;

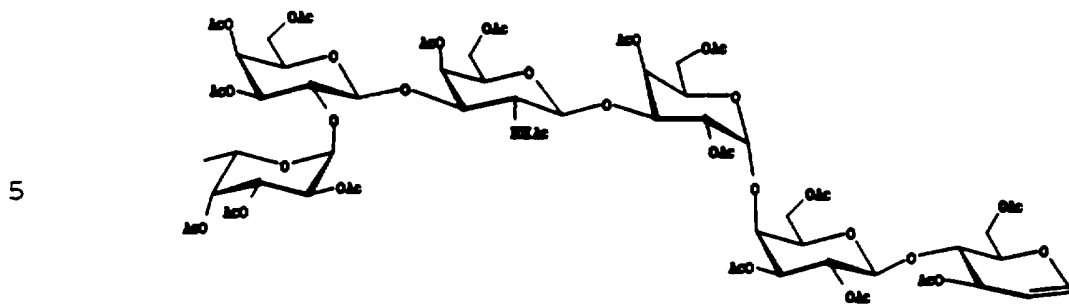
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(ii) de-protecting the hexasaccharide formed in step (b) (i) under suitable conditions to form a deprotected hexasaccharide; and

35

(iii) peracetylating the compound formed in step (b) (ii) under suitable conditions to form a hexasaccharide peracetate having the structure:

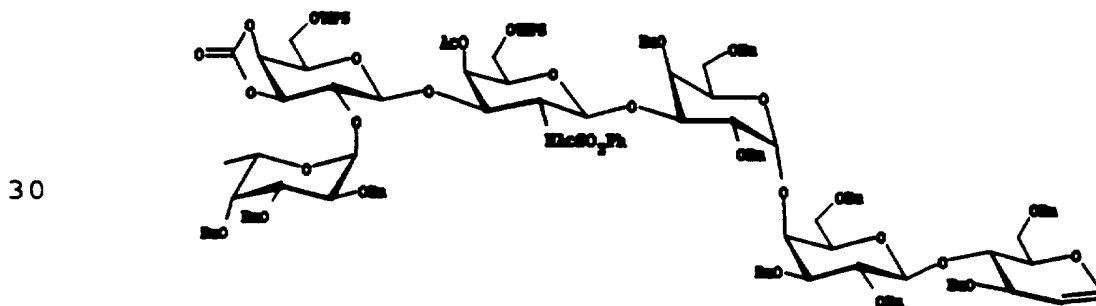


10 (c) (i) reacting the hexasaccharide peracetate formed in step (b)(iii) with an epoxidizing agent under suitable conditions to form an hexasaccharide epoxide peracetate;

15 (ii) treating the hexasaccharide epoxide peracetate formed in step (c)(i) with allyl alcohol under suitable conditions to form an allyl hexasaccharide peracetate; and

20 (iii) saponifying the allyl hexasaccharide peracetate under suitable conditions to form the allyl hexasaccharide.

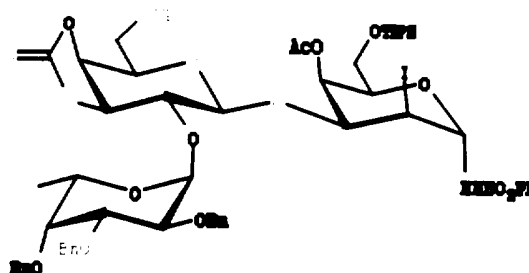
25 7. A process of synthesizing a hexasaccharide having the structure:



which comprises:

(a) coupling a compound having the structure:

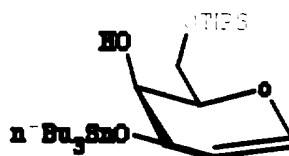
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with a compound having the structure:

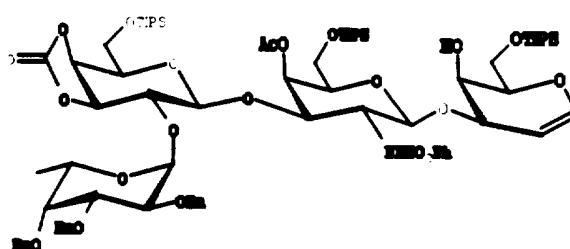
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under suitable conditions to form a compound having the structure:

25



30

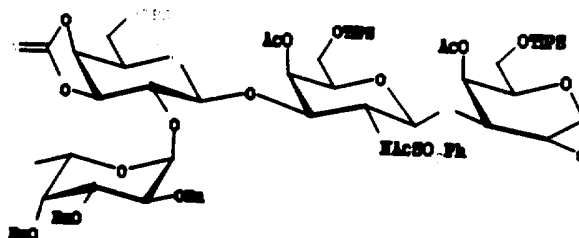
(b) (i) acylating the compound formed in step (a) under suitable conditions; and

35

(ii) reacting the compound formed in step (b) (i) with an epoxidizing agent under suitable

conditions to form an epoxide having the structure:

5



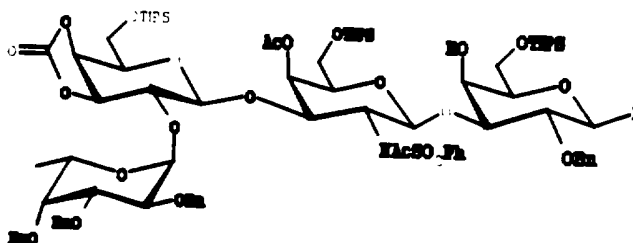
10

(c) (i) treating the epoxide with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group under suitable conditions; and

15

(ii) alkylating the compound formed in step (c) (i) under suitable conditions to form a compound having the structure:

20

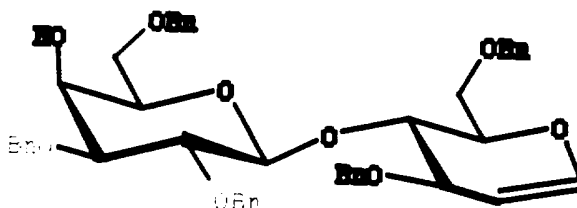


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wherein R is H or acyl;

(d) coupling the compound formed in step (c) (ii) with a compound having the structure:

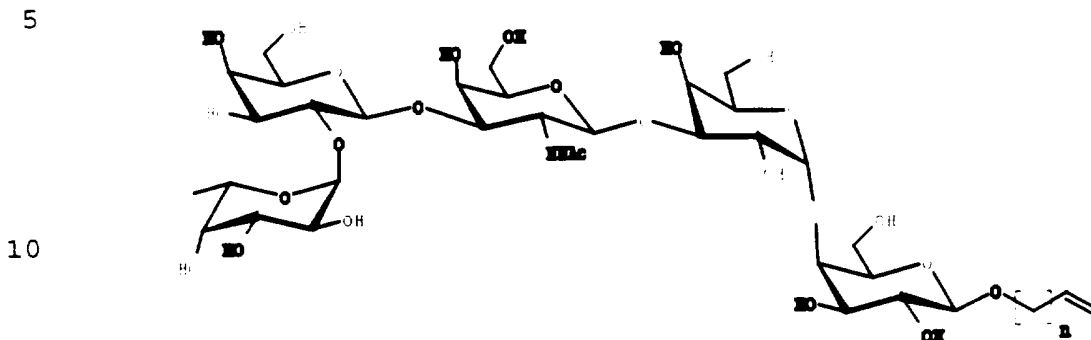
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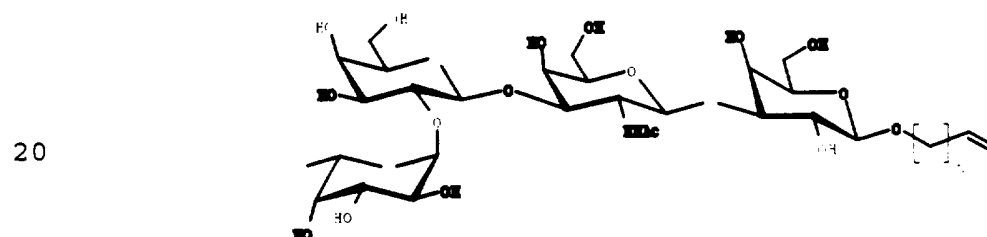
under suitable conditions to form the hexasaccharide.

8. A compound having the structure:



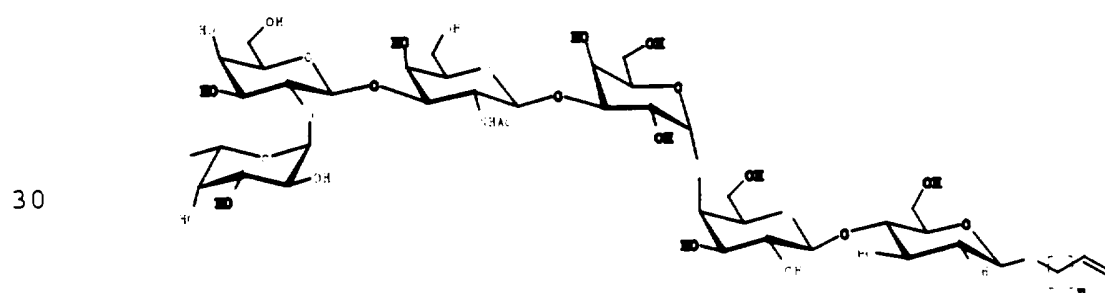
wherein n is an integer between about 0 and about 9.

9. A compound having the structure:



wherein n is an integer between about 0 and about 9.

10. A compound having the structure:

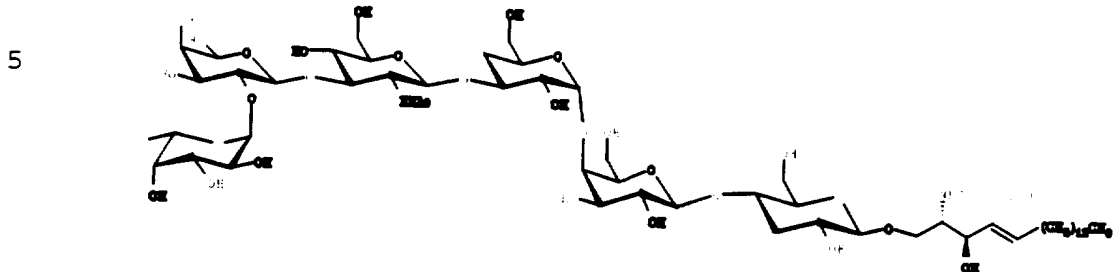


wherein n is an integer between about 0 and about 9.

35 11. A method of inducing antibodies in a human subject, wherein the antibodies are immunoreactive with human breast tumor cells, which comprises administering to

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the subject an amount of a compound having the structure:



- 10 alone or bound to a suitable immunological adjuvant effective to induce the antibodies.
12. The method of claim 11 wherein the antibodies induced are MBr1 antibodies.
- 15
13. The method of claim 11 wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease.
- 20
14. The method of claim 11 wherein the adjuvant is a protein carrier, bacteria or liposomes.
- 25
15. The method of claim 14 wherein the adjuvant is bacille Calmette-Guerin.
- 30
16. A method of inducing antibodies in a subject, wherein the antibodies are immunoreactive with human breast tumor cells, which comprises administering to the subject an amount of the compound of claim 8 alone or bound to a suitable immunological adjuvant effective to induce the antibodies.
- 35
17. The method of claim 16 wherein the antibodies induced are MBr1 antibodies.

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18. The method of claim 16 wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease.
- 5 19. The method of claim 16 wherein the adjuvant is a protein carrier, bacteria or liposomes.
20. The method of claim 19 wherein the adjuvant is bacille Calmette-Guerin.
- 10 21. A method of inducing antibodies in a subject, wherein the antibodies are immunoreactive with human breast tumor cells, which comprises administering to the subject an amount of the compound of claim 9
15 alone or bound to a suitable immunological adjuvant effective to induce the antibodies.
22. The method of claim 21 wherein the antibodies induced are MBr1 antibodies.
- 20 23. The method of claim 21 wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease.
- 25 24. The method of claim 21 wherein the adjuvant is a protein carrier, bacteria or liposomes.
25. The method of claim 24 wherein the adjuvant is bacille Calmette-Guerin.
- 30 26. A method of inducing antibodies in a subject, wherein the antibodies are immunoreactive with human breast tumor cells, which comprises administering to the subject an amount of the compound of claim 10
35 alone or bound to a suitable immunological adjuvant effective to induce the antibodies.

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27. The method of claim 26 wherein the antibodies induced are MBrl antibodies.
- 5 28. The method of claim 26 wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease.
- 10 29. The method of claim 26 wherein the adjuvant is a protein carrier, bacteria or liposomes.
30. The method of claim 29 wherein the adjuvant is bacille Calmette-Guerin.
- 15 31. A method of preventing recurrence of breast cancer in a subject which comprises vaccinating the subject with the compound of claim 8.
- 20 32. A method of preventing recurrence of breast cancer in a subject which comprises vaccinating the subject with the compound of claim 9.
- 25 33. A method of preventing recurrence of breast cancer in a subject which comprises vaccinating the subject with the compound of claim 10.

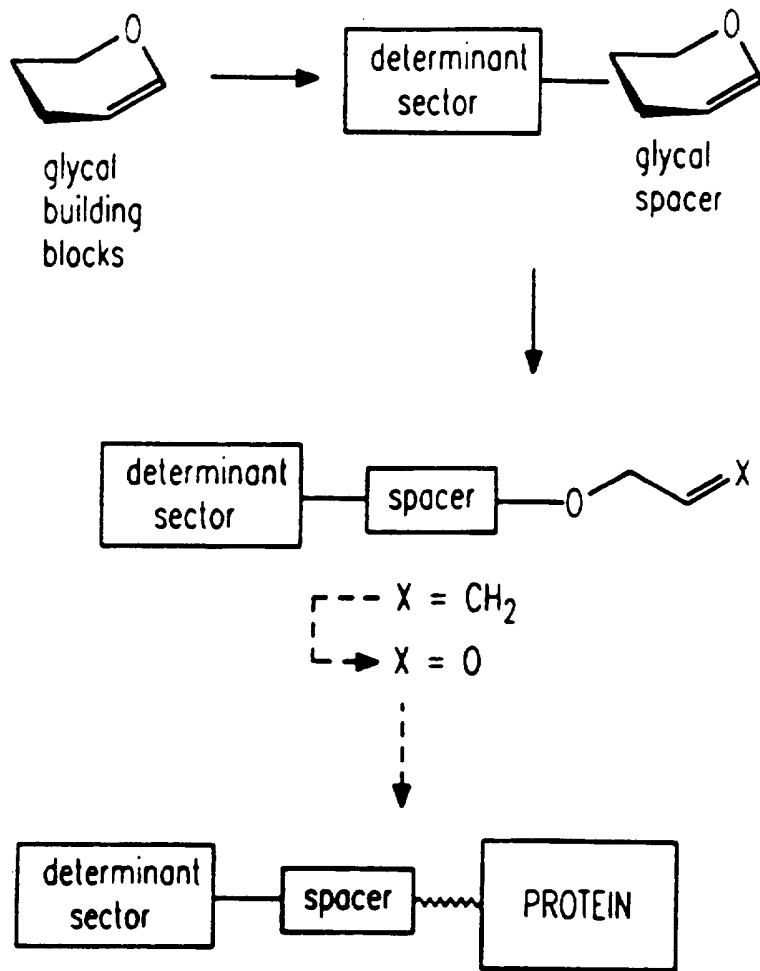


Figure 1

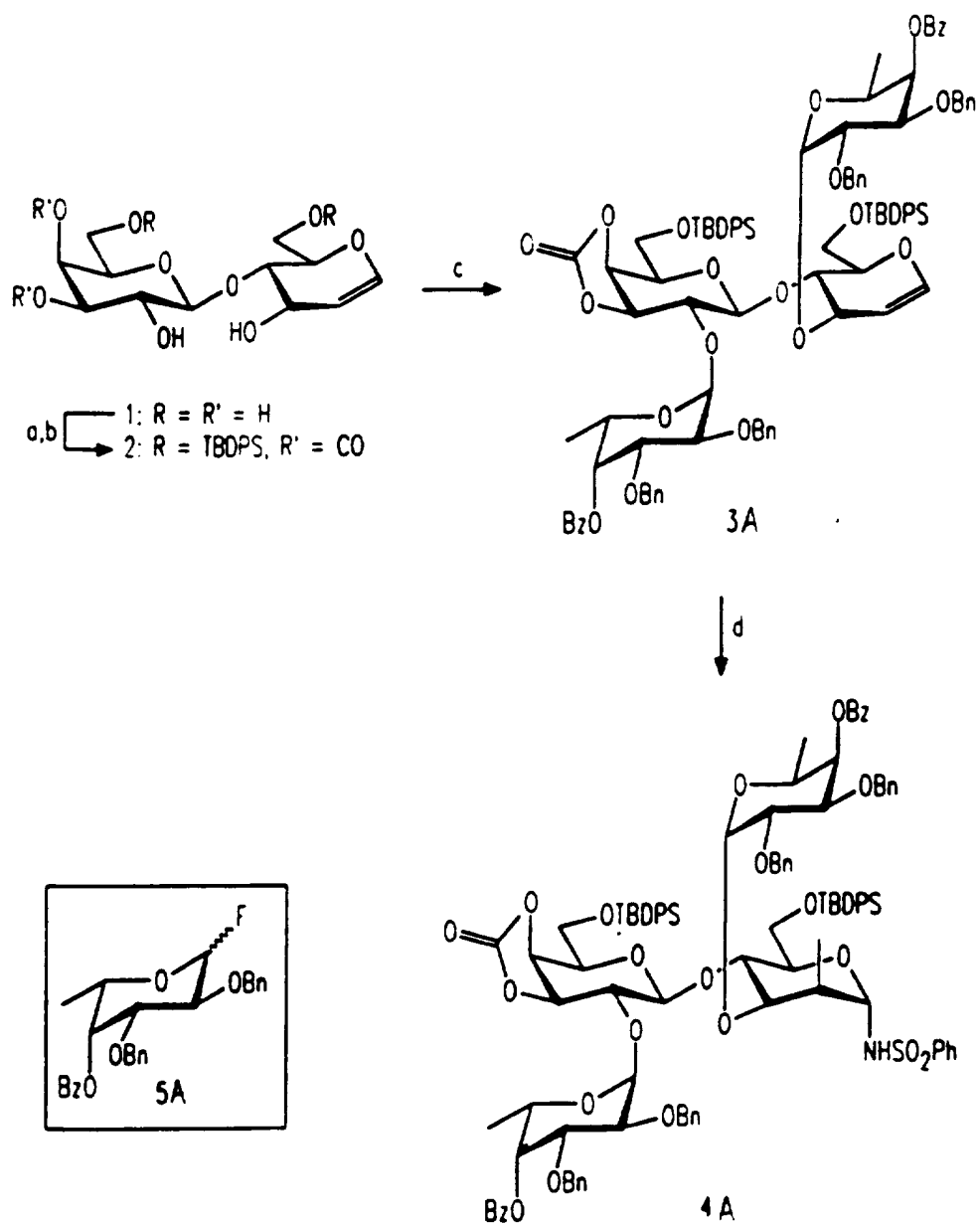


Figure 2

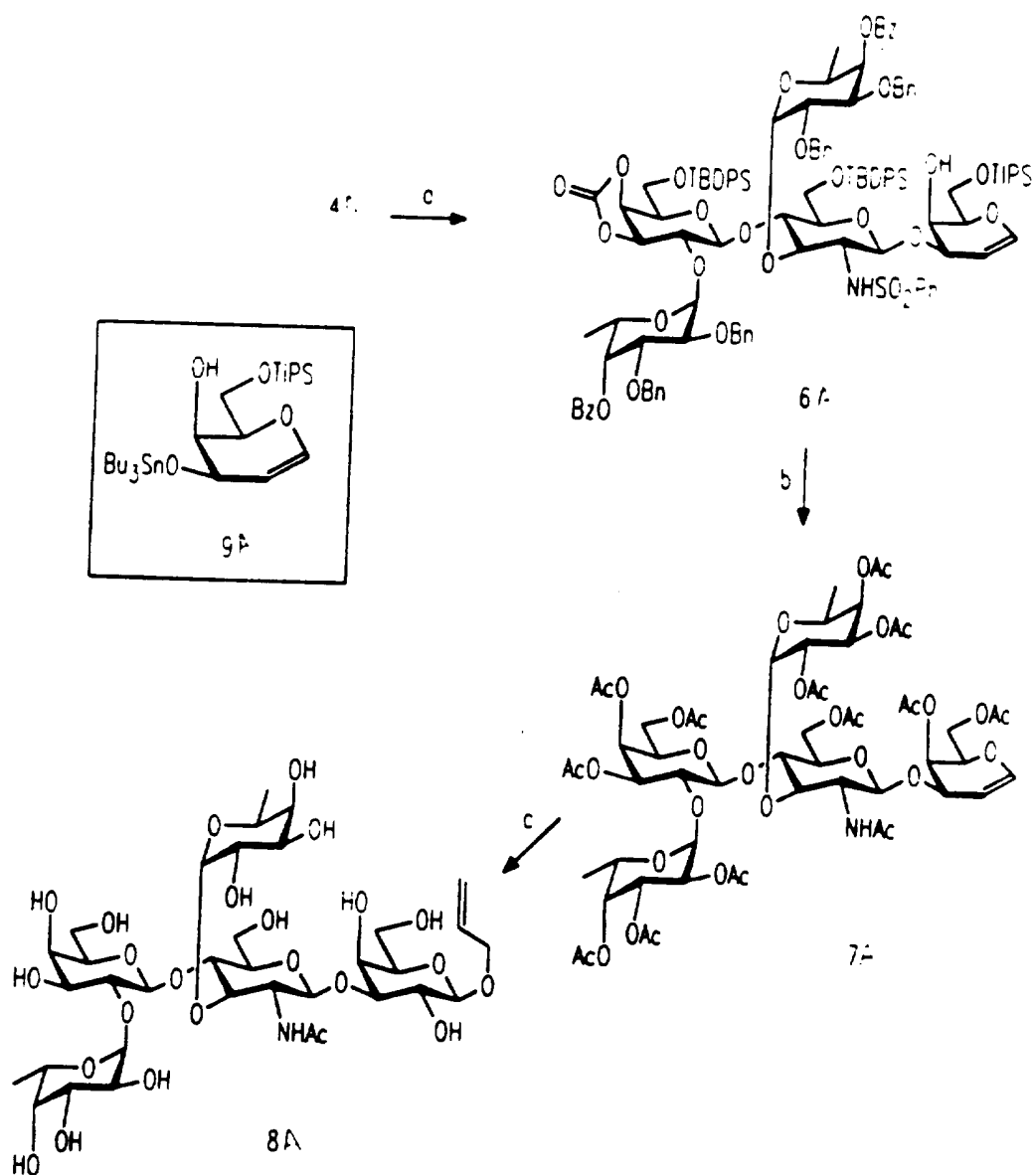


Figure 3

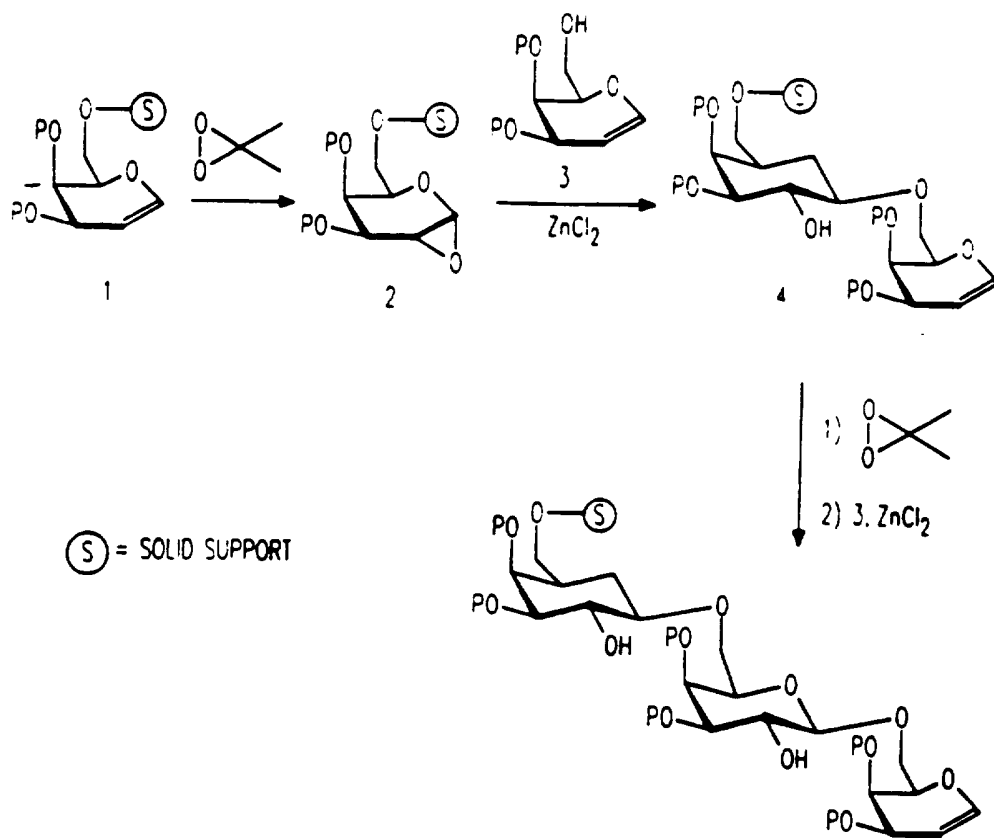


Figure 4

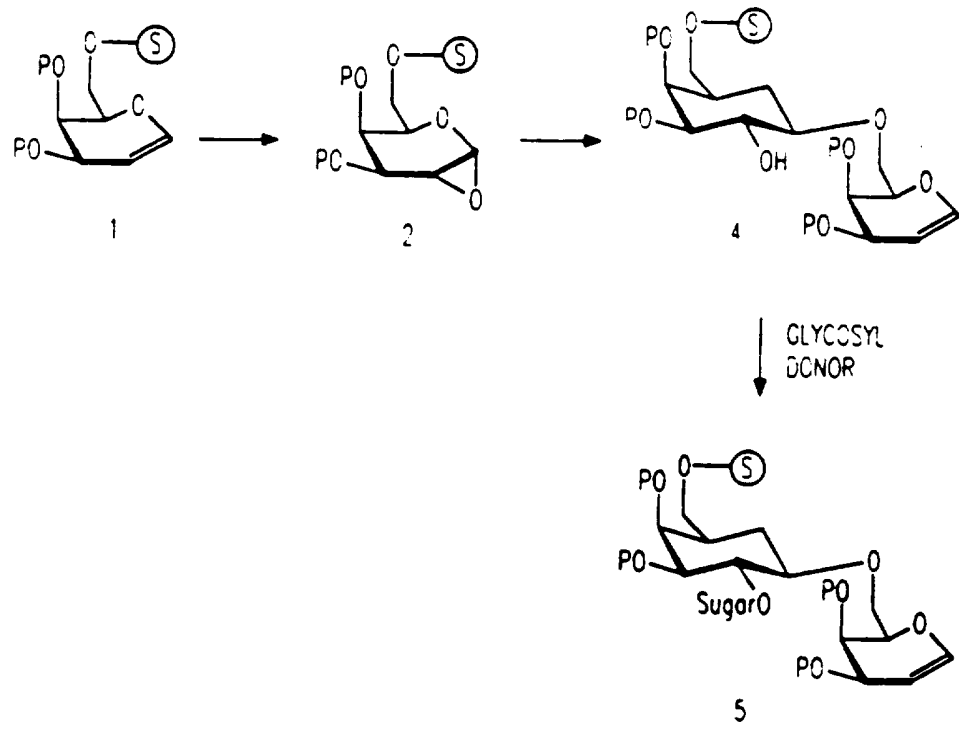


Figure 5

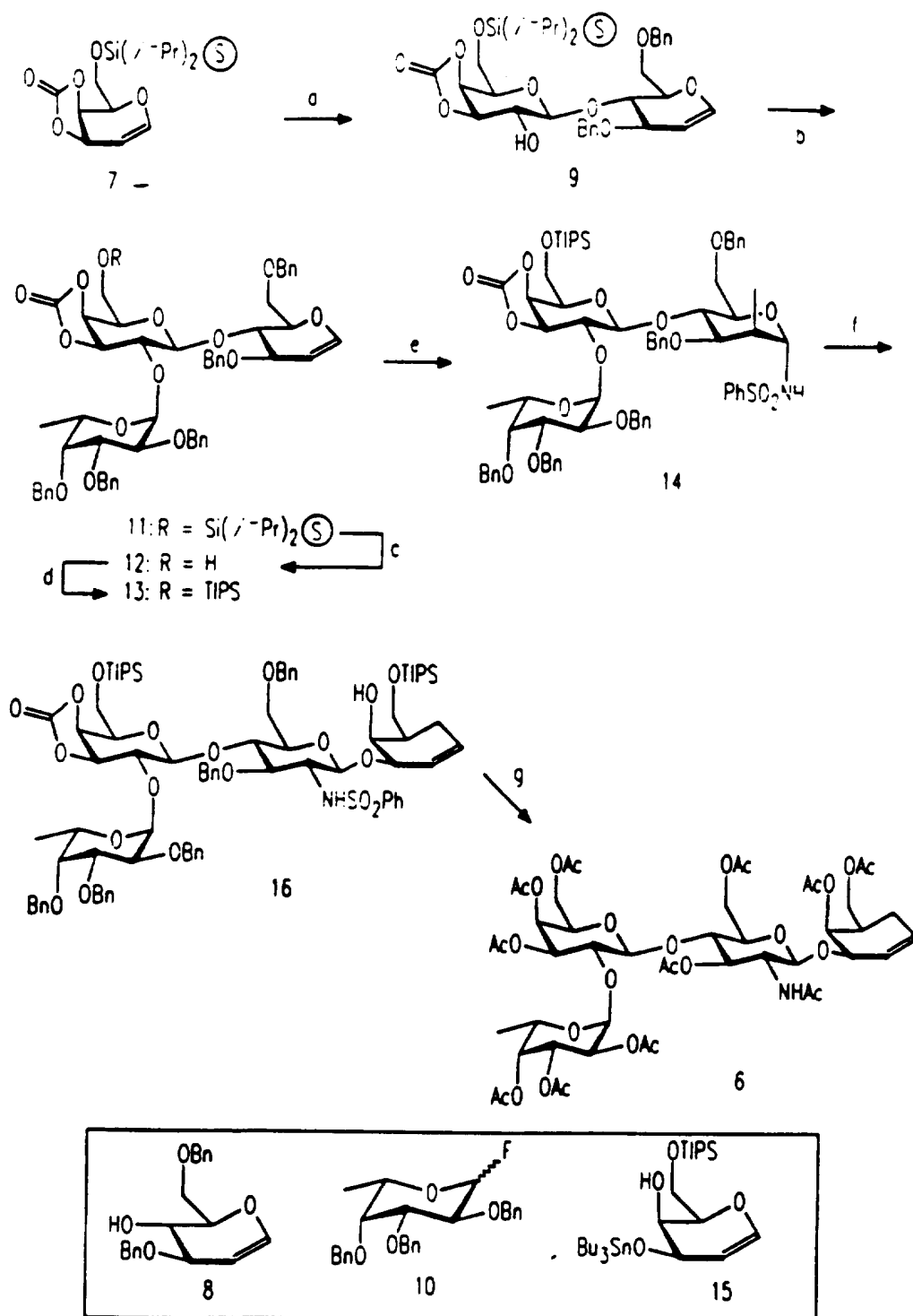


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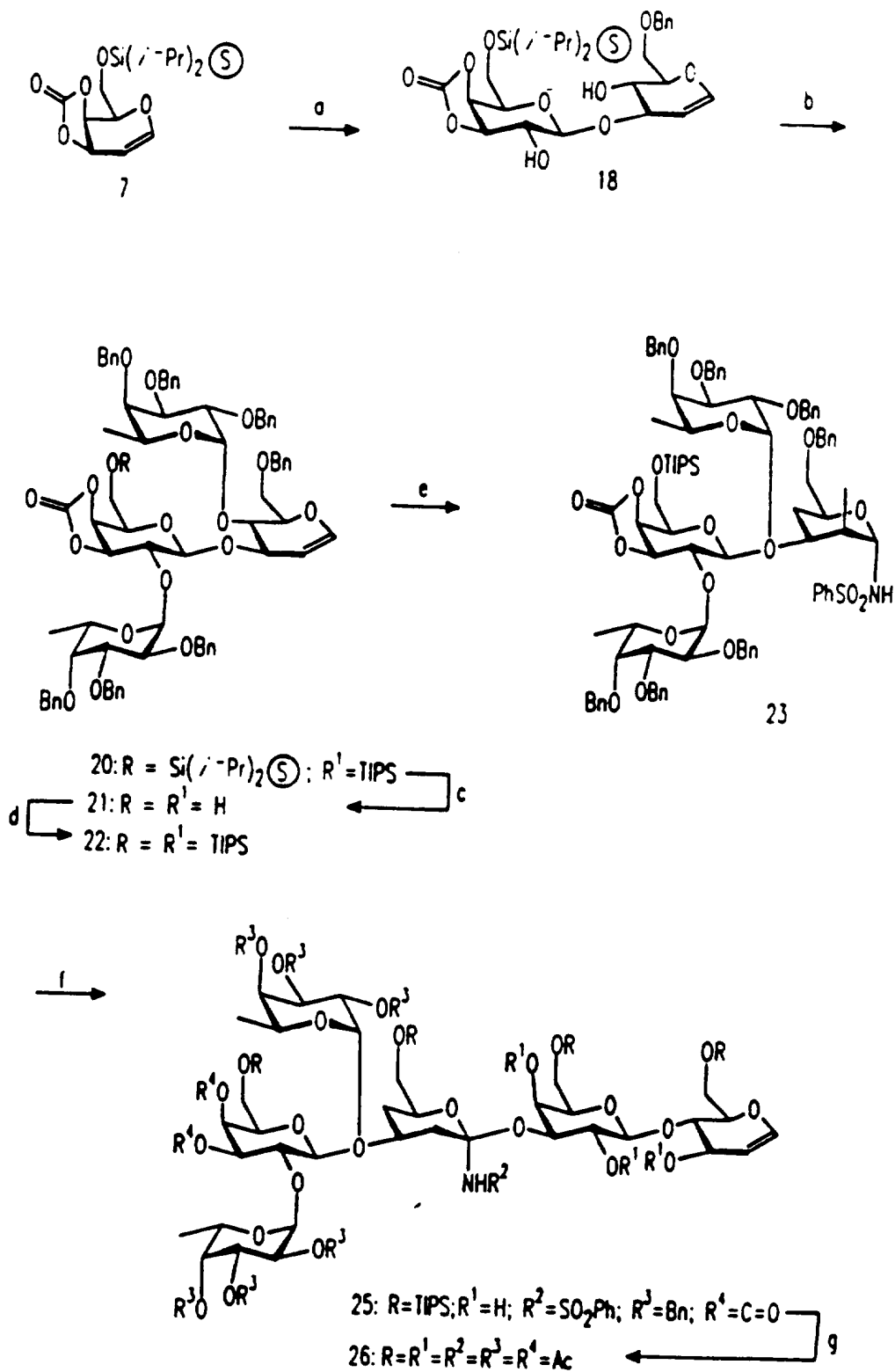


Figure 7a

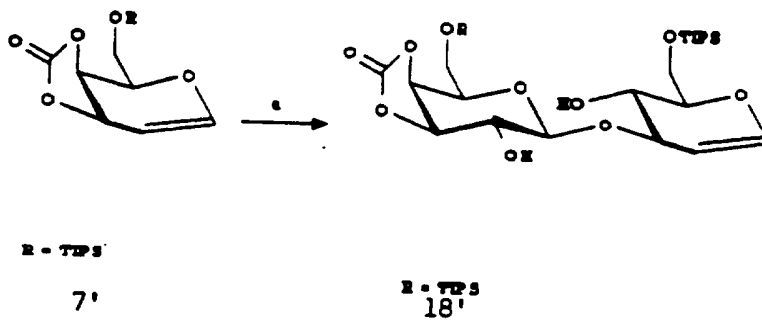
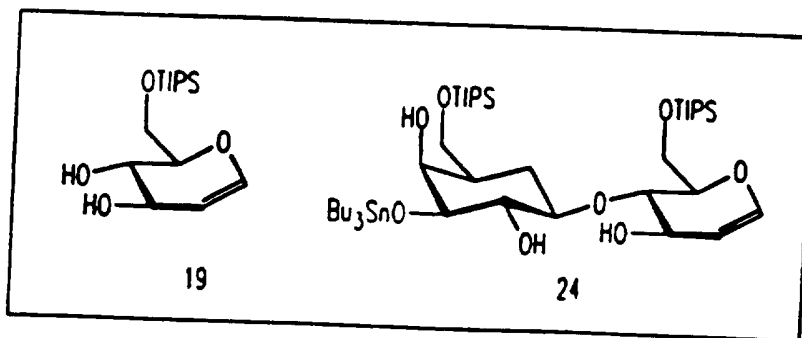
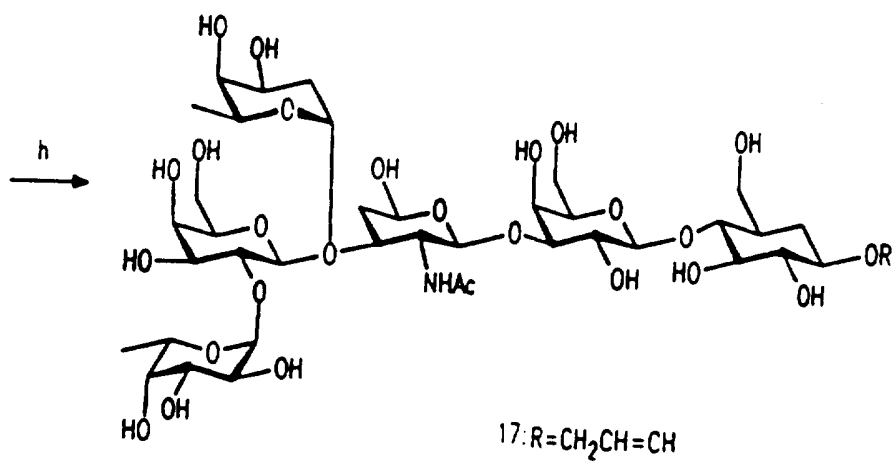


Figure 7b

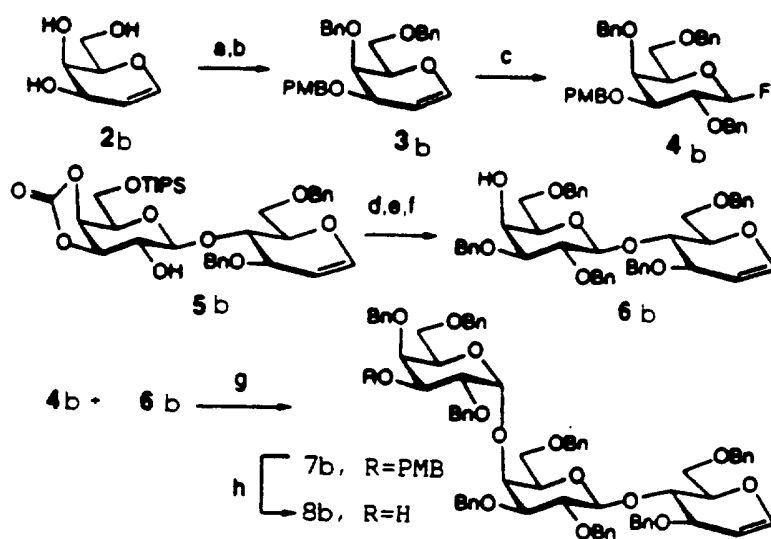
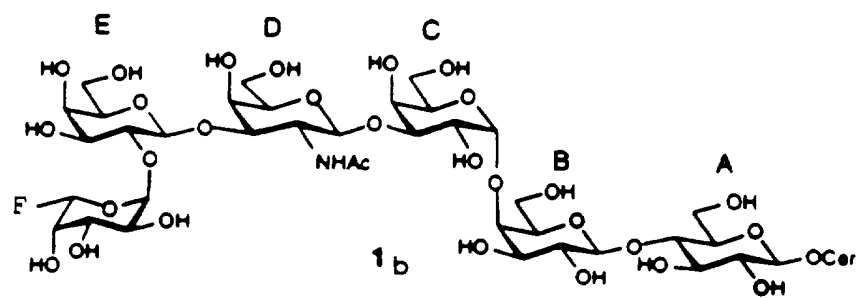


Figure 8

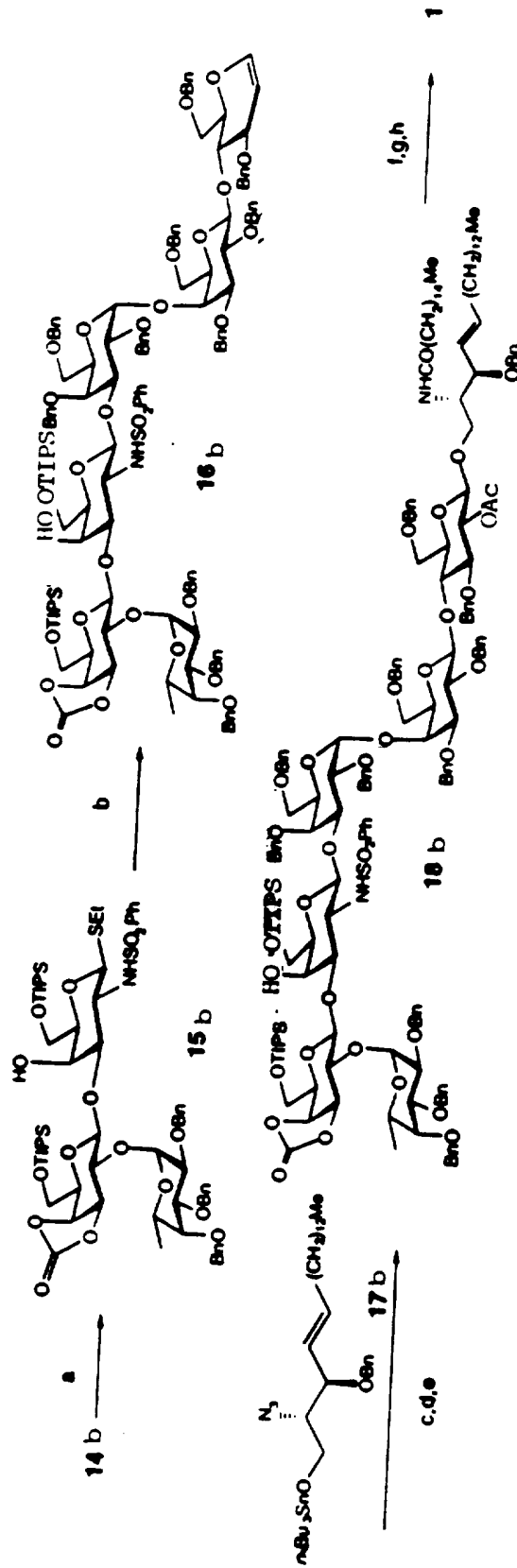


Figure 9

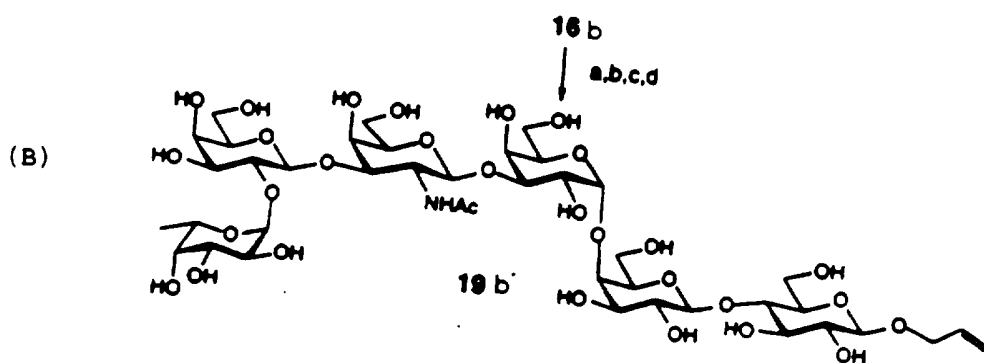
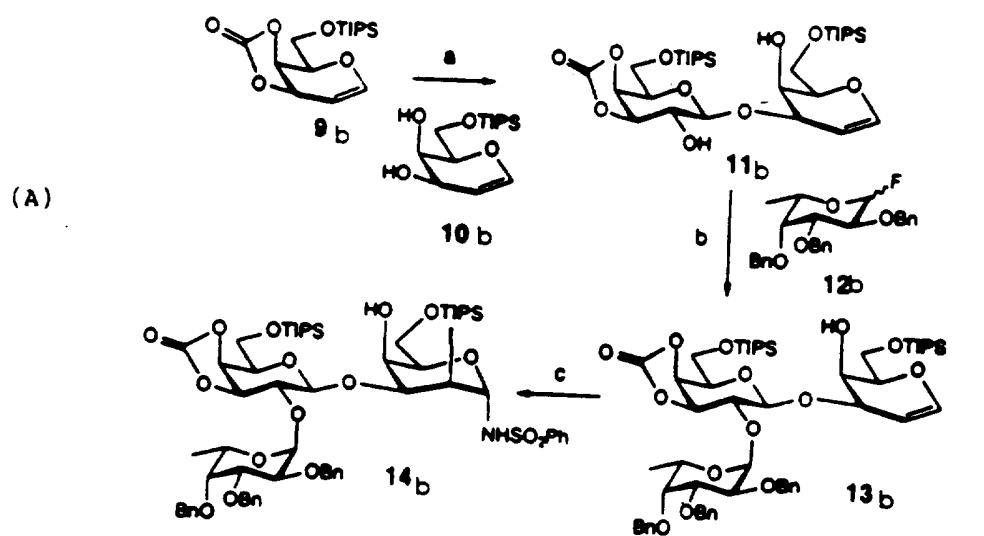


Figure 10

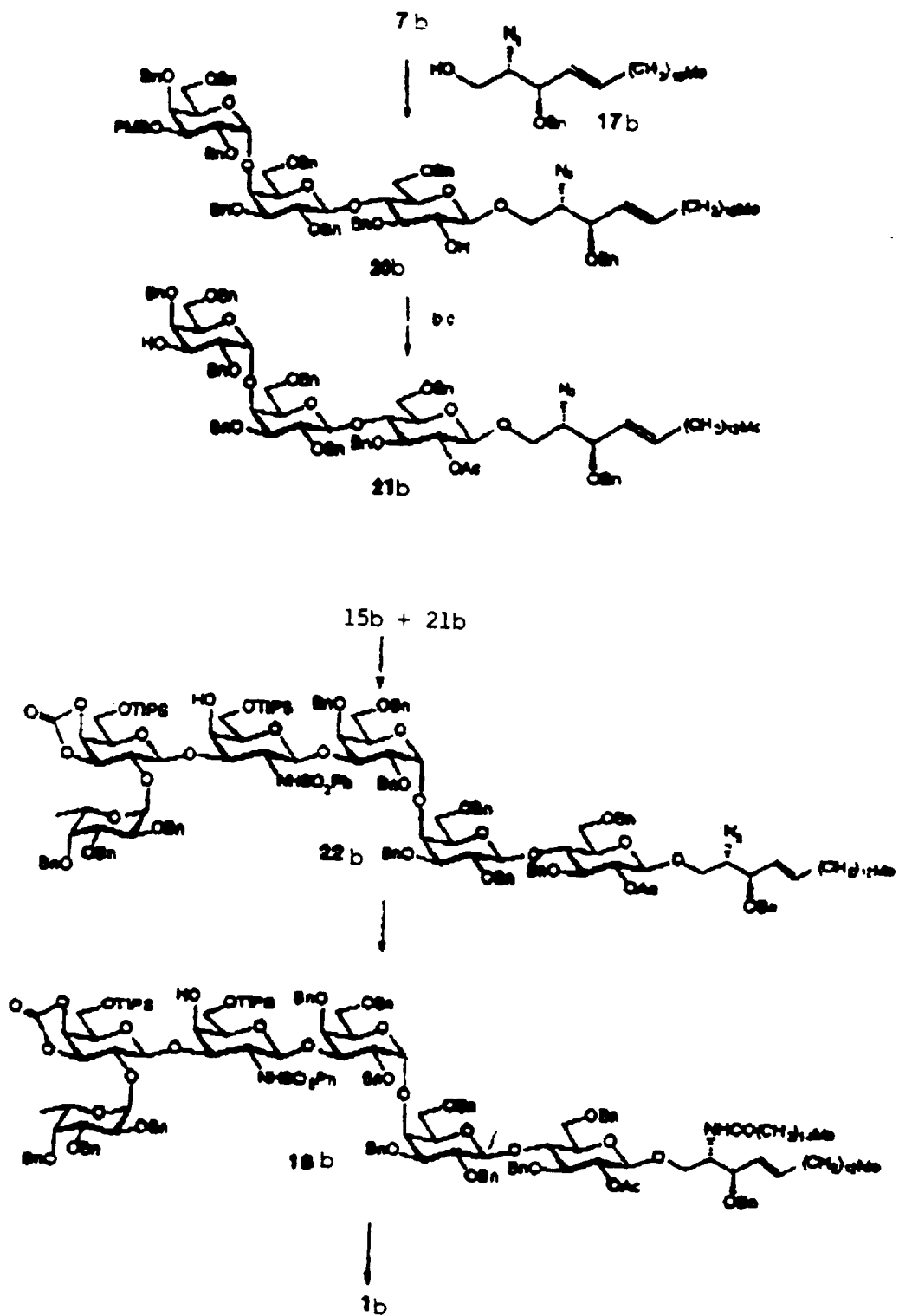


Figure 11

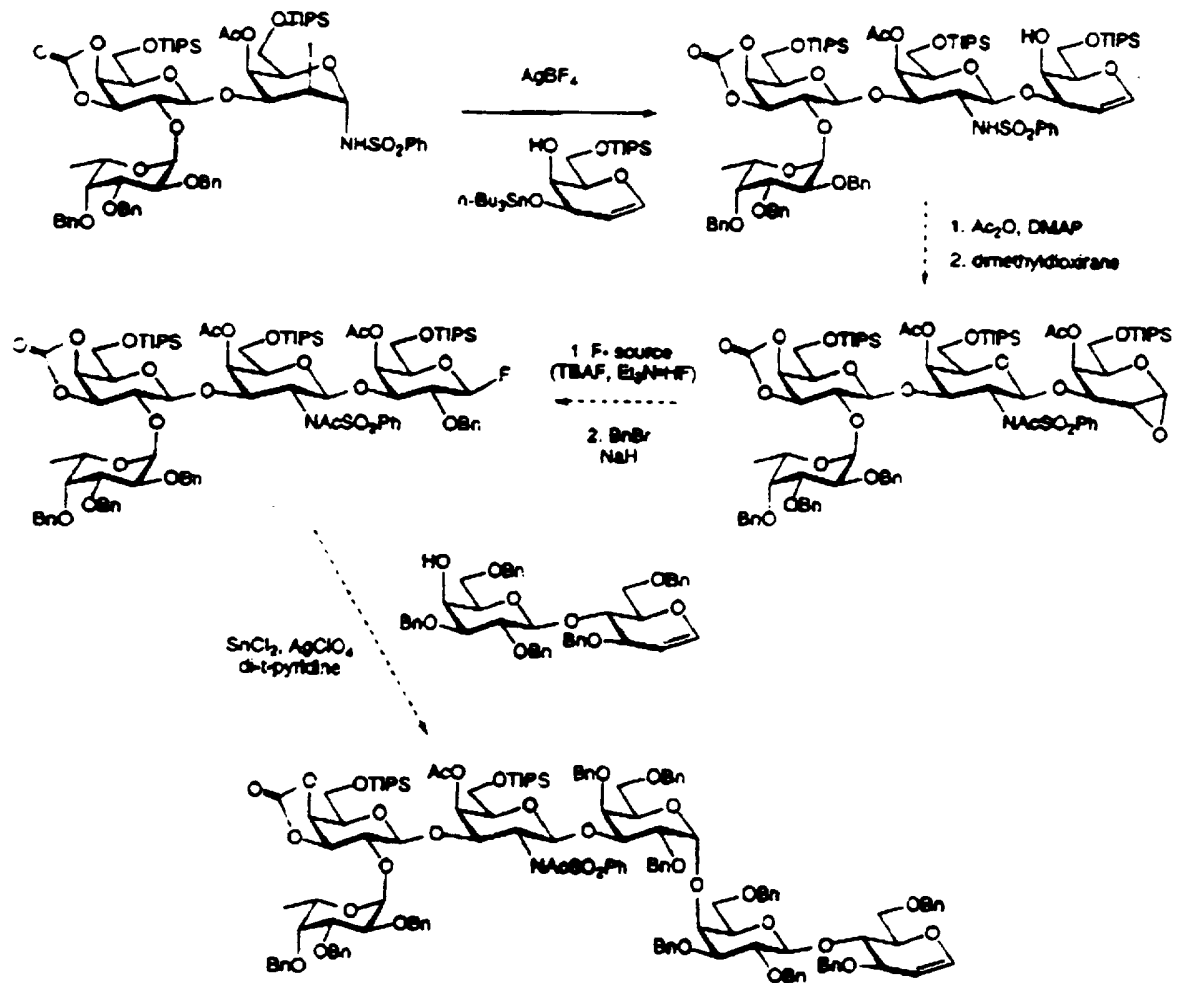


Figure 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06109**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C07H 15/24, 15/00; A61K 39/395, 39/44

US CL : 536/18.1, 18.6; 424/137.1, 138.1

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. : 536/18.1, 18.6; 424/137.1, 138.1

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

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


Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RANDOLPH, J.T. et al. An Interactive Strategy for the Assembly of Complex, Branched Oligosaccharide Domains on a Solid Support: A Concise Synthesis of the Lewis ^b Domain in Bioconjugatable Form. Angew. Chem. Int. Ed. Engl. 1994, Vol. 33, No. 14, pages 1470-1473.	1-10
A, P	BILODEAU, M.T. Total Synthesis of a Human Breast Tumor Associated Antigen. J. Am. Chem. Soc. May, 1995, Vol. 117, pages 7840-7841.	1-10
A	LAY, L. et. al. Oligosaccharides Related to Tumor-Associated Antigens. Helv. Chim. Acta. 1994, Vol. 77, pages 509-514.	1-10

 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 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
"I" 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 12 JULY 1996	Date of mailing of the international search report 30 JUL 1996
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 SUSAN UNGAR  Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06109

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	KIM, I. J. Defining the Molecular Recognition of Globo H (Human Breast Cancer) Antigen through Probe Structures Prepared by Total Synthesis. J. Org. Chem. August 1995, Vol. 60, pages 7716-7717.	1-10
A, P	LIVINGSTON, P.O. Augmenting the immunogenicity of carbohydrate tumor antigens. Seminars in Cancer Biology. December 1995, Vol. 6, pages 357-366.	11-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06109

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN-Bioscience, Structural Searches in STN-REGISTRY, MARPAT, Answer File from REGISTRY AND MARPAT searched in CAPLUS and CAOLD

search terms: oligosaccharide, polysaccharide, vaccine, cancer, MBrl, antibody, immunogen