P/00/001 28/5/91
Section 20

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AUSTRALIA

Patents Act 1990

PATENT REQUEST: STANDARD PATENT/PATENT OF ADDITION

We, being the persons identified below as the Applicant, request the grant of a patent to the person identified below as the Nominated Person, for an invention described in the accompanying standard complete specification.

Full application details follow.

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[70] Nominated Person: HOECHST AKTIENGESELLSCHAFT

Address: D-65926 FRAKFURT AM MAIN, FEDERAL REPUBLIC OF GERMANY

[54] Invention Title: PHYSIOLOGICALLY TOLERATED AND PHYSIOLOGICALLY DEGRADABLE POLYMER-BASED CARBOHYDRATE RECEPTOR BLOCKER, A PROCESS FOR ITS PREPARATION AND ITS USE

[72] Name(s) of actual inventor(s): WILHELM STAHL, MICHAEL AHLERS, AXEL WALCH, ECKHART BARTNIK, GERHARD KRETZSCHMAR, SUSANNE GRABLEY AND RUDOLF SCHLEYERBACH

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BASIC CONVENTION APPLICATION(S) DETAILS

[31] Application Number	[33] Country	Country Code	[32] Date of Application
P42 41 829.1	GERMANY	DE	11TH DECEMBER 1992
P43 26 777.7	GERMANY	DE	10TH AUGUST 1993

Basic Applicant(s): HOECHST AKTIENGESELLSCHAFT

Drawing number recommended to accompany the abstract .....

By our Patent Attorneys,
WATERMARK PATENT & TRADEMARK ATTORNEYS

.....

Darryl B. Mischlewski
Registered Patent Attorney

DATED this 10th day of December 1993.

M25 1 04 . 101293

P/00/008b 12/11/91
Section 29 (1)
Regulation 3.1 (2)

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NOTICE OF ENTITLEMENT

We, **HOECHST AKTIENGESELLSCHAFT** of, D-65926 Frankfurt am Main, Federal Republic of Germany, being the applicant in respect of Application No. **52328/93** state the following:-

The Person nominated for the grant of the patent has entitlement from the actual inventors by assignment.

The person nominated for the grant of the patent is the applicant of the basic applications listed on the patent request form.

The basic applications listed on the request form are the first applications made in a Convention country in respect of the invention.

HOECHST AKTIENGESELLSCHAFT
By our Patent Attorneys,
WATERMARK PATENT & TRADEMARK ATTORNEYS

.....

Karen J. Sinclair
Registered Patent Attorney

12 August 1996

.....

AU9352328

(12) PATENT ABRIDGMENT (11) Document No. AU-B-52328/93
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 672643

(54) Title
PHYSIOLOGICALLY TOLERATED AND PHYSIOLOGICALLY DEGRADABLE POLYMER-BASED CARBOHYDRATE RECEPTOR BLOCKER, A PROCESS FOR ITS PREPARATION AND ITS USE

International Patent Classification(s)
(51)[5] C08F 008/32 A61K 031/70 C07H 015/04

(21) Application No. : 52328/93 (22) Application Date : 10.12.93

(30) Priority Data

(31) Number	(32) Date	(33) Country
4241829	11.12.92	DE GERMANY
4326777	10.08.93	DE GERMANY

(43) Publication Date : 23.06.94

(44) Publication Date of Accepted Application : 10.10.96

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(74) Attorney or Agent
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(56) Prior Art Documents
US 5059654
US 5219926

(57) Claim

1. A carbohydrate-containing polymer comprising:

(i) a hydrophilic polymer selected from the group consisting of poly-D,L-succinimide, poly-α,β-(2-hydroxyethyl)-D,L-aspartamide,poly-D,L-succinimide-poly-α,β-(2-hydroxyethyl)-D,L-aspartamide copolymer, poly-L-lysine methyl ester-fumaramide, polyglutamate, and hydroxyethylstarch;

(ii) a carbohydrate selected from the group consisting of N-acetylglucosamine, sialyl-Lewis X and sialyl-Lewis A; and

(iii) a bifunctional spacer linking said carbohydrate to said hydrophilic polymer.

P/00/011 28/5/91
Regulation 3.2(2)

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ORIGINAL
COMPLETE SPECIFICATION
STANDARD PATENT

Application Number:

Lodged:

Invention Title: PHYSIOLOGICALLY TOLERATED AND PHYSIOLOGICALLY DEGRADABLE POLYMER-BASED CARBOHYDRATE RECEPTOR BLOCKER, A PROCESS FOR ITS PREPARATION AND ITS USE

The following statement is a full description of this invention, including the best method of performing it known to us :-

HOECHST AKTIENGESELLSCHAFT HOE 92/F 400 K Dr.SI/PP

Description

Physiologically tolerated and physiologically degradable polymer-based carbohydrate receptor blocker, a process for its preparation and its use

The invention relates to polymeric carbohydrate receptor blockers which cause no intolerance reactions in vivo either in their entirety or in the form of degradation products, to processes for their preparation and to their use in vivo for blocking carbohydrate-recognizing receptors.

The importance of carbohydrates in physiologically relevant recognition processes has only recently been investigated in detail and elucidated (T. Feizi, Biochem. J. 1987, (245), 1; C.L.M. Stults et al., Meth Enzym. 1989, (179) 167; S. Hakamori, Adv. Cancer Res. 1989, (52), 257; M.P. Bevilacqua et al., Science 1989, (243), 1160).

It was realized that the carbohydrates are the third, besides the proteins and nucleic acids, important information carrier of life because, as a rule, they have a large number of stereocenters which are able to contain a large amount of information. Their arrangement on the cell surface in the form of ligands makes it possible for them to play, on the basis of receptor-ligand bindings, a crucial part in intercellular communication and thus also in intercellular recognition processes.

In principle, intercellular communication is possible on the basis of receptor-ligand binding: water-soluble signal- molecules, that is to say neurotransmitters, protein hormones, growth factors, bind specifically to integral membrane proteins (= receptor proteins) which are located on the cell surface. After the cell surface

receptor has bound the signal molecule (ligand), a signal is transported into the interior of the cell in order thus to induce changes in the metabolism of the receptor-carrying cell. After a varying number of hours, cellular metabolism is returned to the state before complex formation, by metabolic degradation of the receptor-ligand complex (Molecular Biology Of The Cell, 3rd edition, Bruce Alberts et al., Garland Publishing Inc., New York, USA, page 733).

Carbohydrate ligands on the surfaces of mammalian cells (including human cells) are recognition domains for viruses (for example J.C. Paulson, The Receptors, Vol. II, Ed.: P.M. Conn, Academic Press, 1985, 131), bacteria (for example Strömberg et al., EMBO J. 1990, (9), 2001), toxins (for example K. A. Karlsson et al., Sourcebook of Bacterial Protein Toxins, Eds. J.E. Alouf, J.H: Freer, Academic Press, 1991, 435) and lectins (for example M.-F. Michel et al., Appl. Environ. Microbiol. 1990, (56), 3537). Accordingly, they play a crucial part in the initiation of diseases which are based on the recognition of such cell surface determinants (bacterial and viral infections, inflammatory disorders etc.).

The following mechanism takes place in principle:
the first step is the attachment of the virus to the cell membrane of the cell to be infected. This entails the viral spike proteins adhering specifically to ligands on the cell membrane. These spike proteins are generally glycoproteins with a receptor function. In the second step, the viral genome is taken up into the cell by a complex mechanism. For example, it has been shown for infections with influenzaviruses that at the start of infection the virus adheres by means of the glycoprotein hemagglutinin (= receptor) to specific ligands on the cell surface (Virology, Dulbecco, R. and Ginsberg H.S., 2nd edition, page 223).
Bacterial infection also takes place in principle by the mechanism described above.

Receptor-ligand complexes also play a part in inflammatory processes [B.K. Bradley et al., Cell 1990 (63), 861]. Investigations have shown, for example, that the protein ELAM 1 (= receptor) which is synthesized and expressed by endothelial cells in vivo probably mediates the adhesion of leukocytes via a ligand to the inflammatory focus.

The therapy of bacterial or viral disorders with free oligosaccharides which are intended to bind in place of the natural ligands to receptors such as, for example, on viruses or bacterial cells has been impeded by the very large amounts of oligosaccharide to be administered, because the affinity between the receptors and the oligosaccharides is low [K_D = ~ 10^{-4} M for the interaction between a monovalent galactoside and the corresponding lectin; D.T. Connolly et al., J. Biol. Chem. 1982, (257), 939.

It is known that an increased interaction between receptor and ligand is achieved by coupling a plurality of ligands to a "surface". It has been shown, taking the example of the interaction between the viral protein hemagglutinin, which binds to neuraminic acid on the cell surface, how this polyvalent effect due to the use of a polymer affects an interaction of this type [monovalent: K_D = 2 x 10^{-4} M, polyvalent: K_D = 3 x 10^{-7} M; A. Spaltenstein et al., J. Am. Chem. Soc. 1991, (113), 686].

Investigated to date as "surface" have been

a) liposomes [N. Yamazaki, Int. J. Biochem., 1992, (24), 99]
b) polyacrylamides
c) polylysines or
d) sulfated polysaccharides:

a) liposomes are both obtained from cell preparations and prepared from synthetic lipids. The ligand, for

example a carbohydrate, is presented on the liposome surface by a hydrophobic alkyl radical on the carbohydrate being integrated, utilizing hydrophobic interactions, into the liposome membrane. The disadvantages of using liposomes as surface are their low stability in vivo and short residence time in the bloodstream. Liposome preparations, besides other oligomeric and polymeric presentation forms of the carbohydrate portion, are described in WO 91/19501 and WO 91/19502.

b) Polyacrylamide-based polymers which are prepared by polymerization of carbohydrate-containing monomers are unsuitable as carriers of ligands for medical use. The polymer portion is composed entirely of C-C bonds. The crucial disadvantage for in vivo diagnostic/therapeutic use is that they can be degraded in the body to toxic metabolites and thus are not tolerated in vivo.

Examples of such publications are:

- R.C. Rathi et al., [J. Polym. Sci.: Part A: Polym. Chem. 1991, (29), 1895] describe the polymerization of carbohydrate-containing acrylamide monomers for use as carrier of active substances.
- S.-I. Nishimura et al., [Macromolecules 1991, (24), 4236] describe copolymerized pentylglycosides with acrylamide and investigate the interaction of this macromolecule with lectins.

Both R.C. Rathi et al. and S.-I. Nishimura et al. use the polymer only as carrier for a carbohydrate moiety and a pharmacologically active macromolecule. The principle of action is based on a receptor-ligand binding which is as specific as possible so that the pharmacologically active macromolecule can be liberated at the site of action and display its effect.

c) Polylysine is basically composed of physiologically well-tolerated, that is to say nontoxic, units. However, the disadvantage of polylysine is the large number of free NH_2 groups therein which cause non-specific interactions with cells or cell surface structures so that it is normally impossible with polylysine-drug conjugates to achieve a specific effect of the drug at the site of action.

d) Cell adhesion when conventional sulfated polysaccharides are used results from ionic interactions of low specificity so that there is non-specific adhesion to any available cell surfaces. These macromolecules contain no specific receptor blocker.

European Published Specifications 0 089 938, 0 089 939 and 0 089 940 claim sugar compounds of various chain lengths which are identical to ligands located on cell surfaces or receptors located on microorganisms. The inventive idea in the abovementioned published specifications is to block the receptors located on the microorganisms by the claimed sugar compounds in vitro and in vivo and thus to be able to diagnose and treat disorders, especially of a bacterial or viral nature, of mammals, especially of humans. Examples are given of the in vitro diagnosis of bacterial infections, but not of in vivo diagnosis or therapy.

The abovementioned published specifications also claim sugar compounds which are coupled to a carrier, where appropriate via spacers. The complex of sugar compound-(spacer)-carrier or the sugar compound alone is used to prepare antibodies, isolate cell surface structures and disinfect wounds.

Furthermore, the interactions of dextran-fixed β-blockers with β-adrenoreceptors have also been investigated (J. Pitka, J.W. Kusiak, in "Macromolecules as Drugs and as Carriers for biologically active Material", Ed.:

D.A. Tirell, L.G. Donaruma, A.B. Turek; The New York Academy of Sciences, Vol. 446, p. 249). β-Blockers are artificial active substances which, in the present case, are linked via a spacer to the polysaccharide carrier dextran. The blocking of the β-adrenoreceptors takes place not via a specific receptor-ligand binding but via a comparatively non-specific coupling of the active substance.

The Patent Application WO 92/02527 claims a macromolecule which is composed of a carrier and saccharide units and functions as ligand for the ELAM-1 receptor in order to diagnose, by the receptor-ligand binding, inflammatory processes. The diagnosis is carried out in vitro but not in vivo. The saccharide units are carried by a solid carrier. The skilled worker understands a "solid carrier" to mean compounds which must have an inert behavior towards physiological systems since only then are in vitro diagnoses possible.

The object of the invention is to synthesize carbohydrate receptor blockers with improved activity as pharmaceuticals, which are physiologically tolerated as the entire molecule and as degradation products, and which display their diagnostic or therapeutic effect by blocking specific structures.

This object is achieved according to the invention by the synthesis of a polymer-based carbohydrate receptor blocker which is composed of the physiologically well-tolerated components: carbohydrate portion - bifunctional spacer - hydrophilic, biodegradable polymer - potentiator, where the individual components are linked enzymatically, chemically and/or chemoenzymatically, there is no or scarcely any accumulation of the carbohydrate receptor blocker in the body, and it displays its diagnostic or therapeutic effect by blocking specific structures as the entire molecule.

1. A physiologically tolerated and physiologically degradable polymer-based carbohydrate receptor blocker with an HLB* of 10 to 20 containing:
carbohydrate portion - bifunctional spacer - hydrophilic, biodegradable polymer - potentiator, where the carbohydrate portion which is composed of 1 to 20 naturally occurring, identical or different, monosaccharide units is coupled via ~~one or more~~ a bifunctional spacer, which is a natural or synthetic molecule, to a hydrophilic, biodegradable polymer, where the polymer in turn is the carrier of ~~one or more~~ the spacers, and the hydrophilic, biodegradable polymer is ~~additionally~~ also linked to the potentiator which is a crosslinker, one or more groups with hydrophobic, hydrophilic or ionic properties or a solubility improver.

2. Physiologically tolerated and physiologically degradable polymer-based carbohydrate receptor blocker with an HLB* of 10 to 20, obtainable by covalent chemical, chemoenzymatic and/or enzymatic linkage of the following components:

- carbohydrate portion composed of 1-20 naturally occurring, identical or different monosaccharide units,
- bifunctional spacer which is a natural or synthetic molecule,
- hydrophilic, biodegradable polymer and
- potentiator which is a crosslinker, one or more groups with hydrophobic, hydrophilic or ionic properties or a solubility improver.

3. A process for the preparation of the carbohydrate receptor blocker specified under 1., which comprises enzymatic, chemical and/or chemoenzymatic linkage of the carbohydrate portion, bifunctional spacer, hydrophilic or biodegradable polymer and potentiator.

4. The use of the carbohydrate receptor blocker specified under 1. for diagnostic, therapeutic or prophylactic application.

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In particular, the invention relates to a carbohydrate-containing polymer comprising:

(i) a hydrophilic polymer selected from the group consisting of poly-D,L-succinimide, poly-α,β-(2-hydroxyethyl)-D,L-aspartamide,poly-D,L-succinimide-poly-α,β-(2-hydroxyethyl)-D,L-aspartamide copolymer, poly-L-lysine methyl ester-fumaramide, polyglutamate, and hydroxyethylstarch;

(ii) a carbohydrate selected from the group consisting of N-acetylglucosamine, sialyl-Lewis X and sialyl-Lewis A; and

(iii) a bifunctional spacer linking said carbohydrate to said hydrophilic polymer.

It is preferred that the bifunctional spaces is selected from

(i) a 6-aminohexanol-based spacer and

(ii) an N-(6'-hydroxyhexyl)-6-aminohexanoamide-based spacer,

whereby the amino group of said spacer is linked to said polymer via an amide bond, and the hydroxyl group of said spacer is linked to said carbohydrate via a glycosidic bond.

It is also preferred that the carbohydrate-containing polymer includes a potentiator.

The invention also relates to a pharmaceutical comprising a carbohydrate-containing polymer comprising:

(i) a hydrophilic polymer selected from the group consisting of poly-D,L-succinimide, poly-α,β-(2-hydroxyethyl)-D,L-aspartamide,poly-D,L-succinimide-poly-α,β-(2-hydroxyethyl)-D,L-aspartamide copolymer, poly-L-lysine methyl ester-fumaramide, polyglutamate, and hydroxyethylstarch;

(ii) a carbohydrate selected from the group consisting of N-acetylglucosamine, sialyl-Lewis X and sialyl-Lewis A; and

(iii) a bifunctional spacer linking said carbohydrate to said hydrophilic polymer.

AUSTRALIAN PATENT OFFICE

Furthermore, the invention relates to a process for preparing the carbohydrate-containing polymer which comprises the step of coupling the carbohydrate portion with the hydrophilic polymer portion via the functional spacer.

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The invention is described in detail hereinafter, especially in its preferred embodiments.

The carbohydrate receptor blocker is physiologically tolerated and preferably has a molecular weight of ≤ 70 kd. Well tolerated means that the carbohydrate receptor blocker according to the invention does not cause any intolerance reactions which have a considerable influence on the wellbeing of the body.

The term "physiologically tolerated" is defined as follows:
The carbohydrate receptor blocker and the polymer alone must not in vivo be damaging for the body. They must therefore not have hemolytic, antigenic or thrombogenic effects.

In addition, non-specific adsorption onto molecules such as, for example, receptors, cell membrane components, hormones, etc. are undesired because they too may lead to intolerance reactions.
The use of a spacer which causes intolerance reactions is possible if the carbohydrate receptor blocker as a whole or its degradation products resulting in vivo are physiologically tolerated.

The term "physiologically degradable" is defined as follows:
The carbohydrate receptor blocker is in vivo excretable as entire molecule or in vivo degradable to smaller physiologically tolerated units in order to be excretable (= biodegradable) in this form. This particularly applies to the polymer.

The residence time of the carbohydrate receptor blocker on the particular receptor, and thus the duration of the effect of the carbohydrate receptor blocker, is controlled via the degradability of the carbohydrate receptor blocker in vivo. Thus, for example, the

degradability can be controlled by the choice of the polymer units and their linkage. It is thus possible to prevent accumulation in the body when there is an excess of blocker.

The terms "receptor" and "ligand" are defined as follows: The receptor is a macromolecule on the cell surface which, by reason of its configuration, has a binding site for the specific binding of one and rarely a plurality of signal substances. This signal substance is called the ligand. The aim of the specific binding of the ligand to the receptor is the transfer of information into the cell and subsequent alteration in cellular metabolism.

Definition of the HLB*:
The hydrophilicity of the carbohydrate receptor blocker according to the invention is characterized by the HLB*. The HLB* defines the ratio of the hydrophilic and hydrophobic portions of a molecule using a scale from 1 to 20 (R. Heusch, Kolloid-Zeitschrift und Zeitschrift für Polymere, Vol. 236, No. 1, pages 31 et seq.).

The HLB* of the carbohydrate receptor blocker according to the invention is in the present case from 10 to 20, but preferably from 14 to 20.

Oligosaccharides are defined as sugars which are composed of ≤ 20 naturally occurring monosaccharide units.

The term "polymer-based" means that the polymer is a component of the carbohydrate receptor blocker which is linked to the other components of spacer and potentiator.

Definition of the term "degree of loading of the polymer with the carbohydrate portion via spacer": the degree of loading indicates the percentage (in %) of the repeating units of the polymer to which the carbohydrate portion is linked via spacers. The percentage is based on the totality of repeating units.

Repeating units mean the smallest regularly recurring unit which completely describes the degradation of a chain (Makromoleküle, Vol. 1, 5th edition, Hüthig & Wepf Verlag, Basle, Heidelberg, New York, 1990).

"Solubility improver" is a chemical group which improves the solubility of the carbohydrate receptor blocker.

It is characteristic of the carbohydrate receptor blocker according to the invention that it exerts its function as receptor blocker as entire molecule and not on the basis of the activity of substructures. The consequence of this is that the carbohydrate portion is active in coupled form, and the polymer portion acts not only as "transport vehicle" to the receptor but also as active moiety.

The carbohydrate receptor blocker is synthesized on the laboratory scale. This means that the carbohydrate receptor blocker according to the invention is synthesized in milligram quantity to gram quantity while the intermediates required for its synthesis, i.e. the hydrophilic biodegradable polymer, the bifunctional spacer and the potentiator, can be prepared in gram to kilogram quantity. However, the carbohydrate portion forms an exception. It can be synthesized only in a quantity from milligrams up to one gram. The synthetic strategies known from the literature for the preparation of oligosaccharides are in this connection designed so that a reaction which does not normally take place with quantitative yield is followed by purification of the resulting mixture of products by column chromatography on silica gel.

This purification process is generally too costly and elaborate for the preparation of quantities meeting industrial needs and is at the most employed for purifying final products or valuable intermediates. In addition, heavy metal compounds are very often used for the synthesis of oligosaccharides. There are great objections to the use for the synthesis of substances with a

pharmaceutical effect with a view to future authorization of the carbohydrate receptor blocker as pharmaceutical.

CARBOHYDRATE PORTION:

Carbohydrates are defined as all compounds which have a structure corresponding to the formula $C_n(H_2O)_n$, as well as polyhydroxyaldehydes, ketones, acids and amines and their naturally occurring derivatives.

The carbohydrate portion is composed of 1-20 naturally occurring monosaccharide units. Preferably 1-15, and particularly preferably 1-10, naturally occurring monosaccharide units are used.

Naturally occurring monosaccharide units are known to the skilled worker from standard works of organic chemistry or biochemistry, thus, for example from: Beyer/Walter, "Lehrbuch der organischen Chemie" (Textbook of organic chemistry), S. Hirzel Verlag Stuttgart, 19th edition, pages 393 et seq.; Albert L. Lehninger, "Biochemistry", 2nd edition, pages 249 et seq., Worth Publishing Inc., New York or Lubert Stryer, "Biochemie" (Biochemistry), Spektrum-der-Wissenschaft-Verlagsgesellschaft GmbH, Heidelberg, Germany, 1st edition, pages 345 et seq. Examples of monosaccharide units are the D and L configurations of aldoses:

- glyceraldehyde, erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, mannose, gulose, idose, galactose and talose;

and/or

of ketoses:

- dihydroxyacetone, erythrulose, ribulose, xylulose, psicose, fructose, sorbose and/or tagatose.

The term "naturally occurring" monosaccharide units also includes those monosaccharides which represent naturally occurring substitutions of the stated examples. The

skilled worker understands this to mean, preferably, deoxy sugars, very preferably 2-, 3-, 4- or 6-deoxyaldoses such as, for example, fucose, rhamnose, digitoxose and/or very preferably 2-, 3-, 5-, 6-deoxyketoses, preferably deoxyamino sugars such as, for example, glucosamine, mannosamine, galactosamine, preferably deoxyacylamino sugars such as, for example, N-acetylglucosamine, N-acetylmannosamine, N-acetylgalactosamine, preferably aldonic, aldaric and/or uronic acids such as, for example, gluconic acid or glucuronic acid. It is also possible to use: ascorbic acid, amino-acid-carrying monosaccharides and monosaccharides which carry lipid, phosphatidyl or polyol residues.

The skilled worker also understands "naturally occurring" substituted monosaccharides to mean those with a carbon chain which is longer than 6 carbon atoms, as well as representatives thereof substituted in accordance with the criteria detailed above, such as, for example, ketodeoxyoctonic acid, ketodeoxynononic acid, N-acylneuraminic acids, N-acetylmuramic acid.

Preferred carbohydrate portions are those which occur on cell surfaces as component of glycoproteins, glycolipids or proteoglycans, as well as any desired segments thereof.

Particularly preferred carbohydrate portions are those composed of monosaccharides which also occur in the human body, such as glucose, N-acetylglucosamine, galactose, N-acetylgalactosamine, mannose, fucose, N-acetylneuraminic acid and/or glucuronic acid.

The monosaccharide units forming the carbohydrate portion can be identical or different. In addition, the stereochemistry of the glycosidic linkage (axial or equatorial, or α or β) of the individual monosaccharide units can be identical or different.

The complete carbohydrate portion can be composed, for example, of the following sugar residues:

Galß1-4GlcNAc-;

Galß1-3GlcNAc-;

SAα2-6Galß1-4GlcNAc-;
SAα2-3Galß1-4GlcNAc-;
SAα2-3Galß1-3GlcNAc-;
Galß1-4(Fucα1-3)GlcNAc-;
Galß1-3(Fucα1-3)GlcNAc-;
SAα2-3Galß1-3(Fucα1-4)GlcNAc-;
SAα2-3Galß1-4(Fucα1-3)GlcNAc-;

Galß1-4GlcNAcß1-4GlcNAc-;
Galß1-3GlcNAcß1-4GlcNAc-;
SAα2-6Galß1-4GlcNAcß1-4GlcNAc-;
SAα2-3Galß1-4GlcNAcß1-4GlcNAc-;
SAα2-3Galß1-3GlcNAcß1-4GlcNAc-;
Galß1-4(Fucα1-3)GlcNAcß1-4GlcNAc-;
Galß1-3(Fucα1-4)GlcNAcß1-4GlcNAc-;
SAα2-3Galß1-3(Fucα1-4)GlcNAcß1-4GlcNAc-;
SAα2-3Galß1-4(Fucα1-3)GlcNAcß1-4GlcNAc-;
SAα2-3Galß1-3(Fucα1-4)GlcNAcß1-4Gal-;
SAα2-3Galß1-4(Fucα1-3)GlcNAcß1-4Gal-;

SAα2-3Galß1-4GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAc;
SAα2-6Galß1-4GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAc;
SAα2-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4Glc;
SAα2-6Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4Glc;
SAα2-3Galß1-4GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4Glc;
SAα2-6Galß1-4GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4Glc;
SAα2-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4Glc;
SAα2-6Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4Glc;
SAα2-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;
SAα2-6Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;
SAα2-3Galß1-4GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;
SAα2-6Galß1-4GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;

SAα2-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;

SAα2-6Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;

SAα2-3Galß1-3(Fucα1-4)GlcNAc;

SAα2-6Galß1-3(Fucα1-4)GlcNAc;

SAα2-3Galß1-3GlcNAcß1-4Galß1-4(Fucα1-3)GlcNAc;

SAα2-6Galß1-3GlcNAcß1-4Galß1-4(Fucα1-3)GlcNAc;

SAα2-3Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAc;

SAα2-6Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAc;

SAα2-3Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4Glc;

SAα2-6Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4Glc;

SAα2-3Galß1-3GlcNAcß1-4Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4Glc;

SAα2-6Galß1-3GlcNAcß1-4Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4Glc;

SAα2-3Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4Glc;

SAα2-6Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4Glc;

SAα2-3Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;

SAα2-6Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;

SAα2-3Galß1-3GlcNAcß1-4Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;

SAα2-6Galß1-3GlcNAcß1-4Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;

SAα2-3Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;

SAα2-6Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-4(Fucα1-3)Glc;

SA2-3Galß1-4GlcNAcß1-3Galß1-3(Fucα1-4)GlcNAc;

SA2-6Galß1-4GlcNAcß1-3Galß1-3(Fucα1-4)GlcNAc;

SA2-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-3(Fucα1-4)GlcNAc;

SA2-6Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-3(Fucα1-4)GlcNAc;

SAα2-3Galß1-4GlcNAcß1-3Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4Glc;

SAα2-6Galß1-4GlcNAcß1-3Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4Glc;

SAα2-3Galß1-4(Fucα1-3)GlcNAcß1-3Galß1-3(Fucα1-4)GlcNAcß1-3Galß1-4Glc;

SAα2-6Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc;
SAα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-3(Fucα1-4)Glc;
SAα2-6Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-3(Fucα1-4)Glc;
SAα2-3Galβ1-4GlcNAcβ1-3Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc;
SAα2-6Galβ1-4GlcNAcβ1-3Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc;
SAα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc;
SAα2-6Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc.

[GlcNAcβ-1-3Galβ1-4]$_n$GlcNAc-, where n is a number from the series from 1 to 8;
[GlcNAcβ1-3Galβ1-4]$_n$GlcNAcβ1-4GlcNAc-, where n is a number from the series from 1 to 8;
Galβ1-4[GlcNAcβ1-3Galβ1-4]$_n$GlcNAc-, where n is a number from the series from 1 to 8;
Galβ1-3[GlcNAcβ1-3Galβ1-4]$_n$GlcNAc-, where n is a number from the series from 1 to 8;
SAα2-6Galβ1-4[GlcNAcβ1-3Galβ1-4]$_n$GlcNAc-, where n is a number from the series from 1 to 8;
SAα2-3Galβ1-4[GlcNAcβ1-3Galβ1-4]$_n$GlcNAc-, where n is a number from the series from 1 to 8;
SAα2-3Galβ1-3[GlcNAcβ1-3Galβ1-4]$_n$GlcNAc-, where n is a number from the series from 1 to 4;
Galβ1-4(Fucα1-3)GlcNAcβ1-3[Galβ1-4(Fucα1-3)$_m$-GlcNAcβ1-3]$_n$-Galβ1-4GlcNAc-, where m is a number from the series from 0 to 1 and where n is a number from the series from 1 to 4;
Galβ1-3(Fucα1-4)GlcNAcβ1-3[Galβ1-4(Fucα1-3)$_m$-GlcNAcβ1-3]$_n$-Galβ1-4GlcNAc-, where m is a number from the series from 0 to 1 and where n is a number from the series from 1 to 8;
SAα2-3Galβ1-3(Fucα1-4)GlcNAcβ1-3[Galβ1-4GlcNAcβ-3]$_n$-Galβ1-4GlcNA-, where n is a number from the series from 1 to 8;
SAα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3[Galβ1-4(Fucα1-3)$_m$-GlcNAcβ1-3]$_n$-Galβ1-4GlcNAc-, where m is a number from the series from 0 to 1 and where n is a number from the series from 1 to 2.

Galβ1-4[GlcNAcβ1-3Galβ1-4]$_n$GlcNAcβ1-4GlcNAc-, where n is a number from the series from 1 to 8;
Galβ1-3[GlcNAcβ1-3Galβ1-4]$_n$GlcNAcβ1-4GlcNAc-, where n is a number from the series from 1 to 8;
SAα2-6Galβ1-4[GlcNAcβ1-3Galβ1-4]$_n$GlcNAcβ1-4GlcNAc-, where n is a number from the series from 1 to 8;
SAα2-3Galβ1-4[GlcNAcβ1-3Galβ1-4]$_n$GlcNAcβ1-4GlcNAc-, where n is a number from the series from 1 to 8;
SAα2-3Galβ1-3[GlcNAcβ1-3Galβ1-4]$_n$GlcNAcβ1-4GlcNAc-, where n is a number from the series from 1 to 8;
Galβ1-4(Fucα1-3)GlcNAcβ1-3[Galβ1-4(Fucα1-3)$_m$GlcNAcβ1-3]$_n$-Galβ1-4GlcNAcβ1-4GlcNAc-, where m is a number from the series from 0 to 1 and where n is a number from the series from 1 to 4;
Galβ1-3(Fucα1-4)GlcNAcβ1-3[Galβ1-4(Fucα1-3)$_m$GlcNAcβ1-3]$_n$-Galβ1-4GlcNAcβ1-4GlcNAc-, where m is a number from the series from 0 to 1 and where n is a number from the series from 1 to 4;
SAα2-3Galβ1-3(Fucα1-4)GlcNAcβ1-3[Galβ1-4GlcNAcβ1-3]$_n$-Galβ1-4GlcNAcβ1-4GlcNAc-, where n is a number from the series from 1 to 6;
(GlcNAcβ1-3Galβ1-4)$_n$GlcNAcβ1-3Gal, where n is a number from the series from 1 to 8;
SAα2-6Gal-;
SAα2-6Galβ1-4(GlcNAcβ1-3Galβ1-4)$_n$GlcNAcβ1-3Gal, where n is a number from the series from 1 to 10;
SAα2-3Gal-; and
SAα2-3Galβ1-4(GlcNAcβ1-3Galβ1-4)$_n$GlcNAcβ1-3Gal-, where n is a number from the series from 1 to 10.

Examples of preferred embodiments of the carbohydrate portion are:
sialyl-Lewis X, sialyl-Lewis A, VIM-2 and the following blood-group determinants Lewis A, B, X, Y and A type^1, A type^2, B type^1, B type^2 and H type^1, H type^2 (R.U.Lemieux, Chem. Soc. Rev., 1978, page 423 and 1989, page 347).

Examples of particularly preferred embodiments of the

carbohydrate portion are sialyl-Lewis X, sialyl-Lewis A or VIM-2.

The formula of sialyl-Lewis X is: NeuNAcα2-3Galβ1-4(Fucα1-3)GlcNAc and of sialyl-Lewis A: NeuNAcα2-3Galβ1--3(Fucα1-4)GlcNAc. The formula of VIM-2 is: NeuNAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAc;

SPACER:

The spacer is a physiologically tolerated, natural or synthetic compound which has the task of spatially separating the carbohydrate portion and the biodegradable polymer. This spatial separation is necessary to prevent steric interactions between carbohydrate portion and polymer and thus to ensure optimal accessibility of the ligand (carbohydrate portion) for a receptor.

In order to ensure coupling of the spacer to the carbohydrate portion and the polymer, the spacer must be bifunctional.

The term "bifunctional spacer" is defined as follows: a bifunctional spacer carries at both ends reactive functional units which are used for linking the spacer to the polymer portion on the one hand and to the carbohydrate portion on the other hand, with the formation of a covalent bond.

The spacer can be a naturally occurring molecule or a synthetic molecule. By "synthetic" is meant molecules which do not occur in nature.

It is preferred that ~~In principle~~ the spacer has the following formula: (carbohydrate portion)-A_1-[B-C_x]$_y$-D_z-A_2-(biodegradable, hydrophilic polymer-potentiator), where

x is 0 or 1;

y is 1, 2, 3, 4, 5, 6 or 7;

z is 0 or 1, with the proviso that z can be 1 only when x is 1;

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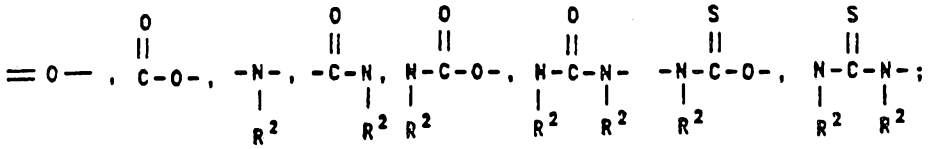
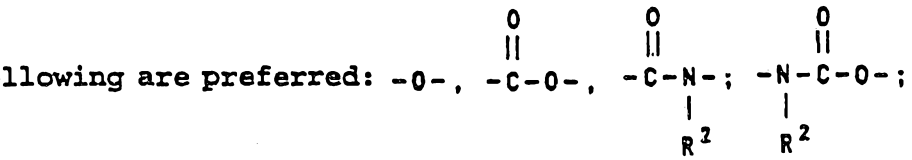
B and D can be identical or different and are alkylene with 0 to 30 carbon atoms,
preferably 0 to 15 carbon atoms, and particularly preferably 0 to 10 carbon atoms, which can be straight-chain, branched or cyclic, are unsubstituted or substituted and can contain single and/or double bonds or 1 to 3 aromatic rings.

B and D can, independently of one another, preferably contain the following substituents: hydroxyl, alkoxy, carboxyl, amino or carboxyalkyl groups of the formula CO_2-R^1 where R^1 is H or C_1-C_6-alkyl, preferably R^1 is H or C_1-C_4-alkyl, and particularly preferably R^1 is H or C_1-C_2-alkyl;
C is a substituent of the following formula:



The following are preferred: 

where R^2 can be, independently of one another, hydrogen, methyl, ethyl, benzyl, propyl, acetyl or benzoyl.

The repetitive units defined by the substituents y can be identical or different.

A_1 and A_2 can be identical or different.
A_1/A_2 is defined as part of the spacer which joins the spacer to the carbohydrate portion and to the biodegradable hydrophilic polymer.

A_1/A_2 arise from the covalent linkage of, in each case, one reactive to one activated group.

The terms "reactive group" and "activated group" are defined as follows:

"reactive groups" are functional groups which act as donor, preferably OH, NH_2 of SH functionalities, which react with "activated groups" to form a covalent bond. Particularly preferred are NH_2 or the OH group.

"activated groups" are functional groups which act as acceptor and react with the "reactive groups" to form a covalent bond. Preferably used are bromides, iodides, active esters, particularly preferably p-nitrophenyl or N-hydroxysuccinimide derivatives. Also preferred are carbonyl chlorides and imidazolides of carboxylic acids, mixed carboxylic anhydrides, particularly preferably composed of the following units:



where R^3 is C_1-C_6, straight-chain or branched, as well as phenyl radicals. Preferably used are aldehydes, acrylic esters, acrylamides, maleimide, succinimide, 2-vinylpyridine, iodoacetic esters, isothiocyanate and/or isocyanate. Particularly preferred are N-hydroxysuccinimide active radicals, maleimide, succinimide, acrylamides, aldehyde or isocyanate.

Reactive groups are components of the polymer because the

polymer is composed of monomers which contain reactive groups.

Activated groups are either already present as part of the polymer or can be prepared by processes known to the skilled worker (see, inter alia, R.C. Larock: Comprehensive Organic Transformations, VCK-Verlagsgesellschaft, 1989, Weinheim/Germany) from reactive groups which are already part of the polymer.

HYDROPHILIC, BIODEGRADABLE POLYMER:

Polymers which, in their function as carriers, are component of carbohydrate receptor blockers and are intended for diagnostic or therapeutic use must in principle meet a number of requirements in order to be physiologically well tolerated:

a) to induce no immune response and

b) in order to avoid non-specific interactions and accumulation in the RES (reticuloendothelial system).

By definition, the biodegradable, hydrophilic polymer is composed of at least two identical or different monomer units which are linked together linearly, in branched fashion, in the form of a ring (for example macrocycles), in the form of a star (dendrimers) or in the form of a ball (for example fullerenes) and may display a molecular weight distribution.

Polymers suitable for the preparation of polymeric carbohydrate receptor blockers are: polyamides, polycarbonates, polyiminocarbonates, polyanhydrides, polyorthoesters, polyesters, polydioxanones, polyhydroxycarboxylic acids, polyamino-acids, polyphosphazenes and polysaccharides.

The biodegradable, hydrophilic polymer is preferably a polyamino-acid, a polyhydroxycarboxylic acid linked as

polyester, polyamide or -anhydride, or a polysaccharide preferably having a molecular weight ≤ 70 kd. The polymer preferably has a minimum size of 2 kd in order to achieve a residence time in the blood which is increased in comparison with low molecular weight carriers.

Suitable polymers which are particularly preferred for the preparation of polymeric carbohydrate receptor blockers are the polyaspartate-amides, polysuccinimides, polyglutamates and polylysine-fumaramides belonging to the polyamino-acids, the functional polyhydroxycarboxylic acids which belong to the polyhydroxycarboxylic acids and are based on malic acid, tartaric acid or citric acid in the form of their polyesters, polyamides or polyanhydrides, as well as substituted chitosans, heparins, hyaluronic acids or starch derivatives.

The degree of loading of the polymer with the carbohydrate portion (via spacer) is between 0.5 and 50%, preferably between 2 and 25%.

The density of occupation of the polymer molecule varies depending on the receptor molecule to be blocked:

- A polymer can be the carrier of a plurality of carbohydrate portions coupled via spacers or carry only one single carbohydrate portion. The carbohydrate portions can be identical or different. The number of spacer-carbohydrate portion complexes carried by the polymer depends on the medical use and activity of the carbohydrate receptor blocker according to the invention.
- It is possible for a plurality of these carbohydrate portion-carrying polymers to be connected together.

POTENTIATOR:

The potentiator is a compound which is covalently linked to the carbohydrate portion-ligand-polymer complex. This compound is an intramolecular crosslinker, a hydrophobic, hydrophilic or ionic compound or else a solubility

improver. It has the task of influencing the conformation of the polymer in order to achieve an improved presentation of the ligand on the polymer and thus also an improved accessibility of the ligand to the receptor. In addition, it is possible with the aid of the potentiator to improve the deficient solubility of the carbohydrate receptor blocker.

Intramolecular crosslinkers carry at both ends reactive, functional units which are used for the covalent linkage to two reactive, functional units which are part of the polymer.

An intramolecular crosslinker has the following formula: (hydrophilic, biodegradable polymer)

$$A_1-[B-C_x]_yD_z-A_2-$$

where

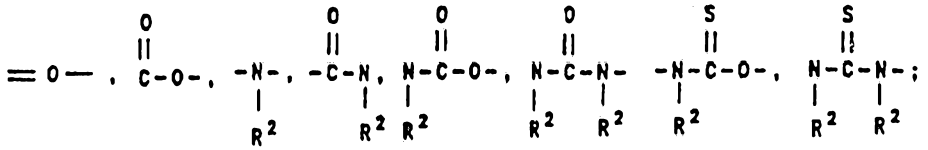
x is 0 or 1;

y is 1, 2;

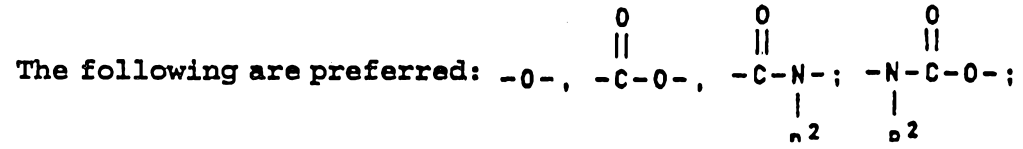
z is 0 or 1 with the proviso that z can only be 1 when x is 1;

B or D are identical or different and are alkylene with 0-20 carbon atoms, preferably 0-15 carbon atoms and particularly preferably 0-10 carbon atoms, which can be straight-chain or branched or cyclic, are unsubstituted or substituted and can contain single and/or double bonds or 1-2 aromatic rings. Preferred substituents for B or D are additionally hydroxyl, alkoxy, amino, carboxyl and carboxyalkyl groups of the formula CO_2R^4 where R^4 is H or C_1-C_6, particularly preferably C_1-C_4, and very particularly preferably C_1-C_2 or preferably carbonylamino and carbonylaminoalkyl groups of the formula $CONR^5R^6$ where R^5 and R^6 can be identical or different and are H or C_1-C_4-alkyl; R is particularly preferably H or C_1-C_2-alkyl;

C is a substituent of the following formula:



where R^2 can, independently of one another, be hydrogen, methyl, ethyl, benzyl, propyl, acetyl or benzoyl.



A_1 and A_2 have the meanings already mentioned for the spacer.

The repetitive units defined by the substituent y can be identical or different.

Hydrophobic, hydrophilic and ionic or ionizable groups are substituents which carry a reactive functional unit and can react with another reactive functional unit of the polymer portion to form a covalent bond. The number of groups employed depends on the extent to which the conformation of the polymer is to be influenced.

Hydrophobic substituents are molecules which have a hydrophobic surface and which carry a reactive functional unit such as, for example, alcohols, amines, active esters or acid chlorides of cyclododecane, adamantane, 2-methylpropane, 2,2-dimethylpropane and the fullerenes.

Hydrophilic substituents are molecules which have a hydrophilic surface and which carry a reactive functional unit such as, for example, cyclodextrin, mono- and oligosaccharides.

Ionic or ionizable substituents are molecules which

contain polar, ionic or ionizable groups and carry a reactive functional unit such as, for example, purines, pyridines, lysines and compounds with a plurality of carboxylic, phosphonic or sulfonic acid groups on a short alkyl chain (C_1-C_8) which can be branched, unbranched or cyclic and contains a reactive group, such as, for example, tartaric acid and aspartic acid.

Solubility enhancers carry a reactive functional unit which is used for covalent linkage to a reactive functional unit which is part of the polymer. They are polar compounds which are readily soluble in water, such as polyols, polyethylene glycols, amino alcohols or compounds with a plurality of carboxylic, phosphonic or sulfonic acid groups on a short alkyl chain, preferably C_1-C_8, which can be branched, unbranched or cyclic.

In addition to the abovementioned compounds, it is possible to couple substituents in the form of a marker group and/or of a drug to the polymer.

The marker group makes it possible to use the carbohydrate receptor blocker for in vivo diagnoses. The coupling of the marker to the polymer takes place via covalent bonds. All the markers known to the skilled worker for in vivo diagnoses can be used for this purpose, such as, for example, radioactive markers which contain a bound radionuclide, for example technetium, X-ray contrast agents which contain, for example, an iodinated compound, as well as magnetic resonance contrast agents, for example based on gadolinium compounds. The proportion of marker in the entire molecule is < 1%.

The drug coupled to the polymer is in each case known for the therapy of the disorder to be treated. The coupling of the drug to the polymer takes place via covalent or ionic bonds.

The following can be used as drugs:

- antitumor agents such as, for example, daunomycin,

doxorubicin, vinblastine, bleomycin;

- antibiotics such as, for example, penicillins, erythromycins, azidamfenicol, cefalotin and griseofulvin;
- immunomodulators such as, for example, FK-506, azathioprine, levamisole;
- antagonists of blood platelet activation factors;
- leukotriene antagonists;
- inhibitors of the cyclooxygenase system such as, for example, salicylic acid compounds;
- lipoxygenase inhibitors,
- antiinflammatory agents such as, for example, indomethacin;
- antirheumatic agents such as, for example, nifenazone.

If the receptor blockers are employed as antiadhesion therapeutic agents, the aim is that, in the case of inflammations, they prevent the ELAM-1 receptors on stimulated endothelial cells binding to sialyl-Lewis X structures on the surface of leukocytes. In the case of influenza therapy, the carbohydrate receptor blockers prevent the adhesion of viruses to the neuraminic acid on the cell surface and thus also the endocytosis of the virus particles.

The carbohydrate receptor blockers are able to react with all naturally occurring receptors which specifically recognize in vivo the carbohydrate portion of ligands.

These are preferably receptors which are expressed on the cell surface, for example by mammalian cells, including human cells, bacterial cells or viruses. Also preferred are hormones and toxins, and recognizing receptors which recognize hormones or toxins.

Particularly preferred cell surface receptors are those which belong to the class of selectins.

Very particularly preferred receptors are those expressed in inflammatory disorders, for example Leu-8 (= L-selectin =gp90^{mel} = LAM-1 = LEC-CAM-1), ELAM-1 (= E-selectin) and GMP-140 (= P-selectin = CD62 = PADGEM).

PREPARATION OF THE CARBOHYDRATE RECEPTOR BLOCKER ACCORDING TO THE INVENTION:

The carbohydrate receptor blocker according to the invention is composed of carbohydrate portion, spacer, polymer and potentiator.

In principle, the carbohydrate receptor blocker is synthesized by forming a covalent bond between carbohydrate portion and bifunctional spacer, subsequent covalent linkage of the carbohydrate portion-spacer complex to the polymer and final covalent linkage to the potentiator.

The linkage can alternatively take place in another sequence:

- Reactions of the polymer with the potentiator on the one hand and of the carbohydrate portion with the bifunctional spacer on the other hand take place to form covalent bonds. The finished carbohydrate receptor blocker results from covalent linkage of the complex polymer-potentiators to the carbohydrate portion-spacer.

- Covalent linkage of polymer and potentiator results in a compound which is reacted with the spacer to form a covalent bond. Covalent reaction of this compound with the carbohydrate portion results in the carbohydrate receptor blocker according to the invention.

- The carbohydrate receptor blocker can also be synthesized in such a way that a complex produced by

covalent linkage of the polymer to the bifunctional spacer is first covalently linked to the carbohydrate portion and subsequently reacted with the potentiator to form a covalent bond.

- Another alternative for the synthesis of the carbohydrate receptor blocker comprises covalent linkage of the polymer to the spacer in the first reaction step, followed by covalent linkages to the potentiator in the next and to the carbohydrate portion in the last step.

The first-mentioned synthetic route and the first-mentioned alternative synthetic route are preferred.

The synthesis of the carbohydrate receptor blocker by covalent linkage of its components takes place by reaction of reactive with activated groups. The distribution of reactive and activated groups on the four components is in this case of no importance for the general practicability of the synthesis.

The reaction between reactive and activated groups takes place by processes known to the skilled worker, by esterification, amidation, addition onto a double bond and alkylation (R.C. Larock: Comprehensive Organic Transformation, VCH-Verlagsgesellschaft, Weinheim, Germany, 1989, L.-F. Tietze and Th. Eichler, Reaktionen und Synthesen im Org.-chem. Praktikum (Reactions and Syntheses in Practical Org.Chem.), G. Thieme Verlag, Stuttgart, Germany, 1981).

CARBOHYDRATE PORTION AND ITS LINKAGE TO THE SPACER:

The carbohydrate portion which is linked via a spacer covalently to the biodegradable polymer which carries a potentiator can originate from natural sources or be prepared chemically, chemoenzymatically or enzymatically.

Suitable natural sources for carbohydrates are known to the skilled worker and can be looked up in the biochemical literature (for example Biochemistry, Biol. Chem. Hoppe-Seyl. J. Biol. Chem. Biochemistry and Cell Biology, Cell). Established processes known to the skilled worker for the purification of oligosaccharides are likewise described therein.

Carbohydrates originating from natural sources are always in the form with a "free reducing end".

Processes for the chemical, enzymatic or chemoenzymatic synthesis of carbohydrates which are recognized by cell surface receptors are known to the skilled worker from the chemical literature and from review articles. For the chemical synthesis for example Carbohydrate Research, Elsevier Science Publishers B.U., Amsterdam, Journal of Carbohydrate Chemistry, Marcel Dekker, Inc., New York; H. Paulsen, Angewandte Chemie 1982 (94) 184; R.R. Schmidt, Angewandte Chemie, 1987 (98) 213; H. Kunz, Angewandte Chemie, 1987 (44) 247; H. Paulsen, Angewandte Chemie, 1990 (102) 851). For the enzymatic synthesis for example Carbohydrate Research, Elsevier Science Publishers B.U., Amsterdam, Journal of Carbohydrate Chemistry, Marcel Dekker, Inc., New York; Advances in Carbohydrate Chemistry and Biochemistry, David, S. et al., Vol. 49, 1991, page 175; Applied Biocatalysis, Nielsson, K.G.I., Vol. 1, 1991, pages 117 et seq.; Synthesis, Drueckhammer, D.G. et al., 1991, pages 499 et seq.).

By chemoenzymatic synthesis is meant the combination of chemical and enzymatic reaction steps for the synthesis of the carbohydrate portion and of a covalent linkage of carbohydrate portion and spacer.

Synthetically prepared oligosaccharides can be obtained both with free reducing end and in a spacer-linked form. The introduction of the spacer takes place by processes known to the skilled worker for chemical of enzymatic

glycosylation (see above). The preparation processes which are preferably used are those which result in an oligosaccharide which is already linked by covalent bond to the spacer.

The coupling of the carbohydrate portion with a free reducing end to the spacer takes place via a covalent bond. The following processes can be carried out for the coupling:

1) The oligosaccharide with free reducing end is converted, for example in analogy to the process of Lundquist et al. (Journal of Carbohydrate Chemistry, 1991 (10) 377) into the free 1-aminoglycoside which is subsequently linked covalently to a spacer by acylation. The compound can be covalently linked, for example in analogy to the process of Kochetkow (Carbohydrate Research 1986 (146) C1), to the spacer using an N-hydroxysuccinimide active ester as activated group on the latter.

2) The free reducing end of the oligosaccharide can be converted in analogy to the process of Isebell et al. in Methods of Carbohydrate Chemistry, Academic Press, New York, with iodine and potassium hydroxide into a lactone. This lactone can be covalently linked, for example in analogy to the process of Isebell et al. in Methods of Carbohydrate Chemistry, Academic Press, New York, to the spacer by means of a primary amino group which is a component of the latter.

3) The formation of a covalent bond between the reducing end of an oligosaccharide and the spacer is also possible by reductive amination using a spacer which has a primary amino group at the appropriate end, for example in analogy to Lane, Synthesis, 1975, 135.

4) If the oligosaccharide contains at its reducing end an amino sugar with free amino group, the latter can be covalently linked, for example in analogy to the process of Kochetkow (see above), to the spacer by means of an N-hydroxysuccinimide active ester of the latter.

SPACER:

The covalent linkage of the polymer to the spacer or to a compound composed of covalently linked spacer and carbohydrate and of a covalent compound of polymer and potentiator with the spacer or with a compound composed of covalently linked spacer and carbohydrate takes place by reaction between a reactive group and an activated group. In this connection it is possible both for the reactive group to be located at the end of the spacer or of a compound composed of covalently linked spacer and carbohydrate and the activated group to be located on the polymer or on a compound composed of covalently linked polymer and potentiator and for the activated group to be located at the end of the spacer or of a compound composed of covalently linked spacer and carbohydrate and the reactive group to be located on the polymer or on a compound composed of covalently linked polymer and potentiator.

The reaction between a reactive group and an activated group takes place by processes known to the skilled worker for esterification, amidation, addition onto a double bond and alkylation. These processes are known to the skilled worker from the literature (for example Larock, R.C., Comprehensive Organic Transformations, 1989, VCH Verlagsgesellschaft Weinheim, Germany; L.-F.-Tietze and Th. Eicher, Reaktionen und Synthesen im Org.-chem. Praktikum (Reactions and Syntheses in Practical Org. Chem.), G. Thieme Verlag, Stuttgart, Germany, 1981).

BIODEGRADABLE HYDROPHILIC POLYMER:

The preparation of biodegradable, hydrophilic polymer takes place by processes known to the skilled worker. These are described, for example, in: H.G. Elias, Makromoleküle (Macromolecules), Vols. 1 and 2, Hüthig & Wepf Verlag, Basle, Switzerland, 1991/92 or D. Braun, H. Cherdron, W. Kern, Praktikum der Makromolekularen, Organischen Chemie (Practical Macromolecular Organic Chemistry), Hüthig-Verlag, 1979.

POTENTIATOR:

The potentiator is a compound which is covalently linked to the polymer portion of the carbohydrate receptor blocker.

Its task is to influence the structure of the entire molecule in such a way that the carbohydrate portion is (optimally) accessible for the receptor to improve the solubility which emerges as deficient in some cases.

The covalent linkage between potentiator and polymer takes place by reaction of a reactive group with an activated group. The reaction between reactive groups and activated groups takes place by processes known to the skilled worker for alkylation, acylation or addition onto a double bond. These processes are known to the skilled worker from the literature. (Larock, R.C., Comprehensive Organic Transformations, 1989, VCH Verlagsgesellschaft Weinheim, Germany; L.-F. Tietze and Th. Eicher, Reaktionen und Synthesen im Org.-chem. Praktikum (Reactions and Syntheses in Practical Org. Chem.), G. Thieme Verlag, Stuttgart, Germany, 1981).

The preparation of the carbohydrate receptor blocker can also, as an alternative to the chemical synthetic routes mentioned above, take place via an enzymatic or chemoenzymatic linkage of the individual components. The enzymes used are glycosidases, glycosyltransferases, transglycosidases and/or lipases. Glycosidases, glycosyltransferases and/or transglycosidases are preferably

employed to construct the carbohydrate portion and to link carbohydrate portion and spacer. Lipases such as, for example, from Pseudomonas, Candida, Mucor or Rhizopus are preferably used for linking the reactive to the activated group. Activated groups employed in this case are: carboxylic acids, methyl esters and/or vinyl esters.

The processes described above for the preparation are embodiments which are mentioned by way of example and which make no claim to completeness. Correspondingly, the preparation of the carbohydrate receptor blockers according to the invention is not confined to the said processes.

DIAGNOSTIC AND THERAPEUTIC USE OF THE CARBOHYDRATE RECEPTOR BLOCKER ACCORDING TO THE INVENTION:

The carbohydrate receptor blockers must not display any adverse side effects on in vivo administration. Thus, in the case of intravenous uses, for example, hemolytic and immunogenic properties must be avoided. The enzymes in the blood coagulation cascade must not be activated, in order to preclude the formation of thrombi. In addition, unused receptor blockers must be metabolized to non-toxic substances or be excreted.

It has additionally emerged that for optimal therapeutic and/or diagnostic use of the carbohydrate receptor blocker both the length of the spacer which links the polymer to the carbohydrate, and the degree of loading of the polymer with the oligosaccharide must be within a defined range of 0.5-50%, preferably of 2-20%. The residence time of the carbohydrate receptor blocker can be controlled by the nature and degradability of the polymer portion and the molecular weight in such a way that the lifetime of the carbohydrate receptor blocker can be from 1 min, several hours up to some days.

The carbohydrate receptor blocker according to the

invention and its physiologically tolerated salts are very suitable, by reason of their valuable pharmacological properties, for use as medicines in mammals, especially humans. The pharmaceuticals are preferably suited for the prophylaxis and/or therapy of bacterial and viral infections and of diseases which develop with the involvement of inflammatory processes, preferably of post-infarct syndrome, shock lung in adults, shock, stroke, acute and chronic organ rejection, vasculitis, sepsis, inflammatory disorders of the skin, rheumatoid arthritis and metastatic tumors.

The pharmaceuticals according to the invention are generally administered intravenously, orally or parenterally or as implants, but rectal use is also possible in principle. Examples of suitable solid or liquid pharmaceutical presentations are granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, aerosols, drops or injectable solutions in ampoule form as well as products with protracted release of active substance, in the production of which it is customary to use excipients and additives and/or aids such as disintegrants, binders, coating agents, swelling agents, glidants or lubricants, flavorings, thickeners or solubilizers. Examples of commonly used excipients or ancillary substances which may be mentioned are magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, lactalbumin, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents such as, for example, sterile water, alcohols, glycerol and polyhydric alcohols.

The pharmaceutical products are preferably produced and administered in dosage units. Preferred in the case of solid dosage units are tablets, capsules and suppositories.

The daily doses necessary for the treatment of a patient

vary depending on the activity of the compound, the mode of administration, the nature and severity of the disorder, and the age and body weight of the patient. However, in some circumstances, higher or lower daily doses may also be appropriate. The daily dose can be administered either by a single administration in the form of a single dosage unit or else a plurality of small dosage units as well as by multiple administration of divided doses at particular intervals. The daily dose to be administered may additionally depend on the number of receptors expressed during the course of the disease. It is conceivable that only a few receptors are expressed on the cell surface in the initial stage of the disease and, accordingly, the daily dose to be administered is less than for seriously ill patients.

The pharmaceuticals according to the invention are produced by converting the carbohydrate receptor blocker with conventional excipients and, where appropriate, additives and/or ancillary substances into the or a suitable dosage form.

A: PRIMARY ASSAYS FOR INVESTIGATING THE EFFECT OF POLYMERIC CARBOHYDRATE RECEPTOR BLOCKERS ON CELL ADHESION TO RECOMBINANT SOLUBLE SELECTIN FUSION PROTEINS

This assay is used to detect the effect of polymer-bound carbohydrate units on cell adhesion of promyelocytic cells by means of selectins:

The assay used to test the activity of polymer-bound carbohydrate units on the interaction between E- and P-selectins (old nomenclature ELAM-1 and GMP-140 respectively) and their ligands is specific for only one of these interactions in each case. The ligands are offered in their natural form as surface structures on promyelocytic HL60 cells. Since HL60 cells have ligands and adhesion molecules which differ widely in specificity, the required specificity of the assay can be provided

only via the binding partner. Used as binding partners were genetically engineered soluble fusion proteins from the extracytoplasmic domain in each case of E- and P-selectin and the constant region of a human immunoglobulin of subclass IgG1.

A1. PREPARATION OF L-SELECTIN-IGG1

The genetic construct "ELAM-Rg" published by Walz et al., 1990, was used to prepare soluble L-selectin-IgG1 fusion protein.

For the expression, the plasmid DNA was transfected into COS-7 cells (ATCC) using DEAE-dextran (Molecular Biological Methods: see Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Struhl, K. and Smith, J.A., 1990. Current Protocols in Molecular Biology, John Wiley, New York). Seven days after the transfection, the culture supernatant is obtained, centrifuged to remove cells and cell fragments and adjusted to 25 mM Hepes pH 7.0, 0.3 mM PMSF, 0.02% sodium azide and stored at +4°C.

Walz, G., Aruffo, A., Kolanus, W., Bevilacqua, M. and Seed, B. 1990. Recognition by ELAM-1 of the sialyl-Lex determinant on myeloid and tumor cells. Science 250, 1132-1135.

A2. PREPARATION OF P-SELECTIN-IGG1

The genetic construct "CD62Rg" published by Aruffo et al., 1991, is used to prepare the soluble P-selectin-IgG1 fusion protein. The subsequent procedure corresponds to the preparation of L-selectin-IgG1 described under A1.

Aruffo, A., Kolanus, W., Walz, G., Fredman, P. and Seed, B. 1991. CD62/P-selectin recognition of myeloid and tumor cell sulfatides. Cell 67, 35-44.

A3. PREPARATION OF CD4-IGG1

The genetic construct "CD4:IgG1 hinge" published by Zettlemeissl et al., 1990, is used to prepare the soluble CD4-IgG1 fusion protein. The subsequent procedure corresponds to the preparation of L-selectin-IgG1 described under A1.

Zettelmeissl, G., Gregersen, J.-P.-, Duport, J.M., Mehdi, S., Reiner, G. and Seed, B. 1990. Expression and characterization of human CD4: Immunoglobulin Fusion Proteins. DNA and Cell Biology 9, 347-353.

A4. PROCEDURE FOR THE HL60 CELL ADHESION ASSAY FOR RECOMBINANT SOLUBLE ADHESION MOLECULES

1. 96-well microtiter assay plates (Nunc Maxisorb) are incubated with 100 µl of a goat anti-human IgG antibody (Sigma) diluted (1+100) in 50 mM Tris pH 9.5 at room temperature for 2 h. Removal of the antibody solution is followed by one wash with PBS.

2. 150 µl of the blocking buffer are left in the wells at room temperature for 1 h. The composition of the blocking buffer is:
0.1% gelatin, 1% BSA, 5% calf serum, 0.2 mM PMSF, 0.02% sodium azide. Removal of the blocking buffer is followed by one wash with PBS.

3. 100 µl of cell culture supernatant from appropriately transfected and expressing COS cells are pipetted into each of the wells. The incubation takes place at room temperature for 2 h. Removal of the cell culture supernatant is followed by one wash with PBS.

4. 20 µl of binding buffer are placed in the wells. A binding buffer has the composition: 50 mM Hepes, pH 7.5; 100 mM NaCl; 1 mg/ml BSA;

2 mM $MgCl_2$; 1 mM $CaCl_2$; 3 mM $MnCl_2$; 0.02% sodium azide; 0.2 mM PMSF. 5 µl of the test substance are added by pipette, mixed by agitating the plate and incubated at room temperature for 10 min.

5. 50 ml of an HL60 cell culture with 200,000 cells/ml are centrifuged at 350 g for 4 min. The pellet is resuspended in 10 ml of RPMI 1640 and the cells are again centrifuged. For labeling of the cells, 50 µg of BCECF-AM (molecular probes) are dissolved in 5 µl of anhydrous DMSO; subsequently 1.5 ml of RPMI 1640 are added to the BCECF-AM/DMSO solution. The cells are resuspended in this solution and incubated at 37°C for 30 min. After centrifugation at 350 g for 2 minutes, the labeled cell pellet is resuspended in 11 ml of binding buffer, and the resuspended cells are distributed in 100 µl aliquots in the wells of the microtiter plate. The plate is left to stand at room temperature for 10 min in order to allow the cells to sediment to the bottom of the test plate. This provides the cells with the opportunity to adhere to the coated plastic.

6. To stop the assay, the microtiter plate is completely immersed at an angle of 45° in the stop buffer (25 mM Tris, pH 7.5; 125 mM NaCl; 0.1% BSA; 2 mM $MgCl_2$; 1 mM $CaCl_2$; 3 mM $MnCl_2$; 0.02% sodium azide). The stop buffer is removed from the wells by inversion, and the procedure is repeated twice more.

7. The BCECF-AM-labeled cells which are firmly adherent in the wells are measured in a cytofluorimeter (Millipore) with a sensitivity setting of 4, an excitation wavelength of 485/22 nm and an emission wavelength of 530/25 nm.

B. SECONDARY ASSAY TO INVESTIGATE THE EFFECT OF POLYMERIC CARBOHYDRATE RECEPTOR BLOCKERS ON CELL ADHESION TO STIMULATED HUMAN ENDOTHELIAL CELLS

The test of the activity of polymeric carbohydrate receptor blockers on cell adhesion to recombinant soluble fusion proteins is a highly specific assay which is based on the interaction of one type of adhesion molecules with the corresponding ligands. In order to simulate the in vivo situation of cell-cell interactions, we use an assay in which HL60 cells adhere to stimulated human umbilical endothelial cells.

1. Obtaining of human umbilical endothelial cells (HUVEC).

Umbilical cords are stored after delivery in PBS containing 100,000 IU/L penicillin, 100 mg/L streptomycin, 50 mg/L gentamicin, 50,000 IU/L mycostatin at +4°C until further processed.

The longest undamaged pieces are cut out of the umbilical cord with a scalpel and placed on fresh aluminum foil. One end of the umbilical cord is closed with a clip. At the other end, a suitable tube is inserted into the umbilical vein and fixed by ligating the end of the umbilical cord.

The vein is filled through the piece of tube with collagenase solution (50 mg collagenase/100 ml 25 mM Hepes, pH 7.0) and incubated at 37°C for 15 min.

In order to increase the cell yield, the umbilical cord is gently massaged after the incubation in order to detach still adherent endothelial cells.

The cell suspension is subsequently allowed to run out of the vein into a culture tube containing cell culture medium and 10% fetal calf serum. The vein is washed with PBS in order to obtain the remaining cells.

The cell suspension is centrifuged at 500 g for 5 min; the cell pellet is subsequently resuspended in 4 ml of culture medium and the cells are plated out. After 3-4 days there is confluent growth of the cells and they can be passaged.

To check the purity of the endothelial cell culture, the culture is stained with an antibody against factor VIII for the immunofluorescence. A positive reaction is shown only by endothelial cells but not by contaminating fibroblasts.

2. Procedure for the assay

20,000 endothelial cells are plated out per well in a 96-well microtiter plate and incubated at 37°C for 24 h. The endothelial cells for this purpose must not have been passaged more than 5-6 times. Four hours before the assay the endothelial cells are stimulated by addition of Il-1 (final concentration: 15 U/ml). After removal of the culture medium, the cells are washed once with RPMI medium without serum. Removal and renewed pipetting of 20 µl of RPMI medium are followed by addition of test substances.

3. The further steps in the assay, labeling of the HL60 cells, introduction of the HL60 cells, are carried out as under A4. Items 5. - 7.

C. SECONDARY ASSAY TO INVESTIGATE THE EFFECT OF POLYMERIC CARBOHYDRATE RECEPTOR BLOCKERS ON CELL ADHESION TO FROZEN SECTIONS OF LYMPHATIC TISSUE

It is possible in vitro to investigate the extent to which leukocytes bind to endothelial cells on frozen sections of lymphatic tissue. These cell-cell interactions are based on the interaction between adhesion molecules on the surface of the endothelial cells in the frozen section and the corresponding ligands on the

surface of leukocytes. It is possible to use as substitute for primary leukocytes HL60 cells whose surface ligands are well described in the scientific literature. It is possible to determine whether adhesion of HL60 cells to lymph node frozen sections takes place from the number of bound HL60 cells.

1. Axillary, cervical or mesenteric lymph nodes are dissected out of freshly sacrificed rats and rapidly frozen in liquid nitrogen.

2. 10 µm-thick cryostat sections are prepared from the frozen lymph nodes, transferred to circular cover glasses (diameter 18 mm) and dried at room temperature for 2 h.

3. 20 µl of binding buffer are pipetted onto the sections. The test substances are added and incubated at room temperature for 10 min. HL60 cells are labeled as under A4. Item 5. 200,000 labeled HL60 cells in 100 µl of binding buffer are added to each cover glass and left to stand for 10 min. This allows the sedimented cells to reach endothelial cells to which some of them adhere.

4. The cover glasses are immersed at an angle of 45° in stop buffer in order to rinse off the non-adherent cells. The cover glasses are subsequently fixed in 4% formaldehyde in PBS at room temperature for 10 min.

5. Cross sections of lymphatic blood vessels are recorded by photography under an immunofluorescence microscope (FITC excitation). The adherent HL60 cells are clearly distinct from the unstained background. The result is expressed as bound HL60 cells per unit area of endothelium.

D. TERTIARY ASSAY TO INVESTIGATE THE EFFECT OF POLYMERIC CARBOHYDRATE RECEPTOR BLOCKERS ON LEUKOCYTE ADHESION IN RATS IN VIVO

The method detailed hereinafter is able to establish the in vivo activity of substances which inhibit the adhesion of leukocytes to the vessel intima.

It is known that some of the circulating white blood cells have the tendency to adhere to the intima of the blood vessels. This tendency is considerably enhanced in inflammatory processes. Leukocytes normally impinge continually on the blood vessel walls, but this collision is elastic so that the cells rebound to a certain extent and return to the circulation. In inflammatory processes, biochemical changes, both in the leukocytes and in the endothelial cells lining the blood vessels, lead to changes in the surface properties of both types of cell. The cells' behavior becomes more adhesive. This adhesiveness is initially expressed by the tendency of the leukocytes after collision with the endothelium to roll on the endothelial cells. This rolling of the leukocytes on the endothelium induces further biochemical reactions on both binding partners, as a consequence of which cell adhesion is enhanced. This further enhancement of the adhesiveness slows down the rolling of the leukocytes until they adhere firmly to the endothelium. The firm adherence is followed by, as further step, the migration of the leukocytes out of the blood vessel. The rolling of the leukocytes, the firm adhesion and the migration out of the blood vessels can be induced with leukocyte-stimulating factors such as FMLP (f-Met-Leu-Phe), LPS (lipopolysaccharides) or TNF (tumor necrosis factor). Microscopic recording of these processes is possible on dissected mesenteric tissue, for example from rats. Substances injected into the bloodstream can therefore be investigated to find whether they are able to influence induced leukocyte adhesion.

Rats are anesthetized with [lacuna] for carrying out this investigation in practice. The abdominal cavity is opened and a section of the small intestine is pulled out. The section of small intestine is continuously kept moist on a heatable microscope stage. For microscopic inspection, a region of mesenteric tissue is covered with liquid paraffin. For the control, all the adherent - non-stimulated-leukocytes in this region are counted every 5 min for a period of 30 min. In parallel with this, the blood pressure, body temperature and flow rate of the blood are recorded. The test substance is administered by continuous venous infusion throughout the test. After introduction of leukocyte stimulants, which are added dropwise to the mesenteric tissue, the adherent leukocytes are counted every 5 min for a period of 90 min.

The investigation is carried out on test groups each composed of three animals. The first animal receives only the vehicle in order to determine the spontaneous adhesion. The second animal receives only the leukocyte stimulation in order to determine the pathogenic control. The third animal receives leukocyte stimulant and test substance.

The number of adherent leukocytes in the pathogenic control is set equal to 100%. The percentage change in the leukocyte adhesion on administration of test substance compared with the pathogenic control indicates the activity of a test substance.

The synthetic procedures are described by way of example hereinafter without limiting the synthesis of the carbohydrate receptor blocker to these procedures.

MONOSACCHARIDE (Schemes 1 and 7)

2-Amino-2-deoxyglucose hydrochloride [1] is converted by the procedure of R.U. Lemieux et al. (ACS Symp. Ser., 39, 90 (1976)) by reaction with phthalic anhydride and

subsequent reaction with acetic anhydride/pyridine into 1,3,4,6-tetraacetyl-2-N-acetyl-2-deoxyglucose [2].

Treatment with tin tetrachloride/thiophenol by the method of K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)) results in the corresponding 1-thiophenyl derivative [3]. The latter is reacted by the procedure of B.A. Silwanis et al. (J. Carbohydr. Chem. 10, 1067 (1991)) with 6-azidohexanol. The acetyl and phthaloyl protective groups are cleaved with hydrazine hydrate in analogy to the procedure of K.C. Nicolaou et al. J. Am. Chem. Soc. 114, 3127 (1992). Before elimination of the benzyl protective groups ($H_2/Pd(OH)_2$, MeOH), the free amino group is selectively acetylated in the presence of the free hydroxyl groups with excess acetic anhydride. The result is [4].

DISACCHARIDE (Schemes 1, 2, 5 and 7)

1) Synthesis of a protected N-acetylglucosamine unit

2-Amino-2-deoxyglucose hydrochloride [1] is converted by the procedure of R.U. Lemieux et al. (ACS Symp. Ser., 39, 90 (1976)) by reaction with phthalic anhydride and subsequent reaction with acetic anhydride/pyridine into 1,3,4,6-tetraacetyl-2-N-acetyl-2-deoxyglucose [2].

Treatment with tin tetrachloride/thiophenol by the method of K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)) results in the corresponding 1-thiophenyl derivative [3]. After elimination of the acetyl protective groups [5], the benzylidene group and the allyl group (benzaldehyde dimethyl acetal/CSA; allyl bromide/NaH) are introduced. Reduction with $NaCNBH_3$ liberates the 4-OH group [6].

2) Synthesis of a protected galactose unit

Galactose [7] is converted by the procedure of K.C. Nicolaou et al. (J. Chem. Soc., Chem. Comm. 870 (1991)) by mixing with acetic anhydride/triethylamine/DMAP and tin tetrachloride/thiophenol into the thioglycoside [8]. After elimination of the acetyl protective groups, [9] is produced by reaction with benzaldehyde dimethyl acetal/CSA. Acetylation (acetic anhydride/triethylamine/-DMAP) followed by reduction with $NaCNBH_3$ results in [10]. After renewed acetylation (acetic anhydride/triethylamine/DMAP), the thioglycoside is converted by NBS/HF into the fluoride [11].

3) Synthesis of the protected N-acetyllactosamine disaccharide

The galactosyl fluoride [11] is linked to the N-acetylglucosamine derivative [5] by the procedure of K.C. Nicolaou et al. (J. Chem. Soc., Chem. Comm. 870 (1991)) in the presence of $AgClO_4/SnCl_2$ to give [12] which is converted by elimination of the allyl protective group ($Ru(PPh_3)_4/H_2$; TsOH/MeOH) into [13]. The latter is reacted with 6-azidohexanol in analogy to the procedure of B.A. Silwanis et al. (J. Carbohydr. Chem. 10, 1067 (1991)).

The cleavage of the acetyl and phthaloyl protective groups with hydrazine hydrate takes place in analogy to the procedure of K.C. Nicolaou et al. J. Am. Chem. Soc. 114, 3127 (1992). Before elimination of the benzyl protective groups ($H_2/Pd(OH)_2$, MeOH), the free amino group is selectively acetylated in the presence of the free hydroxyl groups with excess acetic anhydride. The result is [14].

TRISACCHARIDE (Schemes 1, 2, 3, 5 and 8)

1) Synthesis of a protected N-acetyl glucose unit

2-Amino-2-deoxyglucose hydrochloride [1] is converted by the procedure of R.U. Lemieux et al. (ACS Symp. Ser., 39, 90 (1976)) by reaction with phthalic anhydride and subsequent reaction with acetic anhydride/pyridine into 1,3,4,6-tetraacetyl-2-N-acetyl-2-deoxyglucose [2].

Treatment with tin tetrachloride/thiophenol by the method of K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)) results in the corresponding 1-thiophenyl derivative [4]. After elimination of the acetyl protective groups [5], the benzylidene group and the allyl group (benzaldehyde dimethyl acetal/CSA; allyl bromide/NaH) are introduced. Reduction with $NaCNBH_3$ results in liberation of the 4-OH group [6].

2) Synthesis of a protected galactose unit

Galactose [7] is converted by the procedure of K.C. Nicolaou et al. (J. Chem. Soc., Chem. Comm. 870 (1991)) by mixing with acetic anhydride/triethylamine/DMAP and tin tetrachloride/thiophenol into the thioglycoside [8]. After elimination of the acetyl protective groups, [9] is produced by reaction with benzaldehyde dimethyl acetal/CSA. Acetylation (acetic anhydride/triethylamine/DMAP) followed by reduction with $NaCNBH_3$ results in [10]. After renewed acetylation (acetic anhydride/triethylamine/DMAP), the thioglycoside is converted by NBS/HF into the fluoride [11].

3) Synthesis of a protected fucose unit

Fucose [15] is peracetylated (acetic anhydride/triethylamine/DMAP) by the procedure of K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3694 (1990)) and converted with tin tetrachloride/thiophenol into the thioglycoside [16].

Subsequently, after a replacement of the acetyl by benzyl protective groups (NaOMe/MeOH; NaH/BnBr), the thiophenol is converted with NBS/HF or NBS/DAST into the fluoride [17].

4) Synthesis of the protected N-acetyllactosamine disaccharide

The galactosyl fluoride [11] is linked to the N-acetyl-glucosamine derivative [5] by the procedure of K.C. Nicolaou et al. (J. Chem. Soc., Chem. Comm. 870 (1991)) in the presence of $AgClO_4/SnCl_2$ to give [12] which is converted by elimination of the allyl protective group ($Ru(PPh_3)_4/H_2$; TsOH/MeOH) into [13].

5) Synthesis of the protected Lewis X trisaccharide

The disaccharide [13] is reacted with the fucosyl fluoride [17] ($AgClO_4/SnCl_2$) by the procedure of K.C. Nicolaou et al. (J. Chem. Soc., Chem. Comm. 870 (1991)) to give the Lewis X derivative [18].
The latter is reacted in analogy to the procedure of B.A. Silwanis et al. (J. Carbohydr. Chem. 10, 1067 (1991)) with 6-azidohexanol.

The cleavage of the acetyl and phthaloyl protective groups with hydrazine hydrate takes place in analogy to the procedure of K.C. Nicolaou et al. J. Am. Chem. Soc. 114, 3127 (1992). Before elimination of the benzyl protective groups ($H_2/Pd(OH)_2$, MeOH), the free amino group is selectively acetylated in the presence of the free hydroxyl groups with excess acetic anhydride. The result is [19].

TETRASACCHARIDE (Schemes 1-6 and 8)

1) Synthesis of a protected N-acetyl glucose unit

2-Amino-2-deoxyglucose hydrochloride [1] is converted by the procedure of R.U. Lemieux et al. (ACS Symp. Ser., 39, 90 (1976)) by reaction with phthalic anhydride and subsequent reaction with acetic anhydride/pyridine into 1,3,4,6-tetraacetyl-2-N-acetyl-2-deoxyglucose [2].

Treatment with tin tetrachloride/thiophenol by the method of K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990) results in the corresponding 1-thiophenyl derivative [4]. After elimination of the acetyl protective groups [5], the benzylidene group and the allyl group (benzaldehyde dimethyl acetal/CSA; allyl bromide/NaH) are introduced. Reduction with $NaCNBH_3$ results in liberation of the 4-OH group [6].

2) Synthesis of a protected galactose unit

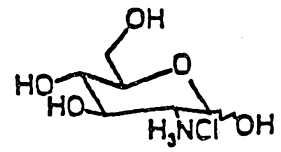
Galactose [7] is converted by the procedure of K.C. Nicolaou et al. (J. Chem. Soc., Chem. Comm. 870 (1991)) by mixing with acetic anhydride/triethylamine/DMAP and tin tetrachloride/thiophenol into the thioglycoside [8]. After elimination of the acetyl protective groups, [9] is produced by reduction with benzaldehyde dimethyl acetal/CSA. Acetylation (acetic anhydride/triethylamine/DMAP) followed by reduction with $NaCNBH_3$ results in [10]. After renewed acetylation (acetic anhydride/triethylamine/DMAP), the thioglycoside is converted by NBS/HF into the fluoride [11].

3) Synthesis of a protected fucose unit

Fucose [15] is peracetylated (acetic anhydride/triethylamine/DMAP) by the procedure of K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3694 (1990)) and converted with tin tetrachloride/thiophenol into the thioglycoside [16].

Subsequently, after a replacement of the acetyl by benzyl protective groups (NaOMe/MeOH; NaH/BnBr), the thiophenol is converted with NBS/HF or NBS/DAST into the fluoride [17].

Scheme 1



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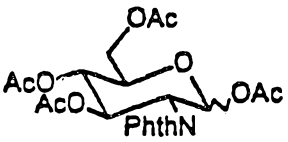

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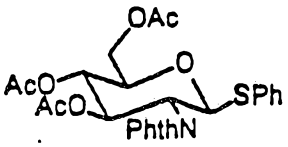

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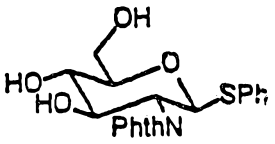

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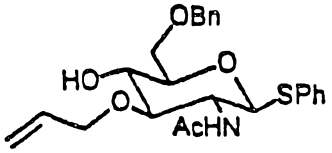

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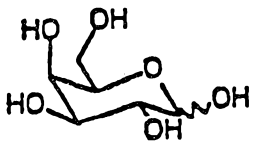
Scheme 2



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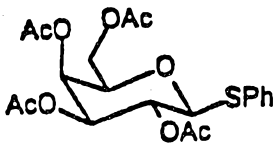

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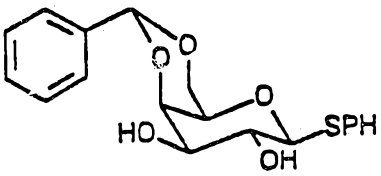

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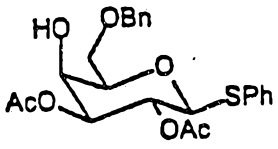

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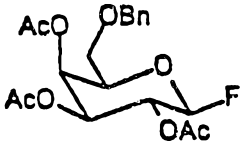

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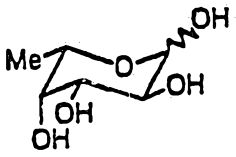
Scheme 3



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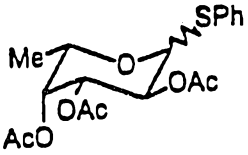

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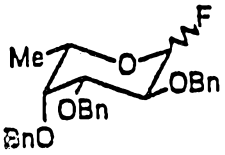

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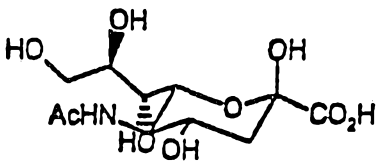
Scheme 4



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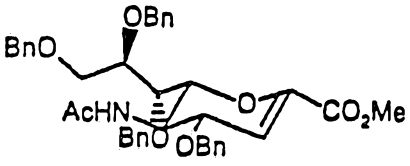

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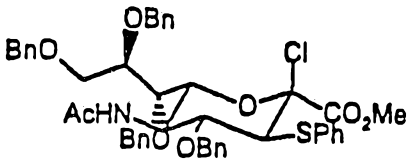

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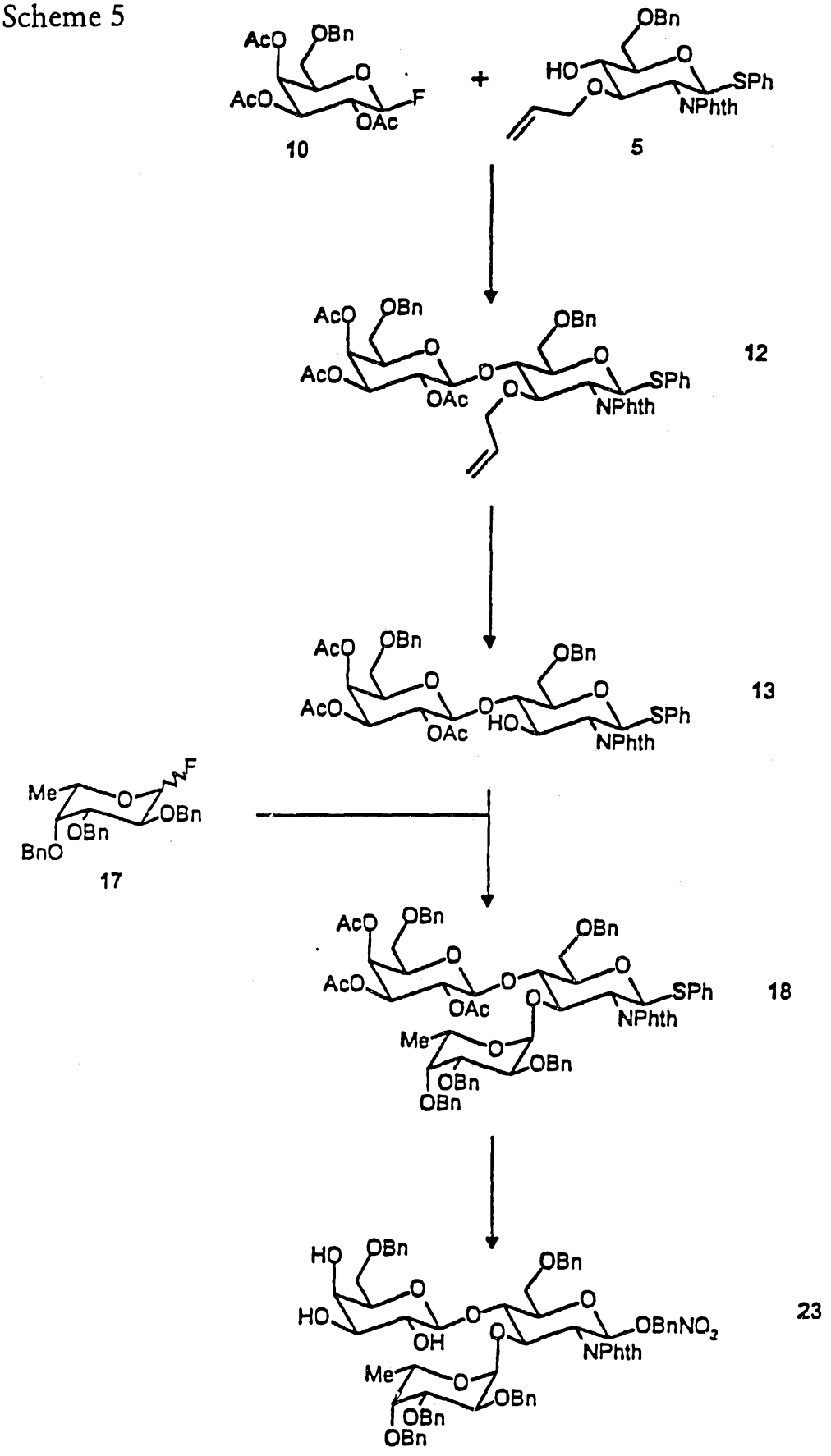
Scheme 5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AcO
OAc
F
10
+
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O
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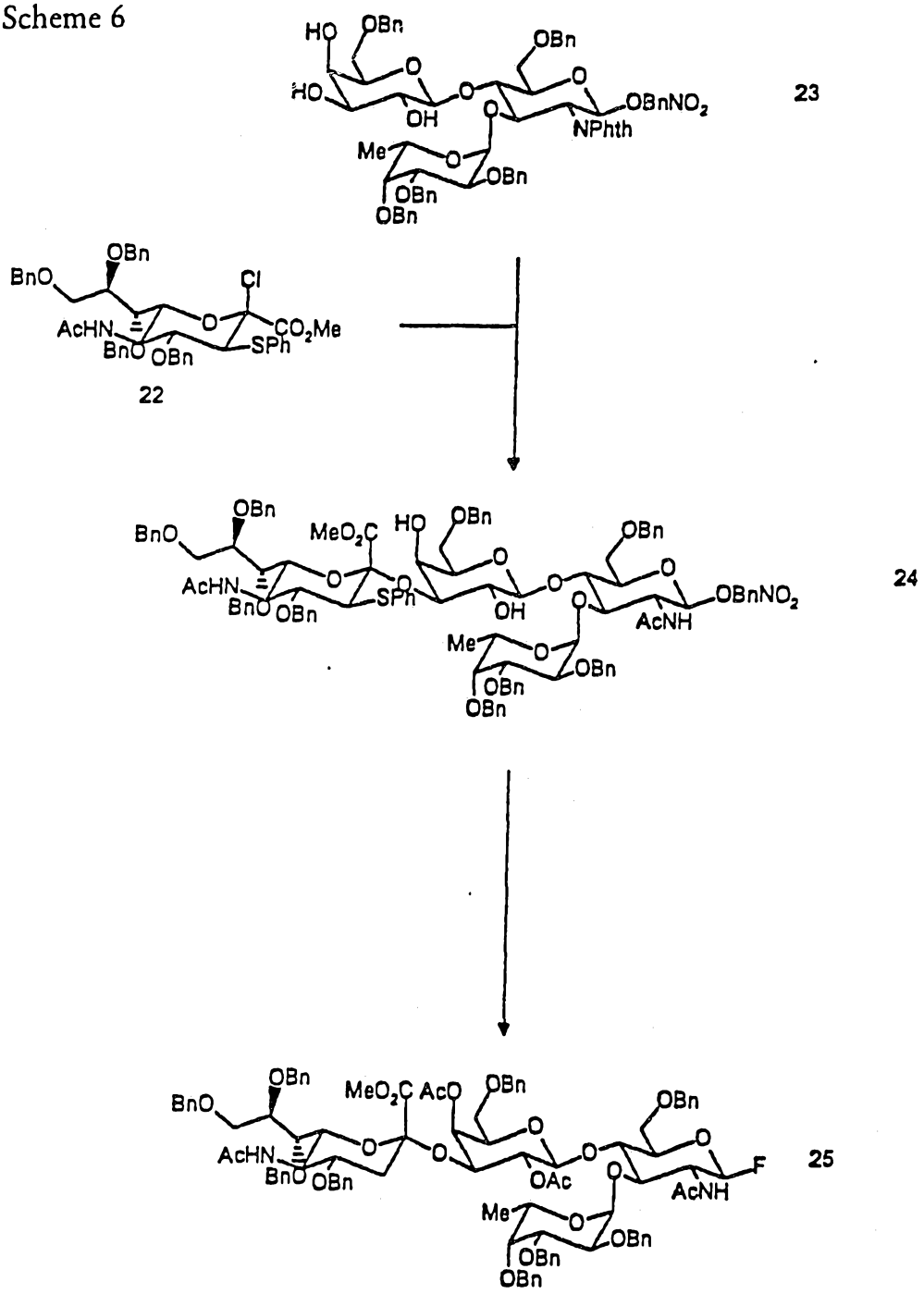
SPh
NPhth
5
12
13
HO
17
Me
BnO
18
23
OH
OBnNO2

Scheme 6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OBn
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Cl
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BnO
OBn
SPh
22
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OBn
MeO2C
HO
OBn
OBn
AcHN
BnO
OBn
SPh
OH
OBnNO2
AcNH
Me
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OBn
OBn
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BnO
OBn
MeO2C
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OBn
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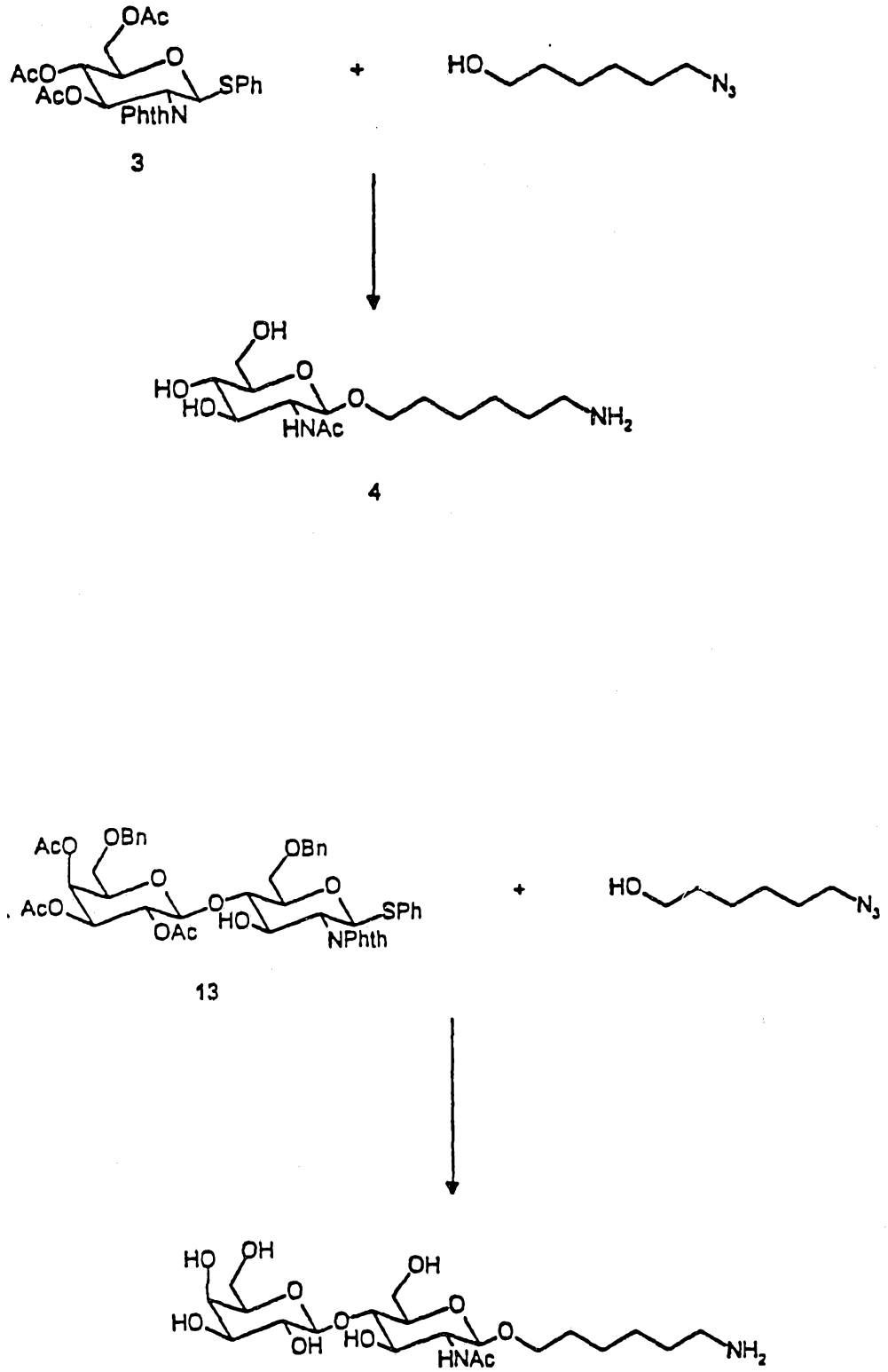
25

Scheme 7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OBn
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SPh
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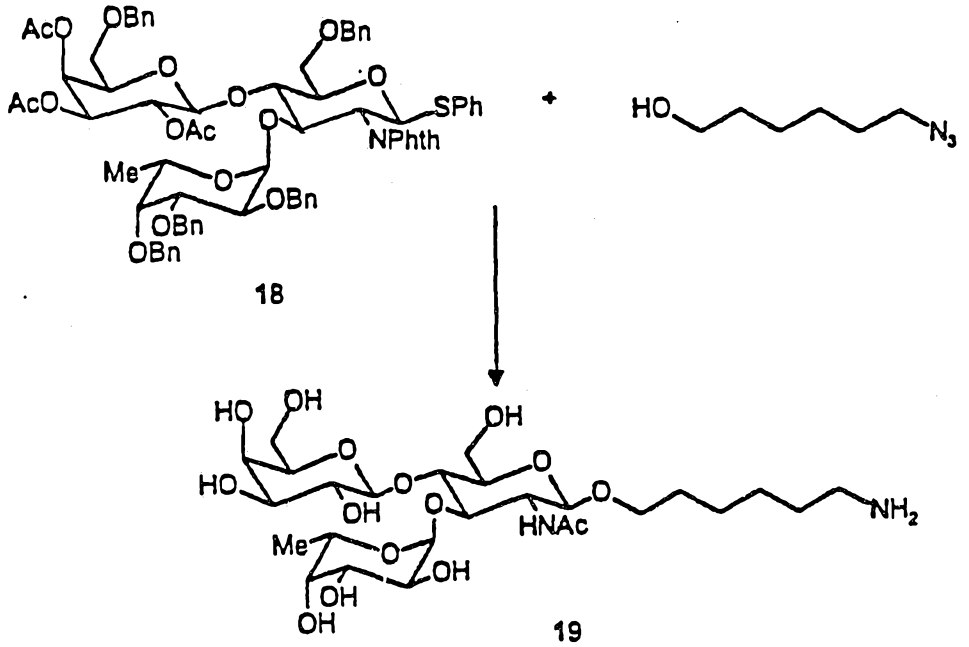
NH2

Scheme 8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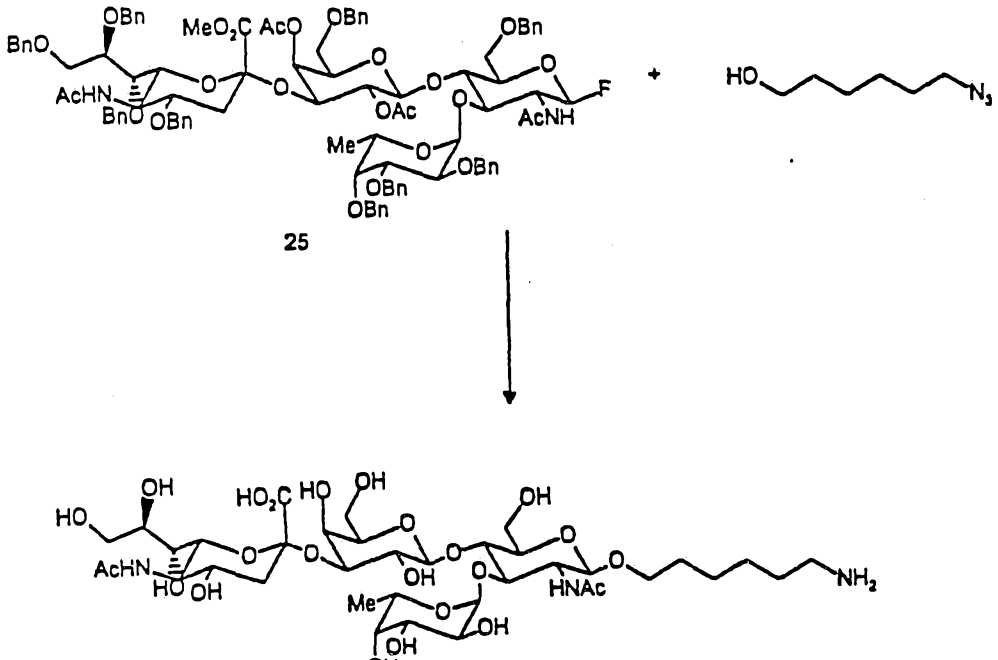
OH
19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OBn
AcHN
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OBn
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HO
N3
25
HO
OH
HO2C
HO
OH
OH
AcHN
HO
OH
HNAc
NH2
Me
OH
OH
OH

4) Synthesis of a protected N-acetylneuraminic acid unit

N-Acetylneuraminic acid [20] is converted by treatment with Dowex 50 in methanol by the procedure of R. Kuhn et al. (Chem. Ber. 99, 611 (1966)) into the corresponding methyl ester [15] and peracetylated with acetic anhydride in the presence of catalytic amounts of sulfuric acid by the procedure of K.C. Nicolaou et al. (J. Chem. Soc., Chem. Comm. 870 (1991)). Subsequent deacetylation with sodium hydroxide solution and perbenzylation with benzyl bromide/NaOH/Bu_4NI gives [21]. Reaction with phenylsulfenyl chloride then results in the neuraminic acid donor [22].

5) Synthesis of the protected N-acetyllactosamine disaccharide

The galactosyl fluoride [11] is linked to the N-acetylglucosamine derivative [5] by the procedure of K.C. Nicolaou et al. (J. Chem. Soc., Chem. Comm. 870 (1991)) in the presence of $AgClO_4/SnCl_2$ to give [12] which is converted by elimination of the allyl protective group ($Ru(PPh_3)_4/H_2$; TsOH/MeOH) into [13].

6) Synthesis of the protected Lewis X trisaccharide

The disaccharide [13] is reacted with the fucosyl fluoride [17] ($AgClO_4/SnCl_2$) by the procedure of K.C. Nicolaou et al. (J. Chem. Soc., Chem. Comm. 870 (1991)) to give the Lewis X derivative [18].

7) Synthesis of the protected sialyl-Lewis X tetrasaccharide

The trisaccharide [18] is converted by the procedure of K.C. Nicolaou et al. J. Am. Chem. Soc. 114, 3127 (1992) with NBS/DAST into the fluoride which, after reaction with o-nitrobenzyl alcohol/$AgClO_4/SnCl_2$, yields the corresponding glycoside. Reaction with NaOMe/HOMe yields

the trihydroxy compound [23].

The latter is linked by the procedure of K.C. Nicolaou et al. (J. Am. Chem. Soc. 114, 3127 (1992)) by reaction with [22] in the presence of $Hg(CN)_2/HgBr_2$ to give the sialyl-Lewis X derivative [24].

In accordance with the procedure of K.C. Nicolaou et al. J. Am. Chem. Soc. 114, 3127 (1992), acetylation is carried out with acetic anhydride/pyridine and the nitrobenzyl group is eliminated by irradiation. Subsequently the thiophenyl group is eliminated by reaction with triphenyltin hydride/AIBN, and conversion into the fluoride [25] takes place by reaction with diethylaminosulfur trifluoride. The latter compound is then reacted in analogy to the above procedure with 6-azidohexanol in the presence of $AgOTf/HfCp_2Cl_2$ to give the corresponding glycoside.

The elimination of the acetyl protective groups and that of the phthaloyl protective group takes place with hydrazine hydrate, and that of the methyl ester takes place with LiI/pyridine in analogy to the procedure of K.C. Nicolaou et al. J. Am. Chem. Soc. 114, 3127 (1992). Before elimination of the benzyl protective groups ($H_2/Pd(OH)_2$, MeOH), the free amino group is selectively acetylated in the presence of the free hydroxyl groups with excess acetic anhydride. The result is [26].

The invention is illustrated further in the examples which follow. Percentage data relate to weight. Mixing ratios of liquids relate to volume unless otherwise indicated.

The biodegradable polymers suitable for coupling of mono-, di- and oligosaccharide units are prepared by described processes of polymer chemistry. The coupling itself takes place by conventional synthetic processes carried out on polymers.

Thus, for example, poly-D,L-succinimide (PSI) is obtained by the procedure of Neri et al. J. Med. Chem., 16, 893 (1973) by the action of 85% strength phosphoric acid on aspartic acid at temperatures of 160°C-180°C. Reaction of PSI polymer with hydroxyethylamine at room temperature or slightly elevated temperature results in poly-α,β-(2-hydroxyethyl)-D,L-aspartamide (PHEA) (Neri et al., ibid). The alcohol groups in PHEA can be esterified by conventional processes (US 5.041.291). Partial reaction of PSI with ethanolamine results in corresponding copolymers (US 5.229.469). Basic hydrolysis of PSI leads to polyaspartic acid (in analogy to Giammona et al., Chem. Pharm. Bull. 37(8), 2245 (1989)).

PSI can also be reacted with other amines in analogy to the reaction with hydroxyethylamine (EP 0 548 794), whereby it is possible to introduce additional functional groups which may act as potentiators.

Another starting polymer, poly-L-lysine methyl ester-fumaramide, is prepared by boundary phase polycondensation from L-lysine methyl ester and fumaryl dichloride (US 4.834.248). The methyl ester groups can be reacted, directly or after partial hydrolysis and subsequent activation, for example as p-nitrophenyl ester, with the mono-, di- and oligosaccharides containing amino groups.

Polymeric carbohydrate receptor blockers based on polyglutamates can be prepared analogously, i.e. by means of the p-nitrophenyl ester (polymer synthesis in analogy to: Anderson in "Macromolecules as Drugs and as Carriers for Biologically active Materials" (Ed: D.A. Tirell), NY Acad. Sci., NY, 1985, pages 67-75).

Hydroxyethylstarch is activated, before the reaction with the carbohydrate derivatives containing amino groups, by periodate oxidation (in analogy to Schacht et al. in "Macromolecules as Drugs and as Carriers for Biologically

active Materials" (Ed: D.A. Tirell), NY Acad. Sci., NY, 1985, 199-212) or by reaction with chlorocarbonic esters such as p-nitrophenyl and N-hydroxysuccinimidyl chloroformates (in analogy to Wilchek, M. and Miron, T.; Biochem. Int. 4, 629 (1982)).

The reaction of the polymer with the potentiator on the one hand and of the carbohydrate portion with the bifunctional spacer on the other hand takes place in homogeneous solution, preferably in water or in water-miscible solvents such as, preferably, dimethylformamide or acetonitrile. Particularly preferably in a mixture of water and water-miscible solvents, preferably in the ratio 1:1.

The reaction is carried out at 0°C - 80°C, preferably at 20°C-40°C and particularly preferably at 35°C-40°C.

Numbers indicated in square brackets in the following examples relate to the numbers indicated in the schemes.

1. Synthesis of the monosaccharide

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose [2]

D-Glucosamine hydrochloride [1] (0.22-5.32 g, 1-20 mmol) is added to a solution of sodium methylate (prepared from 0.02-0.56 g of sodium and 1-20 ml of methanol), shaken for 10 min and filtered to removed precipitated sodium chloride. The filtrate is stirred with phthalic anhydride (0.07-1.48 g, 0.5-10 mmol) for 10 min and then triethylamine (0.10-2.02 g, 1-20 mmol) is added. After the reaction is complete, the solvent is evaporated and the residue is cooled and pyridine (2-40 ml) and acetic anhydride (5-20 ml) are added. After 16 h at room temperature, the mixture is poured into ice and the product is extracted with dichloromethane. Washing with water, dilute hydrochloric acid and saturated sodium bicarbonate

solution results in the crude product which is recrystallized from ether (0.31-8.02 g, about 82% yield).

FAB-MS: $M+H^+$ = 454.
NMR data consistent with R.U. Lemieux et al. (ACS Symp. Ser., 39, 90 (1976)).

1-Thiophenyl-3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose [3]

The product [2] (0.31-8.02 g, 0.68-17.7 mmol) is dissolved in 3.2-100 ml of dry dichloromethane, and thiophenol (0.15-3.9 g, 1.36-35.4 mmol) and tin tetrachloride (0.35-9.22 g, 1.36-35.4 mmol) are added and the mixture is stirred until reaction is complete. The reaction mixture is diluted and then washed with saturated sodium bicarbonate solution. Concentration results in the crude product [3]. Purification takes place by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.27-71.4 g, 0.54-14.2 mmol, about 80%).

FAB-MS: $M+H^+$ = 504.
NMR data consistent with K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)) and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

1-(6-Aminohexyl)-2-deoxy-2-acetamido-β-D-glucopyranose [4]

The crude product [3] (0.27-7.14 g, 0.54-14.2 mmol) and 6-azidohexanol (0.27-3.54 g, 1.08-14.1 mmol) are dissolved in dry acetonitrile, at 0°C, molecular sieves (0.2-5 g, 3 Å) and trimethylsilyl trifluoromethanesulfonate (0.02-1.56 g, 0.1-7 mmol) are added. After the reaction is complete, the mixture is filtered and washed with saturated sodium bicarbonate solution. Concentration results in the crude product, which is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.17-2.23 g, 0.35-4.58 mmol, about

69% yield). The product is then taken up in methanol/hydrazine hydrate (1:1) (1-30 ml) and heated at 80°C until reaction is complete. After evaporation of the solvent, the crude product is taken up in dichloromethane/methanol (1:1) and stirred at 0°C with acetic anhydride (3.5-45.8 mmol) until reaction is complete. After evaporation, the azide is reduced with hydrogen in the presence of catalytic amounts of palladium on carbon (10%) (0.01-1.5 g) in methanol (0.5-40 ml). The crude product resulting after evaporation is purified by gel chromatography on Biogel P2 ([4]; 0.08-1.02 g, 0.25-3.2 mmol, about 70% yield).

FAB-MS: $M+H^+$ = 321.
^{1}H-NMR [300 MHz, DMSO]: 7.68 (HNAc), 4.23 (GlcNAc-H-1), 3.78-3.6 (m), 3.49-3.23 (m), 3.08-3.02 (m) 2.58, 1.79 ($GlcNCOCH_3$), 1.51-1.21 (O-(CH_2)-N).

2. Synthesis of the disaccharide

2.1 Synthesis of the individual sugar units

2.1.1 Synthesis of the N-acetylglucosamine unit [6]

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose [2]

D-Glucosamine hydrochloride [1] (0.22-5.32 g, 1-20 mmol) is added to a solution of sodium methylate (prepared from 0.02-0.56 g of sodium and 1-20 ml of methanol), shaken for 10 min and filtered to removed precipitated sodium chloride. The filtrate is stirred with phthalic anhydride (0.07-1.48 g, 0.5-10 mmol) for 10 min and then triethylamine (0.10-2.02 g, 1-20 mmol) is added. After the reaction is complete, the solvent is evaporated and the residue is cooled and pyridine (2-40 ml) and acetic anhydride (5-20 ml) are added. After 16 h at room temperature, the mixture is poured into ice and the product is extracted with dichloromethane. Washing with water,

dilute hydrochloric acid and saturated sodium bicarbonate solution results in the crude product which is recrystallized from ether (0.31-8.02 g, about 82% yield).

FAB-MS: $M+H^+$ = 454.

NMR data consistent with R.U. Lemieux et al. (ACS Symp. Ser., 39, 90 (1976)).

1-Thiophenyl-2-deoxy-2-phthalimido-β-D-glucopyranose [5]

The product [2] (0.31-8.02 g, 0.68-17.7 mmol) is dissolved in 3.2-100 ml of dry dichloromethane, and thiophenol (0.15-3.9 g, 1.36-35.4 mmol) and tin tetrachloride (0.35-9.22 g, 1.36-35.4 mmol) are added and the mixture is stirred until reaction is complete. The reaction mixture is diluted and then washed with saturated sodium bicarbonate solution. The crude product [3] obtained in this way is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.27-7.14 g, 0.54-14.2 mmol, about 80%) and is dissolved in anhydrous methanol (3-90 ml) and catalytic amounts of sodium methylate (prepared from 0.002-0.05 g of sodium in 0.1-2 ml of methanol) are added. After the reaction is complete, the mixture is neutralized with acetic ion exchanger (0.2-5 g Amberlite IR-120, H^+-form) ([5]; 0.21-5.31 g, 0.54-14.1 mmol, about 99% yield).

FAB-MS: $M+H^+$ = 378.

NMR data consistent with K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)); and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

1-Thiophenyl-3-O-allyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose [6]

The crude product [5] (0.21-5.31 g, 0.54-14.1 mmol) is stirred in anhydrous acetonitrile (3-90 ml) with benzaldehyde dimethyl acetal (0.12-3.22 g, 0.81-21.1 mmol) and catalytic amounts of p-toluenesulfonic acid (4-90 mg)

until the reaction is finished. The crude product resulting after washing with saturated sodium bicarbonate solution is taken up in anhydrous dimethylformamide (2.5-85 ml), and sodium hydride (0.02-0.51 g, 0.81-21.15 mmol) and allyl bromide (0.09-2.37 g, 0.75-19.74 mmol) are added. After the reaction is complete, methanol (0.06-1.55 ml, 2.7-70.5 mmol) is added, and the mixture is diluted with ethyl acetate and washed successively with saturated ammonium chloride and saturated sodium bicarbonate solution. The crude product [5] is purified by chromatography under silica gel with an ethyl acetate/isohexane gradient (0.23-5.98 g, 0.46-12.13 mmol, about 86%) and subsequently taken up in dry tetrahydrofuran (3.5-90 ml), and sodium cyanoborohydride (0.67-17.7 g, 10.8-282 mmol) is added. Subsequently, ethereal hydrochloric acid solution is slowly added until reaction is complete. After filtration, the organic phase is diluted with ethyl acetate and washed with saturated sodium bicarbonate solution to neutrality. The crude product [6] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.17-4.56 g, 0.35-9.17 mmol, about 74% yield).

FAB-MS: $M+H^+$ = 416.
NMR data consistent with K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)); and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

2.1.2 Synthesis of the galactose unit [11]

1-Thiophenyl-2,3,4,6-tetra-O-acetylgalacto-β-D-pyranose [8]

Galactose [7] (0.18-3.6 g, 1-20 mmol) is suspended in dichloromethane (5-90 ml), and acetic anhydride (0.75-15.43 g, 7.5-150 mmol), triethylamine (0.8-16.3 g, 8-160 mmol) and catalytic amounts of dimethylaminopyridine (0.01-0.1 g) are added. After the reaction is complete, the mixture is poured into ice-water and the

product is extracted with dichloromethane. The crude product obtained by washing the extract with dilute hydrochloric acid and saturated sodium bicarbonate solution is dissolved in dry dichloromethane (3.5-110 ml), and thiophenol (0.22-4.41 g, 2-40 mmol) and tin tetrachloride (0.51-10.28 g, 2-40 mmol) are added. After the reaction is complete, the mixture is diluted, and washing with saturated sodium bicarbonate solution results in the crude product [8] which is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.37-7.48 g, 0.85-17 mmol, about 85% yield).

FAB-MS: $M+H^+$ = 441.
NMR data consistent with K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)); and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

1-Thiophenyl-4,6-O-benzylidene-β-D-galactopyranose [9]

The product [8] (0.37-7.48 g, 0.85-17 mmol) is dissolved in anhydrous methanol (4-150 ml), and catalytic amounts of sodium methylate (prepared from 0.003-0.06 g of sodium in 0.1-2.5 ml of methanol) are added. After the reaction is complete, the mixture is neutralized with an acidic ion exchanger (0.25-6 g Amberlite IR-120, H^+-form) and evaporated. The resulting reaction product is stirred in anhydrous acetonitrile (4.5-150 ml) with benzaldehyde dimethyl acetal (0.19-5.05 g, 1.27-33.1 mmol) and catalytic amounts of p-toluenesulfonic acid (4-90 mg) until the reaction is finished. Washing with saturated sodium bicarbonate solution results in the crude product [9] which is purified by chromatography on silica gel with a dichloromethane/methanol gradient (0.28-5.51 g, 0.77-15.3 mmol, about 90% yield).

FAB-MS: $M+H^+$ = 361.
NMR data consistent with K.C. Nicolaou et al. J. Am. Chem. Soc. 114, 3127 (1992) and C.W. Hummel, Ph. D.

Dissertation, University of California, San Diego, 1993.

1-Thiophenyl-2,3-di-O-acetyl-6-O-benzyl-β-D-galacto-pyranose [10]

Product [9] (0.28-5.51 g, 0.77-15.3 mmol) is suspended in dichloromethane (4-60 ml), and acetic anhydride (0.23-4.75 g, 2-40 mmol), triethylamine (0.25-5.02 g, 2.46-49.23 mmol) and catalytic amounts of dimethylamino-pyridine (0.01-0.08 g) are added. After the reaction is complete, the mixture is poured into ice-water and the product is extracted with dichloromethane.

The crude product obtained by washing the extract with dilute hydrochloric acid and saturated sodium bicarbonate solution is dissolved in dry tetrahydrofuran (2.45-130 ml), and sodium cyanoborohydride (0.96-25.3 g, 15.4-402.8 mmol) is added. Subsequently ethereal hydrochloric acid solution is added slowly until the reaction is complete. After filtration, the organic phase is diluted with ethyl acetate and washed with saturated sodium bicarbonate solution to neutrality. The crude product [10] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.27-5.46 g, 0.62-12.24 mmol, about 80% yield).

FAB-MS: $M+H^+$ = 447.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

1-Fluoro-2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galacto-pyranose [11]

The product [9] (0.27-5.46 g, 0.62-12.24 mmol) is suspended in dichloromethane (4-60 ml) and acetic anhydride (0.14-2.91 g, 1.24-24.8 mmol), triethylamine (0.15-3.11 g, 1.53-30.53 mmol) and catalytic amounts of dimethylaminopyridine (0.003-0.04 g) are added. After the

reaction is complete, the mixture is poured into ice-water and the product is extracted with dichloromethane. The crude product obtained by washing the extract with dilute hydrochloric acid and saturated sodium bicarbonate solution is taken up in dry dichloromethane (5-80 ml) and, at -78°C, dimethylaminosulfur trifluoride (0.25-4.89 g, 1.86-36.7 mmol) and N-bromosuccinimide (0.11-3.26 g, 0.93-18.36 mmol) are added, and the mixture is warmed to room temperature. After washing with saturated sodium bicarbonate solution, the mixture is concentrated and the byproducts of the reaction are removed by silica gel chromatography (stepwise gradient: ethyl acetate/isohexane) to produce [11] (0.21-4.14 g, 0.53-10.4 mmol, about 85% yield).

FAB-MS: $M+H^+$ = 399.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

2.2 Assembly of the disaccharide from the N-acetyl-glucosamine unit and the galactose unit

1-Thiophenyl-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galacto-pyranosyl)-(1-4)-3-O-alkyl-2-deoxy-2-acetamido-β-D-glucopyranose [12]

The N-acetylglucosamine derivative [5] (0.17-4.56 g, 0.35-9.17 mmol) and the galactose derivative [11] (0.21-4.14 g, 0.53-10.4 mmol) are dissolved in dichloromethane (3-120 ml) and, at 0°C, 4 Å molecular sieves (0.15-7.8 g), silver perchlorate (0.11-5.39, 0.525-26.0 mmol) and tin(II) chloride (0.1-4.93 g, 0.525-26.0 mmol) are added. After the reaction is complete, the mixture is filtered and washed with saturated sodium bicarbonate solution. The crude product [12] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.23-5.61 g, 0.26-6.42 mmol, about 70% yield).

FAB-MS: $M+H^+$ = 874.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

1-Thiophenyl-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1-4)-2-deoxy-2-acetamido-β-D-glucopyranose [13]

[12] (0.23-5.61 g, 0.26-6.42 mmol) is dissolved in ethanol (30-900 ml) and heated at 95°C with catalytic amounts of tetrakis(triphenylphosphine)ruthenium(II) hydride (0.005-0.04 g). After the reaction is complete, the mixture is filtered, concentrated and taken up in methanol (2.5-85 ml) and catalytic amounts of p-toluenesulfonic acid (0.001-0.08 g) are added. After the reaction is complete, the mixture is washed with saturated sodium bicarbonate solution and, after concentration, the crude product [13] is obtained. Purification takes place by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.2-4.81 g, 0.234-5.78 mmol, about 90% yield).

FAB-MS: $M+H^+$ = 834.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

1-(6-Aminohexyl)-O-(β-D-galactopyranosyl)-(1-4)-2-deoxy-2-acetamido-β-D-glucopyranose [14]

[13] (0.21-2.66 g, 0.54-7.05 mmol) and 6-azidohexanol (0.27-3.54 g, 1.08-14.1 mmol) are dissolved in dry acetonitrile and, at 0°C, molecular sieves (0.2-5 g) and trimethylsilyl trifluoromethanesulfonate (0.02-1.56 g, 1-70 mmol) are added. After the reaction is complete, the mixture is filtered and washed with saturated sodium bicarbonate solution. Concentration results in the crude product which is purified by chromatography on silica gel

with an ethyl acetate/isohexane gradient (0.17-2.23 g, 0.35-4.58 mmol, about 65% yield). The product is subsequently taken up in methanol/hydrazine hydrate (1:1) (1-30 ml) and heated at 80°C until reaction is complete. After evaporation of the solvent, the crude product is taken up in dichloromethane/methanol (1:1) and stirred at 0°C with acetic anhydride (3.5-45.8 mmol) until reaction is complete. Evaporation is followed by reduction of the azide with hydrogen in the presence of catalytic amounts of palladium on carbon (10%) (0.01-1.5 g) in methanol (0.5-40 ml). The crude product obtained after evaporation is purified by gel chromatography on Biogel P2 [14] (0.12-1.54 g, 0.25-3.2 mmol, about 70% yield).

FAB-MS: $M+H^+$ = 483.

^{1}H-NMR [300 MHz, D_2O]: 4.98 (Fuc-H-1), 4.28 (Gal-H-1), 4.33, 3.99-3.38 (m), 2.99, 2.09 ($GlcNCOCH_3$), 1.72-1.32 ($O-(CH_2)_5-CH_2-N$), 1.11 (Fuc-CH_3) ppm.

3. Synthesis of the trisaccharide

3.1 Synthesis of the individual sugar units

3.1.1 Synthesis of the N-acetylglucosamine unit [6]

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose [2]

D-Glucosamine hydrochloride [1] (0.216-5.32 g, 1-20 mmol) is added to a solution of sodium methylate (prepared from 0.023-0.56 g) of sodium and 1-20 ml of methanol), shaken for 10 min and filtered to remove precipitated sodium chloride. The filtrate is stirred with phthalic anhydride (0.074-1.48 g, 0.5-10 mmol) for 10 min and then triethylamine (0.1-2.02 g, 1-20 mmol) is added. After the reaction is complete, the solvent is evaporated and the residue is cooled and pyridine (2-40 ml) and acetic anhydride (5-20 ml) are added. After 16 h at room temperature, the mixture is poured into ice and the product is extracted with dichloromethane. Washing with water,

dilute hydrochloric acid and saturated sodium bicarbonate solution results in the crude product which is recrystallized from ether (0.31-8.02 g, about 82% yield).

FAB-MS: $M+H^+$ = 454.

NMR data consistent with R.U. Lemieux et al. (ACS Symp. Ser., 39, 90 (1976)).

1-Thiophenyl-2-deoxy-2-phthalimido-β-D-glucopyranose [5]

[2] (0.31-8.02 g, 0.68-17.7 mmol) is dissolved in 3.2-100 ml of dry dichloromethane, and thiophenol (0.15-3.9 g, 1.36-35.4 mmol) and tin tetrachloride (0.35-9.22 g, 1.36-35.4 mmol) are added and the mixture is stirred until reaction is complete. The reaction mixture is diluted and then washed with saturated sodium bicarbonate solution. The crude product [3] obtained in this way is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.27-7.14 g, 0.54-14.2 mmol, about 80%) and is dissolved in anhydrous methanol (3-90 ml) and catalytic amounts of sodium methylate (prepared from 0.002-0.05 g of sodium in 0.1-2 ml of methanol) are added. After the reaction is complete, the mixture is neutralized with an acidic ion exchanger (0.2-5 g Amberlite IR-120, H^+-form) (4: 0.21-5.31 g, 0.54-14.1 mmol, about 99% yield).

FAB-MS: $M+H^+$ = 378.

NMR data consistent with K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)); and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

1-Thiophenyl-3-O-allyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose [6]

The crude product [5] (0.21-5.31 g, 0.54-14.1 mmol) is stirred in anhydrous acetonitrile (3-90 ml) with benzaldehyde dimethyl acetal (0.12-3.22 g, 0.81-21.1 mmol) and catalytic amounts of p-toluenesulfonic acid (4-90 mg)

until the reaction is finished. The crude product resulting after washing with saturated sodium bicarbonate solution is taken up in anhydrous dimethylformamide (2.5-85 ml), and sodium hydride (0.02-0.51 g, 0.81-21.15 mmol) and allyl bromide (0.09-2.37 g, 0.75-19.74 mmol) are added. After the reaction is complete, methanol (0.06-1.55 ml, 2.7-70.5 mmol) is added, and the mixture is diluted with ethyl acetate and washed successively with saturated ammonium chloride and saturated sodium bicarbonate solution. The crude product [5] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.23-5.98 g, 0.46-12.13 mmol, about 86%) and subsequently taken up in dry tetrahydrofuran (3.5-90 ml), and sodium cyanoborohydride (0.67-17.7 g, 10.8-282 mmol) is added. Subsequently, ethereal hydrochloric acid solution is slowly added until reaction is complete. After filtration, the organic phase is diluted with ethyl acetate and washed with saturated sodium bicarbonate solution to neutrality. The crude product [6] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.17-4.56 g, 0.35-9.17 mmol, about 74% yield).

FAB-MS: $M+H^+ \approx 416$.

NMR data consistent with K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)); and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

3.1.2 Synthesis of the galactose unit [11]

1-Thiophenyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose [8]

Galactose [7] (0.18-3.6 g, 1-20 mmol) is suspended in dichloromethane (5-90 ml), and acetic anhydride (0.75-15.43 g, 7.5-150 mmol), triethylamine (0.8-16.3 g, 8-160 mmol) and catalytic amounts of dimethylaminopyridine (0.01-0.1 g) are added. After the reaction is complete, the mixture is poured into ice-water and the

product is extracted with dichloromethane. The crude product obtained by washing the extract with dilute hydrochloric acid and saturated sodium bicarbonate solution is dissolved in dry dichloromethane (3.5-110 ml), and thiophenol (0.22-4.41 g, 2-40 mmol) and tin tetrachloride (0.51-10.28 g, 2-40 mmol) are added. After the reaction is complete, the mixture is diluted, and washing with saturated sodium bicarbonate solution results in the crude product [8] which is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.374-7.48 g, 0.85-17 mmol, about 85% yield).

FAB-MS: $M+H^+$ = 441.
NMR data consistent with K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)); and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

1-Thiophenyl-4,6-O-benzylidene-β-D-galactopyranose [9]

[8] (0.374-7.48 g, 0.85-17 mmol) is dissolved in anhydrous methanol (4-150 ml), and catalytic amounts of sodium methylate (prepared from 0.003-0.06 g of sodium in 0.1-2.5 ml of methanol) are added. After the reaction is complete, the mixture is neutralized with an acidic ion exchanger (0.25-6 g Amberlite IR-120, H^+-form) and evaporated. The resulting reaction product is stirred in anhydrous acetonitrile (4.5-150 ml) with benzaldehyde dimethyl acetal (0.19-5.05 g, 1.27-33.1 mmol) and catalytic amounts of p-toluenesulfonic acid (4-90 mg) until the reaction is finished. Washing with saturated sodium bicarbonate solution results in the crude product [9] (0.28-5.51 g, 0.77-15.3 mmol, about 90% yield).

FAB-MS: $M+H^+$ = 361.
NMR data consistent with K.C. Nicolaou et al. J. Am. Chem. Soc. 114, 3127 (1992) and C.W. Hummel, Ph. D. Dissertation, University of California, San Diego, 1993.

1-Thiophenyl-2,3-di-O-acetyl-6-O-benzyl-β-D-galacto-pyranose [10]

[9] (0.28-5.51 g, 0.77-15.3 mmol) is suspended in dichloromethane (4-60 ml), and acetic anhydride (0.23-4.75 g, 2-40 mmol), triethylamine (0.25-5.02 g, 2.46-49.23 mmol) and catalytic amounts of dimethylamino-pyridine (0.01-0.08 g) are added. After the reaction is complete, the mixture is poured into ice-water and the product is extracted with dichloromethane. The crude product obtained by washing the extract with dilute hydrochloric acid and saturated sodium bicarbonate solution is dissolved in dry tetrahydrofuran (2.45-130 ml), and sodium cyanoborohydride (0.96-25.3 g, 15.4-402.8 mmol) is added. Subsequently ethereal hydrochloric acid solution is added slowly until the reaction is complete. After filtration, the organic phase is diluted with ethyl acetate and washed with saturated sodium bicarbonate solution to neutrality. The crude product [10] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.27-5.46 g, 0.62-12.24 mmol, about 80% yield).

FAB-MS: $M+H^+$ = 447.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

1-Fluoro-2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galacto-pyranose [11]

[9] (0.27-5.46 g, 0.62-12.24 mmol) is suspended in dichloromethane (4-60 ml) and acetic anhydride (0.14-2.91 g, 1.24-24.8 mmol), triethylamine (0.15-3.11 g, 1.53-30.53 mmol) and catalytic amounts of dimethyl-aminopyridine (0.003-0.04 g) are added. After the reaction is complete, the mixture is poured into ice-water and the product is extracted with dichloromethane. The crude product obtained by washing the extract with

dilute hydrochloric acid and saturated sodium bicarbonate solution is taken up in dry dichloromethane (5-80 ml) and, at -78°C, dimethylaminosulfur trifluoride (0.25-4.89 g) and N-bromosuccinimide (0.11-3.26 g, 0.93-18.36 mmol) are added, and the mixture is warmed to room temperature. After washing with saturated sodium bicarbonate solution, the mixture is concentrated and the byproducts of the reaction are removed by silica gel chromatography (stepwise gradient: ethyl acetate/isohexane) to produce [11] (0.21-4.14 g, 0.53-10. mmol, about 85% yield).

FAB-MS: $M+H^+$ = 399.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

3.1.3 Synthesis of the fucose unit [17]

1-Thiophenyl-2,3,4-tri-O-acetyl-β-L-fucose [16]

Fucose [15] (0.16-32.8 g, 1-20 mmol) is suspended in dichloromethane (5-90 ml), and acetic anhydride (0.75-15.43 g, , 7.5-150 mmol), triethylamine (0.8-16.3 g, 8-160 mmol) and catalytic amounts of dimethylaminopyridine (0.01-0.1 g) are added. After the reaction is complete, the mixture is poured into ice-water and the product is extracted with dichloromethane. The crude product obtained by washing the extract with dilute hydrochloric acid and saturated sodium bicarbonate solution is dissolved in dry dichloromethane (3.5-110 ml), and thiophenol (0.22-4.41 g, 2-40 mmol) and tin tetrachloride (0.51-10.28 g, 2-40 mmol) are added. After the reaction is complete, the mixture is diluted, and washing with saturated sodium bicarbonate solution results in the crude product [16] which is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.34-6.88 g, 0.9-18 mmol, about 90% yield).

FAB-MS: $M+H^+$ = 382.
NMR data consistent with K.C. Nicolaou et al., (J. Am. Chem. Soc. 112, 3695 (1990)), and T.J. Caulfield, Ph.D. Dissertation, The University of Pennsylvania, 1991.

1-Thiophenyl-2,3,4-tri-O-benzyl-β-L-fucose [17]

[16] (0.34-6.88 g, 0.9-18 mmol) is dissolved in anhydrous methanol (5-180 ml), and catalytic amounts of sodium methylate (prepared from 0.005-0.09 g of sodium in 0.2-2.9 ml of methanol) are added. After the reaction is complete, the mixture is neutralized with an acidic ion exchanger (0.3-6.7 g Amberlite IR-120, H^+-Form) and evaporated. The resulting reaction product is taken up in anhydrous dimethylformamide (5-140 ml) and sodium hydride (0.16-3.21 g, 6.75-135 mmol) and benzyl bromide (0.92-18.46 g, 5.4-108 mmol) are added. After the reaction is complete, methanol (0.16-3.2 g, 5-100 mmol) is added, and the mixture is diluted with ethyl acetate and washed successively with saturated ammonium chloride and saturated sodium bicarbonate solution. The crude product after chromatography on silica gel (ethyl acetate/isohexane gradient) is subsequently taken up in dry dichloromethane (8-120 mol) and, at -78°C, dimethylaminosulfur trifluoride (0.36-7.09 g, 2.69-53.18 mmol) and N-bromosuccinimide (0.16-4.73 g, 1.35-26.6 mmol) are added, and the mixture is warmed to room temperature. After washing with saturated sodium bicarbonate solution, the mixture is concentrated, and the byproducts of the reaction are removed by silica gel chromatography (stepwise gradient: ethyl acetate/isohexane) to result in [17] (0.33-6.47 g, 0.72-14.4 mmol, about 80% yield).

FAB-MS: $M+H^+$ = 450.
NMR data consistent with K. C. Nicolaou et al., (J. Am. Chem. Soc. 112, 3695 (1990)); and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

3.2 Assembly of the disaccharide from the N-acetylglucosamine unit and the galactose unit

1-Thiophenyl-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1-4)-3-O-alkyl-2-deoxy-2-acetamido-β-D-glucopyranose [12]

The N-acetylglucosamine derivative [5] (0.17-4.56 g, 0.35-9.17 mmol) and the galactose derivative [11] (0.21-4.14 g, 0.53-10.4 mmol) are dissolved in dichloromethane (3-120 ml) and, at 0°C, 4 Å molecular sieves (0.15-7.8 g), silver perchlorate (0.11-5.39, 0.525-26.0 mmol) and tin(II) chloride (0.1-4.93 g, 0.525-26 mmol) are added. After the reaction is complete, the mixture is filtered and washed with saturated sodium bicarbonate solution. The crude product [12] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.23-5.61 g, 0.26-6.42 mmol, about 70% yield).

FAB-MS: $M+H^+$ = 874.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992)); and C.W: Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

1-Thiophenyl-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1-4)-2-deoxy-2-acetamido-β-D-glucopyranose [13]

[12] (0.23-5.61 g, 0.26-6.42 mmol) is dissolved in ethanol (3-90 ml) and heated at 95°C with catalytic amounts of tetrakis(triphenylphosphine)ruthenium(II) hydride (0.005-0.04 g). After the reaction is complete, the mixture is filtered, concentrated and taken up in methanol (2.5-85 ml) and catalytic amounts of p-toluenesulfonic acid (0.001-0.08 g) are added. After the reaction is complete, the mixture is washed with saturated sodium bicarbonate solution and, after concentration, the crude product [13] is obtained. Purification takes place by chromatography on silica gel with an ethyl acetate/

isohexane gradient (0.2-4.81 g, 0.234-5.78 mmol, about 90% yield).

FAB-MS: $M+H^+$ = 834.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

3.3 Assembly of the trisaccharide from the disaccharide and the fucose unit

1-Thiophenyl-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1-4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)]-2-deoxy-2-acetamido-β-D-glucopyranose [18]

[13] (0.2-4.81 g, 0.234-5.78 mmol) is dissolved with [17] (0.17-4.15 g, 0.37-9.25 mmol) in dry diethyl ether (5-100 ml) and stirred with 4 Å molecular sieves (0.1-6.8 g), silver perchlorate (0.15-3.59 g, 0.702-17.34 mmol) and tin(II) chloride (0.13-3.29 g, 0.702-17.34 mmol) until the reaction is complete. The mixture is subsequently filtered, and the crude product [18] obtained after concentration is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.25-6.2 g, 0.2-4.91 mmol, about 85% yield).

FAB-MS: $M+H^+$ = 1262.
NMR data consistent with K. C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

1-(6-Aminohexyl)-O-[(-β-D-galactopyranosyl)-(1-4)]-O-[(α-L-fucopyranosyl)-(1-3)-]-2-deoxy-2-acetamido-β-D-glucopyranose [19]

[18] (0.25-6.2 g, 0.2-4.91 mmol) and 6-azidohexanol (0.11-2.47 g, 0.4-9.82 mmol) are dissolved in dry acetonitrile and, at 0°C, 3 Å molecular sieves (0.1-2.5 g) and trimethylsilyl trifluoromethanesulfonate

(0.01-0.78 g, 0.02-7 mmol) are added. After the reaction is complete, the mixture is filtered and washed with saturated sodium bicarbonate solution. Concentration results in the crude product which is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.18-4.48 g, 0.13-3.19 mmol, about 65% yield). It is subsequently taken up in methanol/hydrazine hydrate (1:1) (1-50 ml) and heated at 80°C until reaction is complete. After evaporation of the solvent, the crude product is taken up in dichloromethane/methanol (1:1) and stirred at 0°C with acetic anhydride (1.3-40 mmol) until reaction is complete. Evaporation is followed by reduction of the azide with hydrogen in the presence of catalytic amounts of palladium on carbon (10%) (0.01-1.5 g) in methanol (0.5-35 ml). The crude product obtained after evaporation is purified by gel chromatography on Biogel P2 [19] (0.06-1.35 g, 0.09-2.23 mmol, about 70% yield).

FAB-MS: $M+H^+$ = 608.

^{1}H-NMR [300 MHz, DMSO]: 7.98 ($N\underline{H}_3^+OAc^-$), 4.92 (Fuc-$\underline{H}$-1), 4.65 ($\underline{H}$NAc), 4.36 (GlcNAc-$\underline{H}$-1), 4.28 (Gal-$\underline{H}$-1), 4.1-3.35 (m), 3.26 ($C\underline{H}_2$-$NH_3^+OAc^-$), 1.79 (GlcNCOC$\underline{H}_3$) 1.58-1.18 (O-($C\underline{H}_2$)$_5$-CH_2-$NH_3^+OAc^-$), 0.99 (Fuc-C$\underline{H}_3$) ppm.

4. Synthesis of the tetrasaccharide

4.1 Synthesis of the individual sugar units

4.1.1 Synthesis of the N-acetylglucasamine unit [6]

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose [2]

D-Glucosamine hydrochloride [1] (0.216-5.32 g, 1-20 mmol) is added to a solution of sodium methylate (prepared from 0.023-0.56 g of sodium and 1-20 ml of methanol), shaken for 10 min and filtered to remove precipitated sodium chloride. The filtrate is stirred with phthalic anhydride

(0.074-1.48 g, 0.5-10 mmol) for 10 min and then triethylamine (0.1-2.02 g, 1-20 mmol) is added. After the reaction is complete, the solvent is evaporated and the residue is cooled and pyridine (2-40 ml) and acetic anhydride (5-20 ml) are added. After 16 h at room temperature, the mixture is poured into ice and the product is extracted with dichloromethane. Washing with water, dilute hydrochloric acid and saturated sodium bicarbonate solution results in the crude product which is recrystallized from ether (0.31-8.02 g, about 82% yield).

FAB-MS: $M+H^+$ = 454.
NMR data consistent with R.U. Lemieux et al. (ACS Symp. Ser., 39, 90 (1976)).

1-Thiophenyl-2-deoxy-2-phthalimido-β-D-glucopyranose [5]

[2] (0.31-8.02 g, 0.68-17.7 mmol) is dissolved in 3.2-100 ml of dry dichloromethane, and thiophenol (0.15-3.9 g, 1.36-35.4 mmol) and tin tetrachloride (0.35-9.22 g, 1.36-35.4 mmol) are added and the mixture is stirred until reaction is complete. The reaction mixture is diluted and then washed with saturated sodium bicarbonate solution. The crude product [3] obtained in this way is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.27-7.14 g, 0.54-14.2 mmol, about 80%) and is dissolved in anhydrous methanol (3-90 ml) and catalytic amounts of sodium methylate (prepared from 0.002-0.05 g of sodium in 0.1-2 ml of methanol) are added. After the reaction is complete, the mixture is neutralized with an acidic ion exchanger (0.2-5 g Amberlite IR-120, H^+-form) [4] (0.21-5.31 g, 0.54-14.1 mmol, about 80% yield).

FAB-MS: $M+H^+$ = 378.
NMR data consistent with K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)); and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

1-Thiophenyl-3-O-allyl-6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose [6]

The crude product [5] (0.21-5.31 g, 0.54-14.1 mmol) is stirred in anhydrous acetonitrile (3-90 ml) with benzaldehyde dimethyl acetal (0.12-3.22 g, 0.81-21.1 mmol) and catalytic amounts of p-toluenesulfonic acid (4-90 mg) until the reaction is finished. The crude product resulting after washing with saturated sodium bicarbonate solution is taken up in anhydrous dimethylformamide (2.5-85 ml), and sodium hydride (0.02-0.51 g, 0.81-21.15 mmol) and allyl bromide (0.09-2.37 g, 0.75-19.74 mmol) are added. After the reaction is complete, methanol (0.06-1.55 ml, 2.7-70.5 mmol) is added, and the mixture is diluted with ethyl acetate and washed successively with saturated ammonium chloride and saturated sodium bicarbonate solution. The crude product [5] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.23-5.98 g, 0.46-12.13 mmol, about 86%) and subsequently taken up in dry tetrahydrofuran (3.5-90 ml), and sodium cyanoborohydride (0.67-17.7 g, 10.8-282 mmol) is added. Subsequently, ethereal hydrochloric acid solution is slowly added until reaction is complete. After filtration, the organic phase is diluted with ethyl acetate and washed with saturated sodium bicarbonate solution to neutrality. The crude product [6] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.17-4.56 g, 0.35-9.17 mmol, about 74% yield).

FAB-MS: $M+H^+$ = 416.

NMR data consistent with K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)); and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

4.1.2 Synthesis of the galactose unit [11]

1-Thiophenyl-2,3,4,6-tetra-O-acetylgalacto-β-D-pyranose [8]

Galactose [7] (0.18-3.6 g, 1-20 mmol) is suspended in dichloromethane (5-90 ml), and acetic anhydride (0.75-15.4 g, 7.5-150 mmol), triethylamine (0.8-16.3 g, 8-160 mmol) and catalytic amounts of dimethylaminopyridine (0.01-0.1 g) are added. After the reaction is complete, the mixture is poured into ice-water and the product is extracted with dichloromethane. The crude product obtained by washing the extract with dilute hydrochloric acid and saturated sodium bicarbonate solution is dissolved in dry dichloromethane (3.5-110 ml), and thiophenol (0.22-4.41 g, 2-40 mmol) and tin tetrachloride (0.51-10.28 g, 2-40 mmol) are added. After the reaction is complete, the mixture is diluted, and washing with saturated sodium bicarbonate solution results in the crude product [8] which is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.374-7.48 g, 0.85-17 mmol, about 85% yield).

FAB-MS: $M+H^+$ = 441.
NMR data consistent with K.C. Nicolaou et al. (J. Am. Chem. Soc. 112, 3695 (1990)); and T.J. Caulfield, Ph. D. Dissertation, The University of Pennsylvania, 1991.

1-Thiophenyl-4,6-O-benzylidene-β-D-galactopyranose [9]

[8] (0.374-7.48 g, 0.85-17 mmol) is dissolved in anhydrous methanol (4-150 ml), and catalytic amounts of sodium methylate (prepared from 0.003-0.06 g of sodium in 0.1-2.5 ml of methanol) are added. After the reaction is complete, the mixture is neutralized with an acidic ion exchanger (0.25-6 g Amberlite IR-120, H^+-form) and evaporated. The resulting reaction product is stirred in anhydrous acetonitrile (4.5-150 ml) with benzaldehyde

dimethyl acetal (0.19-5.05 g, 1.27-33.1 mmol) and catalytic amounts of p-toluenesulfonic acid (4-90 mg) until the reaction is finished. Washing with saturated sodium bicarbonate solution results in the crude product [9] (0.28-5.51 g, 0.77-15.3 mmol, about 90% yield).

FAB-MS: $M+H^+$ = 361.
NMR data consistent with K.C. Nicolaou et al. J. Am. Chem. Soc. 114, 3127 (1992) and C.W. Hummel, Ph. D. Dissertation, University of California, San Diego, 1993.

1-Thiophenyl-2,3-di-O-acetyl-6-O-benzyl-β-D-galactopyranose [10]

[9] (0.28-5.51 g, 0.77-15.3 mmol) is suspended in dichloromethane (4-60 ml), and acetic anhydride (0.23-4.75 g, 2-40 mmol), triethylamine (0.25-5.02 g, 2.46-49.23 mmol) and catalytic amounts of dimethylaminopyridine (0.01-0.08 g) are added. After the reaction is complete, the mixture is poured into ice-water and the product is extracted with dichloromethane. The crude product obtained by washing the extract with dilute hydrochloric acid and saturated sodium bicarbonate solution is dissolved in dry tetrahydrofuran (2.45-130 ml), and sodium cyanoborohydride (0.96-25.3 g, 15.4-402.8 mmol) is added. Subsequently ethereal hydrochloric acid solution is added slowly until the reaction is complete. After filtration, the organic phase is diluted with ethyl acetate and washed with saturated sodium bicarbonate solution to neutrality. The crude product [10] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.27-5.46 g, 0.62-12.24 mmol, about 80% yield).

FAB-MS: $M+H^+$ = 447.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

1-Fluoro-2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galacto-pyranose [11]

[9] (0.27-5.46 g, 0.62-12.24 mmol) is suspended in dichloromethane (4-60 ml) and acetic anhydride (0.14-2.91 g, 1.24-24.8 mmol), triethylamine (0.15-3.11 g, 1.53-30.53 mmol) and catalytic amounts of dimethylamino-pyridine (0.003-0.04 g) are added. After the reaction is complete, the mixture is poured into ice-water and the product is extracted with dichloromethane. The crude product obtained by washing the extract with dilute hydrochloric acid and saturated sodium bicarbonate solution is taken up in dry dichloromethane (5-80 ml) and, at -78°C, dimethylaminosulfur trifluoride (0.25-4.89 g, 1.86-36.7 mmol) and N-bromosuccinimide (0.11-3.26 g, 0.93-18.36 mmol) are added, and the mixture is warmed to room temperature. After washing with saturated sodium bicarbonate solution, the mixture is concentrated and the byproducts of the reaction are removed by silica gel chromatography (stepwise gradient: ethyl acetate/isohexane) to produce [11] (0.21-4.14 g, 0.53-10.4 mmol, about 85% yield).

FAB-MS: $M+H^+$ = 399.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

4.1.3 Synthesis of the fucose unit [17]

1-Thiophenyl-2,3,4-tri-O-acetyl-β-L-fucose [16]

Fucose [15] (0.16-32.8 g, 1-20 mmol) is suspended in dichloromethane (5-90 ml), and acetic anhydride (0.75-15.43 g, 7.5-150 mmol), triethylamine (0.8-16.3 g, 8-160 mmol) and catalytic amounts of dimethylamino-pyridine (0.01-0.1 g) are added. After the reaction is complete, the mixture is poured into ice-water and the product is extracted with dichloromethane. The crude

product obtained by washing the extract with dilute hydrochloric acid and saturated sodium bicarbonate solution is dissolved in dry dichloromethane (3.5-110 ml) and thiophenol (0.22-4.41 g, 2-40 mmol) and tin tetrachloride (0.51-10.28 g, 2-40 mmol) are added. After the reaction is complete, the mixture is diluted, and washing with saturated sodium bicarbonate solution results in the crude product [16] which is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.34-6.88 g, 0.9-18 mmol, about 90% yield).

FAB-MS: $M+H^+$ = 382.
NMR data consistent with K.C. Nicolaou et al., (J. Am. Chem. Soc. 114, 3127 (1992); C.W. Hummel, Ph.D. Dissertation, The University of California, San Diego, 1993.

1-Thiophenyl-2,3,4-tri-O-benzyl-β-L-fucose [17]

[16] (0.34-6.88 g, 0.9-18 mmol) is dissolved in anhydrous methanol (5-180 ml), and catalytic amounts of sodium methylate (prepared from 0.005-0.09 g of sodium in 0.2-2.9 ml of methanol) are added. After the reaction is complete, the mixture is neutralized with an acidic ion exchanger (0.3-6.7 g Amberlite IR-120, H^+-form) and evaporated. The resulting reaction product is taken up in anhydrous dimethylformamide (5-140 ml) and sodium hydride (0.16-3.21 g, 6.75-135 mmol) and benzyl bromide (0.92-18.46 g, 5.4-108 mmol) are added. After the reaction is complete, methanol (0.16-3.2 g, 5-100 mmol) is added, and the mixture is diluted with ethyl acetate and washed successively with saturated ammonium chloride and saturated sodium bicarbonate solutions. The crude product after chromatography on silica gel (ethyl acetate/isohexane gradient) is subsequently taken up in dry dichloromethane (8-120 mol) and, at -78°C, dimethylaminosulfur trifluoride (0.36-7.09 g, 2.69-53.18 mmol) and N-bromosuccinimide (0.16-4.73 g, 1.35-26.6 mmol) are added, and the mixture is warmed to room temperature.

After washing with saturated sodium bicarbonate solution, the mixture is concentrated, and the byproducts of the reaction are removed by silica gel chromatography (stepwise gradient: ethyl acetate/isohexane) to result in [17] (0.33-6.47 g, 0.72-14.4 mmol, about 80% yield).

FAB-MS: $M+H^+$ = 450.
NMR data consistent with K. C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); C.W. Hummel, Ph. D. Dissertation, The University of California, San Diego, 1993.

4.1.4 Synthesis of the neuraminic acid unit [22]

Methyl 5-acetamido-4,7,8,9-tetra-O-benzyl-2,3,5-trideoxy-2,3-dehydro-D-glycero-α-D-galacto-2-nonulopyranosylonate [21]

N-Acetylneuraminic acid [20] (0.31-6.18 g, 1-20 mmol) is added to acetic anhydride (3-100 ml), and catalytic amounts of sulfuric acid (0.001-0.5 g) are added. After the reaction is complete, the mixture is poured into ice-water and the crude product is extracted with ethyl acetate. The extracts are washed with saturated sodium bicarbonate solution and concentrated. The crude product is mixed with water and subsequently 1 M sodium hydroxide solution is added. After the reaction is complete, the solvent is removed and the crude product is taken up in dimethylformamide (5-260 ml). Subsequently sodium hydroxide (0.03-7.2 g, 0.8-180 mmol), benzyl bromide (0.14-30.78 g, 0.8-180 mmol) and catalytic amounts of tetrabutylammonium iodide (0.005-0.75 g) are added, and the mixture is heated at 60°C until the reaction is complete. Then methanol (1.5-35 ml) is added and the reaction mixture is adjusted to pH 2 by adding 1 M aqueous hydrochloric acid. The crude product is obtained by extraction with ethyl acetate and washing of the extracts with saturated sodium bicarbonate solution. Purification takes place by chromatography on silica gel with a dichloromethane/methanol gradient [21] (0.29-5.77 g, 0.45-9 mmol,

yield about 45%).

FAB-MS: $M+H^+$ = 642.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W: Hummel, Ph. D. Dissertation, University of California, San Diego, 1993.

Chloromethyl 5-acetamido-4,7,8,9-tetra-O-benzyl-3,5-dideoxy-3-thiophenyl-D-glycero-α-D-galacto-2-nonulopyranosylonate [22]

[21] (0.29-5.77 g, 0.45-9 mmol) is taken up in dichloromethane (2.5-80 ml) and phenylsulfenyl chloride is added. After the reaction is complete, the mixture is washed with saturated sodium bicarbonate solution and concentrated. The crude product [22] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.29-5.83 g, 0.36-7.2 mmol, about 80% yield).

FAB-MS: $M+H^+$ = 810.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); C.W. Hummel, Ph. D. Dissertation, University of California, San Diego, 1993.

4.2 Assembly of the disaccharide from the N-acetylglucosamine unit and the galactose unit

1-Thiophenyl-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galactopyranosyl)-(1-4)-3-O-alkyl-2-deoxy-2-acetamido-β-D-glucopyranose [12]

The N-acetylglucosamine derivative [5] (0.17-4.56 g, 0.35-9.17 mmol) and the galactose derivative [11] (0.21-4.14 g, 0.53-10.4 mmol) are dissolved in dichloromethane (3-120 ml) and, at 0°C, 4 Å molecular sieves (0.15-7.8 g), silver perchlorate (0.11-5.39, 0.525-26 mmol) and tin(II) chloride (0.1-4.93 g, 0.525-26 mmol) are added. After the reaction is complete, the mixture is filtered and washed with saturated sodium bicarbonate solution.

The crude product [12] is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.23-5.61 g, 0.26-6.42 mmol, about 70% yield).

FAB-MS: $M+H^+$ = 874.

NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

1-Thiophenyl-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galacto-pyranosyl)-(1-4)-2-deoxy-2-acetamido-β-D-glucopyranose [13]

[12] (0.23-5.61 g, 0.26-6.42 mmol) is dissolved in ethanol (3-90 ml) and heated at 95°C with catalytic amounts of tetrakis(triphenylphosphine)ruthenium(II) hydride (0.005-0.04 g). After the reaction is complete, the mixture is filtered, concentrated and taken up in methanol (2.5-85 ml) and catalytic amounts of p-toluene-sulfonic acid (0.001-0.08 g) are added. After the reaction is complete, the mixture is washed with saturated sodium bicarbonate solution and, after concentration, the crude product [13] is obtained. Purification takes place by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.2-4.8 g, 0.234-5.78 mmol, about 90% yield).

FAB-MS: $M+H^+$ = 834.

NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

4.3 Assembly of the trisaccharide from the disaccharide and the fucose unit

1-Thiophenyl-(2,3,4-tri-O-acetyl-6-O-benzyl-β-D-galacto-pyranosyl)-(1-4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)]-2-deoxy-2-acetamido-β-D-glucopyranose [18]

[13] (0.2-4.81 g, 0.234-5.78 mmol) is dissolved with [17] (0.17-4.15 g, 0.37-9.25 mmol) in dry diethyl ether (5-100 ml) and stirred with 4 Å molecular sieves (0.1-6.8 g), silver perchlorate (0.15-3.59 g, 0.702-17.34 mmol) and tin(II) chloride (0.13-3.29 g, 0.702-17.34 mmol) until the reaction is complete. The mixture is subsequently filtered, and the crude product [18] obtained after concentration is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.25-6.2 g, 0.2-4.91 mmol, about 85% yield).

FAB-MS: $M+H^+$ = 1262.
NMR data consistent with K. C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); C.W: Hummel, Ph.D. Dissertation, University of California, San Diego, 1993.

4.4 Assembly of the tetrasaccharides from the trisaccharide and the N-acetylglucosamine unit

1-(o-Nitrobenzyl)-(6-O-benzyl-β-D-galactopyranosyl)-(1-4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)]-2-deoxy-2-acetamido-β-D-glucopyranose [23]

[18] (0.25-6.2 g, 0.2-4.91 mmol) is taken up in dry dichloromethane (3-85 ml) and, at -78°C, dimethylaminosulfur trifluoride (0.08-1.93 g, 0.6-14.73 mmol) and N-bromosuccinimide (0.05-1.23 g, 0.28-6.87 mmol) are added, and the mixture is warmed to room temperature. After washing with saturated sodium bicarbonate solution, the mixture is concentrated and the byproducts of the reaction are removed by silica gel chromatography (stepwise gradient: ethyl acetate/isohexane) [22] (0.33-6.47 g, 0.72-14.4 mmol, about 80% yield). The reaction product is dissolved in dichloromethane and, at -78°C, o-nitrobenzyl alcohol (0.08-1.88 g, 0.5-12.28 mmol), 4 Å molecular sieves (0.1-7.5 g), silver trifluoromethanesulfonate (0.18-4.42 g, 0.7-17.19 mmol) and hafnocene dichloride (0.27-6.53 g, 0.7-17.19 mmol) are added, and the mixture is allowed to warm to room temperature.

Filtration is followed by washing with saturated sodium bicarbonate solution and concentration. The crude product obtained in this way is purified by chromatography on silica gel (dichloromethane/methanol gradient) and dissolved in dry methanol (3-80 ml), and catalytic amounts of sodium methylate (prepared from 0.001-0.09 g of sodium in 0.2-2.5 ml of methanol) are added. After the reaction is complete, the mixture is neutralized with an acidic ion exchanger (0.2-6.5 g Amberlite IR-120, H^+-form). Evaporation results in [23].

FAB-MS: $M+H^+$ = 1186.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph. D. Dissertation, University of California, San Diego, 1993.

1-(O-N-Nitrobenzyl)-[(methyl-5-acetamido-4,7,8,9-tetra-O-benzyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonatyl)-(2-3)-(6-O-benzyl-β-D-galactopyranosyl)-(1-4)]-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)]-2-deoxy-2-acetamido-β-D-glucopyranose [24]

The resulting reaction product [23] is dissolved with [22] (0.1-2.81 g, 0.12-3.47 mmol) in tetrachloromethane and, at 0°C, 4 Å molecular sieves (0.08-0.7 g), mercury(II) cyanide (0.13-2.67 g, 0.36-10.43 mmol) and mercury(II) bromide (0.14-1.25 g, 0.12-3.47 mmol) are added, and the mixture is slowly warmed to room temperature. After washing with saturated sodium bicarbonate solution, the mixture is concentrated and the crude product is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient [24] (0.14-3.36 g, 0.07-1.72 mmol, about 35% yield).

FAB-MS: $M+H^+$ = 1959.
NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph. D. Dissertation, University of California, San Diego, 1993.

1-Fluoro-[(methyl-5-acetamido-4,7,8,9-tetra-O-benzyl-3,5-dideoxy-D-glucero-α-D-galacto-2-nonulopyranosylonatyl)-(2-3)-(6-O-benzyl-2,4-di-O-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1-3)-2-deoxy-2-acetamido-β-D-glucopyranose

[24] (0.14-3.36 g, 0.07-1.72 mmol) is dissolved in acetic anhydride (0.5-10 ml) and pyridine (0.5-10 ml), and catalytic amounts of dimethylaminopyridine (0.001-0.1 g) are added. After the reaction is complete, the mixture is poured into ice-water and the crude product is extracted with ethyl acetate. The solution is subsequently washed successively with dilute hydrochloric acid and saturated sodium bicarbonate solution. The reaction product is taken up in a 10:1 tetrahydrofuran/water mixture (0.5-30 ml) and irradiated at 0°C with a UV lamp. After the reaction is complete, the mixture is concentrated, taken up in toluene (0.5-60 ml) and heated under reflux with triphenyltin hydride (0.25-6.04 g; 0.7-17.2 mmol) and azoisobutyronitrile (0.01-0.25 g, 0.06-1.55 mmol). After the reaction is complete, the mixture is concentrated and the reaction product is chromatographed on silica gel with a dichloromethane/methanol gradient (0.036-0.92 g, 0.02-0.52 mmol, about 30% yield).

FAB-MS: $M+H^+$ = 1644.

NMR data consistent with K.C. Nicolaou et al., J. Am. Chem. Soc. 114, 3127 (1992); and C.W. Hummel, Ph. D. Dissertation, University of California, San Diego, 1993.

The purified product is dissolved in dry dichloromethane (0.1-10 ml) and, at -78°C, diethylaminosulfur trifluoride (0.016-0.41 g, 0.12-3.1 mmol) is added. After the reaction is complete, the mixture is extracted with saturated sodium bicarbonate solution to result, after concentration, in the crude product [25] which is purified by chromatography on silica gel with a dichloromethane/methanol gradient.

1-(6-Aminohexyl)-O-[(methyl-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonatyl)-(2-3)-(-β-D-galactopyranosyl)-(1-4)-(α-L-fucopyranosyl)-(1-3)-2-deoxy-2-acetamido-β-D-glucopyranose [26]

[25] and 6-azidohexanol (0.017-0.44 g, 0.12-3.1 mmol) are dissolved in dry acetonitrile and, at 0°C, 4 Å molecular sieves (0.06-1.5 g) and trimethylsilyl trifluoromethanesulfonate (0.001-1.4 g, 0.005-5.1 mmol) are added. After the reaction is complete, the mixture is filtered and washed with saturated sodium bicarbonate solution. Concentration results in the crude product which is purified by chromatography on silica gel with an ethyl acetate/isohexane gradient (0.021-0.51 g, 0.01-0.27 mmol, about 55% yield). It is subsequently taken up in methanol/hydrazine hydrate (1:1) (1-30 ml) and heated at 80°C until reaction is complete. After evaporation of the solvent, the crude product is taken up in dichloromethane/methanol (1:1) and stirred at 0°C with acetic anhydride (0.03-4.1 mmol) until the reaction is complete. Evaporation is followed by reduction of the azide with hydrogen in the presence of catalytic amounts of palladium on carbon (10%) (0.001-0.15 g) in methanol (1-45 ml). The crude product obtained after evaporation is purified by gel chromatography on Biogel P2 [26] (0.0064-0.17 g, 0.007-0.18 mmol, about 65% yield).

FAB-MS: $M+H^+ = 920$.

^{1}H-NMR [300 MHz, D_2O]: 4.92 (Fuc-H-1), 4.36 (GlcNAc-H-1), 4.28 (Gal-H-1), 4.1-3.35 (m), 3.29 (CH_2-$NH_3^+OAc^-$), 2.19 (NANA-H-3eq.), 1.93 (NANA-NCOCH_3), 1.81 (GlcNCOCH_3), 1.77 (NANA-H-3ax.), 1.58-1.18 (O-(CH_2)$_5$-CH_2-$NH_3^+OAc^-$), 0.99 (Fuc-CH_3) ppm.

Reaction of the polymer with the potentiator on the one hand and of the carbohydrate portion with the bifunctional spacer on the other hand to form covalent bonds.

5. Poly-D,L-succinimide-co-aspartamido-C_6-GlcNAc (degree of loading: 12%) (= Poly-D,L-succinimide-co-α,β-D,L-aspartamido-6-hexyl-2-deoxy-2-acetamido-β-D-glucopyranose)

PSI (1 g, 10.3 µmol, MW: 24,200) is introduced into DMF (= dimethylformamide, 4 ml, dried and distilled), and 1-(6-aminohexyl)-2-deoxy-2-acetamido-β-D-glucopyranose (H_2N-C_6-GlcNAc) (0.45 g, 1.41 mmol) in 4 ml of DMF is added. The mixture is stirred under N_2 at room temperature for 4 h. It is then precipitated with 80 ml of 1-butanol, and the resulting polymer is washed with methanol. After a second precipitation from DMF in 1-butanol, the product is again washed with methanol and subsequently dried under oil pump vacuum.

Yield: 1.2 g (86%)
Degree of substitution (DS) according to NMR: 12%

6. PHEA-co-aspartamido-C_6-GlcNAc (degree of loading: 12%) (= Poly-α,β-(2-hydroxyethyl)-D,L-aspartamide-co-α,β-D,L-aspartamido-6-hexyl-2-deoxy-2-acetamido-β-D-glucopyranose)

PSI-co-aspartamido-C_6-GlcNAc from Example 5 (0.8 g, 5.9 µmol) is dissolved in 6 ml of DMF, and freshly distilled hydroxyethylamine (325 mg, 5.3 mmol) is added. After stirring at room temperature under N_2 for 4 h, the mixture is precipitated in monobutanol. The resulting white flocculent polymer is washed with methanol, dissolved in H_2O and freeze-dried. After the second precipitation from the DMF/H_2O (15:1) in 1-butanol, the product is again washed with methanol and freeze-dried from H_2O.

Yield: 1 g (90%)
DS according to NMR: 12% H_2N-C_6-GlcNAc, 88% HEA
^{1}H-NMR (300 MHz, D_2O): 1.3 (-$(CH_2)_2$-), 1.5 (-CH_2-CH_2-NHCO- and -CH_2-CH_2-O-GlcNAc), 2.04 (GlcNCOCH_3), 2.8 (Asp.

$-CH_2-CO$), 3.35 ($-CONH-CH_2-CH_2-OH$), 3.65 ($-CONH-CH_2-CH_2-OH$), 4.5 (GlcNAc-H-1), 4.7 (Asp. -CH(NH-)-CO).

7. Poly-succinimide-co-HEA-co-aspartamido-C_6-GlcNAc (degree of loading: 12%) (= Poly-D,L-succinimide-co-α,β-(2-hydroxyethyl)-D,L-aspartamide-co-D,L-aspartamido-6-hexyl-2-deoxy-2-acetamido-β-D-glucopyranose)

PSI-co-aspartamido-C_6-GlcNAc from Example 5 (0.4 g, 3 μmol) and hydroxyethylamine (0.1 g, 1.6 mmol) are reacted in analogy to Example 6.

Yield: 380 mg (78%)
DS according to NMR: 53% HEA, 12% H_2N-C_6-GlcNAc

8 Poly-succinimide-co-aspartamido-C_6-GlcNAc (degree of loading: 22%) (= Poly-D,L-succinimide-co-α,β-D,L-aspartamido-6-hexyl-2-deoxy-2-acetamido-β-D-glucopyranose)

In analogy to Example 5, 0.32 g (3.3 mmol) of PSI is reacted with 0.3 g (1.0 mmol) of H_2N-C_6-GlcNAc and worked up by precipitation.

Yield: 400 mg (72%)
DS according to NMR: 22%

9. Poly-succinimide-co-HEA-co-aspartamido-C_6-GlcNAc (degree of loading: 22%) (= Poly-D,L-succinimide-co-α,β-(2-hydroxyethyl)-D,L-aspartamide-co-D,L-aspartamido-6-hexyl-2-deoxy-2-acetoamido-β-D-glucopyranose)

PSI-co-aspartamido-C_6GlcNAc from Example 8 (0.4 g, 2.39 mmol) and hydroxyethylamine (0.1 g, 1.67 mmol) are reacted in analogy to Example 6.

Yield: 410 mg (84%)
DS according to NMR: 22% 4, 60% HEA

10. Poly-HEA-co-3-dimethylamino-1-propylamidoaspartamide-co-aspartamido-C_6-GlcNAc (degree of loading: 12%) (= Poly-α,β-(2-hydroxyethyl)-D,L-aspartamide-co-α,β-(3-dimethylamino-1-propylamido-D,L-aspartamide-co-D,L-aspartamido-6-hexyl-2-deoxy-2-acetamido-β-D-glucopyranose)

PSI (0.1 g, 1.0 µmol) is dissolved in 1 ml of DMF, and H_2N-C_6-GlcNAc (45 mg, 0.14 mmol) and hydroxyethylamine (12 mg, 0.2 mmol) are added. After 3 h at 35°C, freshly distilled 3-dimethylamino-1-propylamine (105 mg, 0.1 mmol) and, after a further 3 h at 35°C, hydroxyethylamine (37 mg, 0.6 mmol) are added. The mixture is stirred for 3 h and worked up in analogy to Example 6.

Yield: 160 mg (82%)
DS according to NMR: 78% HEA, 10% dimethylaminopropylamide, 12% H_2N-C_6-GlcNAc

11 Poly-HEA-co-4-aminobutyl-1-amidoaspartamide-co-aspartamido-C_6-GlcNAc (degree of loading: 12%) (= Poly-α,β-(2-hydroxyethyl)-D,L-aspartamide-co-α,β-4-aminobutyl-1-amido-D,L-aspartamide-co-D,L-aspartamido-6-hexyl-2-deoxy-2-acetamido-β-D-glucopyranose)

PSI (0.75 g, 7.7 µmol) is dissolved in dried and distilled DMF (150 ml), and 1,4-diaminobutane (34 mg, 0.39 mmol) is added and the mixture is stirred at room temperature for 2 days. It is concentrated to about 1 ml under oil pump vacuum, H_2N-C_6-GlcNAc (34 mg, 1.1 mmol) is added and the mixture is stirred at 35°C for 3 h. After addition of hydroxyethylamine (0.47 g, 7.7 mmol) the mixture is stirred at 35°C for a further 3 h and subsequently worked up in analogy to Example 6.

Yield: 100 mg (70%)
DS according to NMR: 83% HEA, 12% H_2N-C_6-GlcNAc, 5% diaminobutane

12. Poly-HEA-co-aspartamido-C_6-LacNAc (= Poly-α,β-(2-hydroxyethyl)-D,L-aspartamide-co-D,L-aspartamido-6-hexyl-O-(β-D-galactopyranosyl)-(1-4)-2-deoxy-2-acetamido-β-D-glycopyranose)

PSI (70 mg, 0.72 µmol) is dissolved in 0.5 ml of DMF, and a solution of hydroxyethylamine (9 mg, 0.147 mmol) and H_2N-C_6-LacNAc (41 mg, 0.086 mmol) in 0.5 ml of DMF/NMP (10:1) is added and the mixture is stirred at 35°C for 6 h. After addition of hydroxyethylamine (35 mg, 0.58 mmol), the mixture is stirred for a further 3 h. Working up is carried out in analogy to Example 6.

Yield: 90 mg (66%)
DS according to NMR: 93% HEA, 7% H_2N-C_6-LacNAc
^{1}H-NMR (300 MHz, D_6DMSO): 1.2 (-$(CH_2)_2$-), 1.4 (-CH_2-CH_2-NHCO- and -CH_2-CH_2-O-LacNAc), 1.80 (GlcNCOCH_3), 3.15 (-CONH-CH_2-CH_2-OH), 4.2 (Gal-H-1), 4.3 (GlcNAc-H-1), 4.7 (Asp. -CH(NH)-CO).

13. Poly-HEA-co-aspartamido-C_6-sialyl Lewis X (= Poly-α,β-(2-hydroxyethyl)-D,L-aspartamide-co-D,L-aspartamido-6-hexyl-O-[(methyl-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonatyl)-(2-3)-(β-D-galactopyranosyl)-(1-4)]-(α-L-fucopyranosyl)-(1-3)-2-deoxy-2-acetamido-β-D-glucopyranose)

PSI (70 mg, 0.72 µmol) is dissolved in 0.5 ml of DMSO, hydroxyethylamine (18 mg, 0.3 mmol) is added and the mixture is stirred at 35°C for 2 h. Compound No. 26 (see page 91) (46 mg, 0.05 mmol) is dissolved with triethylamine (5 mg) in 0.2 ml of H_2O, added to the polymer mixture and stirred at 35°C for 6 h. The mixture is then left to react with the addition of H_2O and with additional hydroxylamine (27 mg, 0.45 mmol) for a further 4 h. Working up is carried out in analogy to Example 6.

Yield: 92 mg (64%)
DS according to NMR: 95% HEA, 5% compound No. 26

14) Poly-L-lysine-(2-hydroxyethyl)amide-co-L-lysinamido-C_6-GlcNAc-fumaramide (degree of loading: 13%) (= Poly-L-lysine-(2-hydroxyethyl)-amide-co-L-lysin-amido-6-hexyl-2-deoxy-2-acetamido-β-D-glucopyranose-fumaramide)

Poly-L-lysine methyl ester fumaramide (240 mg, 1 μmol) is dissolved in 5 ml of DMSO at 80°C. After cooling to room temperature, H_2N-C_6-GlcNAc (48 mg, 0.15 mmol) is added, and the mixture is stirred at 35°C for 4 h. Hydroxyethyl-amine (101 mg, 1 mmol) is added and the mixture is stirred at room temperature for a further 3 h. The working up comprises precipitation twice in acetone and freeze-drying from H_2O.

Yield: 166 mg (55%)

DS according to NMR: 87% HEA, 13% H_2N-C_6-GlcNAc

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A carbohydrate-containing polymer comprising:

(i) a hydrophilic polymer selected from the group consisting of poly-D,L-succinimide, poly-α,β-(2-hydroxyethyl)-D,L-aspartamide,poly-D,L-succinimide-poly-α,β-(2-hydroxyethyl)-D,L-aspartamide copolymer, poly-L-lysine methyl ester-fumaramide, polyglutamate, and hydroxyethylstarch;

(ii) a carbohydrate selected from the group consisting of N-acetylglucosamine, sialyl-Lewis X and sialyl-Lewis A; and

(iii) a bifunctional spacer linking said carbohydrate to said hydrophilic polymer.

2. A carbohydrate-containing polymer according to claim 1, wherein said bifunctional spacer is selected from

(i) a 6-aminohexanol-based spacer and

(ii) an N-(6'-hydroxyhexyl)-6-aminohexanoamide-based spacer,

whereby the amino group of said spacer is linked to said polymer via an amide bond, and the hydroxyl group of said spacer is linked to said carbohydrate via a glycosidic bond.

3. A carbohydrate-containing polymer according to claim 2, wherein said hydrophilic polymer is poly-D,L-succinimide, and said carbohydrate is N-acetylglucosamine.

4. A carbohydrate-containing polymer according to claim 3, wherein said polymer consists of poly-D,L-succinimide-co-α,β-D,L-aspartamido-6-hexyl-2-deoxy-2-acetamido-β-D-glucopyranose.

5. A carbohydrate-containing polymer according to claim 2, further comprising a potentiator.

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6. A carbohydrate-containing polymer according to claim 5, wherein said potentiator is a 2-aminoethanol-based potentiator, whereby said 2-aminoethanol is linked to said polymer via an amide linkage.

7. A carbohydrate-containing polymer according to claim 6, wherein said carbohydrate is N-acetyl glucosamine.

8. A carbohydrate-containing polymer according to claim 7, wherein said hydrophilic polymer is poly-D,L-succinimide.

9. A carbohydrate-containing polymer according to claim 7, wherein said carbohydrate-containing polymer consists of poly-α,β-(2-hydroxyethyl)-D,L-aspartamide-co-α,β-D,L-aspartamido-6-hexyl-2-deoxy-2-acetamido-β-D-glucopyranose.

10. A carbohydrate-containing polymer according to claim 6, wherein said hydrophilic polymer is poly-D,L-succinimide, and said carbohydrate is sialyl Lewis X.

11. A carbohydrate-containing polymer according to claim 10, wherein said carbohydrate-containing polymer consists of poly-α,β-(2-hydroxyethyl)-D,L-aspartamide-co-D,L-aspartamido-6-hexyl-O-[(methyl-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonatyl)-(2-3)-(β-D-galacto-pyranosyl)-(1-4)]-(α-L-fucopyranosyl)-(1-3)-2-deoxy-2-acetamido-β-D-glucopyranose.

12. A carbohydrate-containing polymer according to claim 7, wherein said hydrophilic polymer is poly-L-lysine methyl ester-fumaramide.

13. A carbohydrate-containing polymer according to claim 12, wherein said carbohydrate-containing polymer consists of poly-L-lysine-(2-hydroxyethyl)-amide-co-L-lysinamido-6-hexyl-2-deoxy-2-acetamido-β-D-glucopyranose-fumaramide.

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14. A pharmaceutical composition comprising a compound according to claim 1.

15. A process for preparing a compound according to claim 1, comprising the step of coupling said carbohydrate portion to said hydrophilic polymer portion via said bifunctional spacer.

16. The process according to claim 15, wherein said process comprises the steps of:

(i) forming a covalent bond between said carbohydrate and said bifunctional spacer to form a carbohydrate-spacer complex;

(ii) forming a covalent bond between the carbohydrate portion-spacer complex and said hydrophilic polymer.

17. A process for preparing a compound according to claim 5, comprising the steps of coupling said carbohydrate to said hydrophilic polymer via said bifunctional spacer and coupling the resulting carbohydrate-spacer-polymer complex with a potentiator.

18. The process according to claim 17, wherein said process comprises the steps of:

(i) forming a covalent bond between said carbohydrate and said bifunctional spacer;

(ii) forming a covalent bond between the carbohydrate-spacer complex and said hydrophilic polymer; and

(iii) forming a covalent bond between the carbohydrate-spacer-polymer complex and said potentiator.

19. The process according to claim 15, wherein said coupling step comprises a reaction selected from the group consisting of an alkylation, an acylation or an addition reaction with a double bond.

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20. The process according to claim 15, wherein said coupling step comprises a reaction comprising one or more enzymes selected from the group consisting of glycosidase, glycosyltransferases, transglycosidases and lipases.

21. The process according to claim 20, wherein said one or more enzymes assemble said carbohydrate portion from monosaccharide units and links said carbohydrate portion and said bifunctional spacer.

22. The process according to claim 18, wherein a lipase is employed to link a reactive group of said spacer to an activated group of said potentiator.

23. The process according to claim 22, wherein said activated group is a carboxylic acid, a methyl ester or a vinyl ester.

DATED this 12th day of August, 1996.

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Abstract of the disclosure

Physiologically tolerated and physiologically degradable polymer-based carbohydrate receptor blocker, a process for its preparation and its use

The invention relates to physiologically tolerated and physiologically degradable polymer-based carbohydrate receptor blockers with an HLB* of 10 to 20 containing: carbohydrate portion - bifunctional spacer - hydrophilic, biodegradable polymer - potentiator, where the carbohydrate portion which is composed of 1 to 20 naturally occurring, identical or different, monosaccharide units is coupled via one or more bifunctional spacers, which is a natural or synthetic molecule, to a hydrophilic, biodegradable polymer, where the polymer in turn is the carrier of one or more spacers, and the hydrophilic, biodegradable polymer is additionally linked to the potentiator which is a crosslinker, one or more groups with hydrophobic, hydrophilic or ionic properties or a solubility improver, to a process for the preparation and to their use in vivo for blocking carbohydrate-recognizing receptors.

The carbohydrate receptor blocker causes no intolerance reaction in vivo either in its entirety or in the form of degradation products.