CARBOHYDRATE-GLYCOLIPID CONJUGATE VACCINES

Specification

The present invention relates to the field of synthesizing and biologically evaluating of a novel class of carbohydrate-based vaccines. The new vaccines consist of a multi-modular structure which allows applying the vaccine to a whole variety of pathogenes. This method allows preparing vaccines against all pathogens expressing immunogenic carbohydrate antigens. As conjugation of antigenic carbohydrates to proteins is not required the conjugate vaccine is particularly heat stable. No refrigeration is required, a major drawback of protein-based vaccines.

Background of the invention

High prevalence of many infectious diseases, such as invasive pneumococcal disease (IPD) and increasing antibiotic resistance of the related pathogens requires urgent development of protective vaccines. Especially as existing vaccines exhibit major drawbacks such as variable immunogenicity and the lack of development of immunological memory.

Vaccines have traditionally consisted of live attenuated pathogens, whole inactivated organisms or inactivated toxins. In many cases, these approaches have been successful at inducing immune protection based on antibody mediated responses. However, certain pathogens, e.g., HIV, HCV, TB, and malaria, require the induction of cell-mediated immunity (CMI). Non-live vaccines have generally proven ineffective in producing CMI. In addition, although live vaccines may induce CMI, some live attenuated vaccines may cause disease in immunosuppressed subjects.
In contrast to older vaccines which were typically based on live attenuated or non-replicating inactivated pathogens, modern vaccines are composed of synthetic, recombinant, or highly purified subunit antigens. Subunit-vaccines are designed to include only the antigens required for protective immunization and are believed to be safer than whole inactivated or live-attenuated vaccines. However, the purity of the subunit antigens and the absence of the self-adjuvanting immunomodulatory components associated with attenuated or killed vaccines often result in weaker immunogenicity.

The immunogenicity of a relatively weak antigen can be enhanced by the simultaneous or more generally conjoined administration of the antigen with an "adjuvant", usually a substance that is not immunogenic when administered alone, but will evoke, increase and/or prolong an immune response to an antigen. In the absence of adjuvant, reduced or no immune response may occur, or worse the host may become tolerized to the antigen.

Adjuvants can be found in a group of structurally heterogeneous compounds (Gupta et al., 1993, Vaccine, 11: 293-306). Classically recognized examples of adjuvants include oil emulsions (e.g., Freund's adjuvant), saponins, aluminium or calcium salts (e.g., alum), non-ionic block polymer surfactants, lipopolysaccharides (LPS), mycobacteria, tetanus toxoid, and many others. Theoretically, each molecule or substance that is able to favor or amplify a particular situation in the cascade of immunological events, ultimately leading to a more pronounced immunological response can be defined as an adjuvant.

A galactosylceramide (α-GalCer) is a glycolipid, more specifically a glycosylceramide, originally isolated from Okinawan marine sponges (Natori et al., Tetrahedron, 50: 2771-2784, 1994), or its synthetic analog KRN7000 [(2S,3S,4R)-1-O-(α-D-galactopyranosyl)-2-(N-hexacosanoylamino)-l,3,4-octadecanetriol], which can be obtained from Pharmaceutical Research Laboratories, Kirin Brewery (Gumna, Japan) or synthesized as described previously (see, e.g., Kobayashi et al., 1995, Oncol. Res., 7:529-534; Kawano et al., 1997, Science, 278: 1626-9; Burdin et al., 1998, J. Immunol., 161:3271; Kitamura et al., 1999, J. Exp. Med., 189:1121; U.S. Patent No. 5,936,076).

It was shown that α-GalCer can stimulate natural killer (NK) activity and cytokine production by natural killer T (NKT) cells and exhibits potent antitumor activity in vivo (Kawano et al., 1998, Proc. Natl Acad. Sci. USA, 95:5690). After intake by antigen presenting cell (APC), which is represented by dendritic cell (DC) and the like, α-galactosylceramide is presented on the cellular membrane by a CD1d protein similar to major histocompatible complex (MHC) class I molecule. NKT cells are activated by recognition using TCR (T cell receptor) of the thus-presented complex of CD1d protein and α-galactosylceramide, which triggers various immune reactions. Invariant Natural Killer T cells have been also shown to induce B cell activation, enhancing B cell proliferation and antibody production (Galli *et al, Vaccine,* 2003, 21: 2148-S2154; Galli *et al, J* Exp. *Med,* 2003, 197: 1051-1057).

These studies open the possibility that α-GalCer may play an equally important role in bridging not only innate immunity mediated by NKT cells, but also adaptive immunity mediated by B cells, T helper (Th) cells and T cytotoxic (Tc) cells. Recently,

α-GalCer has been shown to act as an adjuvant for a variety of co-administered protein antigens and saccharide antigens (W003/0098 12).

The development so far exhibits the simultaneous use of the vaccine and an adjuvant that produces the desired immunogenicity. A major drawback of protein-based vaccines, where a conjugation of antigenic carbohydrates to proteins is required, is that the vaccine is particularly heat unstable and a refrigeration of the vaccine is required. Moreover the use of at least two components to achieve a sufficient vaccination is also a significant drawback, since the procedure of administration is rather complex, e.g. the point in time where the adjuvant is administered is essential to achieve the desired immunogenicity (WO03009812).

Description of the invention

To fulfill these requirements and to overcome the disadvantages of current vaccines the invention exhibits a new type of conjugate vaccine, wherein the carbohydrate antigen is covalently bound to the glycolipid adjuvant.
Protection against an infectious disease is provided by neutralization of virulence factors or opsonizing antibodies. The antibodies (**Abs**.) have to be directed against the carbohydrate antigen of the pathogen, e.g from capsules composed of polysaccharides or viral glycoproteins. Therefore, an ideal efficient vaccine has to induce high affinity and complement-fixing anti-carbohydrate antibodies. This is actually fulfilled by the conjugates of the present invention.

The novel carbohydrate-glycolipid conjugate derivatives according to the present invention are represented by the following general formula (I). It was surprisingly found that extraordinary potent and stable vaccine can be derived when a polysaccharide antigen is bound via a linker and a carbohydrate moiety to a ceramide moiety. Thus the present invention relates to compounds of the general formula (I)

$$\mathbf{A[L—CH—CA]_p}$$

(I)

wherein
A represents a carbohydrate antigen of 1 to 10.000 carbohydrate monomers, wherein the carbohydrate monomers of the carbohydrate antigen are optionally modified to carry amide, carbonate, carbamate, carbonyl, thiocarbonyl, carboxy,

thiocarboxy, ester, thioester, ether, epoxy, hydroxyalkyl, alkylenyl, phenylene, alkenyl, imino, imide, isourea, thiocarbamate, thiourea and/or urea moieties,

p is the number of residues **–L–CH–CA** which are bound to the carbohydrate antigen **A**, and

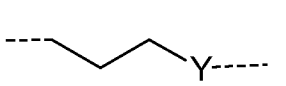
p is an integer defined as follows:

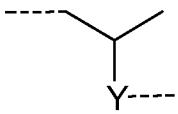
p is 1 or 2		if u is 1
p is 1, 2, 3 or 4		if u is 2
p is 1, 2, 3, 4, 5 or 6		if u is 3
p is 1, 2, 3, 4, 5, 6, 7 or 8		if u is 4
$1 \leq p \leq 10$	if	$5 \leq u \leq 10$
$2 \leq p \leq 50$	if	$11. \leq u \leq 100$
$20 \leq p \leq 200$	if	$101 \leq u \leq 1000$
$50 \leq p \leq 400$	if	$1001 \leq u \leq 10000$

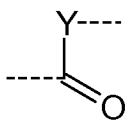
u is the number of carbohydrate monomers of the carbohydrate antigen **A**

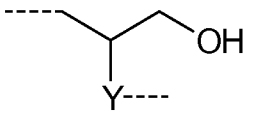
L represents $-L^1-L^2-$, $-L^2-$, $-L^2-L^3-$ or $-L^1-L^2-L^3-$;

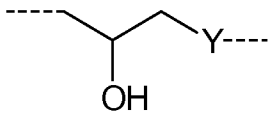
L^1 represents one of the following residues:

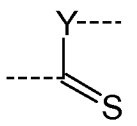


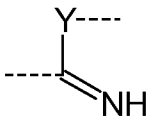
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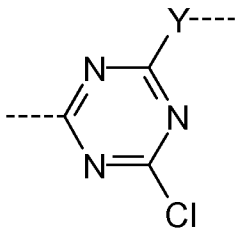
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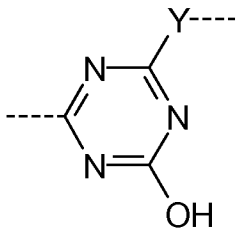
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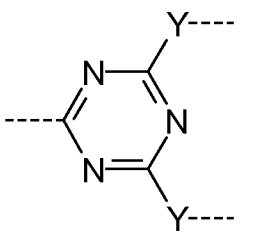
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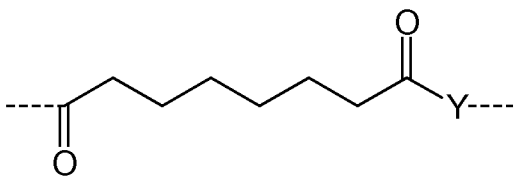
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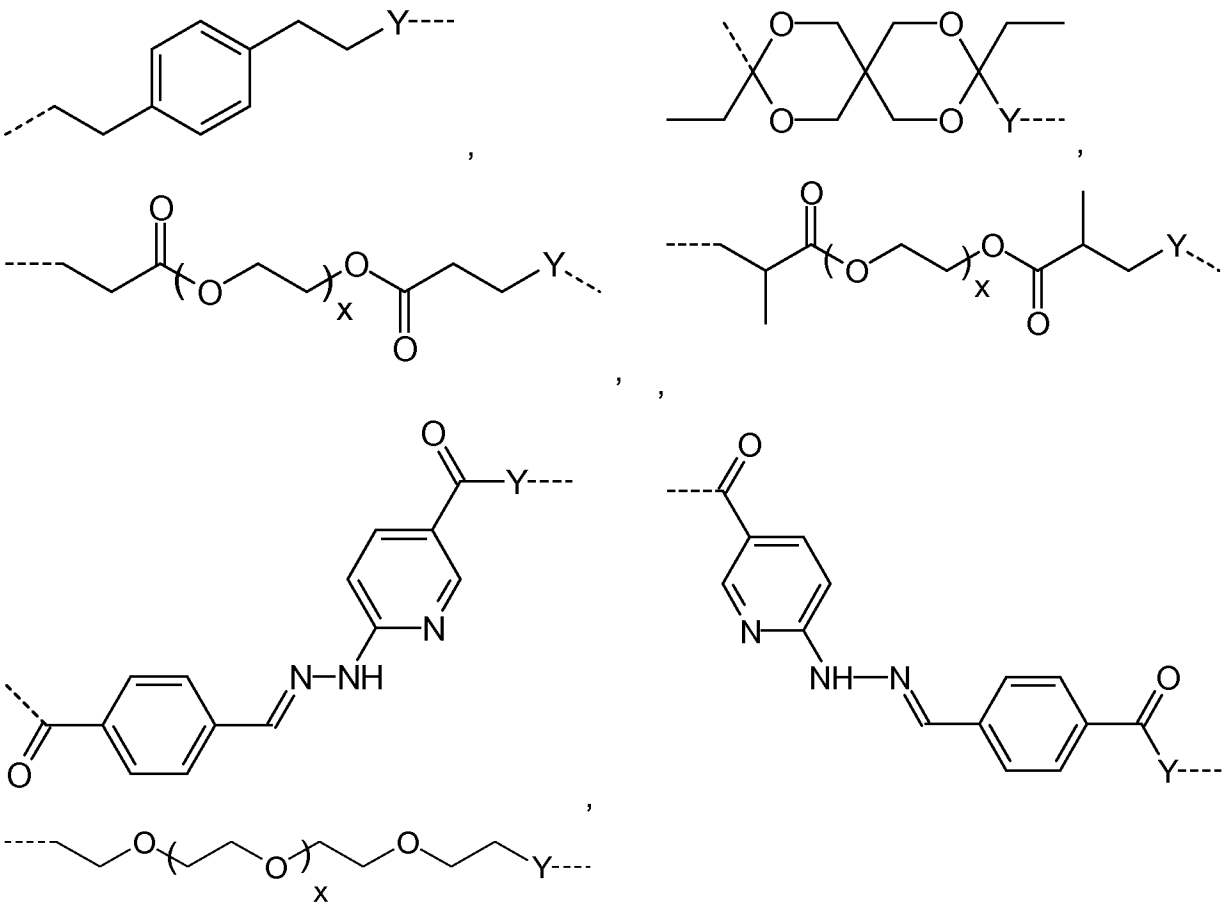
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wherein x is in integer from 1 to 60;

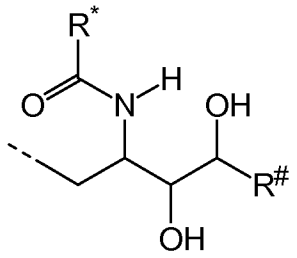
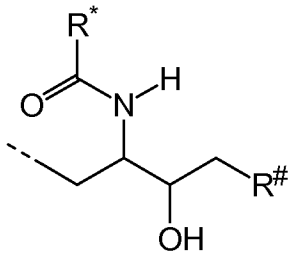
Y represents a bond, –NH–, –O–, –S–;

L^2 represents –CH_2–, –C_2H_4–, –C_3H_6–, –C_4H_8–, –C_5H_{10}–, –C_6H_{12}–, –C_7H_{14}–, –C_8H_{16}–, –C_9H_{18}–, –$C_{10}H_{20}$–, –$CH(CH_3)$–, –$C[(CH_3)_2]$–, –CH_2–$CH(CH_3)$–, –$CH(CH_3)$–CH_2–, –$CH(CH_3)$–C_2H_4–, –CH_2–$CH(CH_3)$–CH_2–, –C_2H_4–$CH(CH_3)$–, –CH_2–$C[(CH_3)_2]$–, –$C[(CH_3)_2]$–CH_2–, –$CH(CH_3)$–$CH(CH_3)$–, –$C[(C_2H_5)(CH_3)]$–, –$CH(C_3H_7)$–, –$(CH_2$–CH_2–$O)_n$–CH_2–CH_2–, –CO–CH_2–, –CO–C_2H_4–, –CO–C_3H_6–, –CO–C_4H_8–, –CO–C_5H_{10}–, –CO–C_6H_{12}–, –CO–C_7H_{14}–, –CO–C_8H_{16}–, –CO–C_9H_{18}–, –CO–$C_{10}H_{20}$–, –CO–$CH(CH_3)$–, –CO–$C[(CH_3)_2]$–, –CO–CH_2–$CH(CH_3)$–, –CO–$CH(CH_3)$–CH_2–, –CO–$CH(CH_3)$–C_2H_4–, –CO–CH_2–$CH(CH_3)$–CH_2–, –CO–C_2H_4–$CH(CH_3)$–, –CO–CH_2–$C[(CH_3)_2]$–, –CO–$C[(CH_3)_2]$–CH_2–, –CO–$CH(CH_3)$–$CH(CH_3)$–, –CO–$C[(C_2H_5)(CH_3)]$–, –CO–$CH(C_3H_7)$–, –CO–$(CH_2$–CH_2–$O)_n$–CH_2–CH_2–;

n represents an integer from 1 to 60;

L^3 represents –CO–, –O–CO–, –NH–CO–, –NH(C=NH)–, –SO_2–, –O–SO_2–;

CH represents a monosaccharide, a disaccharide or a trisaccharide;

CA represents  or 

R* and **R#** represent independently of each other a linear or branched or cyclic, substituted or unsubstituted, saturated or unsaturated carbon residue consisting of 1 to 30 carbon atoms;

and enantiomers, stereoisomeric forms, mixtures of enantiomers, diastereomers, mixtures of diastereomers, prodrugs, hydrates, solvates, tautomers, and racemates of the above mentioned compounds and pharmaceutically acceptable salts thereof.

Antigen

A represents a carbohydrate antigen consisting of 1 to 10.000 carbohydrate monomers.

The term “antigen” as used herein refers to a substance which cause after introduction into the organism of humans and animals, a specific immune response. This manifests itself either in the formation of antibodies (humoral response) and the development of cell-mediated immunity (cellular immune response) or a specific immune tolerance. Depending on whether the formation of the immune response involving T-lymphocytes (T cells) is required, it is called thymus-dependent or-independent antigen. A prerequisite for an immune response (for the immunogenicity of the antigen) is that the antigen is recognized as foreign by the organism, that it has a molecular weight of at least 1000 and that it belongs to the class of proteins or polysaccharides, rare deoxyribonucleic acids or lipids. More complex structures such as bacteria, viruses, or erythrocytes (particulate antigens) are generally more effective antigens. At the molecular level, an antigen is characterized by its ability to be "bound" at the antigen-binding site of an antibody.

Foreign substances that do not stimulate an immune response by themselves, but by the chemical binding to immunogenic macromolecules, are called haptens. For the

efficacy of immunogenic antigens the route of administration (single or multiple dose, dose intradermally or intravenously, with or without adjuvant) is determining. Repeated attacks by the same antigens accelerate the immune response and may result in the worst case of a specific hypersensitivity (allergy, where the antigens are often called allergens). In the presence of large amounts of antigen or chronic persistent amounts of antigen the formation of soluble immune complexes may occur, which can cause anaphylaxis.

An immunogen is a specific type of antigen. An immunogen is a substance that is able to provoke an adaptive immune response if injected on its own. An immunogen is able to induce an immune response, whereas an antigen is able to combine with the products of an immune response once they are made. Immunogenicity is the ability to induce a humoral and/or cell-mediated immune response

The term “antigen” may shortly be described as a substance, belonging to the class of proteins or polysaccharides, generally comrising parts (coats, capsules, cell walls, flagella, fimbrae, and toxins) of bacteria, viruses, and other microorganisms, and also rare deoxyribonucleic acids or lipids, smaller molecules or ions (haptens), which are recognized as foreign by the organism of humans and animals and which may cause after introduction into the organism of humans and animals, a specific immune response, which comprises a humoral and/or or a cellular immune response, which leads to the formation of antibodies (humoral response) and/or the development of cell-mediated immunity (cellular response), wherein the mentioned antibodies may lead to a specific binding of the antigen.

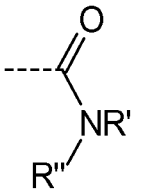
Specifically, the term “antigen” can be described as a substance, which is recognized as foreign by the organism of humans and animals and which may cause after introduction into the organism of humans and animals, a specific immune response, which comprises a humoral and/or or a cellular immune response.

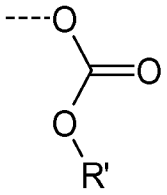
Preferably **A** represents an isolated, a semi-synthetic or a synthetic carbohydrate antigen. The isolated carbohydrate antigen consists of 1 to 10,000 carbohydrate monomers, preferably of 10 to 5,000 carbohydrate monomers, and more preferably of 20 to 3,000. The semi-synthetic carbohydrate antigen preferably consists of 1 to 1.000 carbohydrate monomers, more preferably of 5 to 900 and still more preferably of 10 to 800 carbohydrate monomers and the synthetic carbohydrate antigen preferably consists of 1 to 1.000 carbohydrate monomers, more preferably of 5 to 900 and still more preferably of 10 to 800 carbohydrate monomers.

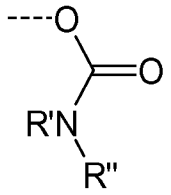
The antigens and especially the isolated antigens are normally mixtures of antigens having a certain range of carbohydrate monomers so that the term "antigen consisting of 500 carbohydrate monomers" refers to a mixture of antigens having in average the number of 500 carbohydrate monomers. Such a mixture might contain 10% of the antigens with 450 to 470 carbohydrate monomers, 10% of the antigens with 530 to 550 carbohydrate monomers, 20% of the antigens with 471 to 490 carbohydrate monomers, 20% of the antigens with 510 to 529 carbohydrate monomers and 40% of the antigens with a number of 491 to 509 carbohydrate monomers.

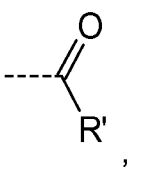
Preferably the carbohydrate monomers belong to heptoses, hexoses, pentoses, tetroses or sialic acids, wherein the carbohydrate monomers are connected to each other via α/β glycosidic bonds which belong to the group consisting of 1,2; 1,3; 1,4; 1,5; 1,6; 2,2; 2,3; 2,4; 2,5; or 2,6 glycosidic bonds. Also, the carbohydrate monomers can be more specifically derivatives of peptidoglycanes such as *N*-acetylmuramic acid, *N*-acetyl-D-glocosamine or *N*-acetyl talosaminuronic acid.

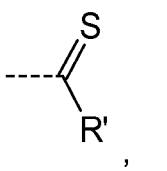
Some of the hydroxyl groups (–OH) of the carbohydrate monomers of the antigen **A** can independently of each other optionally be substituted with the following substituents $–CH_3$, $–C_2H_5$, $–SO_3H$, $–SO_3^-$, $–CH_2–COOH$, $–CH_2–COO^-$, $–C_2H_4–COOH$, $–C_2H_4–COO^-$ or some of the hydroxyl groups (–OH) of the carbohydrate monomers can be replaced by the following moieties:
–H, $–O–CH_3$, $–O–SO_3H$, $–O–SO_3^-$, $–CH_3$, $–NH_2$, $–NH–CO–CH_3$, $–O–CH_2–COOH$, $–O–CH_2–COO^-$, $–O–C_2H_4–COOH$, $–O–C_2H_4–COO^-$, $–NH–SO_3H$, $–NH–SO_3^-$,

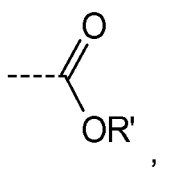


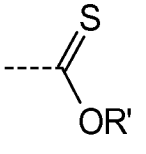
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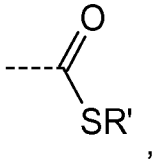
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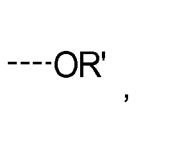
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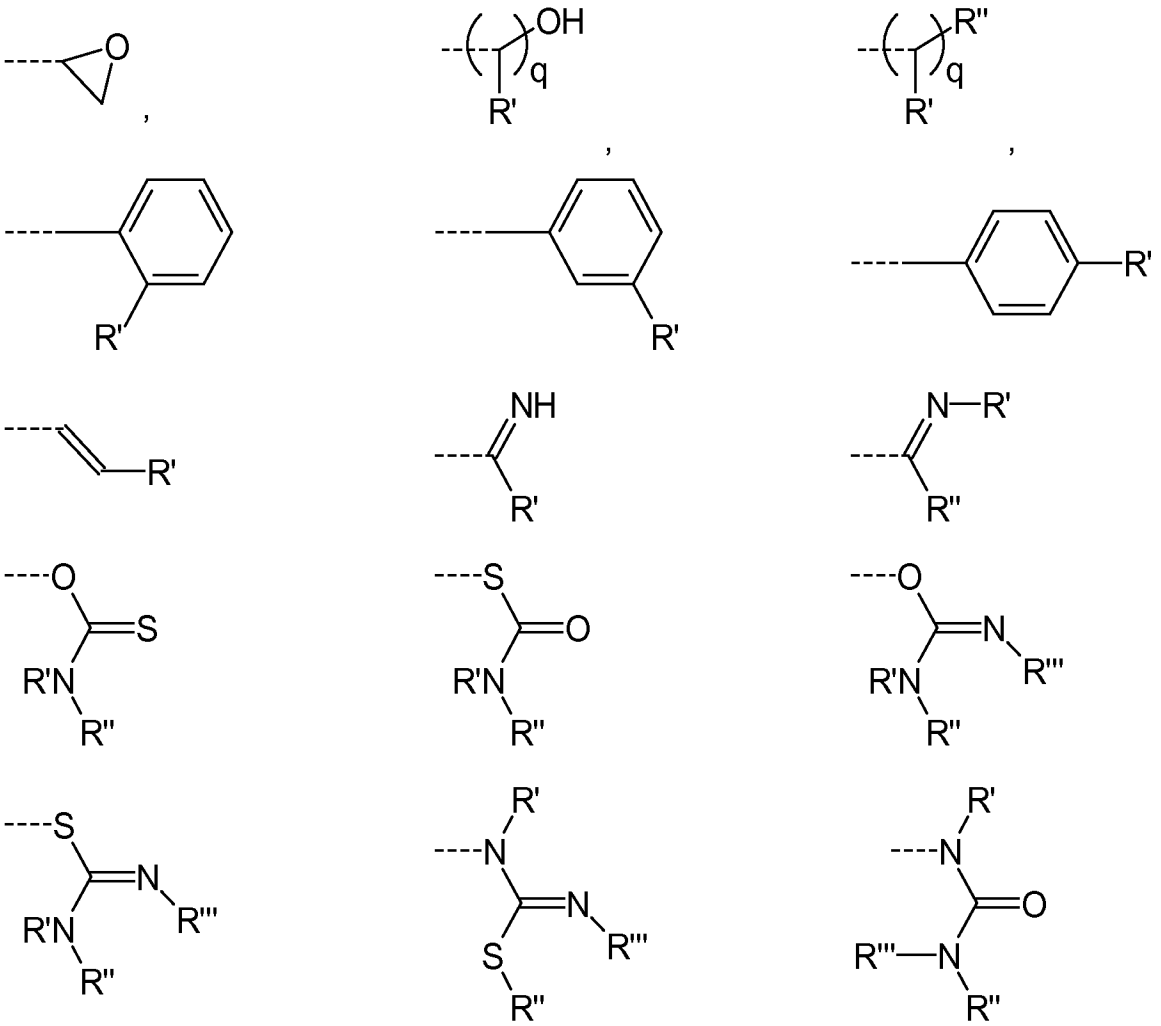
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wherein

q is an integer from 1 to 4, and

R', R'' and R''' independently of each other represent one of following residues:

–H, $–CH_3$, $–C_2H_5$, $–C_3H_7$, –cyclo-C_3H_5, $–CH(CH_3)_2$, $–C(CH_3)_3$, $–C_4H_9$, –Ph, $–CH_2–Ph$, $–CH_2–OCH_3$, $–C_2H_4–OCH_3$, $–C_3H_6–OCH_3$, $–CH_2–OC_2H_5$, $–C_2H_4–OC_2H_5$, $–C_3H_6–OC_2H_5$, $–CH_2–OC_3H_7$, $–C_2H_4–OC_3H_7$, $–C_3H_6–OC_3H_7$, $–CH_2–O–$cyclo-C_3H_5, $–C_2H_4–O–$cyclo-C_3H_5, $–C_3H_6–O–$cyclo-C_3H_5, $–CH_2–OCH(CH_3)_2$, $–C_2H_4–OCH(CH_3)_2$, $–C_3H_6–OCH(CH_3)_2$, $–CH_2–OC(CH_3)_3$, $–C_2H_4–OC(CH_3)_3$, $–C_3H_6–OC(CH_3)_3$, $–CH_2–OC_4H_9$, $–C_2H_4–OC_4H_9$, $–C_3H_6–OC_4H_9$, $–CH_2–OPh$, $–C_2H_4–OPh$, $–C_3H_6–OPh$, $–CH_2–OCH_2–Ph$, $–C_2H_4–OCH_2–Ph$, $–C_3H_6–OCH_2–Ph$.

These groups are naturally occurring subsituents which can be present in the carbohydrate antigens.

The carbohydrate monomers of the carbohydrate antigen can therefore be optionally modified or can be modified to carry amide, carbonate, carbamate, carbonyl,

thiocarbonyl, carboxy, thiocarboxy, ester, thioester, ether, epoxy, hydroxyalkyl, alkylenyl, phenylene, alkenyl, imino, imide, isourea, thiocarbamate, thiourea and/or urea moieties.

The term "hydroxylalkyl" refers preferably to linear or branched C_1-C_4 hydroxyalkyl residues which consist in total of 1 to 4 carbon atoms including the carbon atoms of the branches wherein one of the hydrogen atoms is substituted by a hydroxyl group such as –CH_2OH, –C_2H_4OH, –$CHOHCH_3$, –$CH_2CH_2CH_2OH$, –$CH_2CHOHCH_3$, –$CHOHCH_2CH_3$, –cyclo-C_3H_4OH, –$COH(CH_3)_2$, –$CH(CH_3)CH_2OH$, –$CH_2CH_2CH_2CH_2OH$, –$CH_2CH_2CHOHCH_3$, –$CH_2CHOHCH_2CH_3$, –$CHOHCH_2CH_2CH_3$, –$C(CH_3)_2CH_2OH$, –$CHOH–CH(CH_3)_2$, –$CH(CH_3)–CHOHCH_3$, –$CCH_3OH–C_2H_5$, –$CH_2–C(CH_3)_2OH$.

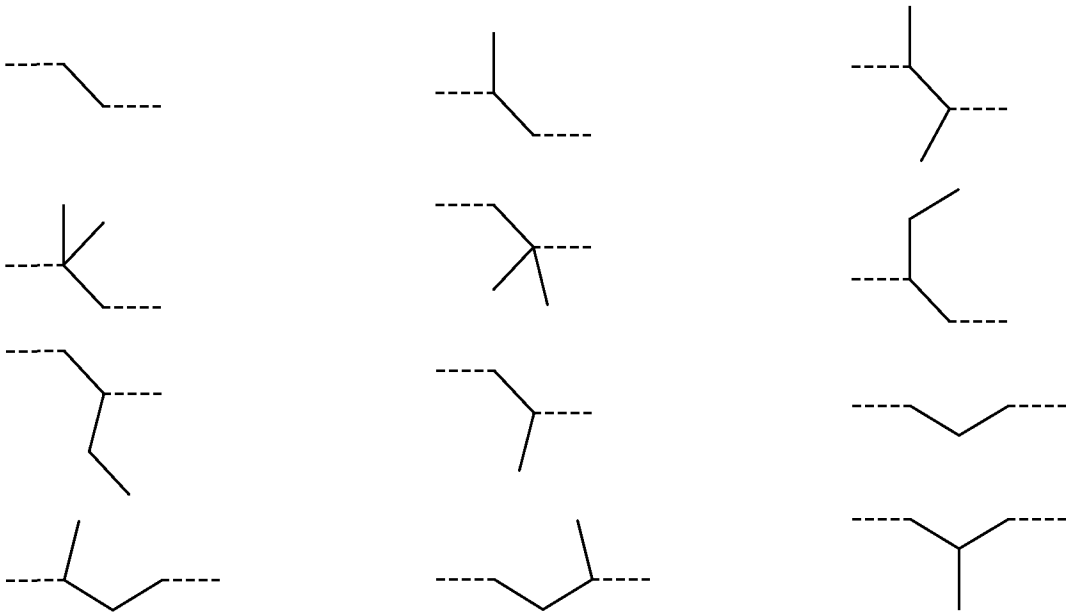
As used herein, the term alkenyl refers preferably to "linear or branched C_2–C_8–alkenyl" such as –$CH=CH_2$, –$CH_2–CH=CH_2$, –$C(CH_3)=CH_2$, –$CH=CH–CH_3$, –$C_2H_4–CH=CH_2$, –$CH=CH–C_2H_5$, –$CH_2–C(CH_3)=CH_2$, –$CH(CH_3)–CH=CH$, –$CH=C(CH_3)_2$, –$C(CH_3)=CH–CH_3$, –$CH=CH–CH=CH_2$, –$C_3H_6–CH=CH_2$, –$C_2H_4–CH=CH–CH_3$, –$CH_2–CH=CH–C_2H_5$, –$CH=CH–C_3H_7$, –$CH_2–CH=CH–CH=CH_2$, –$CH=CH–CH=CH–CH_3$, –$CH=CH–CH_2–CH=CH_2$, –$C(CH_3)=CH–CH=CH_2$, –$CH=C(CH_3)–CH=CH_2$, –$CH=CH–C(CH_3)=CH_2$, –$C_2H_4–C(CH_3)=CH_2$, –$CH_2–CH(CH_3)–CH=CH_2$, –$CH(CH_3)–CH_2–CH=CH_2$, –$CH_2–CH=C(CH_3)_2$, –$CH_2–C(CH_3)=CH–CH_3$, –$CH(CH_3)–CH=CH–CH_3$, –$CH=CH–CH(CH_3)_2$, –$CH=C(CH_3)–C_2H_5$, –$C(CH_3)=CH–C_2H_5$, –$C(CH_3)=C(CH_3)_2$, –$C(CH_3)_2–CH=CH_2$, –$CH(CH_3)–C(CH_3)=CH_2$, –$C(CH_3)=CH–CH=CH_2$, –$CH=C(CH_3)–CH=CH_2$, –$CH=CH–C(CH_3)=CH_2$, –$C_4H_8–CH=CH_2$, –$C_3H_6–CH=CH–CH_3$, –$C_2H_4–CH=CH–C_2H_5$, –$CH_2–CH=CH–C_3H_7$, –$CH=CH–C_4H_9$, –$C_3H_6–C(CH_3)=CH_2$, –$C_2H_4–CH(CH_3)–CH=CH_2$, –$CH_2–CH(CH_3)–CH_2–CH=CH_2$, –$CH_2–CH=CH–CH_3$, –$CH(CH_3)–C_2H_4–CH=CH_2$, –$C_2H_4–CH=C(CH_3)_2$, –$C_2H_4–C(CH_3)=CH–CH_3$, –$CH_2–CH(CH_3)–CH=CH–CH_3$, –$CH(CH_3)–CH_2–CH=CH–CH_3$, –$C(C_4H_9)=CH_2$, –$CH_2–CH=CH–CH(CH_3)_2$, –$CH_2–CH=C(CH_3)–C_2H_5$, –$CH_2–C(CH_3)=CH–C_2H_5$, –$CH(CH_3)–CH=CH–C_2H_5$, –$CH=CH–CH_2–CH(CH_3)_2$, –$CH=CH–CH(CH_3)–C_2H_5$, –$CH=C(CH_3)–C_3H_7$, –$C(CH_3)=CH–C_3H_7$, –$CH_2–CH(CH_3)–C(CH_3)=CH_2$, –$CH(CH_3)–CH_2–C(CH_3)=CH_2$, –$CH(CH_3)–CH(CH_3)–CH=CH_2$, –$CH_2–C(CH_3)_2–CH=CH_2$, –$C(CH_3)_2–CH_2–CH=CH_2$, –$CH_2–C(CH_3)=C(CH_3)_2$, –$CH(CH_3)–CH=C(CH_3)_2$, –$C(CH_3)_2–CH=CH–CH_3$, –$CH(CH_3)–C(CH_3)=CH–CH_3$, –$CH=C(CH_3)–CH(CH_3)_2$, –$C(CH_3)=CH–CH(CH_3)_2$,

$-C(CH_3)=C(CH_3)-C_2H_5$, $-CH=CH-C(CH_3)_3$, $-C(CH_3)_2-C(CH_3)=CH_2$, $-CH(C_2H_5)-C(CH_3)=CH_2$, $-C(CH_3)(C_2H_5)-CH=CH_2$, $-CH(CH_3)-C(C_2H_5)=CH_2$, $-CH_2-C(C_3H_7)=CH_2$, $-CH_2-C(C_2H_5)=CH-CH_3$, $-CH(C_2H_5)-CH=CH-CH_3$, $-C(C_3H_7)=CH-CH_3$, $-C(C_2H_5)=CH-C_2H_5$, $-C(C_2H_5)=C(CH_3)_2$, $-C[C(CH_3)_3]=CH_2$, $-C[CH(CH_3)(C_2H_5)]=CH_2$, $-C[CH_2-CH(CH_3)_2]=CH_2$, $-C_2H_4-CH=CH-CH=CH_2$, $-CH_2-CH=CH-CH_2-CH=CH_2$, $-CH=CH-C_2H_4-CH=CH_2$, $-CH_2-CH=CH-CH=CH-CH_3$, $-CH=CH-CH_2-CH=CH-CH_3$, $-CH=CH-CH=CH-C_2H_5$, $-CH_2-CH=CH-C(CH_3)=CH_2$, $-CH_2-CH=C(CH_3)-CH=CH_2$, $-CH_2-C(CH_3)=CH-CH=CH_2$, $-CH(CH_3)-CH=CH-CH=CH_2$, $-CH=CH-CH_2-C(CH_3)=CH_2$, $-CH=CH-CH(CH_3)-CH=CH_2$, $-CH=C(CH_3)-CH_2-CH=CH_2$, $-C(CH_3)=CH-CH_2-CH=CH_2$, $-CH=CH-CH=C(CH_3)_2$, $-CH=CH-C(CH_3)=CH-CH_3$, $-CH=C(CH_3)-CH=CH-CH_3$, $-C(CH_3)=CH-CH=CH-CH_3$, $-CH=C(CH_3)-C(CH_3)=CH_2$, $-C(CH_3)=CH-C(CH_3)=CH_2$, $-C(CH_3)=C(CH_3)-CH=CH_2$, $-CH=CH-CH=CH-CH=CH_2$, $-C_5H_{10}-CH=CH_2$, $-C_4H_8-CH=CH-CH_3$, $-C_3H_6-CH=CH-C_2H_5$, $-C_2H_4-CH=CH-C_3H_7$, $-CH_2-CH=CH-C_4H_9$, $-C_4H_8-C(CH_3)=CH_2$, $-C_3H_6-CH(CH_3)-CH=CH_2$, $-C_2H_4-CH(CH_3)-CH_2-CH=CH_2$, $-CH_2-CH(CH_3)-C_2H_4-CH=CH_2$, $-C_3H_6-CH=C(CH_3)_2$, $-C_3H_6-C(CH_3)=CH-CH_3$, $-C_2H_4-CH(CH_3)-CH=CH-CH_3$, $-CH_2-CH(CH_3)-CH_2-CH=CH-CH_3$, $-C_2H_4-CH=CH-CH(CH_3)_2$, $-C_2H_4-CH=C(CH_3)-C_2H_5$, $-C_2H_4-C(CH_3)=CH-C_2H_5$, $-CH_2-CH(CH_3)-CH=CH-C_2H_5$, $-CH_2-CH=CH-CH_2-CH(CH_3)_2$, $-CH_2-CH=CH-CH(CH_3)-C_2H_5$, $-CH_2-CH=C(CH_3)-C_3H_7$, $-CH_2-C(CH_3)=CH-C_3H_7$, $-C_2H_4-CH(CH_3)-C(CH_3)=CH_2$, $-CH_2-CH(CH_3)-CH_2-C(CH_3)=CH_2$, $-CH_2-CH(CH_3)-CH(CH_3)-CH=CH_2$, $-C_2H_4-C(CH_3)_2-CH=CH_2$, $-CH_2-C(CH_3)_2-CH_2-CH=CH_2$, $-C_2H_4-C(CH_3)=C(CH_3)_2$, $-CH_2-CH(CH_3)-CH=C(CH_3)_2$, $-CH_2-C(CH_3)_2-CH=CH-CH_3$, $-CH_2-CH(CH_3)-C(CH_3)=CH-CH_3$, $-CH_2-CH=C(CH_3)-CH(CH_3)_2$, $-CH_2-C(CH_3)=CH-CH(CH_3)_2$, $-CH_2-C(CH_3)=C(CH_3)-C_2H_5$, $-CH_2-CH=CH-C(CH_3)_3$, $-CH_2-C(CH_3)_2-C(CH_3)=CH_2$, $-CH_2-CH(C_2H_5)-C(CH_3)=CH_2$, $-CH_2-C(CH_3)(C_2H_5)-CH=CH_2$, $-CH_2-CH(CH_3)-C(C_2H_5)=CH_2$, $-C_2H_4-C(C_3H_7)=CH_2$, $-C_2H_4-C(C_2H_5)=CH-CH_3$, $-CH_2-CH(C_2H_5)-CH=CH-CH_3$, $-CH_2-C(C_4H_9)=CH_2$, $-CH_2-C(C_3H_7)=CH-CH_3$, $-CH_2-C(C_2H_5)=CH-C_2H_5$, $-CH_2-C(C_2H_5)=C(CH_3)_2$, $-CH_2-C[C(CH_3)_3]=CH_2$, $-CH_2-C[CH(CH_3)(C_2H_5)]=CH_2$, $-CH_2-C[CH_2-CH(CH_3)_2]=CH_2$, $-C_3H_6-CH=CH-CH=CH_2$, $-C_2H_4-CH=CH-CH_2-CH=CH_2$, $-CH_2-CH=CH-C_2H_4-CH=CH_2$, $-C_2H_4-CH=CH-CH=CH-CH_3$, $-CH_2-CH=CH-CH_2-CH=CH-CH_3$, $-CH_2-CH=CH-CH=CH-C_2H_5$, $-C_2H_4-CH=CH-C(CH_3)=CH_2$, $-C_2H_4-CH=C(CH_3)-CH=CH_2$, $-C_2H_4-C(CH_3)=CH-CH=CH_2$, $-CH_2-CH(CH_3)-CH=CH-CH=CH_2$, $-CH_2-CH=CH-CH_2-C(CH_3)=CH_2$, $-CH_2-CH=CH-CH(CH_3)-CH=CH_2$, $-CH_2-CH=C(CH_3)-CH_2-CH=CH_2$, $-CH_2-C(CH_3)=CH-CH_2-CH=CH_2$, $-CH_2-CH=CH-CH=C(CH_3)_2$, $-CH_2-CH=CH-C(CH_3)=CH-CH_3$, $-CH_2-CH=C(CH_3)-CH=CH-CH_3$, $-CH_2-C(CH_3)=CH-CH=CH-$

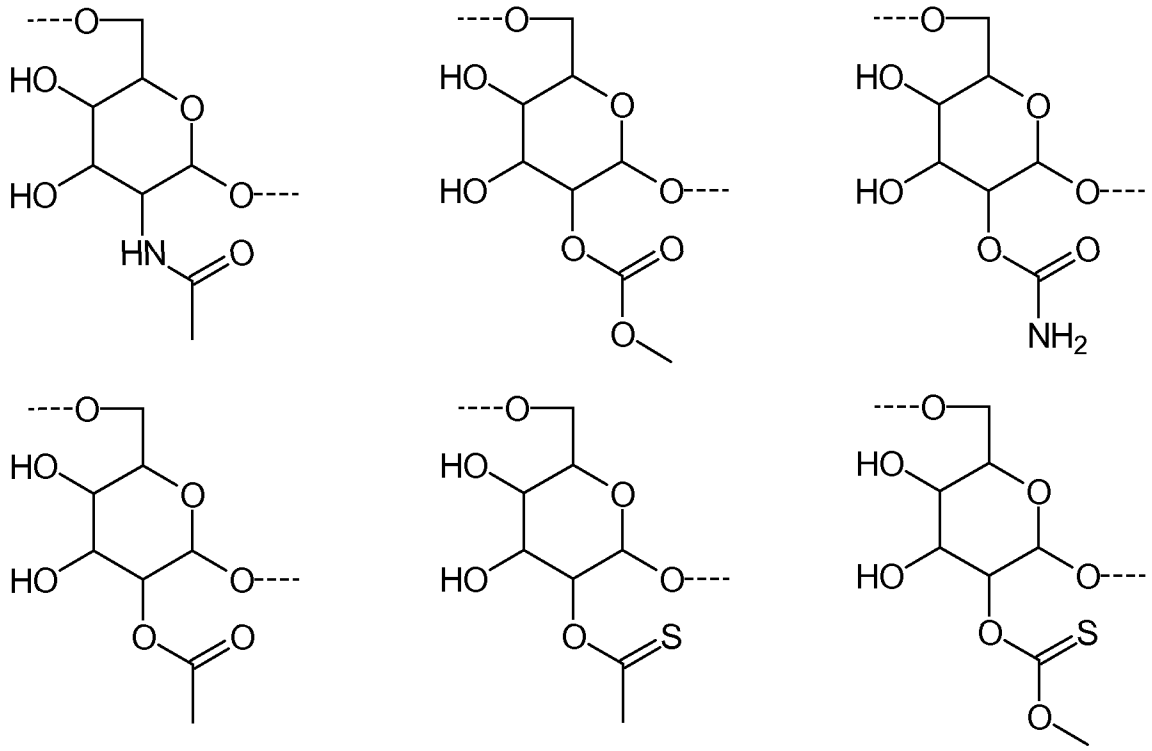
CH_3, $–CH_2–CH=C(CH_3)–C(CH_3)=CH_2$, $–CH_2–C(CH_3)=CH–C(CH_3)=CH_2$, $–CH_2–C(CH_3)=C(CH_3)–CH=CH_2$, $–CH_2–CH=CH–CH=CH–CH=CH_2$, $–C_6H_{12}–CH=CH_2$, $–C_5H_{10}–CH=CH–CH_3$, $–C_4H_8–CH=CH–C_2H_5$, $–C_3H_6–CH=CH–C_3H_7$, $–C_2H_4–CH=CH–C_4H_9$, $–C_5H_{10}–C(CH_3)=CH_2$, $–C_4H_8–CH(CH_3)–CH=CH_2$, $–C_3H_6–CH(CH_3)–CH_2–CH=CH_2$, $–C_2H_4–CH(CH_3)–C_2H_4–CH=CH_2$, $–C_4H_8–CH=C(CH_3)_2$, $–C_4H_8–C(CH_3)=CH–CH_3$, $–C_3H_6–CH(CH_3)–CH=CH–CH_3$, $–C_2H_4–CH(CH_3)–CH_2–CH=CH–CH_3$, $–C_3H_6–CH=CH–CH(CH_3)_2$, $–C_3H_6–CH=C(CH_3)–C_2H_5$, $–C_3H_6–C(CH_3)=CH–C_2H_5$, $–C_2H_4–CH(CH_3)–CH=CH–C_2H_5$, $–C_2H_4–CH=CH–CH_2–CH(CH_3)_2$, $–C_2H_4–CH=CH–CH(CH_3)–C_2H_5$, $–C_2H_4–CH=C(CH_3)–C_3H_7$, $–C_2H_4–C(CH_3)=CH–C_3H_7$, $–C_3H_6–CH(CH_3)–C(CH_3)=CH_2$, $–C_2H_4–CH(CH_3)–CH_2–C(CH_3)=CH_2$, $–C_2H_4–CH(CH_3)–CH(CH_3)–CH=CH_2$, $–C_3H_6–C(CH_3)_2–CH=CH_2$, $–C_2H_4–C(CH_3)_2–CH_2–CH=CH_2$, $–C_3H_6–C(CH_3)=C(CH_3)_2$, $–C_2H_4–CH(CH_3)–CH=C(CH_3)_2$, $–C_2H_4–C(CH_3)_2–CH=CH–CH_3$, $–C_2H_4–CH(CH_3)–C(CH_3)=CH–CH_3$, $–C_2H_4–CH=C(CH_3)–CH(CH_3)_2$, $–C_2H_4–C(CH_3)=CH–CH(CH_3)_2$, $–C_2H_4–C(CH_3)=C(CH_3)–C_2H_5$, $–C_2H_4–CH=CH–C(CH_3)_3$, $–C_2H_4–C(CH_3)_2–C(CH_3)=CH_2$, $–C_2H_4–CH(C_2H_5)–C(CH_3)=CH_2$, $–C_2H_4–C(CH_3)(C_2H_5)–CH=CH_2$, $–C_2H_4–CH(CH_3)–C(C_2H_5)=CH_2$, $–C_3H_6–C(C_3H_7)=CH_2$, $–C_3H_6–C(C_2H_5)=CH–CH_3$, $–C_2H_4–CH(C_2H_5)–CH=CH–CH_3$, $–C_2H_4–C(C_4H_9)=CH_2$, $–C_2H_4–C(C_3H_7)=CH–CH_3$, $–C_2H_4–C(C_2H_5)=CH–C_2H_5$, $–C_2H_4–C(C_2H_5)=C(CH_3)_2$, $–C_2H_4–C[C(CH_3)_3]=CH_2$, $–C_2H_4–C[CH(CH_3)(C_2H_5)]=CH_2$, $–C_2H_4–C[CH_2–CH(CH_3)_2]=CH_2$, $–C_4H_8–CH=CH–CH=CH_2$, $–C_3H_6–CH=CH–CH_2–CH=CH_2$, $–C_2H_4–CH=CH–C_2H_4–CH=CH_2$, $–C_3H_6–CH=CH–CH=CH–CH_3$, $–C_2H_4–CH=CH–CH_2–CH=CH–CH_3$, $–C_2H_4–CH=CH–CH=CH–C_2H_5$, $–C_3H_6–CH=CH–C(CH_3)=CH_2$, $–C_3H_6–CH=C(CH_3)–CH=CH_2$, $–C_3H_6–C(CH_3)=CH–CH=CH_2$, $–C_2H_4–CH(CH_3)–CH=CH–CH=CH_2$, $–C_2H_4–CH=CH–CH_2–C(CH_3)=CH_2$, $–C_2H_4–CH=CH–CH(CH_3)–CH=CH_2$, $–C_2H_4–CH=C(CH_3)–CH_2–CH=CH_2$, $–C_2H_4–C(CH_3)=CH–CH_2–CH=CH_2$, $–C_2H_4–CH=CH–CH=C(CH_3)_2$, $–C_2H_4–CH=CH–C(CH_3)=CH–CH_3$, $–C_2H_4–CH=C(CH_3)–CH=CH–CH_3$, $–C_2H_4–C(CH_3)=CH–CH=CH–CH_3$, $–C_2H_4–CH=C(CH_3)–C(CH_3)=CH_2$, $–C_2H_4–C(CH_3)=CH–C(CH_3)=CH_2$, $–C_2H_4–C(CH_3)=C(CH_3)–CH=CH_2$ and $–C_2H_4–CH=CH–CH=CH–CH=CH_2$,

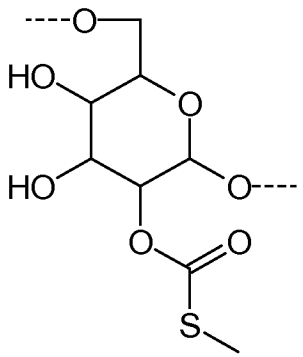
As used herein, the term alkylenyl refers to preferably "**linear or branched C_1–C_4–alkylenyl**" such as

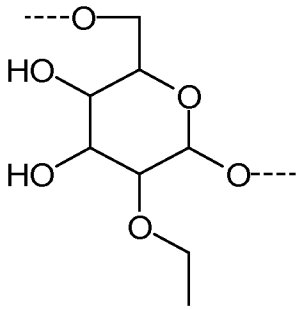


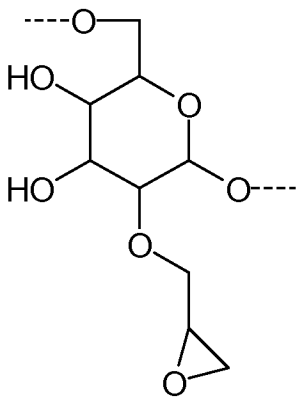


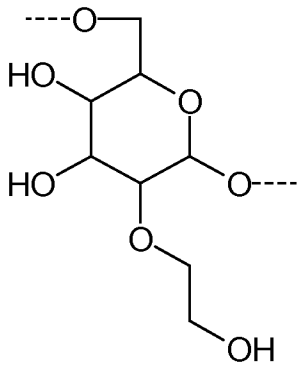
Preferred examples of modified hydroxylgroups of carbohydrate monomers of the carbohydrate antigen **A** are

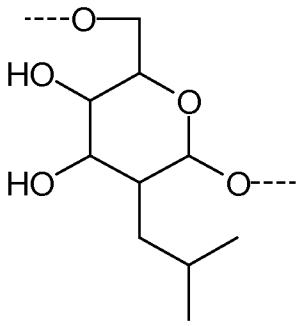


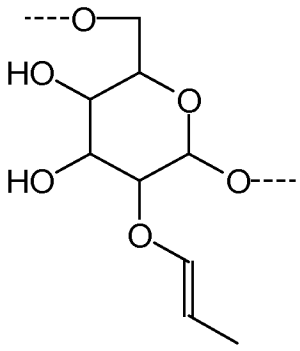


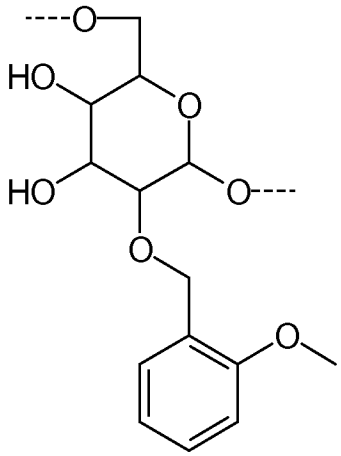


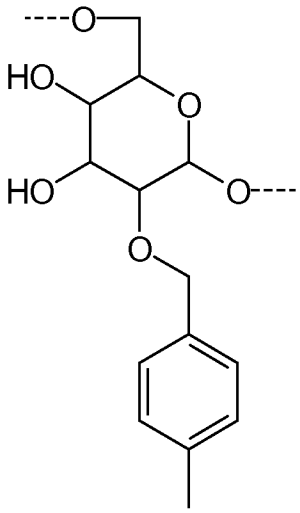


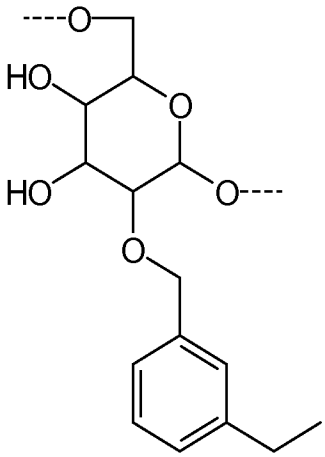


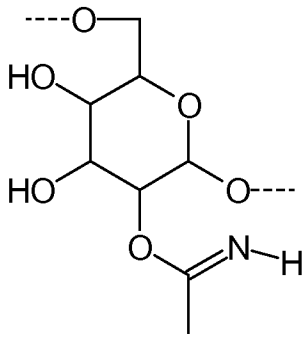


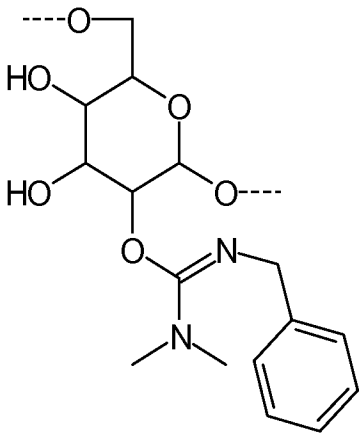


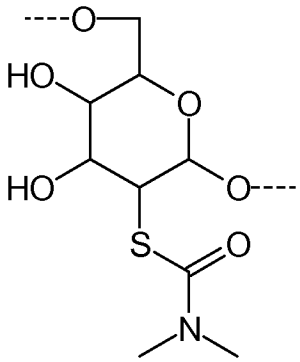


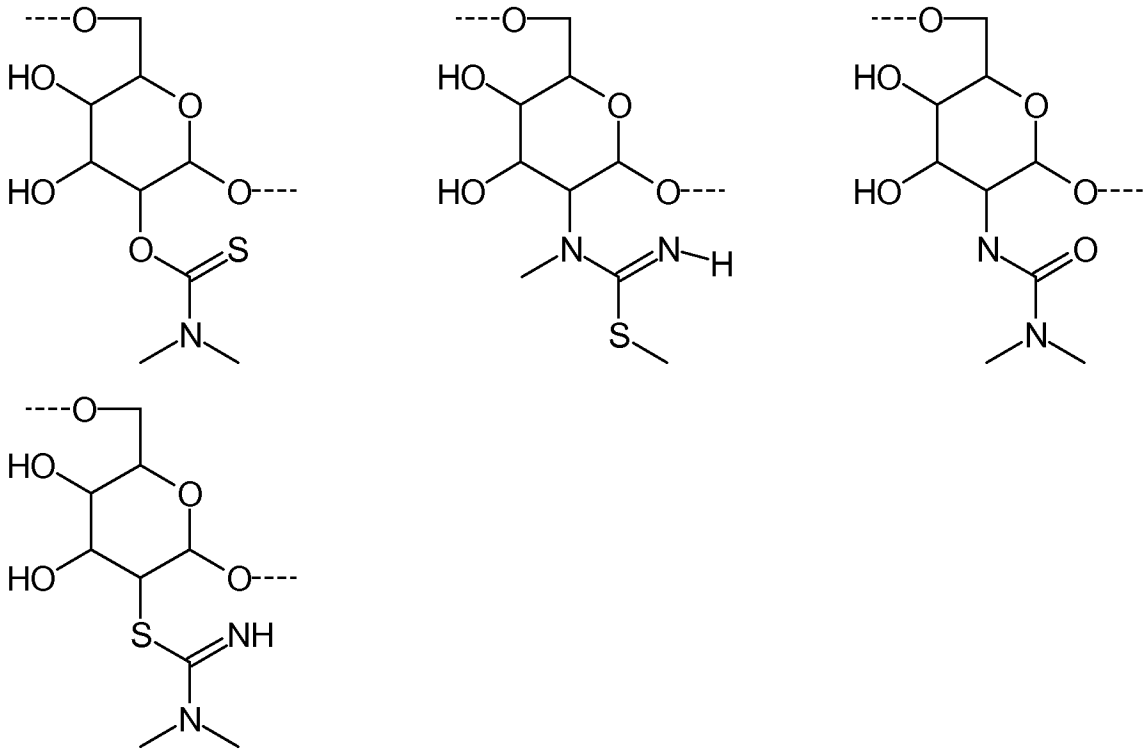












Modified hydroxylgroups of carbohydrate monomers of the carbohydrate antigen **A** may be formed by the activiation of the carbohydrate antigen in order to couple the residues –L–CH–CA to the carbohydrate antigen. Since not all activated groups of the carbohydrate antigen are thereafter coupled to one of the residues –L–CH–CA, activated groups of the carbohydrate antigen remain which are not converted to an antigen linker (A–L) linkage. Such activated but not converted groups of the carbohydrate antigen are normally hydrolyzed during work-up of the $A[L—CH—CA]_p$ complex and remain on the carbohydrate antigen A as amide, carbonate, carbamate, carbonyl, thiocarbonyl, carboxy, thiocarboxy, ester, thioester, ether, epoxy, hydroxyalkyl, alkylenyl, phenylene, alkenyl, imino, imide, isourea, thiocarbamate, thiourea and/or urea moieties.

That means, in case the carbohydrate antigen A is activated to form the covalent bond to the residues –L–CH–CA, the originally isolated or synthesized antigen is modified to bear such amide, carbonate, carbamate, carbonyl, thiocarbonyl, carboxy, thiocarboxy, ester, thioester, ether, epoxy, hydroxyalkyl, alkylenyl, phenylene, alkenyl, imino, imide, isourea, thiocarbamate, thiourea and/or urea moieties.

Only in case the residue –L–CH–CA is activated at the L-terminus to form the covalent bond to the carbohydrate antigen A, the functional groups of the carbohydrate antigen A which are not linked to the residues –L–CH–CA remain unaltered.

Generally the carbohydrate antigen consists of a plurality of carbohydrate monomers, wherein each carbohydrate monomer has further more than one functionality which could be used for a covalent linkage of the residue –L–CH–CA, thus more than one residue –L–CH–CA and generally a larger number of resudues –L–CH–CA is bound to the carbohydrate antigen A. It is clear to a skilled person that the more residues –L–CH–CA can be bound to one carbohydrate antigen the more carbohydrate monomers are contained in said carbohydrate antigen. For instance, a carbohydrate antigen consisting of 2 (u=2) carbohydrate monomers can bear 1, 2, 3 or 4 residues –L–CH–CA, while a carbohydrate antigen consisting of 50 (u=50) carbohydrate monomers might bear between 2 and 50 residues –L–CH–CA, and a carbohydrate antigen consisting of 3,000 (u=3000) carbohydrate monomers might have between 50 and 400 residues –L–CH–CA.

The bonding mode is represented by the integer p. p is the number of residues –L–CH–CA which are bound to the carbohydrate antigen A.
p represents an integer from 1 to (**Φ***u), wherein **Φ** represents the following intergers: **Φ** = 2 (if **u** is 1 to 4); **Φ** = 1 (if **u** is 5 to 10); **Φ** = 0.5 (if **u** is 11 to 100); **Φ** = 0.2 (if **u** is 101 to 1000); **Φ** = 0.04 (if **u** is 1001 to 10000); wherein u is the number of carbohydrate monomers of the carbohydrate antigen **A**.

In another preferred embodiment of the invention **p** is an integer and is defined as follows:

p is 1 or 2		if u is 1
p is 1, 2, 3 or 4		if u is 2
p is 1, 2, 3, 4, 5 or 6		if u is 3
p is 1, 2, 3, 4, 5, 6, 7 or 8		if u is 4
$1 \leq p \leq 10$	if	$5 \leq u \leq 10$
$2 \leq p \leq 50$	if	$11. \leq u \leq 100$
$20 \leq p \leq 200$	if	$101 \leq u \leq 1000$
$50 \leq p \leq 400$	if	$1001 \leq u \leq 10000$

wherein u is the number of carbohydrate monomers of the carbohydrate antigen **A**.

In a preferred embodiment of this invention **p** is an integer falling within the range from $0{,}02\mathbf{u} \leq \mathbf{p} \leq (0{,}7\mathbf{u} + 3)$ with the proviso that $\mathbf{p} \geq 1$, wherein **u** is an integer from 1 to 10000, representing the total number of carbohydrate monomers within the carbohydrate antigen **A**.

In order to connect the linker L or respectively the moiety **–L–CH–CA** to the carbohydrate antigen, two ways are possible. On the one hand the antigen could be activated and than reacted with the linker L or the moiety **–L–CH–CA** or on the other hand the linker L could be activated and than reacted with the antigen.

In case the linker **L** is activated in order to form a covalent bond with the carbohydrate antigen the number **p** of **–L–CH–CA** moieties present in the carbohydrate antigen depends on the molar equivalents of the moieties **–L–CH–CA** in regard to the number **u** of carbohydrate monomers present in the carbohydrate antigen. Thus, if **u** = 100, i.e. the carbohydrate antigen **A** consists of 100 carbohydrate monomers, one molar equivalent of the moiety **–L–CH–CA** means that each carbohydrate antigen **A** bears only one moiety **–L–CH–CA**, while 50 molar equivalents of the moiety **–L–CH–CA** means, that in average every second carbohydrate monomer of the carbohydrate antigen **A** has one moiety **–L–CH–CA**, while 200 molar equivalents means that in average each carbohydrate monomer of the carbohydrate antigen **A** has two moieties **–L–CH–CA**.

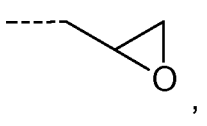
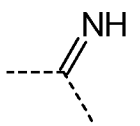
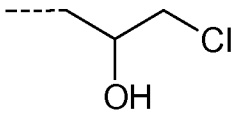
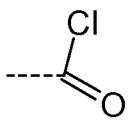
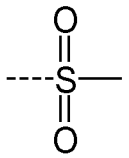
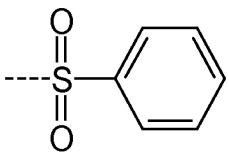
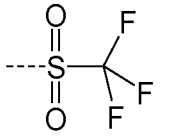
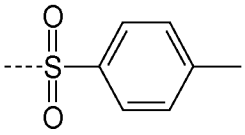
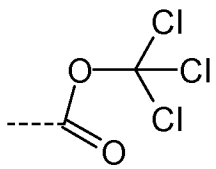
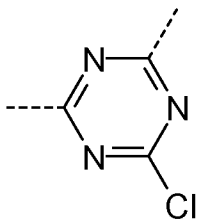
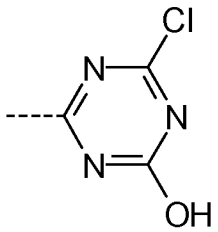
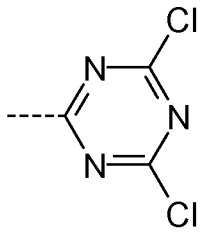
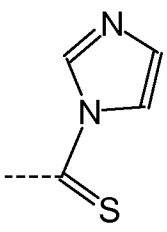
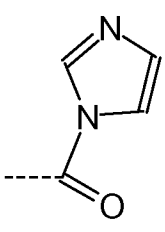
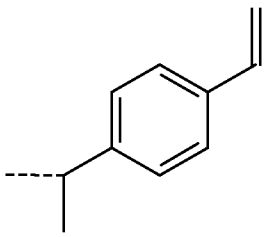
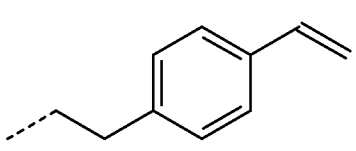
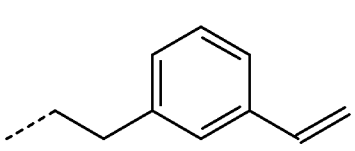
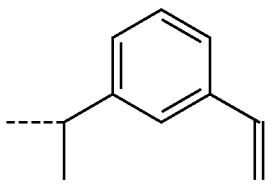
In case the carbohydrate antigen **A** is activated and not the linker **L**, the carbohydrate antigen normally comprises a larger number of activated groups which are theoretically all possible to form a covalent bond with the linker **L** or respectively with the moiety **–L–CH–CA**. Generally not all activated groups of the carbohydrate antigen **A** are reacted with the linker **L** or respectively with the moiety **–L–CH–CA**, thus several activated groups remain in the carbohydrate antigen after reaction with the linker **L** or respectively with the moiety **–L–CH–CA**. These remaining activated groups normally react during workup of the reaction product of the activated carbohydrate antigen with the linker **L** or respectively with the moiety **–L–CH–CA**. Thus during workup these remaining activated groups of the carbohydrate antigen **A** are, for instance, hydrolyzed, oxidized, isomerized, cyclized and/or crosslinked. During work up and especially during aqueous workup these remaining activated groups are, for instance, converted to amide, carbonate, carbamate, carbonyl, thiocarbonyl, carboxy, thiocarboxy, ester, thioester, ether, epoxy, hydroxyalkyl, alkylenyl, phenylene, alkenyl, imino, imide, isourea, thiocarbamate, thiourea and/or urea moieties.

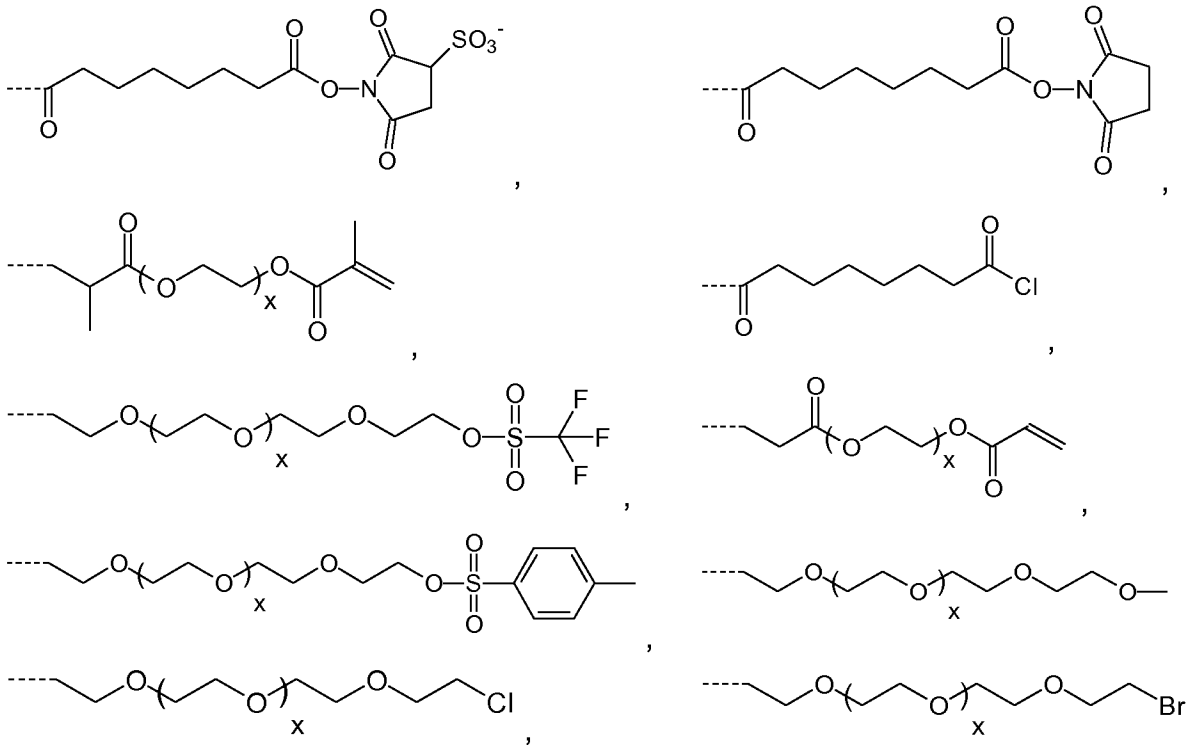
The activated groups which can be converted to the amide, carbonate, carbamate, carbonyl, thiocarbonyl, carboxy, thiocarboxy, ester, thioester, ether, epoxy, hydroxyalkyl, alkylene, phenylene, alkenyl, imino, imide, isourea, thiocarbamate, thiourea and urea moieties are, for instance, cyano, chloro, bromo, iodo, azido, imino groups, vinyl, styryl and allyl groups, anhydrides, oxiranes, cyanates, isocyanates,

thiocyanates, isothiocyanates, triazines and especially 1,3,5-triazines, imidazoles, methoxy ethers as well as sulfonyl groups such as *para*-toluenesulfonyl (Ts–), trifluoromethanesulfonyl (Tf–, CF_3SO_2–), benzenesulfonyl ($C_6H_5SO_2$–) or methanesulfonyl (Ms–).

In the following more specific examples for such activated groups are given. The activation method comprising the modification of the functional groups of the carbohydrate monomers of the carbohydrate antigen may lead to the formation of activated moieties which are covalently bound to heteroatoms (N, O, S) of the functionalities of the carbohydrate antigen, wherein the activated moieties preferably belong to the following group comprising or consisting of:

–N_3, –CN, –CH_2–CH=CH_2, –CH=CH_2, –OCH_3, –Cl, –Br, –I, –OCN, –NCO, –SCN, –NCS, –CO–O–CO–CH_3, –CO–O–CO–C_2H_5,


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wherein x is in integer from 1 to 60.

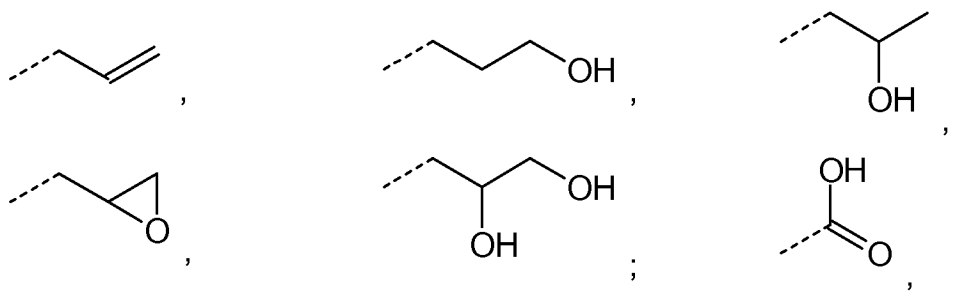
Thus, the modification of the carbohydrate monomers of the carbohydrate antigen also implies that the carbohydrate monomers comprise or contain amide, carbonate, carbamate, carbonyl, thiocarbonyl, carboxy, thiocarboxy, ester, thioester, ether, epoxy, hydroxyalkyl, alkylenyl, phenylene, alkenyl, imino, imide, isourea, thiocarbamate, thiourea and/or urea moieties. That means, that the modification of the carbohydrate monomers of the carbohydrate antigen **A** implies that the functional groups of the carbohydrate monomers are modified to amide, carbonate, carbamate, carbonyl, thiocarbonyl, carboxy, thiocarboxy, ester, thioester, ether, epoxy, hydroxyalkyl, alkylenyl, phenylene, alkenyl, imino, imide, isourea, thiocarbamate, thiourea and/or urea moieties.

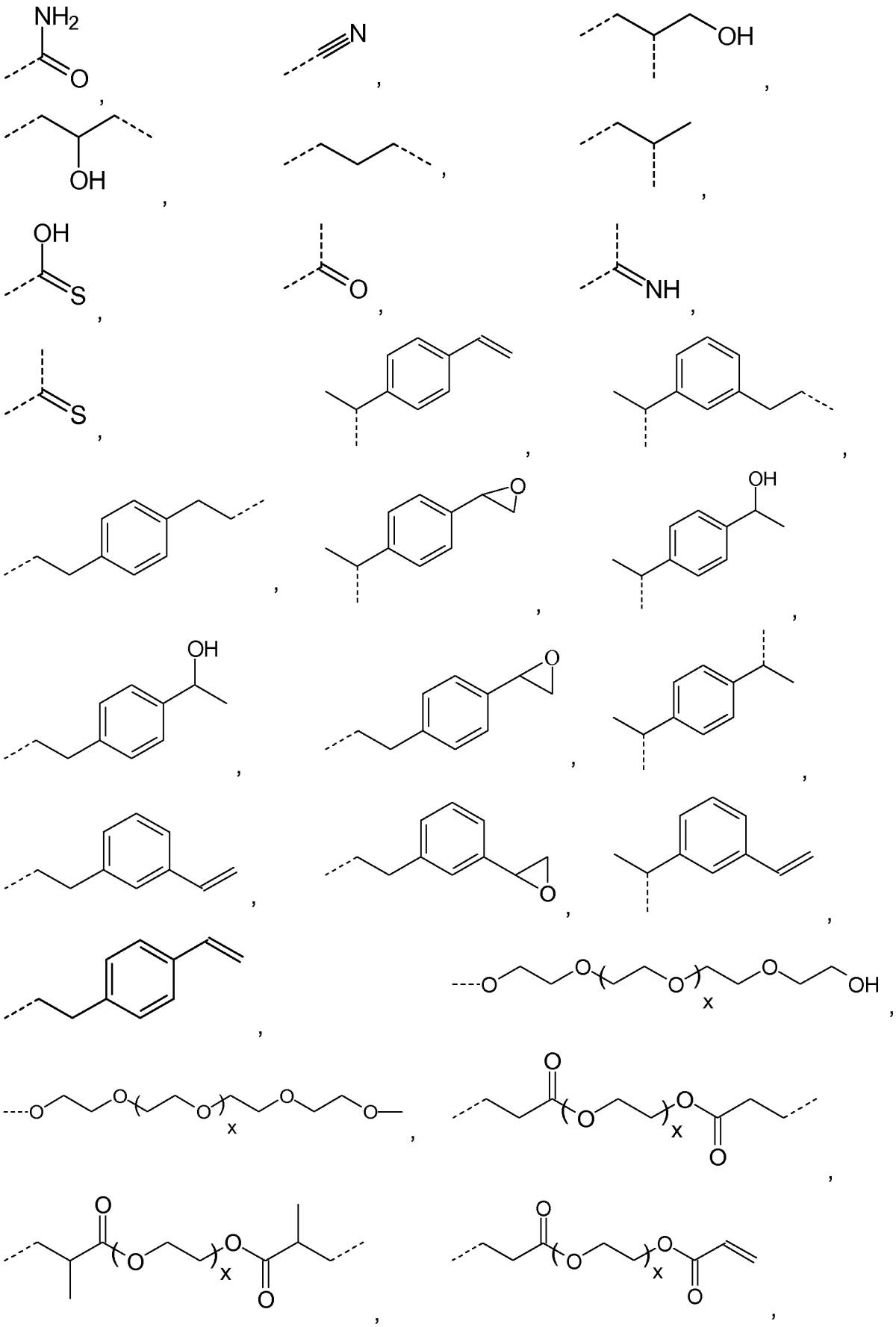
Therefore, the optional modification of the carbohydrate monomers of the carbohydrate antigen may be the result of an activation method which comprises the reaction of the carbohydrate functionalities with one activation agent or several activation agents and wherein the activation agent or the activation agents may form especially after hydrolysis, oxidation, isomerization, cyclization and/or crosslinking amide, carbonate, carbamate, carbonyl, thiocarbonyl, carboxy, thiocarboxy, ester, thioester, ether, epoxy, hydroxyalkyl, alkylenyl, phenylene, alkenyl, imino, imide, isourea, thiocarbamate, thiourea and/or urea moieties.

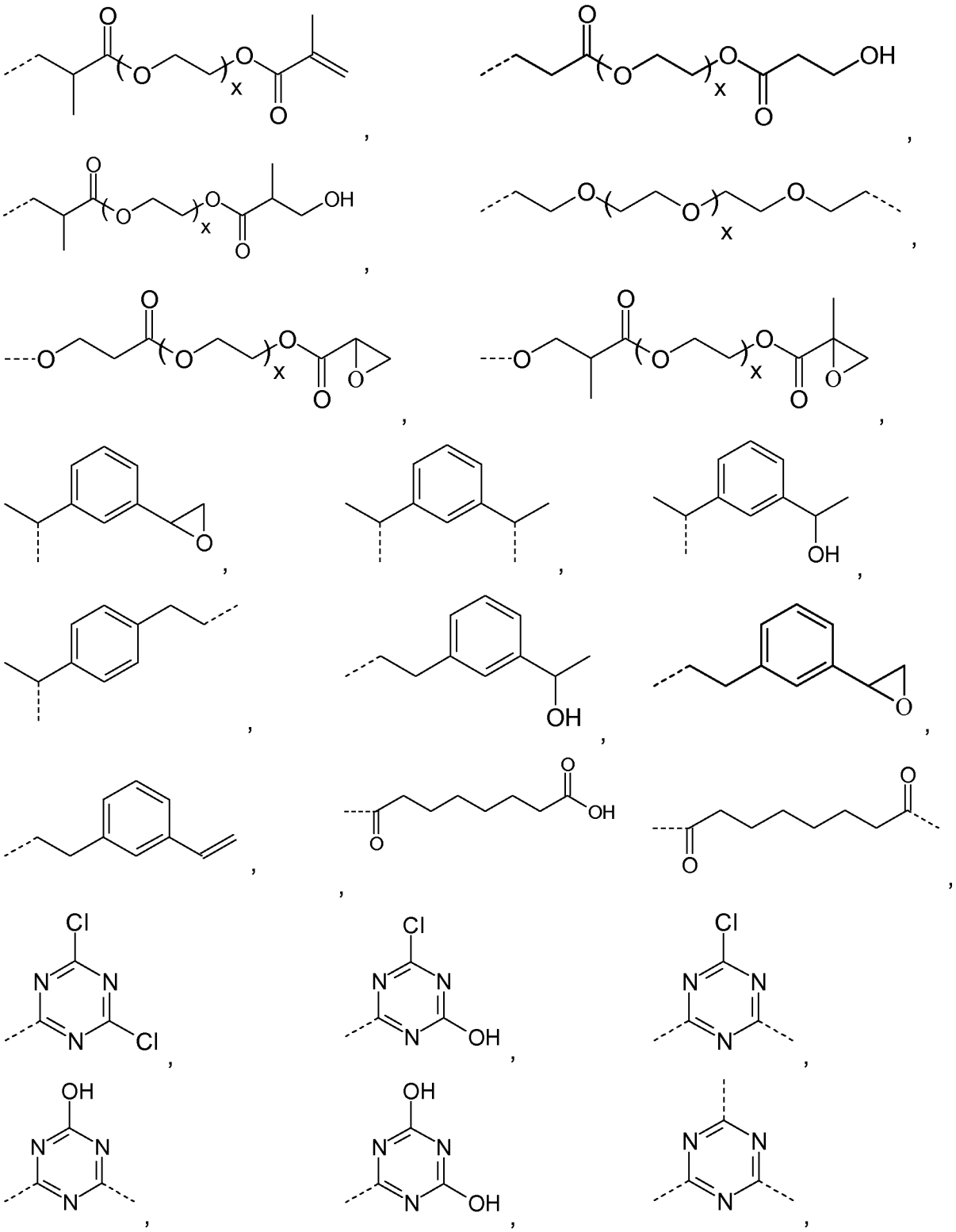
The mentioned activation agent or agents can be used for the coupling of the carbohydrate antigen to the linker **L** or respectively to the residues **–L–CH–CA** and preferably belong to the group comprising:
allylbromide, allylchloride, bis-NHS-esters like bis[sulfosuccinimidyl] suberate, cyanogen bromide, 1,4-cyclohexanedimethanol divinyl ether, 1,1'-carbonyldiimidazole (CDI), *N,N'*-(1,2-dihydroxyethylene)bisacrylamide, divinylbenzene, epichlorhydrin (ECH), ethylene-glycol-di(meth)acrylates, ethylene-glycol-diacrylates, *N*-hydroxysuccinimide (NHS), *N*-(1-hydroxy-2,2-dimethoxyethyl)-acrylamide, methylenebisacrylamides, 4,4′-methylenebis(cyclohexylisocyanate), 1,4-phenylenediacryloylchloride, phosgene, diphosgene, triphosgene, polyethylene-glycol-di(meth)acrylates, polyethylene-glycol-diacrylates, tetraethylene glycol dimethyl ether, 1,1'-thiocarbonyldiimidazol (TCDI), thiophosgene, 2,4,6-trichlorotriazine (TCT).

In case the carbohydrate antigen **A** is activated, the activation method leads to a conversion of the functionalities of the carbohydrate monomers of the carbohydrate antigen into activated species which react with the residues **–L–CH–CA** in a further step.

Not all of the activated groups of the carbohydrate antigen **A** react with the residues **–L–CH–CA** and may therefore hydrolize, oxidize, isomerize, cyclize or crosslink with other sugar moieties of the carbohydrate antigen during workup to form hydrolized, oxidized, isomerized, cyclized or crosslinked residues. These hydrolized, oxidized, isomerized, cyclized or crosslinked residues derive from the activation agent itself and their chemistry due to hydrolysis, oxidation, isomerization, cyclization or crosslinking reactions. The hydrolized, oxidized, isomerized, cyclized or crosslinked residues are covalently bound to any hetero atom (N, O, S) of the functionalities of the carbohydrate monomers of the carbohydrate antigen, and belong preferably to the group comprising or consisting of:







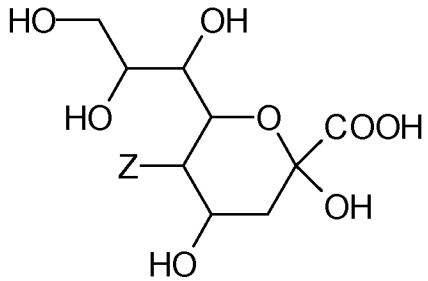
wherein x is in integer from 1 to 60.

The modification of the functionalities of the carbohydrate monomers of the carbohydrate antigen comprises the reaction of the functionalities of the carbohydrate monomers of the carbohydrate antigen with one activation agent or activation agents and/or with the activated linker **L** or respectiviely the activated linker **L** in **–L–CH–CA**

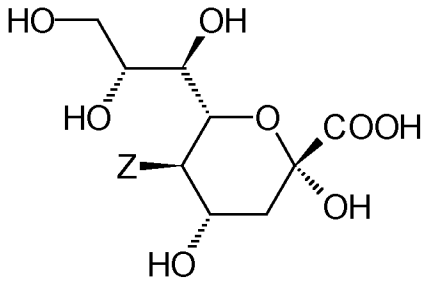
or when the carbonhydrate monomers of the carbohydrate antigen with the non-activated linker to form a covalent bond between the hetero atom (N, O, S) of the functionality of the carbohydrate monomer or the modified carbohydrate monomer and the activation agent and/or with the activated or non-activated linker **L**. The formation of this covalent bond is accompanied by the cleavage of a N-H, O-H or S-H bond and the loss of a H-atom. Possible reactions for the formation of this covalent bond are belonging to the group comprising nucleophilic substitution, esterification, etherification, amidation, acylation.

The carbohydrate monomers of the carbohydrate antigen **A** preferably belong to hexoses, pentoses, tetroses or sialic acids.

In a preferred embodiment of the invention, the sialic acids belong to the group of N- or O-substituted derivatives of neuraminic acid of the following formula:



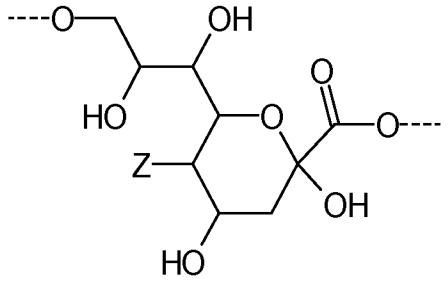
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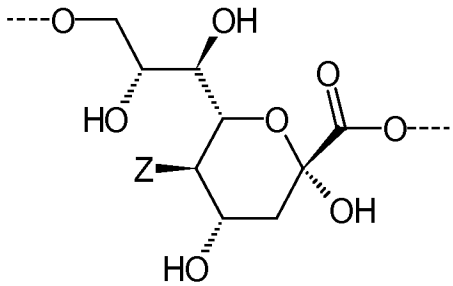
wherein Z represents $-NH_2$, –NHAc, or –OH.

In case such a sialic acid carbohydrate monomer is present in the carbohydrate antigen **A**, linkage to the subsequent carbohydrate monomer is achieved through a glycosidic bond (and replacement of the corresponding hydrogen atom at the glycosidic hydroxyl group) and/or through linkage of another carbohydrate monomer to one of the hydroxyl groups of the sialic acid by replacement of the corresponding hydrogen atom at this hydroxyl group.

In a preferred embodiment the sialic acid carbohydrate monomer represents within the building block **A** as follows:



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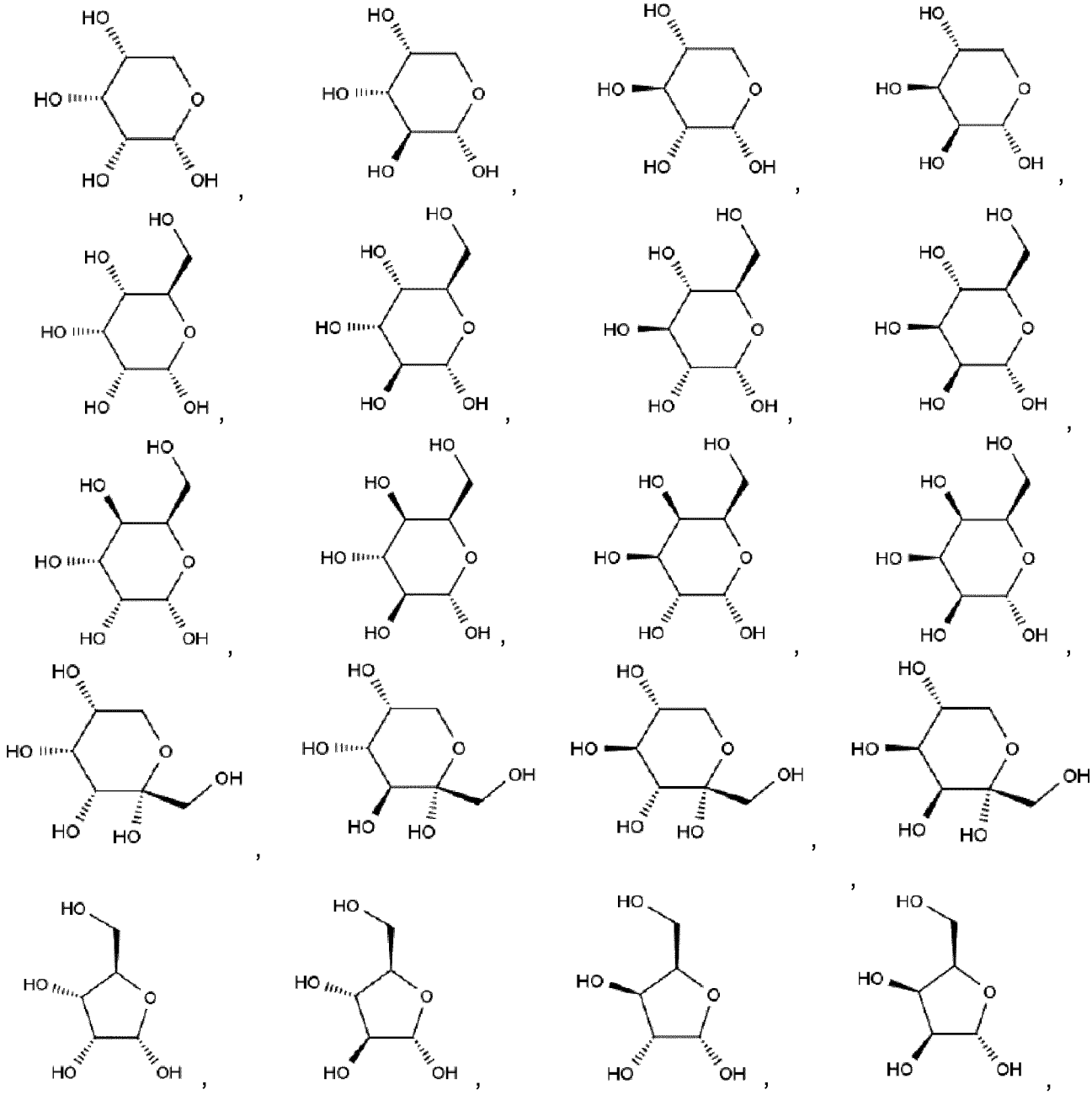
wherein Z represents $-NH_2$, –NHAc, or –OH.

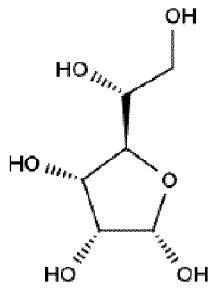
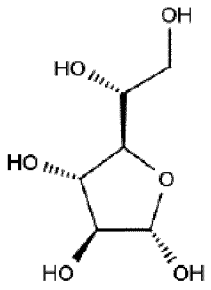
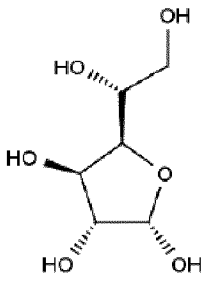
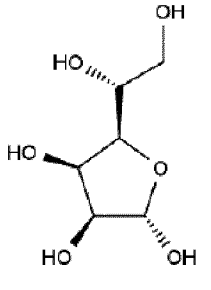
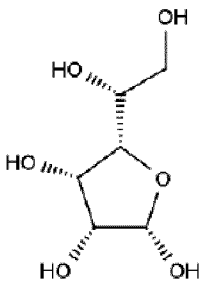
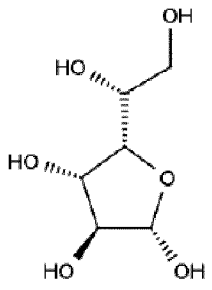
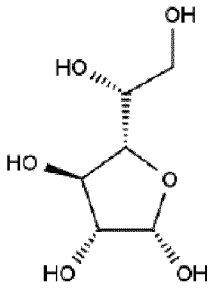
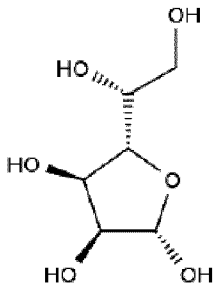
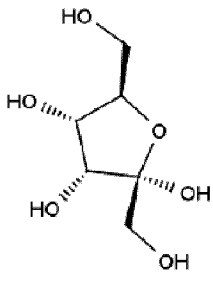
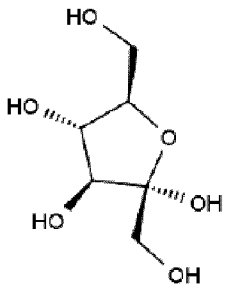
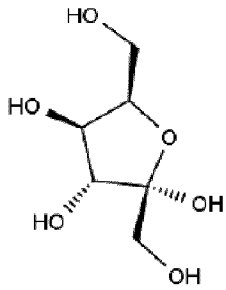
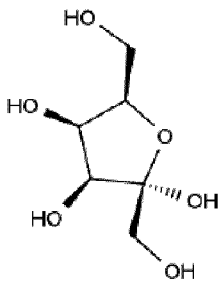
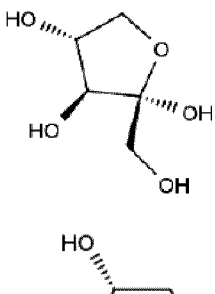
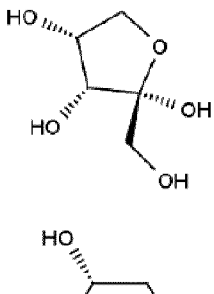
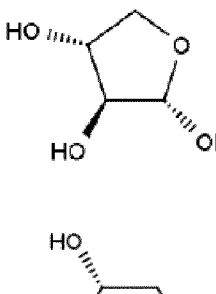
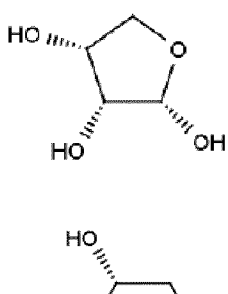
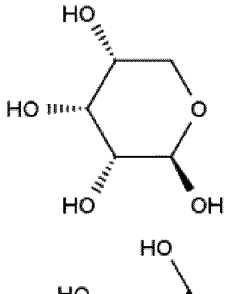
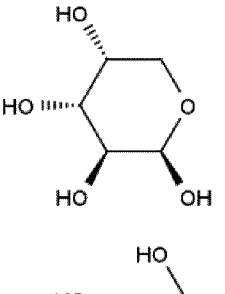
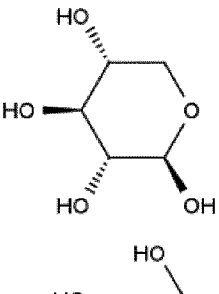
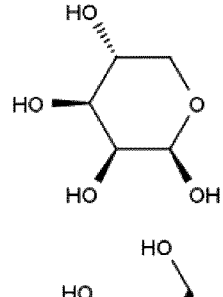
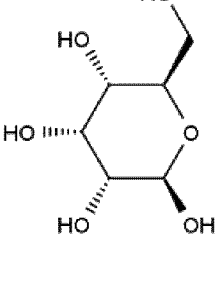
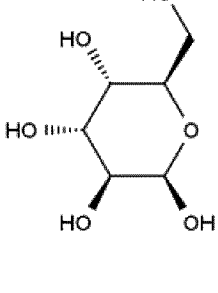
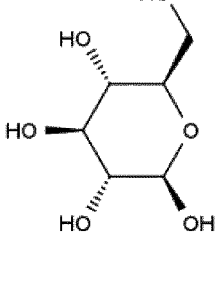
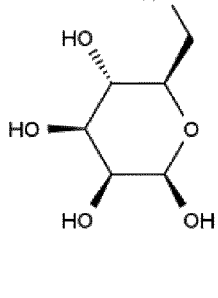
In a preferred embodiment of the invention, the used carbohydrate monomers of the **A**-moiety belong to the following group of α- and β-D/L-carbohydrates comprising or consisting of:

α-D-ribopyranose, α-D-arabinopyranose, α-D-xylopyranose, α-D-lyxopyranose, α-D-allopyranose, α-D-altropyranose, α-D-glucopyranose, α-D-mannpyranose, α-D-glucopyranose, α-D-idopyranose, α-D-galactopyranose, α-D-talopyranose, α-D-psicopyranose, α-D-fructopyranose, α-D-sorbopyranose, α-D-tagatopyranose, α-D-ribofuranose, α-D-arabinofuranose, α-D-xylofuranose, α-D-lyxofuranose, α-D-Allofuranose, α-D-Altrofuranose, α-D-Glucofuranose, α-D-Mannofuranose, α-D-gulofuranose, α-D-idofuranose, α-D-galactofuranose, α-D-talofuranose, α-D-psicofuranose, α-D-fructofuranose, α-D-sorbofuranose, α-D-tagatofuranose, α-D-xylulofuranose, α-D-ribulofuranose, α-D-threofuranose, α-D-rhamnopyranose, α-D-erythrofuranose, α-D-glucosamine, α-D-glucopyranuronic acid, β-D-ribopyranose, β-D-arabinopyranose, β-D-xylopyranose, β-D-lyxopyranose, β-D-allopyranose, β-D-altropyranose, β-D-glucopyranose, β-D-mannpyranose, β-D-glucopyranose, β-D-idopyranose, β-D-galactopyranose, β-D-talopyranose, β-D-psicopyranose, β-D-fructopyranose, β-D-sorbopyranose, β-D-tagatopyranose, β-D-ribofuranose, β-D-arabinofuranose, β-D-xylofuranose, β-D-lyxofuranose, β-D-rhamnopyranose, β-D-allofuranose, β-D-altrofuranose, β-D-glucofuranose, β-D-mannofuranose, β-D-gulofuranose, β-D-idofuranose, β-D-galactofuranose, β-D-talofuranose, β-D-psicofuranose, β-D-fructofuranose, β-D-sorbofuranose, β-D-tagatofuranose, β-D-xylulofuranose, β-D-ribulofuranose, β-D-threofuranose, β-D-erythrofuranose, β-D-glucosamine, β-D-glucopyranuronic acid, α-L-ribopyranose, α-L-arabinopyranose, α-L-xylopyranose, α-L-lyxopyranose, α-L-allopyranose, α-L-altropyranose, α-L-glucopyranose, α-L-mannpyranose, α-L-glucopyranose, α-L-idopyranose, α-L-galactopyranose, α-L-talopyranose, α-L-psicopyranose, α-L-fructopyranose, α-L-sorbopyranose, α-L-tagatopyranose, α-L-rhamnopyranose, α-L-ribofuranose, α-L-arabinofuranose, α-L-xylofuranose, α-L-lyxofuranose, α-L-Allofuranose, α-L-Altrofuranose, α-L-Glucofuranose, α-L-Mannofuranose, α-L-gulofuranose, α-L-idofuranose, α-L-galactofuranose, α-L-talofuranose, α-L-psicofuranose, α-L-fructofuranose, α-L-sorbofuranose, α-L-tagatofuranose, α-L-xylulofuranose, α-L-ribulofuranose, α-L-threofuranose, α-L-erythrofuranose, α-L-glucosamine, α-L-glucopyranuronic acid, β-L-ribopyranose, β-L-arabinopyranose, β-L-xylopyranose, β-L-lyxopyranose, β-L-allopyranose, β-L-altropyranose, β-L-glucopyranose, β-L-mannpyranose, β-L-glucopyranose, β-L-idopyranose, β-L-galactopyranose, β-L-talopyranose, β-L-psicopyranose, β-L-fructopyranose, β-L-sorbopyranose, β-L-tagatopyranose, β-L-ribofuranose, β-L-arabinofuranose,

β-L-xylofuranose, β-L-lyxofuranose, β-L-allofuranose, β-L-altrofuranose, β-L-glucofuranose, β-L-mannofuranose, β-L-gulofuranose, β-L-idofuranose, β-L-galactofuranose, β-L-talofuranose, β-L-psicofuranose, β-L-fructofuranose, β-L-sorbofuranose, β-L-tagatofuranose, β-L-xylulofuranose, β-L-ribulofuranose, β-L-threofuranose, β-L-erythrofuranose, β-L-glucosamine, β-L-glucopyranuronic acid, and β-L-rhamnopyranose.

In another preferred embodiment of the invention, the carbohydrate monomers of the **A**-moiety and the CH moiety are selected independently of each other from the group comprising or consisting of the following α- and β-D-carbohydrates:



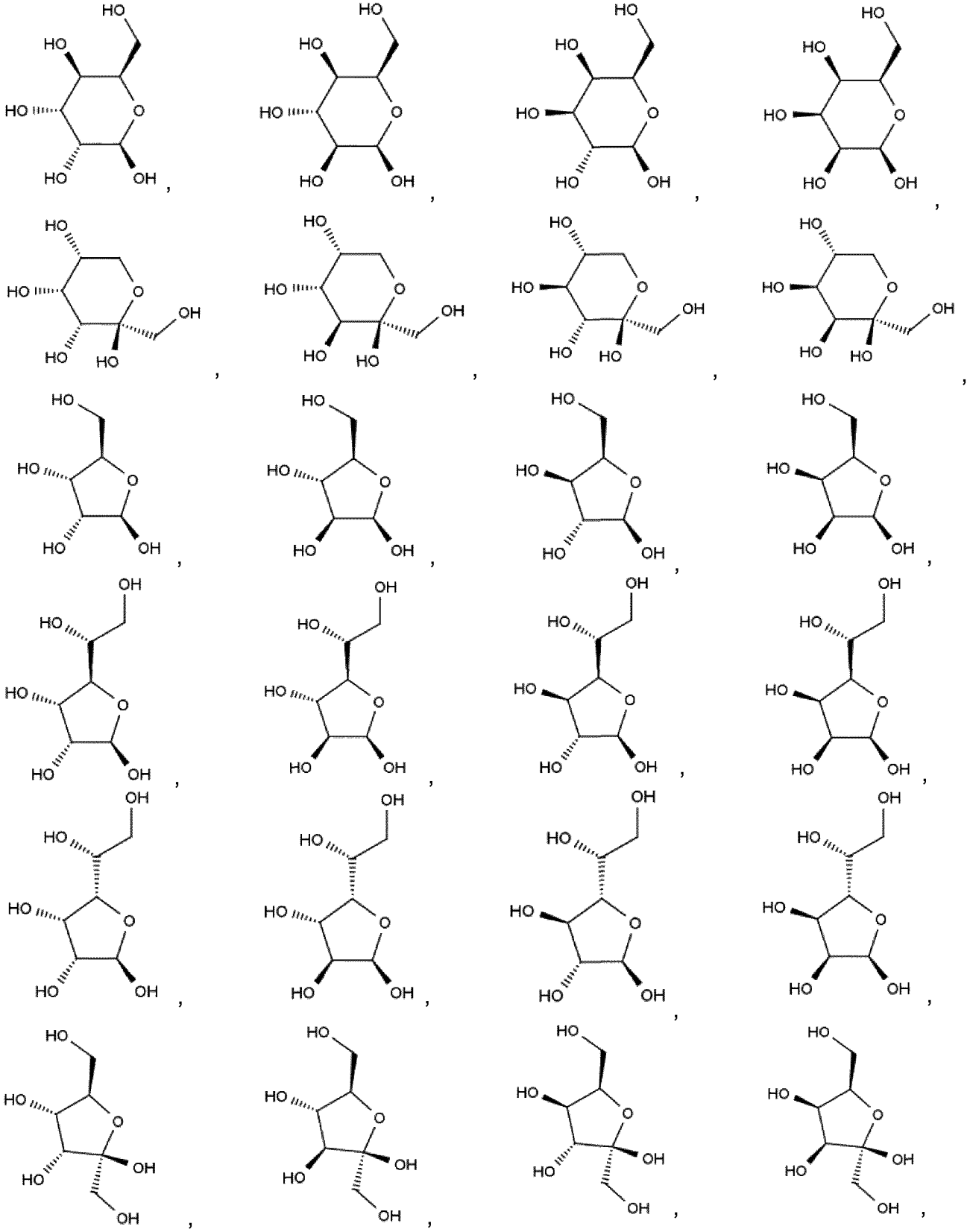

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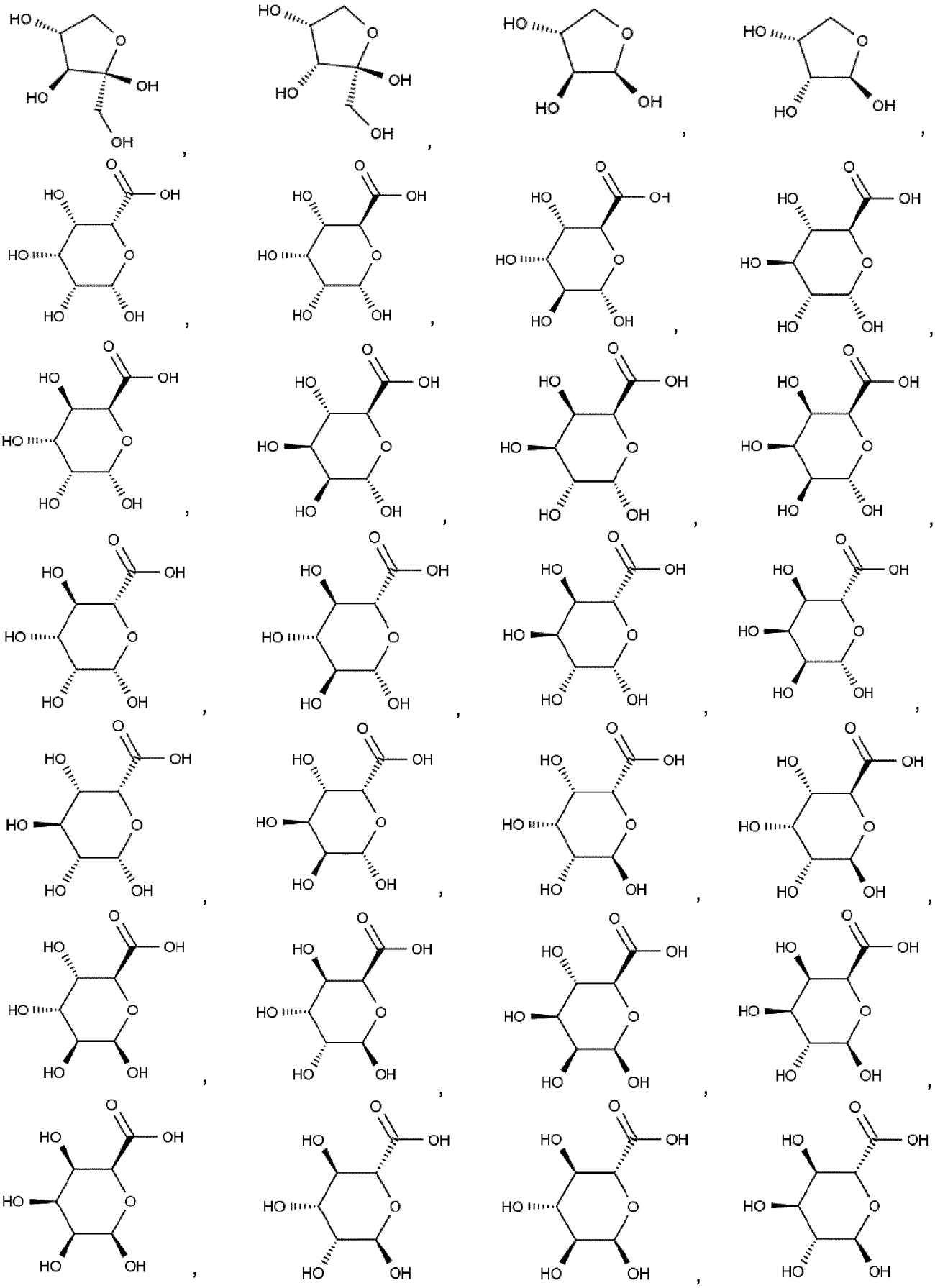
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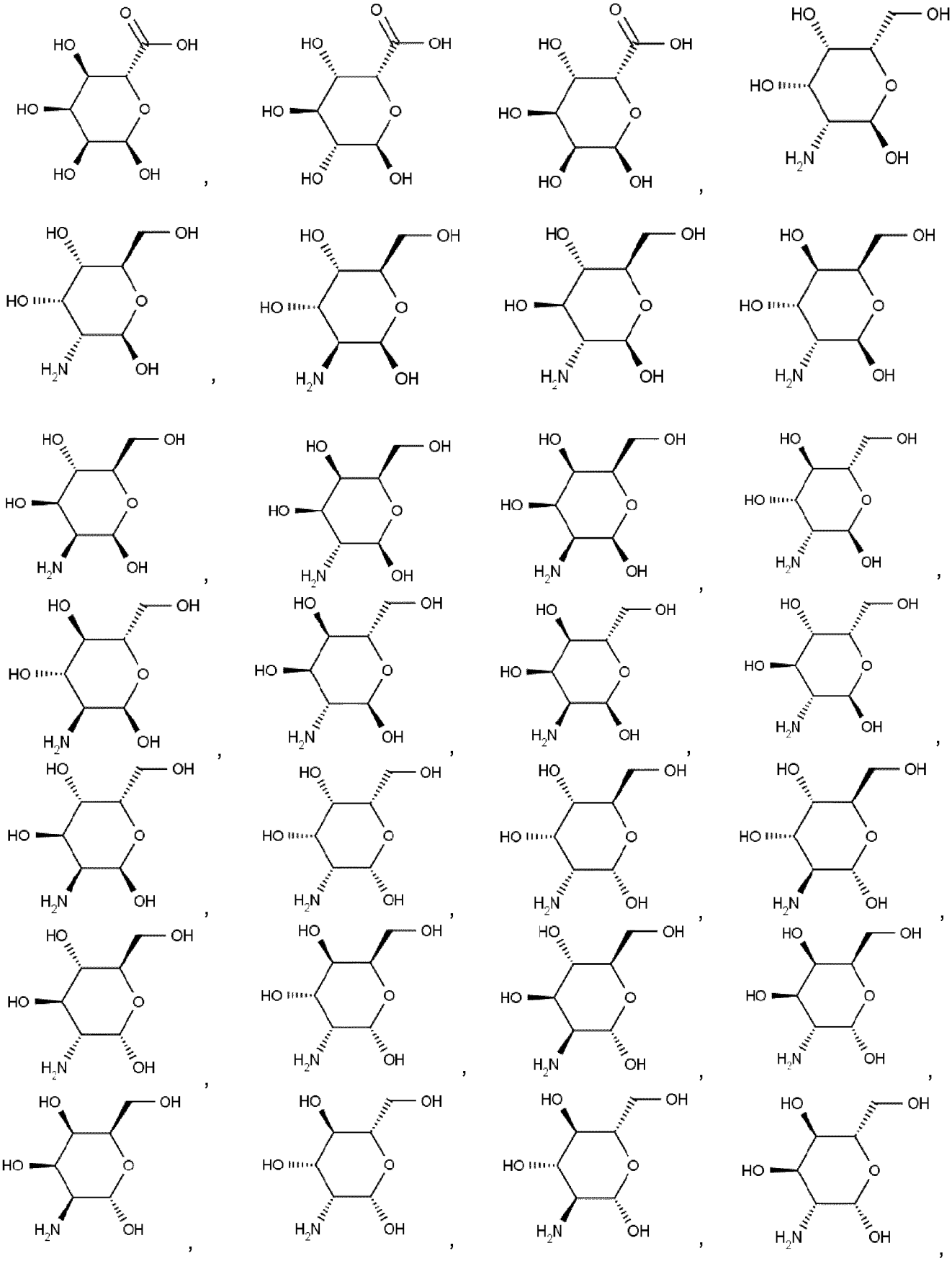
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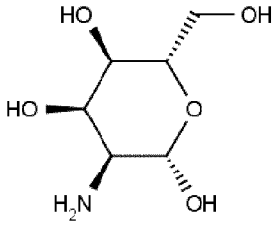
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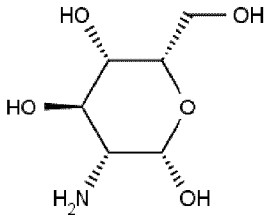
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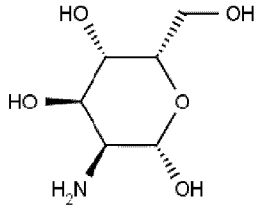








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According to the present invention these carbohydrate monomers as defined herein are abundant in an antigen and occur as linking building block by deprotonation of two hydrogen atoms of different hydroxyl groups and formation of a bond to the rest of the molecule of the antigen A and to the moiety **L**, respectively.

L represents a linker moiety which is covalently bound to any atom, especially any hetero atom and most preferably any oxygen atom of a former hydroxyl group of the carbohydrate monomers of the carbohydrate antigen. Moreover the linker **L** is covalently bound to any hetero atom of **CH** and especially any oxygen atom of a hydroxyl group of **CH**. Thus, the linker molecule interconnects between the antigen **A** and the carbonhydrate moiety **CH**. Further, according to the present invention the interconnection between the the antigen **A** and the carbonhydrate moiety **CH** occurs as described herein preferably by activation of the carbohydrate monomers of the carbohydrate antigen a and/or by activation of the linker molecule. Thereby, in a preferred embodiment of the present invention it is not merely connected the antigen **A** with the carbonhydrate moiety **CH** via the linker **L**, but it is the interconnection between the antigen **A** and the carbonhydrate moiety **CH** already bond to the ceramid **CA** forming the inventive compounds of the general formula (I)

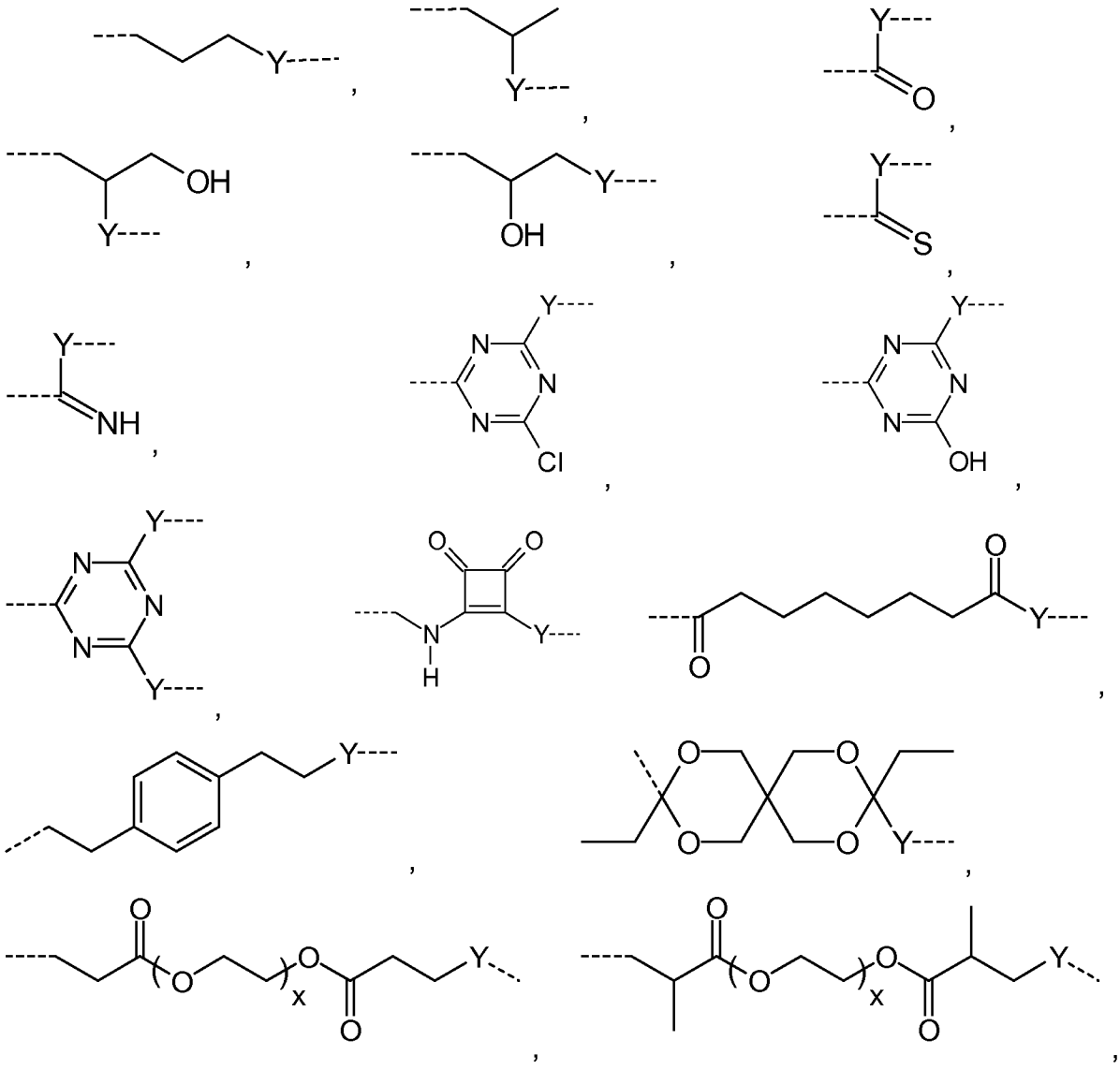
$$\mathbf{A[L—CH—CA]_p}$$

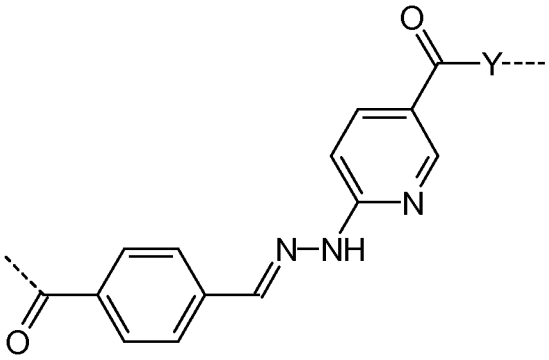
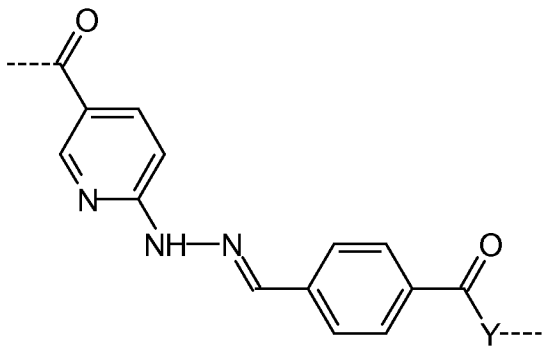
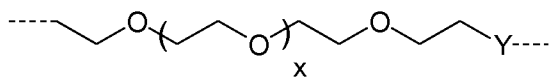
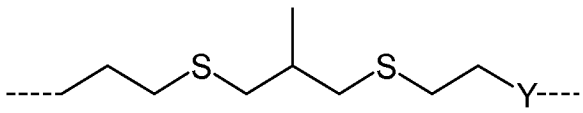
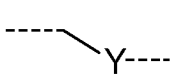
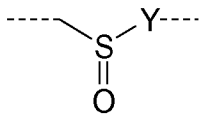
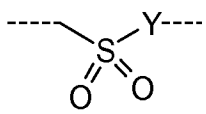
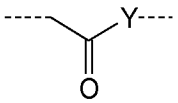
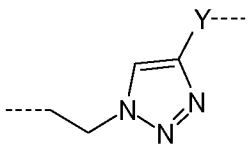
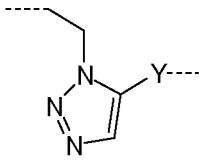
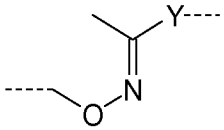
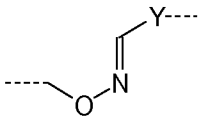
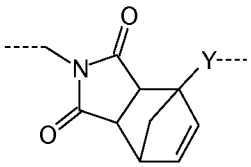
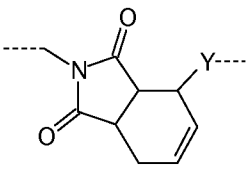
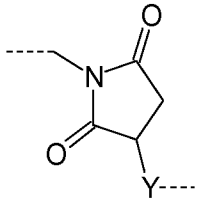
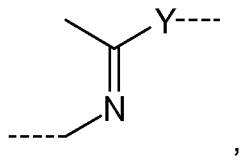
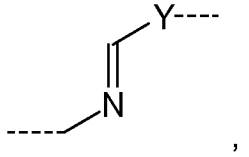
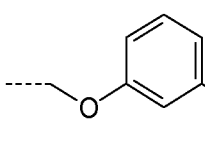
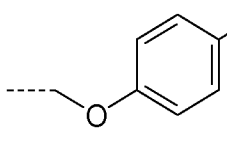
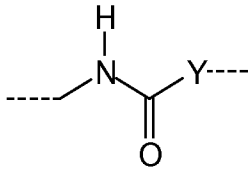
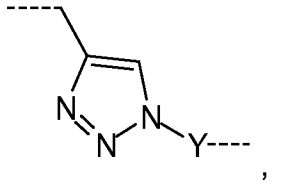
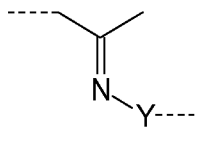
(I).

The linker **L** can be subdivided into subunits $-L^1-$, $-L^2-$ and $-L^3-$ and can be formed of the subunits alone or of combinations thereof. Therefore, **L** may represent $-L^1-L^2-$, $-L^2-$, $-L^2-L^3-$ or $-L^1-L^2-L^3-$. The preferred order of connectivity in the above cases with **A** and **CH** is as follows: $A-L^1-L^2-CH-$, $A-L^2-CH-$, $A-L^2-L^3-CH-$ or $A-L^1-L^2-L^3-CH-$. However, it is also possible that the different fragments such as $-L^1-L^2-$, $-L^2-$, $-L^2-L^3-$, $-L^3-L^2-L^3-$, $-L^2-L^3-L^2-$ or $-L^1-L^2-L^3-$ are aligned in all possible orders as long as the connection between the different parts is chemically reasonable and possible.

The linker **L** may be bound to the carbohydrate moiety in such a manner that this bond can be cleaved in cell, e.g. a B help cell, a T help cell, in order to release the fragment **A–L** on the one hand and the fragment **–CH-CA** on the other hand.

L may include the functionality or a fragment of the functionality derived from the activation of the carbohydrate monomers of the carbohydrate antigen. **L** is preferably covalently bound to any hetero atom (N, O, S) of the carbohydrate monomers of the carbohydrate antigen **A**. $\mathbf{L^1}$ if present is covalently bound to the linker subunit $\mathbf{L^2}$ preferably through the moiety **Y**, which could also be a chemical bond. $\mathbf{L^1}$ is preferably selected from the following residues:

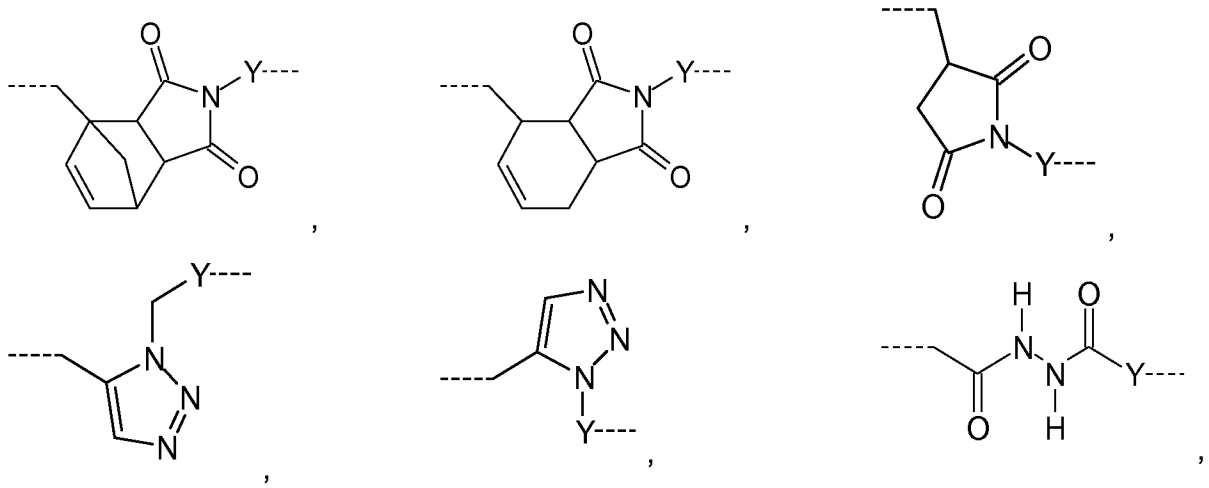



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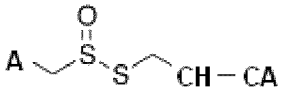
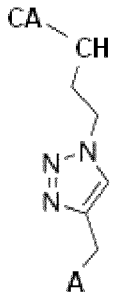
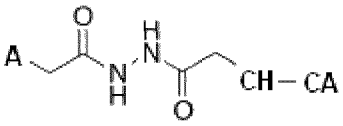
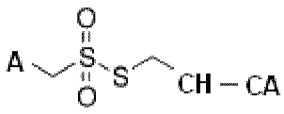
x is an integer from 1 to 60;
Y represents a bond, –NH–, –O–, –S–, –S–S–;

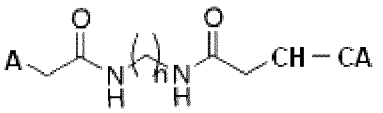
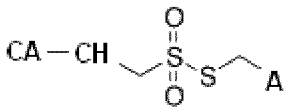
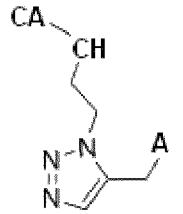
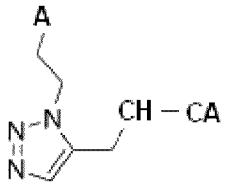
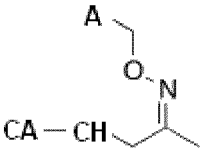
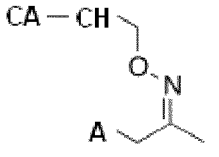
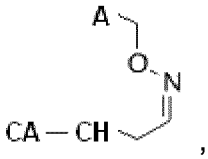
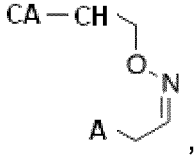
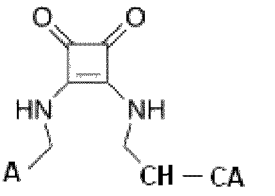
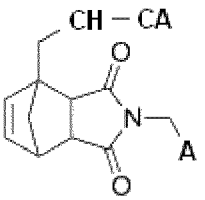
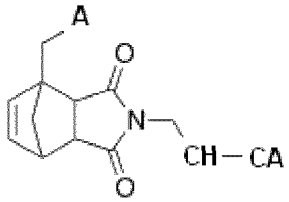
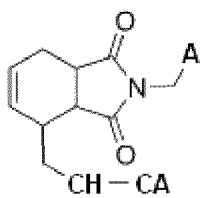
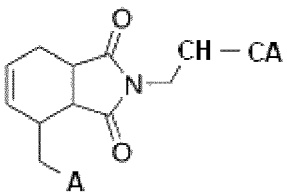
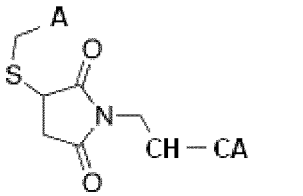
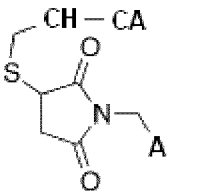
L^2 represents –CH_2–, –C_2H_4–, –C_3H_6–, –C_4H_8–, –C_5H_{10}–, –C_6H_{12}–, –C_7H_{14}–, –C_8H_{16}–, –C_9H_{18}–, –$C_{10}H_{20}$–, –$CH(CH_3)$–, –$C[(CH_3)_2]$–, –CH_2–$CH(CH_3)$–, –$CH(CH_3)$–CH_2–, –$CH(CH_3)$–C_2H_4–, –CH_2–$CH(CH_3)$–CH_2–, –C_2H_4–$CH(CH_3)$–, –CH_2–$C[(CH_3)_2]$–, –$C[(CH_3)_2]$–CH_2–, –$CH(CH_3)$–$CH(CH_3)$–, –$C[(C_2H_5)(CH_3)]$–, –$CH(C_3H_7)$–, –$(CH_2$–CH_2–$O)_n$–CH_2–CH_2–, –CO–CH_2–, –CO–C_2H_4–, –CO–C_3H_6–, –CO–C_4H_8–, –CO–C_5H_{10}–, –CO–C_6H_{12}–, –CO–C_7H_{14}–, –CO–C_8H_{16}–, –CO–C_9H_{18}–, –CO–$C_{10}H_{20}$–, –CO–$CH(CH_3)$–, –CO–$C[(CH_3)_2]$–, –CO–CH_2–$CH(CH_3)$–, –CO–$CH(CH_3)$–CH_2–, –CO–$CH(CH_3)$–C_2H_4–, –CO–CH_2–$CH(CH_3)$–CH_2–, –CO–C_2H_4–$CH(CH_3)$–, –CO–CH_2–$C[(CH_3)_2]$–, –CO–$C[(CH_3)_2]$–CH_2–, –CO–$CH(CH_3)$–$CH(CH_3)$–, –CO–$C[(C_2H_5)(CH_3)]$–, –CO–$CH(C_3H_7)$–, –CO–$(CH_2$–CH_2–$O)_n$–CH_2–CH_2–. **L^2** is in case **L^3** is not present preferably linked to an oxygen atom of a former hydroxyl group of the carbohydrate residue **CH**.

n represents an integer from 1 to 60;

L^3 represents –CO–, –O–CO–, –NH–CO–, –NH(C=NH)–, –SO_2–, –O–SO_2–, –NH–, –NH–CO–CH_2–. **L^3** if present is preferably linked to an oxygen atom of a former hydroxyl group of the carbohydrate residue **CH**.

Preferred examples for linker moieties L of the moiety **A–L–CH–CA** as at least one representative of all moieties in the compounds of the general formula (I) as defined herein are

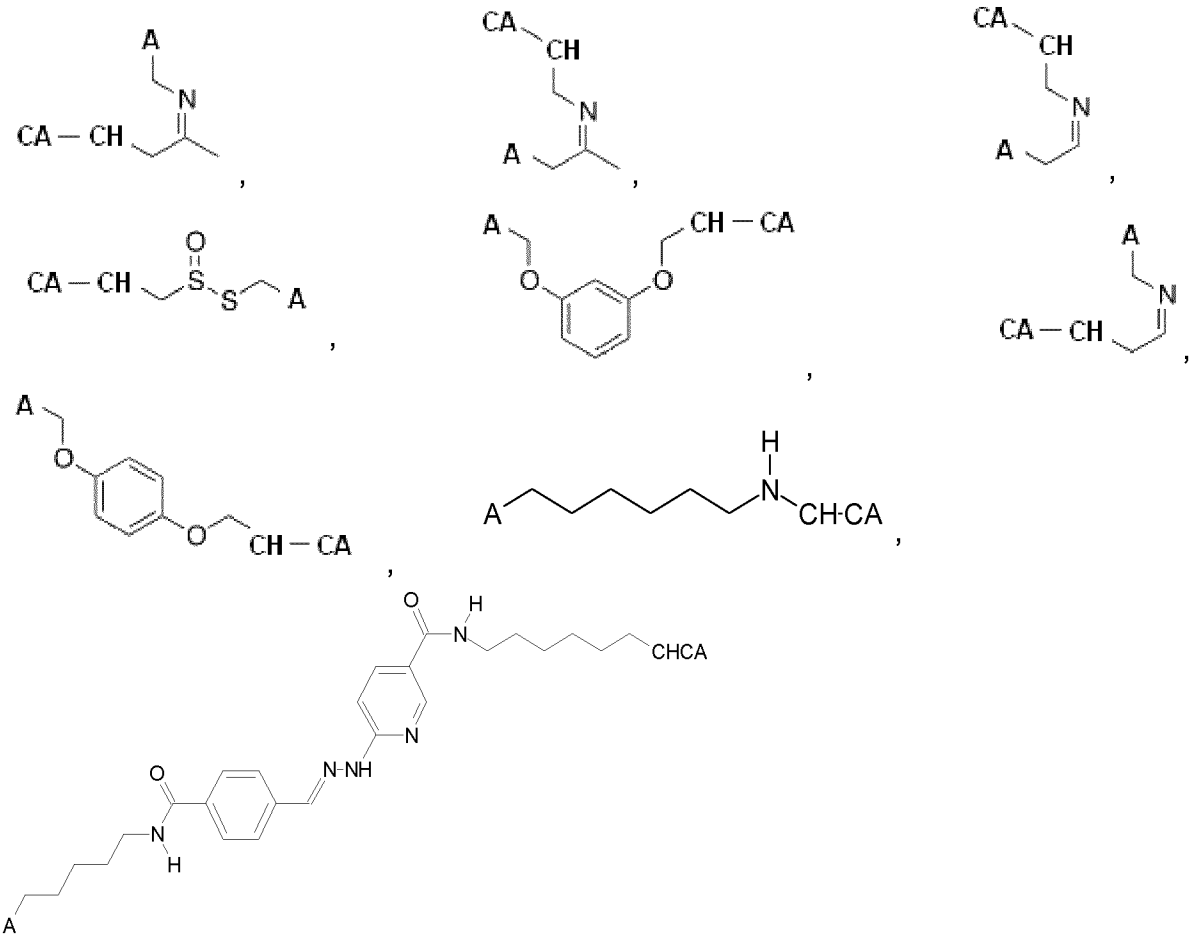

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wherein n is as defined herein, and A, CH and CA represent an antigen, a carbohydrate moiety and a ceramid as defined herein.

The linker molecule **L** may optionally be further substituted with 1 to 3 of the substituents Z^6, Z^7, Z^8. However, it is clear to a skilled person that the term "can be substituted" refers to the replacement of a hydrogen atom by one of the substituents Z^6, Z^7, Z^8.

The substituents Z^6, Z^7 and Z^8 represent independently of each other –OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, –O–cyclo-C_3H_5, $-OCH(CH_3)_2$, $-OC(CH_3)_3$, $-OC_4H_9$, –OPh, $-OCH_2$–Ph, $-OCPh_3$, $-CH_2-OCH_3$, $-C_2H_4-OCH_3$, $-C_3H_6-OCH_3$, $-CH_2-OC_2H_5$, $-C_2H_4-OC_2H_5$, $-C_3H_6-OC_2H_5$, $-CH_2-OC_3H_7$, $-C_2H_4-OC_3H_7$, $-C_3H_6-OC_3H_7$, $-CH_2$–O–cyclo-C_3H_5, $-C_2H_4$–O–cyclo-C_3H_5, $-C_3H_6$–O–cyclo-C_3H_5, $-CH_2-OCH(CH_3)_2$, $-C_2H_4-OCH(CH_3)_2$, $-C_3H_6-OCH(CH_3)_2$, $-CH_2-OC(CH_3)_3$, $-C_2H_4-OC(CH_3)_3$, $-C_3H_6-OC(CH_3)_3$, $-CH_2-OC_4H_9$, $-C_2H_4-OC_4H_9$, $-C_3H_6-OC_4H_9$, $-CH_2$–OPh, $-C_2H_4$–OPh, $-C_3H_6$–OPh, $-CH_2-OCH_2$–Ph, $-C_2H_4-OCH_2$–Ph, $-C_3H_6-OCH_2$–Ph, $-NO_2$, –F, –Cl, –Br, $-COCH_3$, $-COC_2H_5$, $-COC_3H_7$, –CO–cyclo-C_3H_5, $-COCH(CH_3)_2$, $-COC(CH_3)_3$, –COOH, $-COOCH_3$, $-COOC_2H_5$, $-COOC_3H_7$, –COO–cyclo-C_3H_5, $-COOCH(CH_3)_2$, $-COOC(CH_3)_3$, $-OOC-CH_3$,

–OOC–C_2H_5, –OOC–C_3H_7, –OOC–cyclo-C_3H_5, –OOC–$CH(CH_3)_2$, –OOC–$C(CH_3)_3$, –$CONH_2$, –$CONHCH_3$, –$CONHC_2H_5$, –$CONHC_3H_7$, –CONH–cyclo-C_3H_5, –$CONH[CH(CH_3)_2]$, –$CONH[C(CH_3)_3]$, –$CON(CH_3)_2$, –$CON(C_2H_5)_2$, –$CON(C_3H_7)_2$, –CON(cyclo-$C_3H_5)_2$, –$CON[CH(CH_3)_2]_2$, –$CON[C(CH_3)_3]_2$, –$NHCOCH_3$, –$NHCOC_2H_5$, –$NHCOC_3H_7$, –NHCO–cyclo-C_3H_5, –NHCO–$CH(CH_3)_2$, –NHCO–$C(CH_3)_3$, –NH_2, –$NHCH_3$, –NHC_2H_5, –NHC_3H_7, –NH–cyclo-C_3H_5, –$NHCH(CH_3)_2$, –$NHC(CH_3)_3$, –$N(CH_3)_2$, –$N(C_2H_5)_2$, –$N(C_3H_7)_2$, –N(cyclo-$C_3H_5)_2$, –$N[CH(CH_3)_2]_2$, –$N[C(CH_3)_3]_2$, –OCF_3, –CH_2–OCF_3, –C_2H_4–OCF_3, –C_3H_6–OCF_3, –OC_2F_5, –CH_2–OC_2F_5, –C_2H_4–OC_2F_5, –C_3H_6–OC_2F_5, –CH_2F, –CHF_2, –CF_3, –CH_2Cl, –CH_2Br, –CH_2–CH_2F, –CH_2–CHF_2, –CH_2–CF_3, –CH_2–CH_2Cl, –CH_2–CH_2Br.

The carbohydrate moiety CH

CH represents a monosaccharide, a disaccharide or a trisaccharide, wherein the carbohydrate monomers thereof preferably belong to hexoses, pentoses, tetroses. In case **CH** represents a monosaccharide, the carbohydrate monomer is identical to the monosaccharide. The disaccharide contains two carbohydrate monomers and the trisaccharide contains three carbohydrate monomers. In the disaccharide and trisaccharide the carbohydrate monomers are connected to each other via α/β glycosidic bonds which preferably belong to the group consisting of 1,2; 1,3; 1,4; 1,5; 1,6; 2,2; 2,3; 2,4; 2,5; or 2,6 glycosidic bonds.

The monosaccharide, the disaccharide and the trisaccharide **CH** are covalently bound to **L** and also to **CA** via a heteroatom (N, O, S) of the **CH** moiety and most preferably through an oxygen atom of a former hydroxyl group of **CH**.

As used herein the term “former hydroxyl group” means that the oxygen atom of a carbohydrate monomer which is now linked to **L** or **CA** was the oxygen atom of a hydroxyl group and linked to a hydrogen atom which is now replaced by the residue **L** or **CA**.

In a preferred embodiment of this invention, the monosaccharide, the disaccharide or the trisaccharide **CH** is covalently bound by one oxygen atom to **L** and through another oxygen atom to **CA**.

In another preferred embodiment of this invention, the monosaccharide, the disaccharide or the trisaccharide **CH** is covalently bound by one hydroxyl oxygen atom to **L** and through another hydroxyl oxygen atom to **CA**.

In another preferred embodiment of this invention, **L** or **CA** is bound to **CH**, i.e. to the monosaccharide, the disaccharide or the trisaccharide, by a glycosidic bond at C1 of the saccharide.

In a more preferred embodiment of this invention, **L** is bound by a glycosidic bond to C1 of the monosaccharide, the disaccharide or the trisaccharide and **CA** is bound by the oxygen at C6 of a hexose or by the oxygen at C5 of a pentose or by the oxygen at C4 of a tetrose.

In another more preferred embodiment of this invention, **CA** is bound by a glycosidic bond to C1 of the monosaccharide, the disaccharide or the trisaccharide and **L** is bound by the oxygen at C6 of a hexose or by the oxygen at C5 of a pentose or by the oxygen at C4 of a tetrose.

In a preferred embodiment of the invention, the monosaccharide, the disaccharide or the trisaccharide **CH** consists of one, two or respectively 3 carbohydrates selected from the following group comprising or consisting of the following α- and β-D/L-carbohydrates:

α-D-ribopyranose, α-D-arabinopyranose, α-D-xylopyranose, α-D-lyxopyranose, α-D-allopyranose, α-D-altropyranose, α-D-glucopyranose, α-D-mannpyranose, α-D-glucopyranose, α-D-idopyranose, α-D-galactopyranose, α-D-talopyranose, α-D-psicopyranose, α-D-fructopyranose, α-D-sorbopyranose, α-D-tagatopyranose, α-D-ribofuranose, α-D-arabinofuranose, α-D-xylofuranose, α-D-lyxofuranose, α-D-Allofuranose, α-D-Altrofuranose, α-D-Glucofuranose, α-D-Mannofuranose, α-D-gulofuranose, α-D-idofuranose, α-D-galactofuranose, α-D-talofuranose, α-D-psicofuranose, α-D-fructofuranose, α-D-sorbofuranose, α-D-tagatofuranose, α-D-xylulofuranose, α-D-ribulofuranose, α-D-threofuranose, α-D-erythrofuranose, α-D-glucosamine, α-D-glucopyranuronic acid, α-D-rhamnopyranose, β-D-ribopyranose, β-D-arabinopyranose, β-D-xylopyranose, β-D-lyxopyranose, β-D-allopyranose, β-D-altropyranose, β-D-glucopyranose, β-D-mannpyranose, β-D-glucopyranose, β-D-idopyranose, β-D-galactopyranose, β-D-talopyranose, β-D-psicopyranose, β-D-fructopyranose, β-D-sorbopyranose, β-D-tagatopyranose, β-D-ribofuranose, β-D-arabinofuranose, β-D-xylofuranose, β-D-lyxofuranose, β-D-allofuranose, β-D-altrofuranose, β-D-glucofuranose, β-D-mannofuranose, β-D-gulofuranose, β-D-idofuranose, β-D-galactofuranose, β-D-talofuranose, β-D-psicofuranose, β-D-fructofuranose, β-D-sorbofuranose, β-D-tagatofuranose, β-D-xylulofuranose, β-D-ribulofuranose, β-D-threofuranose, β-D-erythrofuranose, β-D-rhamnopyranose, β-D-glucosamine, β-D-glucopyranuronic acid, α-L-ribopyranose, α-L-arabinopyranose, α-L-xylopyranose, α-L-lyxopyranose, α-L-allopyranose, α-L-altropyranose, α-L-glucopyranose, α-L-mannpyranose, α-L-glucopyranose, α-L-idopyranose, α-L-galactopyranose, α-L-talopyranose, α-L-psicopyranose, α-L-fructopyranose, α-L-sorbopyranose, α-L-tagatopyranose, α-L-ribofuranose, α-L-arabinofuranose, α-L-xylofuranose, α-L-lyxofuranose, α-L-Allofuranose, α-L-Altrofuranose, α-L-Glucofuranose, α-L-Mannofuranose, α-L-gulofuranose,

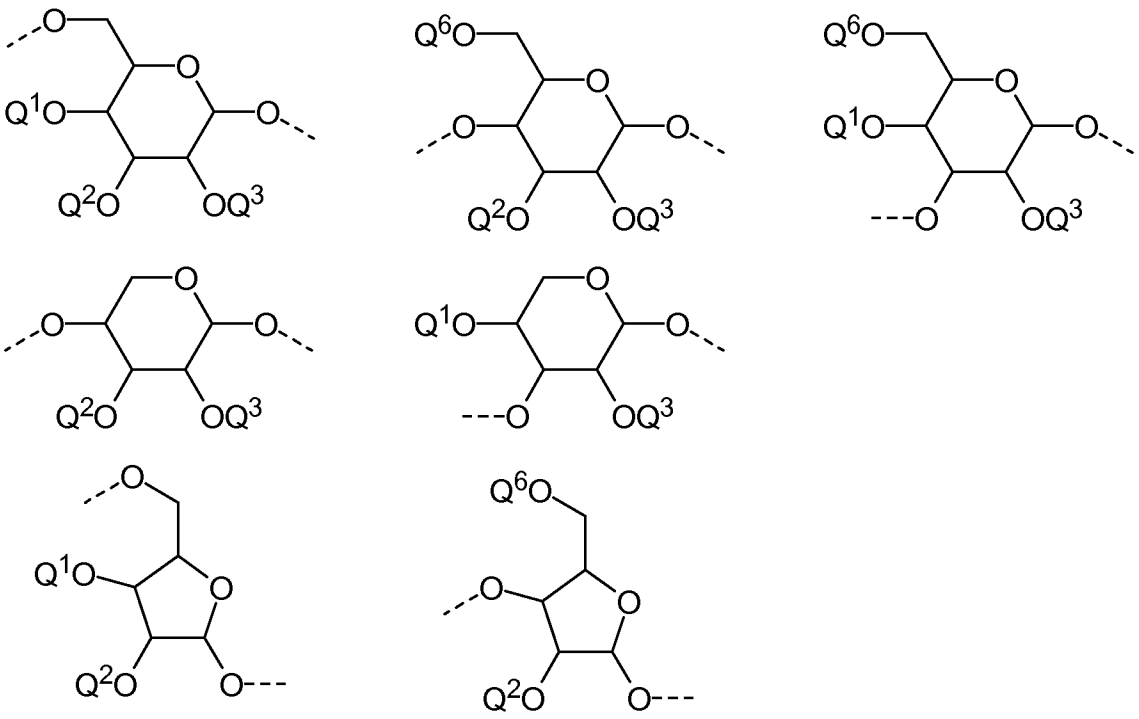
α-L-idofuranose, α-L-galactofuranose, α-L-talofuranose, α-L-psicofuranose, α-L-fructofuranose, α-L-sorbofuranose, α-L-tagatofuranose, α-L-xylulofuranose, α-L-ribulofuranose, α-L-rhamnopyranose α-L-threofuranose, α-L-erythrofuranose, α-L-glucosamine, α-L-glucopyranuronic acid, β-L-ribopyranose, β-L-arabinopyranose, β-L-xylopyranose, β-L-lyxopyranose, β-L-allopyranose, β-L-altropyranose, β-L-glucopyranose, β-L-mannpyranose, β-L-glucopyranose, β-L-idopyranose, β-L-galactopyranose, β-L-talopyranose, β-L-psicopyranose, β-L-fructopyranose, β-L-sorbopyranose, β-L-tagatopyranose, β-L-ribofuranose, β-L-arabinofuranose, β-L-xylofuranose, β-L-lyxofuranose, β-L-allofuranose, β-L-altrofuranose, β-L-glucofuranose, β-L-mannofuranose, β-L-gulofuranose, β-L-idofuranose, β-L-galactofuranose, β-L-talofuranose, β-L-psicofuranose, β-L-fructofuranose, β-L-sorbofuranose, β-L-tagatofuranose, β-L-xylulofuranose, β-L-ribulofuranose, β-L-threofuranose, β-L-erythrofuranose, β-L-glucosamine, β-L-glucopyranuronic acid, and β-L-rhamnopyranose.

In another preferred embodiment of the invention, the monosaccharide, the disaccharide or the trisaccharide **CH** consists of one, two or respectively 3 carbohydrates selected from the α- and β-D/L-carbohydrates as mentioned on pages **25 – 29** and as defined for the **A**-moiety.

The monosaccharide, the disaccharide or the trisaccharide **CH** according to the present invention may further be substituted at specific positions, preferably at hydroxyl groups not involved in the bonding to the moieties A and L, or at an amino group if present in the saccharide moiety. In a preferred embodiment of the present invention the monosaccharide, the disaccharide or the trisaccharide **CH** bear one of the following substituents, preferably instead of a hydrogen atom at a hydroxyl groups one of the following substituents:

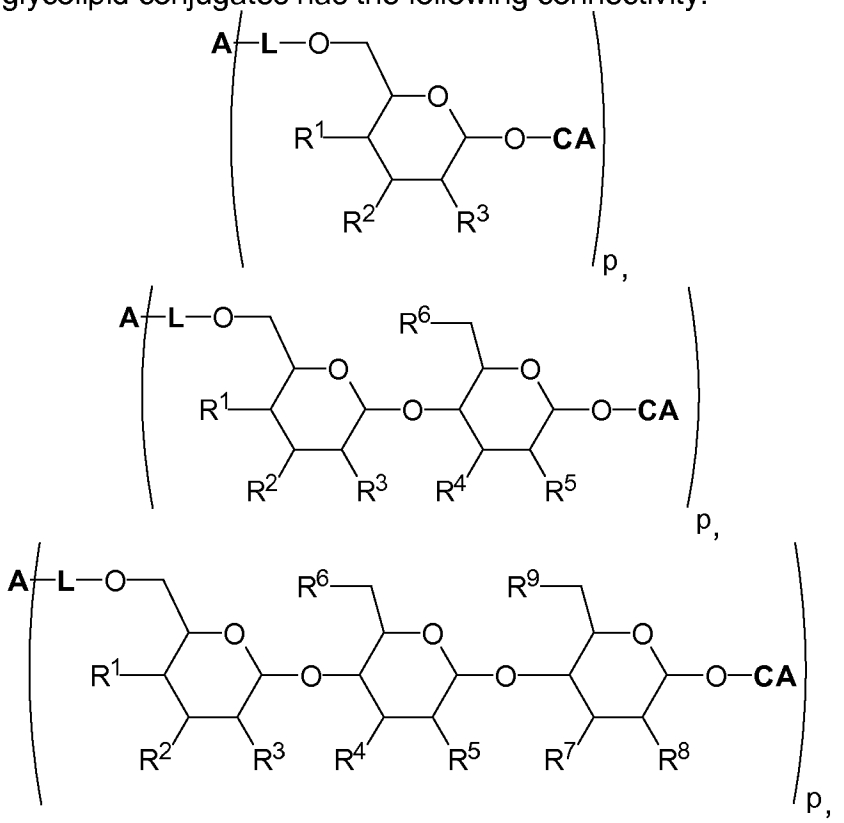
$-CH_3$, $-C_2H_5$, $-C_3H_7$, –cyclo-C_3H_5, $-CH(CH_3)_2$, $-C(CH_3)_3$, $-C_4H_9$, –Ph, $-CH_2$–Ph, $-CH_2-OCH_3$, $-C_2H_4-OCH_3$, $-C_3H_6-OCH_3$, $-CH_2-OC_2H_5$, $-C_2H_4-OC_2H_5$, $-C_3H_6-OC_2H_5$, $-CH_2-OC_3H_7$, $-C_2H_4-OC_3H_7$, $-C_3H_6-OC_3H_7$, $-CH_2$–O–cyclo-C_3H_5, $-C_2H_4$–O–cyclo-C_3H_5, $-C_3H_6$–O–cyclo-C_3H_5, $-CH_2-OCH(CH_3)_2$, $-C_2H_4-OCH(CH_3)_2$, $-C_3H_6-OCH(CH_3)_2$, $-CH_2-OC(CH_3)_3$, $-C_2H_4-OC(CH_3)_3$, $-C_3H_6-OC(CH_3)_3$, $-CH_2-OC_4H_9$, $-C_2H_4-OC_4H_9$, $-C_3H_6-OC_4H_9$, $-CH_2$–OPh, $-C_2H_4$–OPh, $-C_3H_6$–OPh, $-CH_2-OCH_2$–Ph, $-C_2H_4-OCH_2$–Ph, $-C_3H_6-OCH_2$–Ph.

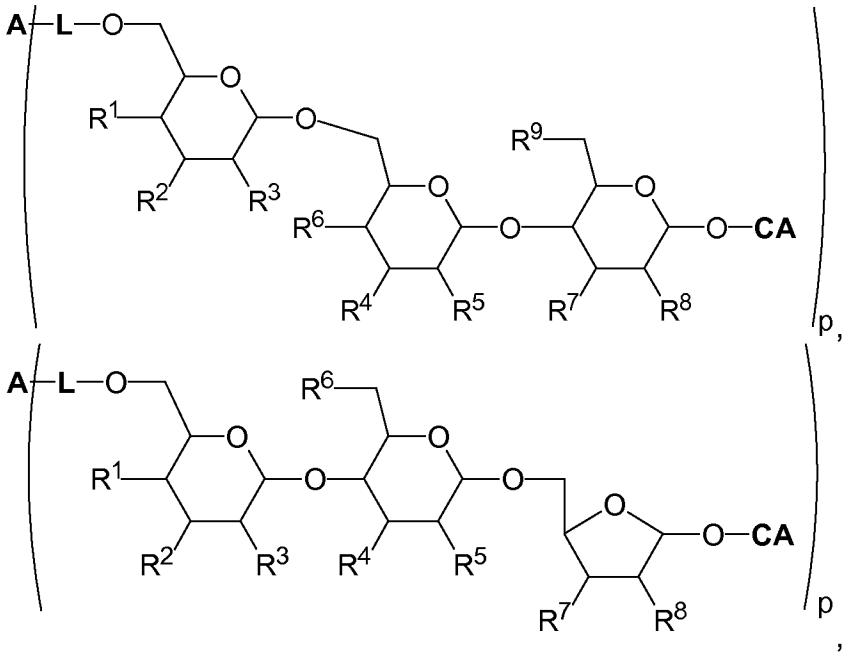
Preferred α- and β-D/L-carbohydrates for the moiety CH with indicated connectivity by the dottet lines are the following residues:



The substituents Q^1, Q^2, Q^3 and Q^6 have the meanings as defined herein.

In other preferred embodiments of the invention the **CH** moiety of the inventive carbohydrate-glycolipid conjugates has the following connectivity:

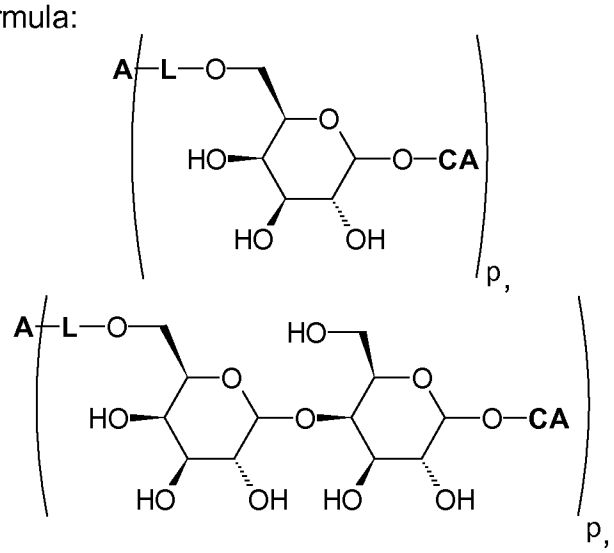


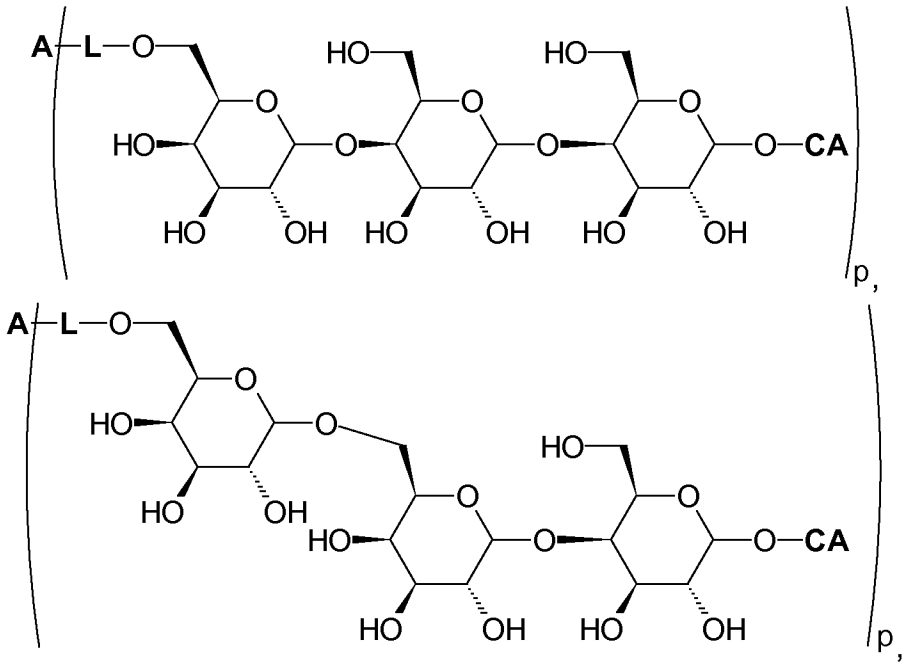


wherein the **A**, **L**, **p** and **CA** are defined as disclosed herein.

R^1, **R**^{2}, **R**^{3}, **R**^{4}, **R**^{5}, **R**^{6}, **R**^{7}, **R**^{8}, **R**^{9} represent independently of each other: –H, –OH, –OCH_3, –OC_2H_5, –OC_3H_7, –O–SO_2–CH_3, –O–SO_2–C_2H_5, –O–SO_2–C_3H_7, –O–$COOCH_3$, –$NHCOCH_3$, or –NH_2.

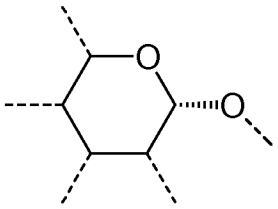
In more preferred embodiments of the invention the **CH** moiety of the inventive carbohydrate-glycolipid conjugates has the following connectivity as shown in the following preferred formula:



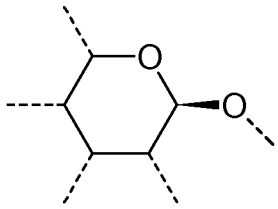


wherein the **A**, **L**, **p** and **CA** are defined as disclosed herein.

The glycosidic bonds within **CH** belong preferably to the group of glycosidic bonds wherein the hydroxyl function of the anomeric carbon is condensed with another hydroxyl fuction of another carbohydrate or of the **CA** moiety respectively. The glycosidic bond between two carbohydrates comprises the glycosidic bond between the anomeric carbon of one carbohydrate and the non-anomeric carbon of the other carbohydrate. Due to the stereochemistry of the anomeric carbon there is the possibility to form α or β-glycosidic bonds such as:



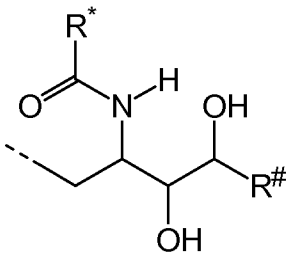
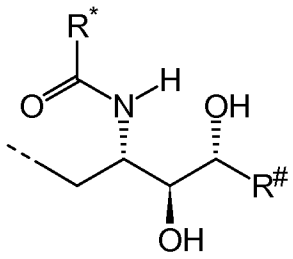
α-glycosidic bond

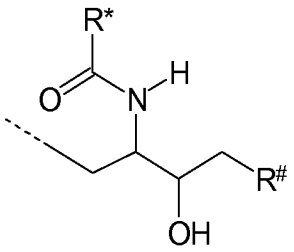
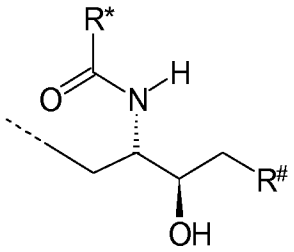


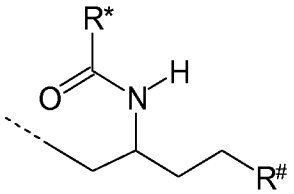
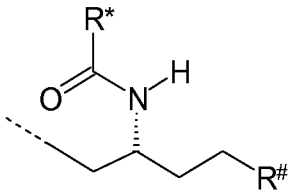
β-glycosidic bond

The Greek letters α and β are applicable only when the anomeric carbon atom has a lower locant than the anomeric reference atom. If this is not the case then the anomeric configuration is described by normal R/S-symbols.

The ceramide moiety CA

CA represents  and more preferably 

or
CA represents  and more preferably 

or
CA represents  and more preferably 

R* and **R#** represent independently of each other a linear or branched or cyclic, substituted or unsubstituted, saturated or unsaturated carbon residue consisting of 1 to 30 carbon atoms and up to 5 hetero atoms selected from N, O, S, F, Br and Cl.

Thus, **R*** and **R#** represent independently of each other a carbon residue of 1 – 30 carbon atoms, wherein the carbon residue may be a linear carbon chain or a branched carbon chain. The carbon residue may also contain carbocyclic structures or heterocyclic structures. The carbon residue may furthermore contain heteroatoms such as N, O, S and/or may have functional groups such as halogen like F, Cl and Br or functional groups containing the hetero atoms N, O, and/or S or functional groups such as double bonds and triple bonds.

The carbon residue or the carbon chain may contain one or more C=C double bonds and/or one or more C≡C triple bonds. The carbocyclic structures which might be present in the carbon residue or the carbon chain are, for instance, saturated 3-membered or 4-membered carbocyclic rings, saturated or unsaturated 5-membered carbocyclic rings or saturated, unsaturated or aromatic 6-membered carbocyclic rings which can be present as substituents on the carbon residue or carbon chain or can be incorporated into the carbon residue or carbon chain.

The heterocyclic structures which might be present in the carbon residue or the carbon chain are, for instance, saturated 3-membered or 4-membered heterocyclic

rings containing one N or O atom, saturated or unsaturated 5-membered heterocyclic rings containing 1, 2, 3 or 4 N atoms or 1 or 2 S or O atoms or 1 O or S atom together with 1 or 2 N atoms or saturated, unsaturated or aromatic 6-membered heterocyclic rings containing 1, 2, 3 or 4 N atoms or 1 or 2 S or O atoms or 1 O or S atom together with 1 or 2 N atoms which can be present as substituents on the carbon residue or carbon chain or can be incorporated into the carbon residue or carbon chain.

The term "carbon residue of 1 to 30 carbon atoms" refers to one carbon atom or a chain of 2 to 30 carbon atoms which can be straight aligned (linear) by a suitable chemical bond or arranged in such an order that from 1 carbon atom two or three individual carbon atoms are bound (branched), and optionally proceed in different directions from the branching carbon atom. Further, the arrangement of the carbon atoms may also form a ring shape (cyclic). Also, any of the above mentioned arrangements of carbon atoms forming a carbon residue may include one or more double or triple bonds (unsaturated). In case the chain of carbon atoms does not include any double or triple bond the carbon residue is considered saturated. Optionally the "carbon residue of 1 to 30 carbon atoms" can be further substituted with 1 to 5 of the substituents Z^1, Z^2, Z^3, Z^4, Z^5. However it is clear to a skilled person that the term "can be substituted" refers to the replacement of a hydrogen atom by one of the substituents Z^1, Z^2, Z^3, Z^4, Z^5 each. In case the carbon residue of 1 to 30 carbon atoms does not contain any of the additional substituents Z^1, Z^2, Z^3, Z^4, Z^5 the residue is considered as unsubstituted.

More preferably **R*** and **R#** represent independently of each other linear or branched C_1–C_{30}–alkyl residue, a linear or branched C_2–C_{30}–alkenyl residue, a linear or branched C_2–C_{30}–alkynyl residue, a C_3–C_{10}–carbocycloalkyl residue, a C_4–C_{30}–alkylcycloalkyl, a C_4–C_{30}–alkylheterocycloalkyl residue, or a substituted C_1–C_{30}–carbon residue containing 1 to 5 of the substituents Z^1, Z^2, Z^3, Z^4, Z^5.

The substituents Z^1, Z^2, Z^3, Z^4, and Z^5 represent independently of each other –OH, –OCH_3, –OC_2H_5, –OC_3H_7, –O–cyclo-C_3H_5, –$OCH(CH_3)_2$, –$OC(CH_3)_3$, –OC_4H_9, –OPh, –OCH_2–Ph, –$OCPh_3$, –CH_2–OCH_3, –C_2H_4–OCH_3, –C_3H_6–OCH_3, –CH_2–OC_2H_5, –C_2H_4–OC_2H_5, –C_3H_6–OC_2H_5, –CH_2–OC_3H_7, –C_2H_4–OC_3H_7, –C_3H_6–OC_3H_7, –CH_2–O–cyclo-C_3H_5, –C_2H_4–O–cyclo-C_3H_5, –C_3H_6–O–cyclo-C_3H_5, –CH_2–$OCH(CH_3)_2$, –C_2H_4–$OCH(CH_3)_2$, –C_3H_6–$OCH(CH_3)_2$, –CH_2–$OC(CH_3)_3$, –C_2H_4–$OC(CH_3)_3$, –C_3H_6–$OC(CH_3)_3$, –CH_2–OC_4H_9, –C_2H_4–OC_4H_9, –C_3H_6–OC_4H_9, –CH_2–OPh, –C_2H_4–OPh, –C_3H_6–OPh, –CH_2–OCH_2–Ph, –C_2H_4–OCH_2–Ph,

–C_3H_6–OCH_2–Ph, –NO_2, –F, –Cl, –Br, –$COCH_3$, –COC_2H_5, –COC_3H_7, –CO–cyclo-C_3H_5, –$COCH(CH_3)_2$, –$COC(CH_3)_3$, –COOH, –$COOCH_3$, –$COOC_2H_5$, –$COOC_3H_7$, –COO–cyclo-C_3H_5, –$COOCH(CH_3)_2$, –$COOC(CH_3)_3$, –OOC–CH_3, –OOC–C_2H_5, –OOC–C_3H_7, –OOC–cyclo-C_3H_5, –OOC–$CH(CH_3)_2$, –OOC–$C(CH_3)_3$, –$CONH_2$, –$CONHCH_3$, –$CONHC_2H_5$, –$CONHC_3H_7$, –CONH–cyclo-C_3H_5, –$CONH[CH(CH_3)_2]$, –$CONH[C(CH_3)_3]$, –$CON(CH_3)_2$, –$CON(C_2H_5)_2$, –$CON(C_3H_7)_2$, –CON(cyclo-$C_3H_5)_2$, –$CON[CH(CH_3)_2]_2$, –$CON[C(CH_3)_3]_2$, –$NHCOCH_3$, –$NHCOC_2H_5$, –$NHCOC_3H_7$, –NHCO–cyclo-C_3H_5, –$NHCO–CH(CH_3)_2$, –$NHCO–C(CH_3)_3$, –NH_2, –$NHCH_3$, –NHC_2H_5, –NHC_3H_7, –NH–cyclo-C_3H_5, –$NHCH(CH_3)_2$, –$NHC(CH_3)_3$, –$N(CH_3)_2$, –$N(C_2H_5)_2$, –$N(C_3H_7)_2$, –N(cyclo-$C_3H_5)_2$, –$N[CH(CH_3)_2]_2$, –$N[C(CH_3)_3]_2$, –OCF_3, –CH_2–OCF_3, –C_2H_4–OCF_3, –C_3H_6–OCF_3, –OC_2F_5, –CH_2–OC_2F_5, –C_2H_4–OC_2F_5, –C_3H_6–OC_2F_5, –CH_2F, –CHF_2, –CF_3, –CH_2Cl, –CH_2Br, –CH_2–CH_2F, –CH_2–CHF_2, –CH_2–CF_3, –CH_2–CH_2Cl, –CH_2–CH_2Br.

The term “linear or branched C_1–C_{30}–alkyl residue” refers to a residue which is linked through a carbon atom and which consists in total of 1 to 30 carbon atoms including the carbon atoms of the branches. The same definition applies accordingly to the terms “linear C_{20}–C_{30}–alkyl residue”, “linear C_1–C_{10}–alkyl residue” and “linear C_{10}–C_{19}–alkyl residue”,

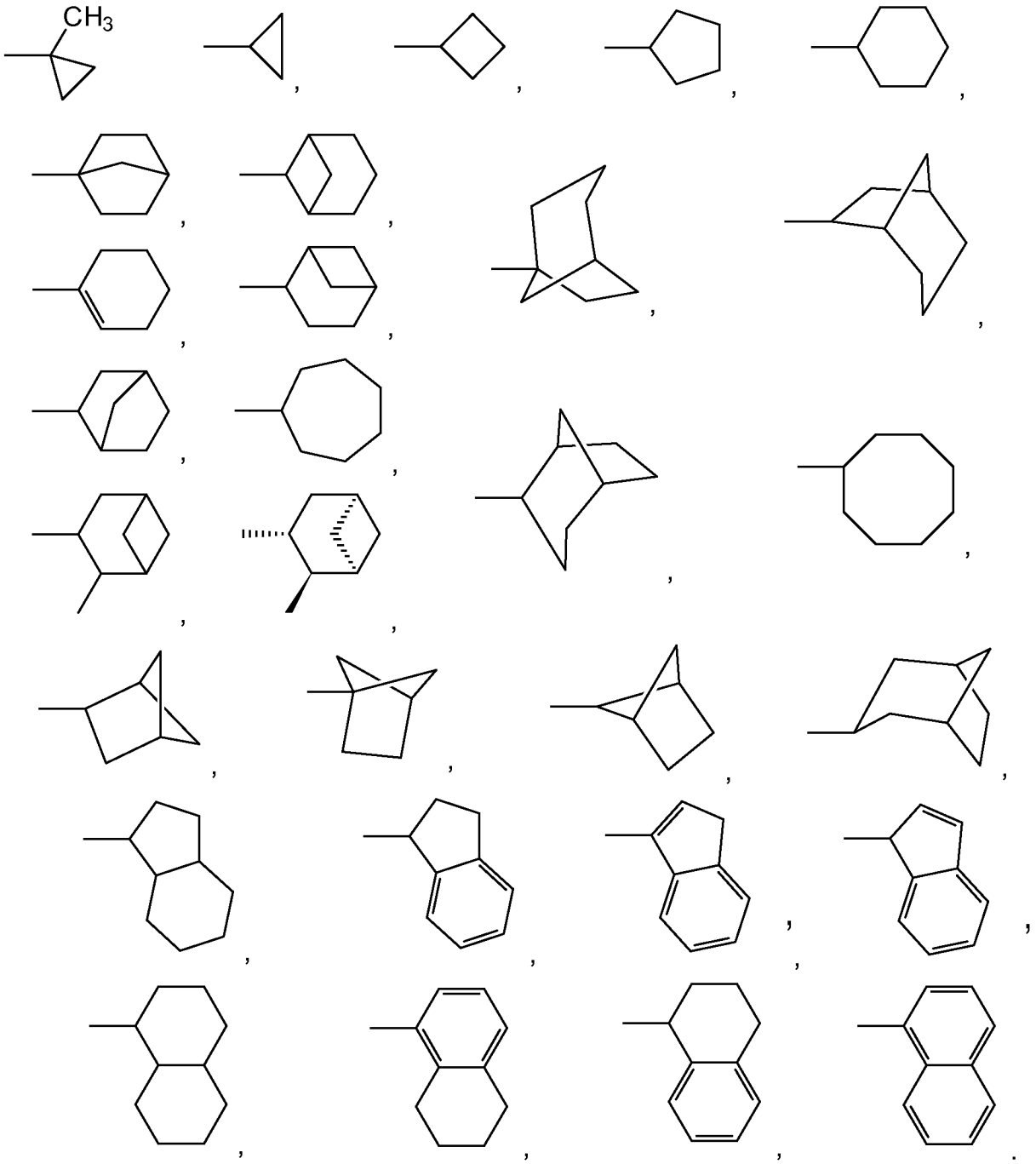
The term “linear or branched C_2–C_{30}–alkenyl residue” refers to a residue which is linked through a carbon atom and which consists in total of 2 to 30 carbon atoms including the carbon atoms of the branches and which has at least one but not more than 15 double bonds. If branched, the longest carbon chain is the main chain while the side chains are the branches. The 1 to 15 C=C double bonds may be present in the main chain and/or the side chain(s).

The term “linear or branched C_2–C_{30}–alkynyl residue” refers to a residue which is linked through a carbon atom and which consists in total of 2 to 30 carbon atoms including the carbon atoms of the branches and which has at least one but not more than 15 triple bonds and preferably 1, 2 or 3 triple bonds. If branched, the longest carbon chain is the main chain while the side chains are the branches. The 1 to 15 C≡C triple bonds may be present in the main chain and/or the side chain(s).

The term “C_3–C_{10}–carbocycloalkyl residue” refers to a residue which is linked through a ring carbon atom and contains at least one carbocyclic ring and which consists in total of 3 to 10 carbon atoms including the carbon atoms of any alkyl, alkenyl or alkinyl substituent. The carbocyclic ring in the C_3–C_{10}–carbocycloalkyl residue can

be saturated, partly unsaturated or fully unsaturated and might be aromatic. If the carbocyclic ring is part of a bicyclic ring or is connected to another ring, both carbocyclic rings may be saturated or unsaturated and might be aromatic or one ring is saturated and the second ring is partly or fully unsatured.

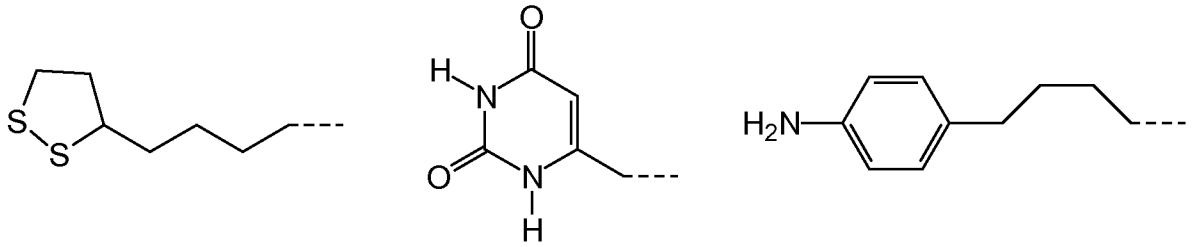
Examples for preferred C_3–C_{10}–carbocycloalkyl residues to which it is also referred to as substituents M^1 are as follows:



The term "C_4–C_{30}–alkylcycloalkyl" refers to a residue which is linked through a carbon atom not part of the carbocyclic ring and contains at least one carbocyclic ring

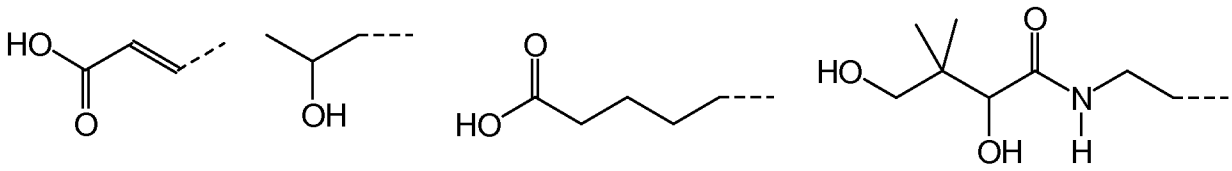
and which consists in total of 4 to 30 carbon atoms including the carbon atoms of any alkyl, alkenyl or alkinyl substituent. The carbocyclic ring in the C_4–C_{30}–carbocycloalkyl residue can be saturated, partly unsaturated or fully unsaturated and might be aromatic. If the carbocyclic ring is part of a bicyclic ring or is connected to another ring, both carbocyclic rings may be saturated or unsaturated and might be aromatic or one ring is saturated and the second ring is partly or fully unsatured.

The term "C_4–C_{30}–alkylheterocycloalkyl residue" refers to a residue which is linked through a carbon atom not part of the heterocyclic ring and contains at least one heterocyclic ring and which consists in total of 4 to 30 carbon atoms including the carbon atoms of any alkyl, alkenyl or alkinyl substituent. The heterocyclic ring in the C_4–C_{30}–alkylheterocycloalkyl residue can be saturated, partly unsaturated or fully unsaturated and might be aromatic. 1 or 2 oxigen atoms can be attached to the heterocyclic ring thus forming one or two carbonyl groups. If the heterocyclic ring is part of a bicyclic ring or is connected to another ring which can be a carbocyclic or heterocyclic ring, both rings may be saturated or unsaturated and might be aromatic or one ring is saturated and the second ring is partly or fully unsatured and might be aromatic. The heterocyclic ring contains 1 or 2 O atoms, 1 or 2 S atoms, 1, 2, 3, or 4 N atoms, 1 O and 1 or 2 N atoms or 1 S and 1 or 2 N atoms. Examples for such C_4–C_{30}–alkylheterocycloalkyl residues are:



The term "substituted C_1–C_{30}–carbon residue containing 1 to 5 of the substituents Z^1, Z^2, Z^3, Z^4, Z^5" refers to a residue which is linked through a carbon atom and which consists in total of 1 to 30 carbon atoms including the carbon atoms of any substituent such as alkyl, alkenyl, alkinyl, Z^1, Z^2, Z^3, Z^4, and/or Z^5 substituent. The residue bears 1 to 5 of the substituents Z^1, Z^2, Z^3, Z^4, Z^5 and can be linear or branched and saturated or unsaturated. Thus in addition to the at least one substituent Z^1, the residue may contain one or more C=C double bonds and/or one or more C≡C triple bonds. Moreover the substituted C_1–C_{30}–carbon residue may contain 1 to 10 hetero atoms N, O, S in the carbon chain or attached to the carbon chain. One or more oxygen atoms might be attached to the carbon chain thus forming one or more carbonyl groups. If branched, the longest chain is the main chain while the side chains are the branches. The carbonyl functionalities, the

double bonds, the triple bonds as well as the substituents Z^1, Z^2, Z^3, Z^4, Z^5 can be present in or on the main chain and also in or on the side chain(s).
Examples for such substituted C_1–C_{30}–carbon residue are:



In a preferred embodiment of the invention the residues **R*** and **R#** represent independently of each other:
$-CH_3$, $-(CH_2)_r-CH_3$, $-CH(OH)-(CH_2)_s-CH_3$, $-CH=CH-CH_3$, $-CH=CH-(CH_2)_t-CH_3$, $-CH(OH)-(CH_2)_v-CH(CH_3)_2$, $-CH(OH)-(CH_2)_w-CH(CH_3)-CH_2-CH_3$, $-(CH_2)_a-CH=CH-(CH_2)_b-CH_3$, $-(CH_2)_c-CH=CH-(CH_2)_d-CH=CH-(CH_2)_e-CH_3$, $-(CH_2)_f-CH=CH-(CH_2)_g-CH=CH-(CH_2)_h-CH=CH-(CH_2)_i-CH_3$, $-(CH_2)_j-CH=CH-(CH_2)_k-CH=CH-(CH_2)_l-CH=CH-(CH_2)_o-CH=CH-(CH_2)_qCH_3$, wherein a, b, c, d, e, f, g, h, i, j, k, l, o, q are integers from 1 to 26 with the proviso that: $(a+b) \leq 27$; $(c+d+e) \leq 25$; $(f+g+h+i) \leq 23$; $(j+k+l+o+q) \leq 21$; and wherein r is an integer from 1 to 29, s is an integer from 1 to 28, t is an integer from 1 to 27, v is an integer from 1 to 26, and w is an integer from 1 to 25 and furthermore $-(CH=CH-CH_2)_q-CH_3$, $-(CH_2-CH=CH)_q-CH_3$, $-(CH=CH)_A-CH_3$, wherein q is an integer from 1 to 9, A is an integer from 1 to 14 and furthermore $-(CH=CH-CH_2)_B-(CH_2)_C-CH_3$, $-(CH_2-CH=CH)_B-(CH_2)_C-CH_3$, $-(CH=CH)_D-(CH_2)_E-CH_3$, $-(CH_2)_E-(CH=CH)_D-CH_3$, $-(CH_2)_F-(CH=CH)_G-(CH_2)_H-CH_3$, $-(CH_2)_J-(CH=CH-CH_2)_K-(CH_2)_N-CH_3$, $-(CH_2)_P-(CH=CH)_Q-(CH_2)_R-(CH=CH)_S-(CH_2)_T-CH_3$, $-(CH_2)_U-(CH=CH-CH_2)_V-(CH_2)_W-(CH=CH-CH_2)_X-(CH_2)_Y-CH_Z$, wherein B, C, D, E, F, G, H; I, J, K, L, M, N, P, Q, R, S, T, U, V, W, X, Y and Z represent independently from each other an integer between 1 and 26 with the proviso that the total number of carbon atoms of the afore-mentioned residues does not exceed 30.

In another preferred embodiment of the invention the residues **R*** and **R#** represent independently of each other:
ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, dodecyl, tetradecyl, cis-9-tetradecenyl, cis-9-hexadecenyl, cis-6-octadecenyl, cis-9-octadecenyl, cis-11-octadecenyl, cis-9-eicosenyl, cis-11-eicosenyl, cis-13-docosenyl, cis-15-tetracosenyl, trans-9-octadecenyl, trans-11-octadecenyl, trans-3-hexadecenyl, 9,12-octadecadienyl, 6,9,12-octadecatrienyl, 8,11,14-eicosatrienyl, 5,8,11,14-eicosatetraenyl, 7,10,13,16-docosatetraenyl, 4,7,10,13,16-docosapentaenyl, 9,12,15-

octadecatrienyl, 6,9,12,15-octadecatetraenyl, 8,11,14,17-eicosatetraenyl, 5,8,11,14,17-eicosapentaenyl, 7,10,13,16,19-docosapentaenyl, 4,7,10,13,16,19-docosahexaenyl, 5,8,11-eicosatrienyl, 9c 11t 13t eleostearyl, 8t 10t 12c calendyl, 9c 11t 13c catalpyl, cis-9 tetradecenyl, cis-9-hexadecenyl, cis-6-octadecenyl, cis-9-octadecenyl, cis-11-octadecenyl, cis-9-eicosenyl, cis-11-eicosenyl, cis-13-docosenyl, cis-15-tetracosenyl, 9,12-octadecadienyl, 6,9,12-octadecatrienyl, 8,11,14-eicosatrienyl, 5,8,11,14-eicosatetraenyl, 7,10,13,16-docosatetraenyl, 4,7,10,13,16 docosapentaenyl, 9,12,15-octadecatrienyl, 6,9,12,15-octadecatetraenyl, 8,11,14,17-eicosatetraenyl, 5,8,11,14,17-eicosapentaenyl, 7,10,13,16,19-docosapentacnyl, 4,7,10,13,16,19-docosahexaenyl, 5,8,11-eicosatrienyl, 1,2-dithiolane-3-pentanyl, 6,8-dithiane octanyl, docosaheptadecanyl, eleostearyl, calendyl, catalpyl, taxoleyl, pinolenyl, sciadonyl, retinyl, 14-methyl pentadecanyl, pristanyl, phytanyl, 11,12-methyleneoctadecanyl, 9,10-methylenehexadecanyl, 9,10-epoxystearyl, 9,10-epoxyoctadec-12-enyl, 6-octadecynyl, t11-octadecen-9-ynyl, 9-octadecynyl, 6-octadecen-9-ynyl, t10-heptadecen-8-ynyl, 9-octadecen-12-ynyl, t7,t11-octadecadiene-9-ynyl, t8,t10-octadecadiene-12-ynyl, 5,8,11,14-eicosatetraynyl, 2-hydroxytetracosanyl, 2-hydroxy-15-tetracosenyl, 12-hydroxy-9-octadecenyl or 14-hydroxy-11-eicosenyl, 4,7,9,11,13,16,19-docosaheptadecanyl, 6-octadecynyl, t11-octadecen-9-ynyl, isopalmityl, 9,10-methylenhexadecyl, coronaryl, (*R,S*)-lipoyl, 6,8-bis(methylsulfanyl)-octanyl, 4,6-bis(methylsulfanyl)-hexanyl, 2,4-bis(methylsulfanyl)-butanyl, 1,2-dithiolanyl, cerebronyl, hydroxynervonyl, ricinyl, lesqueryl, brassylyl, thapsyl, dodecyl, hexadecyl, octadecyl, eicosanyl, docosanyl, tetracosanyl, cis-9-tetradecenyl, cis-9-hexadecenyl, cis-6-octadecenyl, cis-9-octadecenyl, cis-11-octadecenyl, cis-9-eicosenyl, cis-11-eicosenyl, cis-13-docosenyl, cis-15-tetracosenyl, 9,12-octadecadienyl, 6,9,12-octadecatrienyl, 8,11,14-eicosatrienyl, 5,8,11,14-eicosatetraenyl, 7,10,13,16-docosatetraenyl, 4,7,10,13,16-docosapentaenyl, 9,12,15-octadecatrienyl, 6,9,12,15-octadecatetraenyl, 8,11,14,17-eicosatetraenyl, 5,8,11,14,17-eicosapentaenyl, 7,10,13,16,19-docosapentaenyl, 4,7,10,13,16,19-docosahexaenyl, 5,8,11-eicosatrienyl, 1,2-dithiolane-3-pentanyl, 6,8-dithiane octanyl, docosaheptadecanyl, eleostearyl, calendyl, catalpyl, taxoleyl, pinolenyl, sciadonyl, retinyl, 14-methyl pentadecanyl, pristanyl, phytanyl, 11,12-methyleneoctadecanyl, 9,10-methylenehexadecanyl, 9,10-epoxystearyl, 9,10-epoxyoctadec-12-enyl, 6-octadecynyl, t11-octadecen-9-ynyl, 9-octadecynyl, 6-octadecen-9-ynyl, t10-heptadecen-8-ynyl, 9-octadecen-12-ynyl, t7,t11-octadecadiene-9-ynyl, t8,t10-octadecadiene-12-ynyl, 5,8,11,14-eicosatetraynyl, 2-hydroxytetracosanyl, 2-hydroxy-15-tetracosenyl, 12-hydroxy-9-octadecenyl, and 14-hydroxy-11-eicosenyl.

In another preferred embodiment of the invention the residues **R*** and **R#** are independently of each other substituted with a phenyl ring, preferably an unsubstituted phenyl ring. Further, it is preferred that said phenyl ring is positioned at the residues **R*** and **R#** at the opposite end where the residues **R*** and **R#** are bond to the rest of the moiety **CA**.

Also, in a preferred embodiment of the present invention the residues **R*** and **R#** are the same, preferably a linear alkyl residue, and more preferably a linear C_{10}–C_{30}–alkyl residue, and most preferably a linear $–C_{14}H_{29}$.

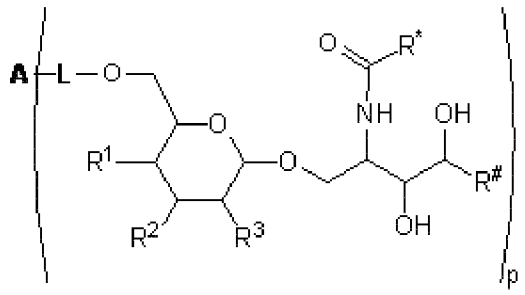
In another preferred embodiment of the present invention the residues **R*** and **R#** are different from each other and represent different linear alkyl residues, preferably the residue **R*** represents a linear C_{20}–C_{30}–alkyl residue and the residue **R#** represents a linear C_{10}–C_{19}–alkyl residue, and more preferably **R*** represents a linear $–C_{25}H_{51}$ residue and the residue **R#** represents a linear $–C_{14}H_{29}$ residue.

In another preferred embodiment of the present invention the residues **R*** and **R#** are different from each other and represent different linear alkyl residues, preferably the residue **R*** represents a linear C_1–C_{10}–alkyl residue and the residue **R#** represents a linear C_{10}–C_{19}–alkyl residue, and more preferably **R*** represents a linear $–C_4H_9$ residue and the residue **R#** represents a linear $–C_{14}H_{29}$ residue.

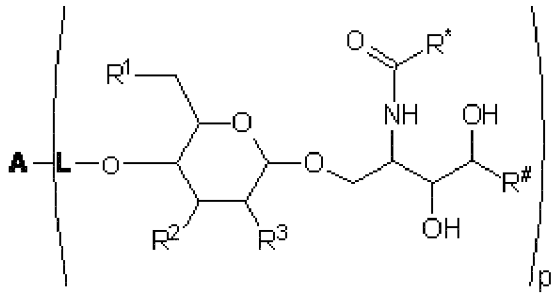
Yet, in another preferred embodiment of the present invention the residues **R*** and **R#** are different from each other and represent different linear alkyl residues, wherein the residues **R*** is further substituted with a phenyl ring, preferably the residue **R*** represents a phenyl-substituted linear C_1–C_{10}–alkyl residue and the residue **R#** represents a linear C_{10}–C_{19}–alkyl residue, and more preferably **R*** represents a linear $–C_6H_{12}$-Ph residue and the residue **R#** represents a linear $–C_{14}H_{29}$ residue.

In another preferred embodiment of the present invention the residues **R*** and **R#** are different from each other and represent different linear alkyl residues, preferably the residue **R*** represents a linear C_{20}–C_{30}–alkyl residue and the residue **R#** represents a linear C_1–C_{10}–alkyl residue, and more preferably **R*** represents a linear $–C_{25}H_{51}$ residue and the residue **R#** represents a linear $–C_5H_{11}$ residue.

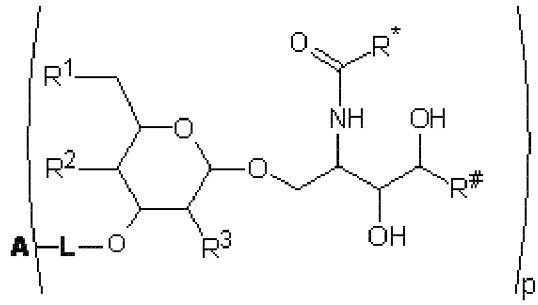
The following formulas (II, III, IV and V) of the general formula (I) are preferred:



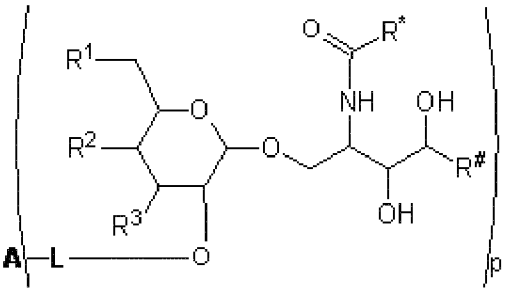
(II)



(III)



(IV)



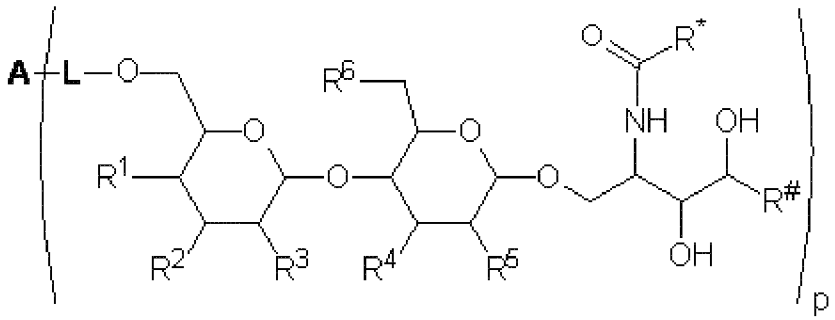
(V)

wherein

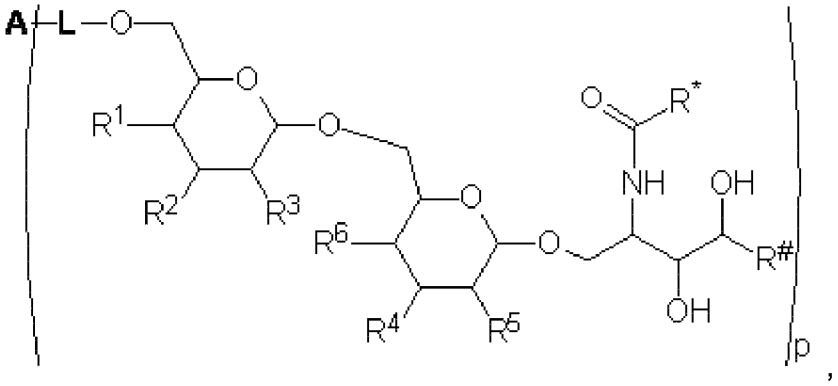
A, **L**, **R***, **R#** and **p** have the meanings as defined herein.

R1, **R2**, **R3** represent independently of each other:
–H, –OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-O-SO_2-CH_3$, $-O-SO_2-C_2H_5$, $-O-SO_2-C_3H_7$, $-O-COOCH_3$, $-NHCOCH_3$, $-NH_2$,

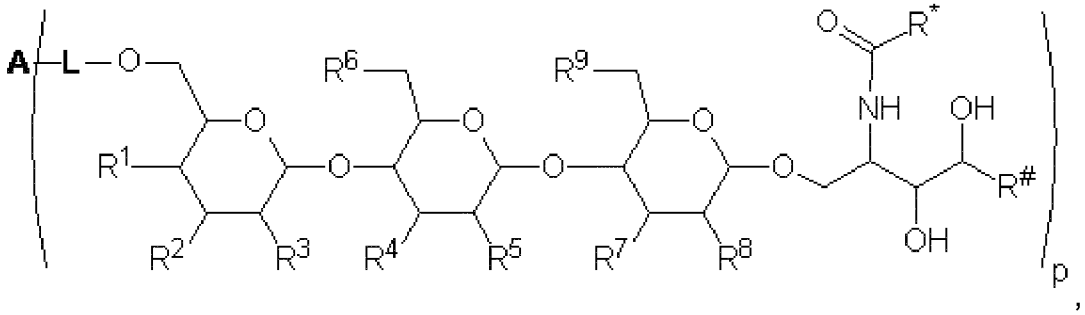
Furthermore, the following formulas (VI, VII, VIII, IX) of the general formula (I) are preferred:



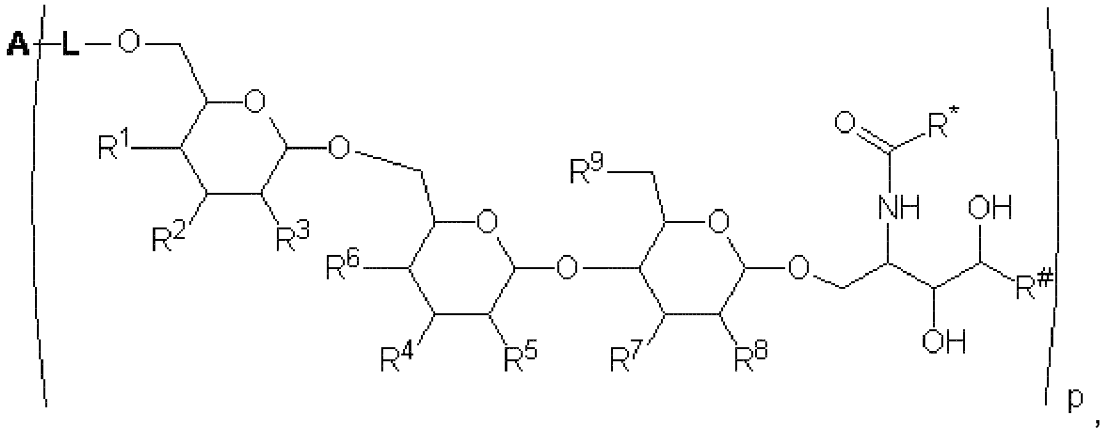
(VI)



(VII)



(VIII)



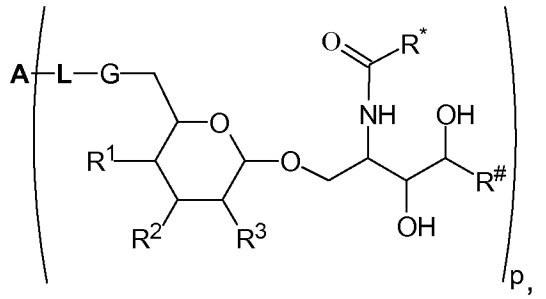
(IX)

wherein

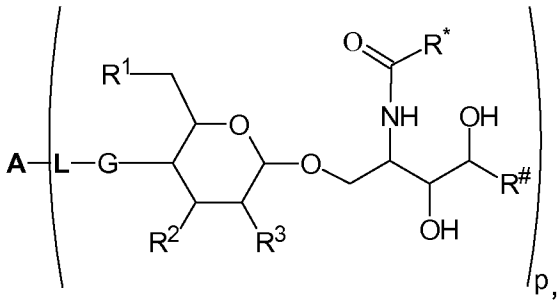
A, **L**, **R*** , **R#** and **p** have the meanings as defined herein.

R1, **R2**, **R3**, **R4**, **R5**, **R6**, **R7**, **R8**, **R9** represent independently of each other:
–H, –OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-O-SO_2-CH_3$, $-O-SO_2-C_2H_5$, $-O-SO_2-C_3H_7$, $-O-COOCH_3$, $-NHCOCH_3$, $-NH_2$,

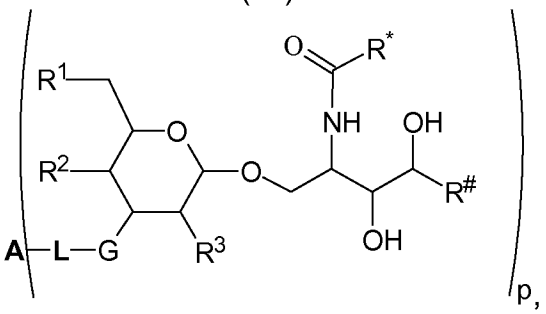
In a specifically preferred embodiment of the present invention the following subformulas (IIb, IIIb, IVb and Vb) of the general formula (I) are preferred:



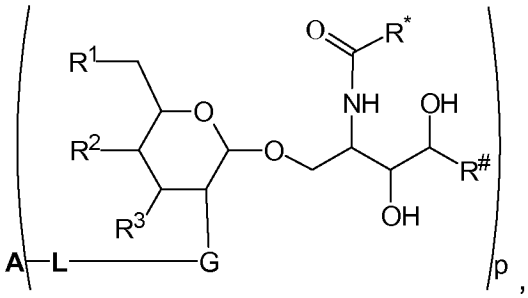
(IIb)



(IIIb)



(IVb)



(Vb)

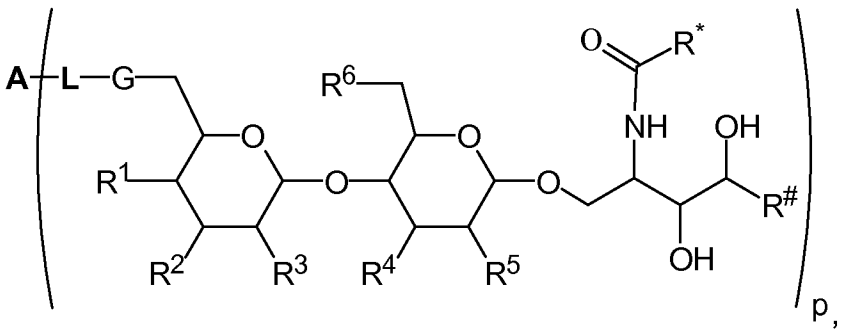
wherein

A, **L**, **R***, **R#** and **p** have the meanings as defined herein.

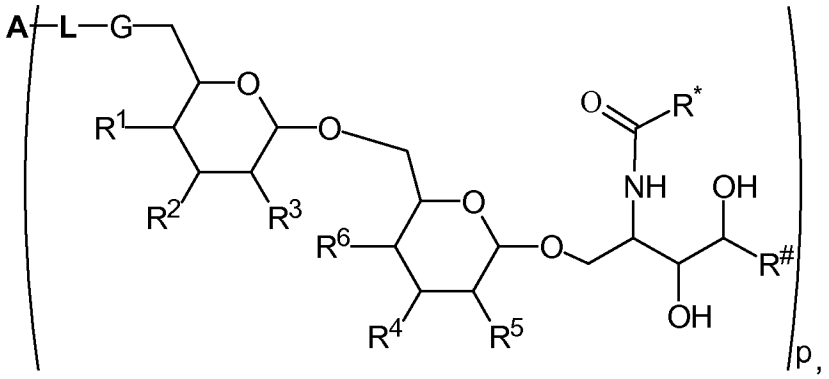
R1, **R2**, **R3** represent independently of each other:
–H, –OH, –OCH_3, –OC_2H_5, –OC_3H_7, –O–SO_2–CH_3, –O–SO_2–C_2H_5, –O–SO_2–C_3H_7, –O–$COOCH_3$, –$NHCOCH_3$, –NH_2,

G represents –NH–, –O–, –S–,

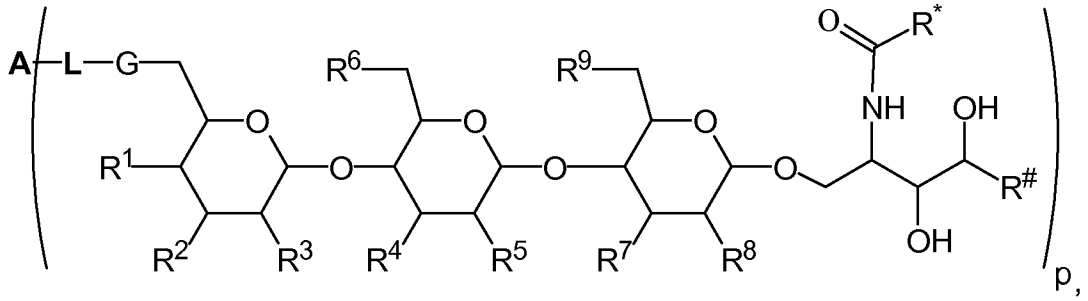
Furthermore, the following subformulas (VIb, VIIb, VIIIb, IXb) of the general formula (I) are preferred:



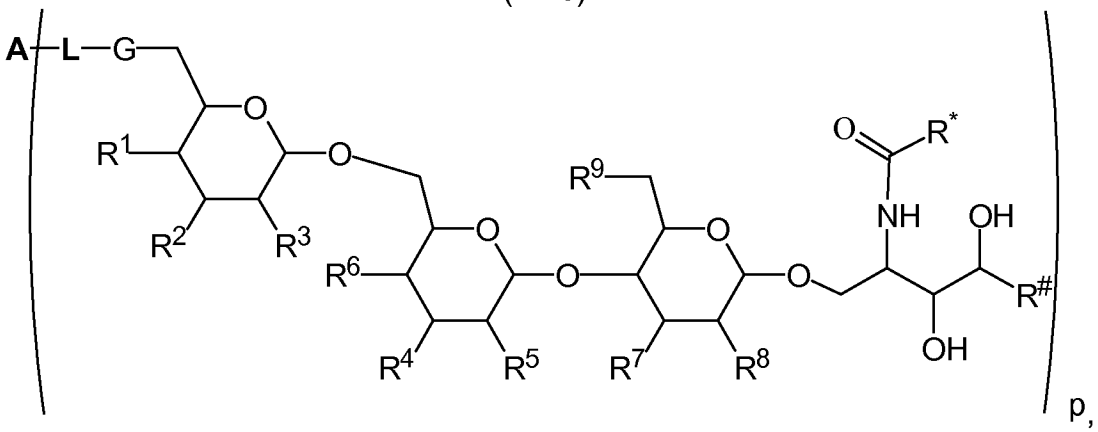
(VIb)



(VIIb)



(VIIIb)



(IXb)

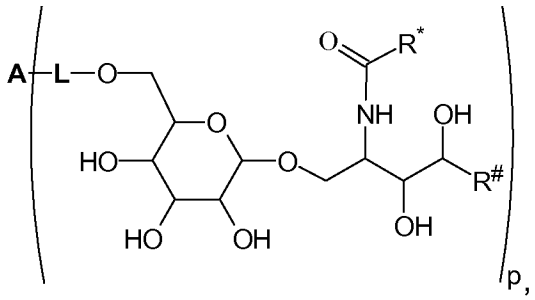
wherein

A, **L**, **R*** , **R#** and **p** have the meanings as defined herein.

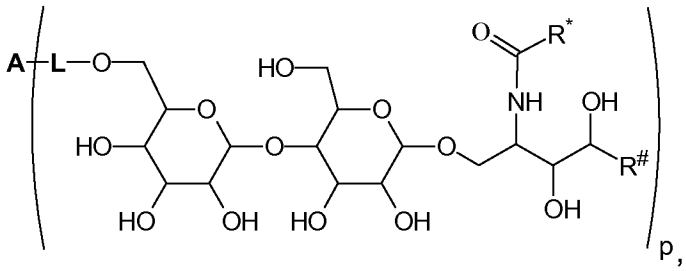
R1, **R2**, **R3**, **R4**, **R5**, **R6**, **R7**, **R8**, **R9** represent independently of each other: –H, –OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-O-SO_2-CH_3$, $-O-SO_2-C_2H_5$, $-O-SO_2-C_3H_7$, $-O-COOCH_3$, $-NHCOCH_3$, $-NH_2$,

G represents –NH–, –O–, –S–,

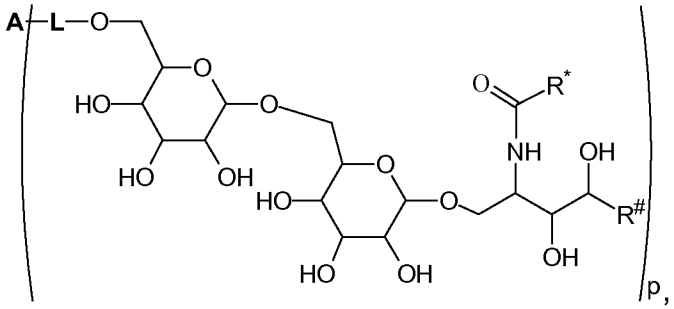
Furthermore the following substructures (X, XI, XII, XIII) of the general structure (I) are preferred:



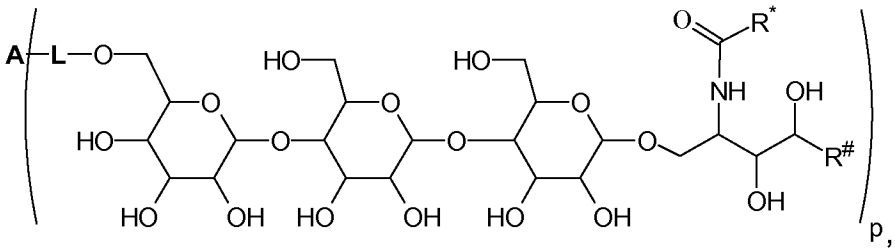
(X)



(XI)



(XII)

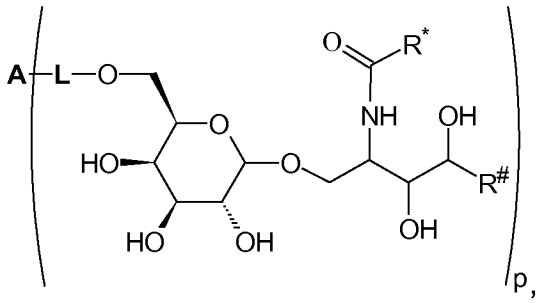


(XIII)

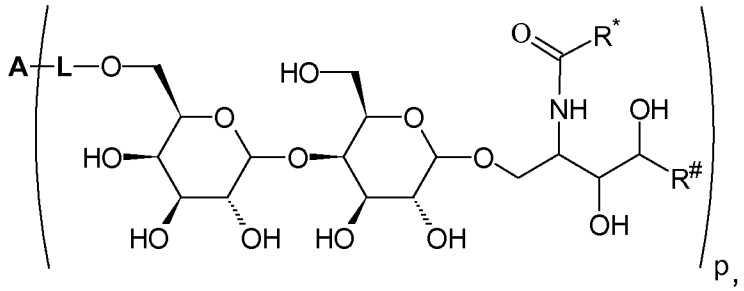
wherein

A, **L**, **R***, **R#** and **p** have the meanings as defined herein.

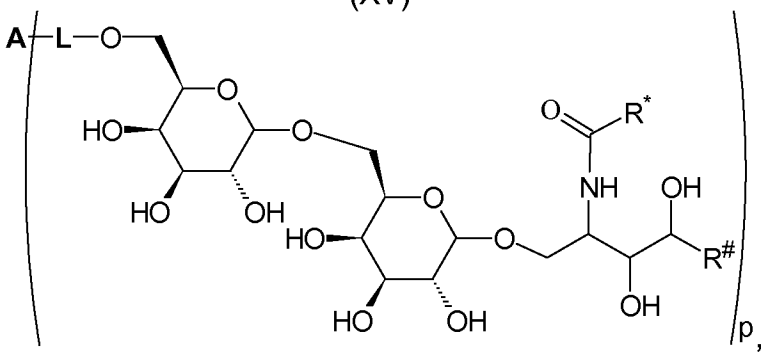
Furthermore the following substructures (XIV, XV, XVI, XVII) of the general structure (I) are preferred:



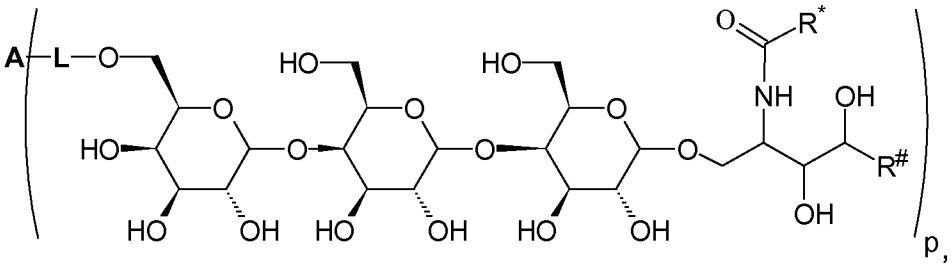
(XIV)



(XV)



(XVI)

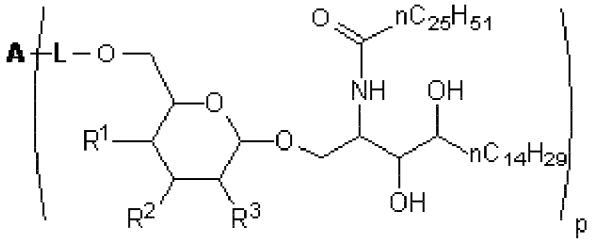


(XVII)

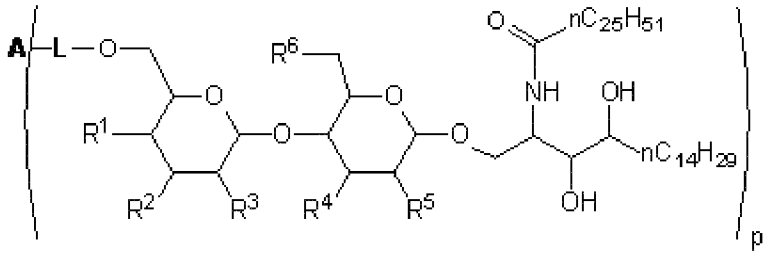
wherein

A, **L**, **R***, **R**$^{\#}$ and **p** have the meanings as defined herein.

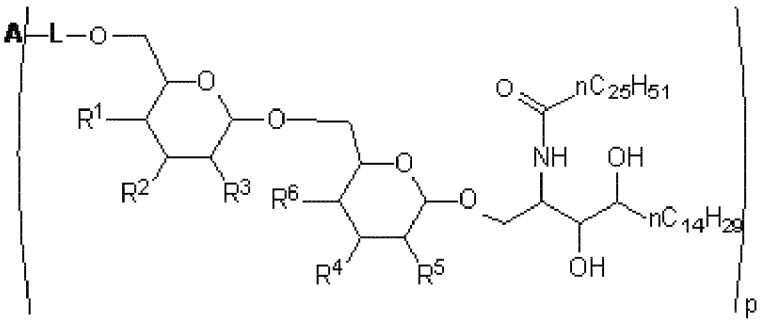
Furthermore the following substructures (XVIII, XIX, XX) of the general structure (I) are preferred:



(XVIII)



(XIX)



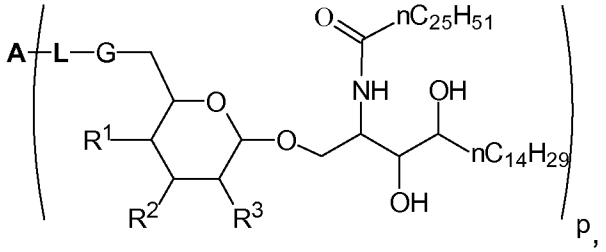
(XX)

wherein

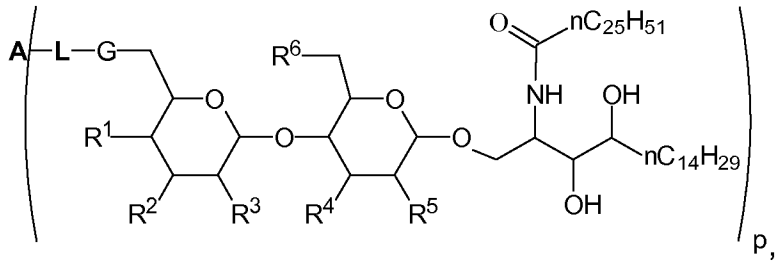
A, **L** and **p** have the meanings as defined herein.

R^1, **R^2**, **R^3**, **R^4**, **R^5**, **R^6**, **R^7**, **R^8**, **R^9** represent independently of each other: –H, –OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, $-O-SO_2-CH_3$, $-O-SO_2-C_2H_5$, $-O-SO_2-C_3H_7$, $-O-COOCH_3$, $-NHCOCH_3$, $-NH_2$,

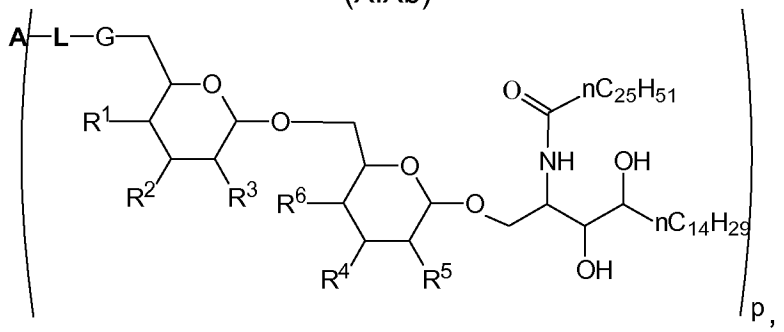
In a specifically preferred embodiment of the present invention the following substructures (XVIIIb, XIXb, XXb) of the general structure (I) are preferred:



(XVIIIb)



(XIXb)



(XXb)

wherein

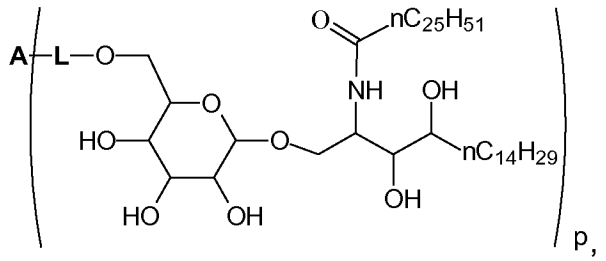
A, **L** and **p** have the meanings as defined herein.

R^1, **R^2**, **R^3**, **R^4**, **R^5**, **R^6**, **R^7**, **R^8**, **R^9** represent independently of each other:

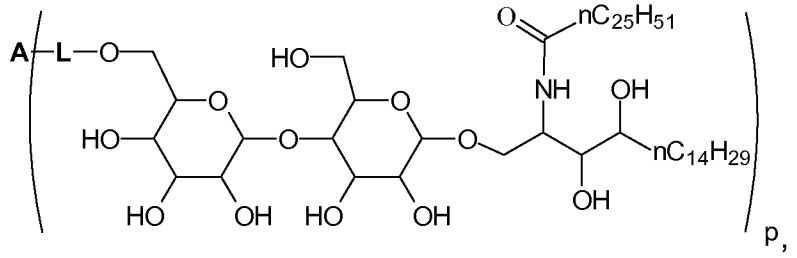
–H, –OH, $–OCH_3$, $–OC_2H_5$, $–OC_3H_7$, $–O–SO_2–CH_3$, $–O–SO_2–C_2H_5$, $–O–SO_2–C_3H_7$, $–O–COOCH_3$, $–NHCOCH_3$, $–NH_2$,

G represents –NH–, –O–, –S–,

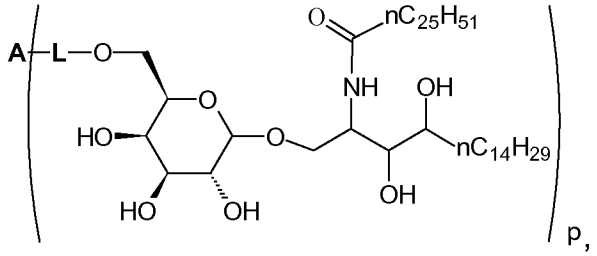
Furthermore the following substructures (XXI, XXII, XXIII, XXIV) of the general structure (I) are preferred:



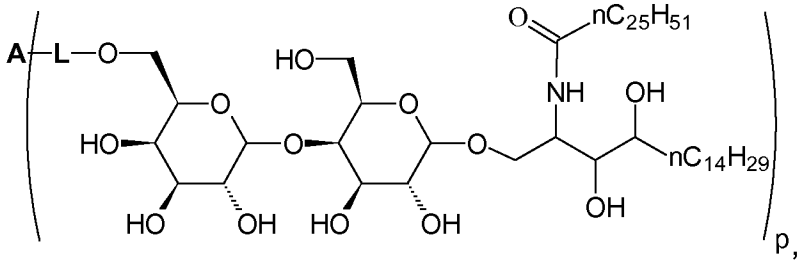
(XXI)



(XXII)



(XXIII)

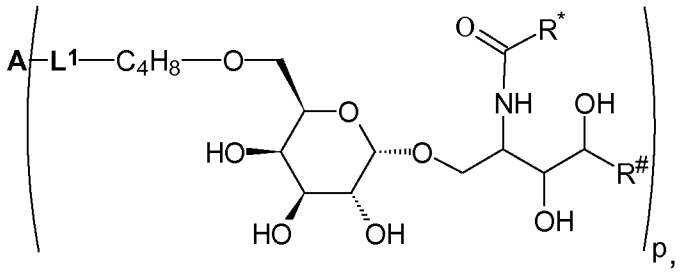


(XXIV)

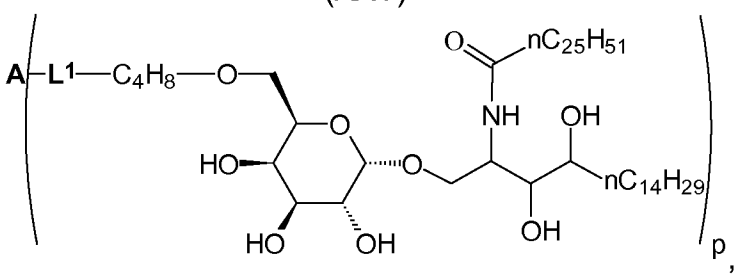
wherein

A, **L** and **p** have the meanings as defined herein.

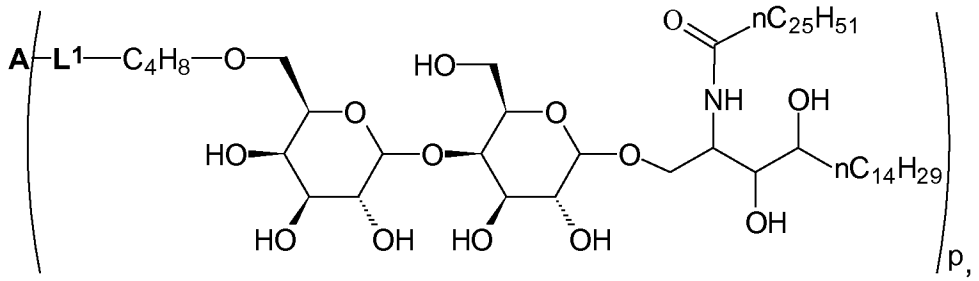
Especially preferred are compounds of the subformulas (XXV), (XXVI) and (XXVII) of the general formula (I):



(XXV)



(XXVI)



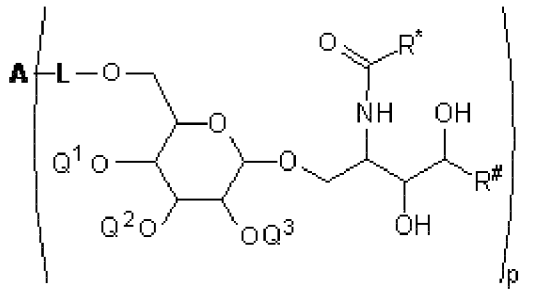
(XXVII)

wherein

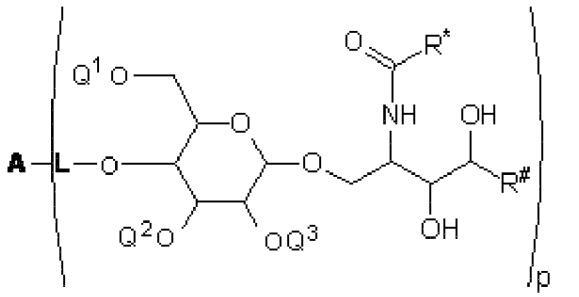
A, **L**[1] and **p** have the meanings as defined herein.

Yet in another preferred embodiment of the present invention the compounds of the present invention refer to the following subformulas

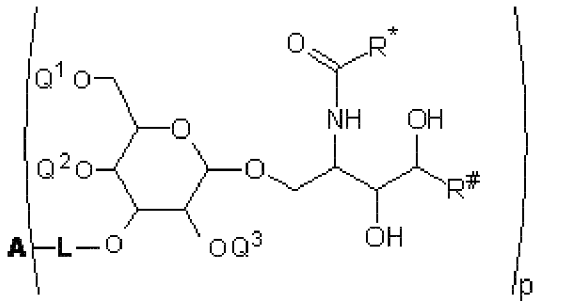
The following subformulas (IIc, IIIc, IVc and Vc) of the general formula (I) are preferred:



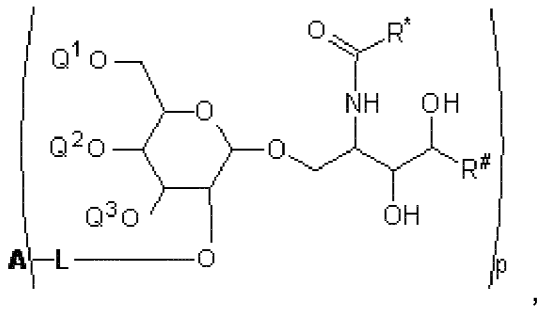
(IIc)



(IIIc)



(IVc)



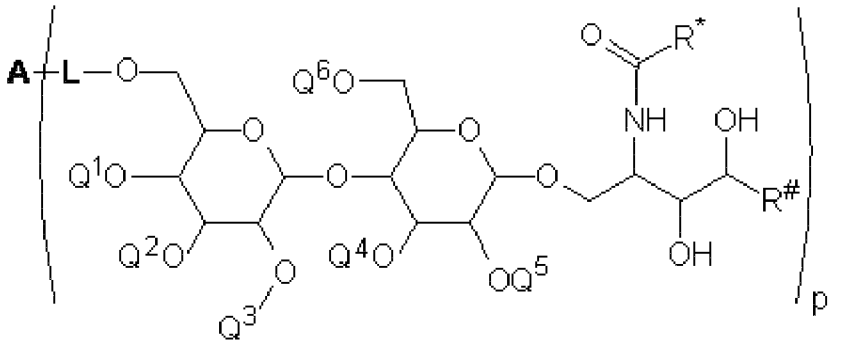
(Vc)

wherein

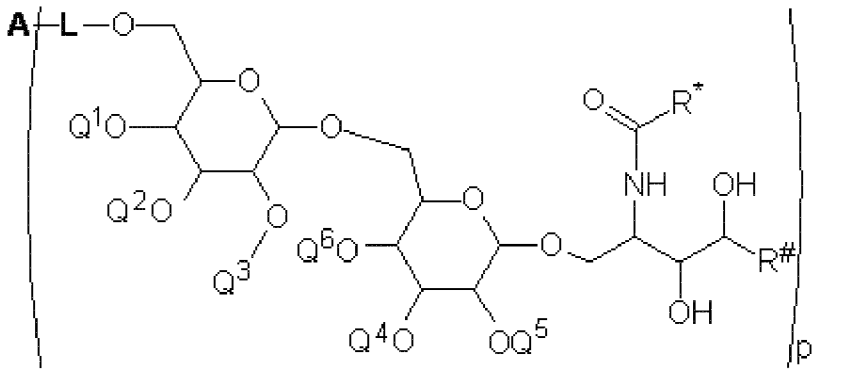
A, **L**, **R***, **R**[#] and **p** have the meanings as defined herein.

Q^1, **Q^2**, **Q^3** represent independently of each other:
–H, $-CH_3$, $-C_2H_5$, $-C_3H_7$, $-SO_2-CH_3$, $-SO_2-C_2H_5$, $-SO_2-C_3H_7$, $-COCH_3$,

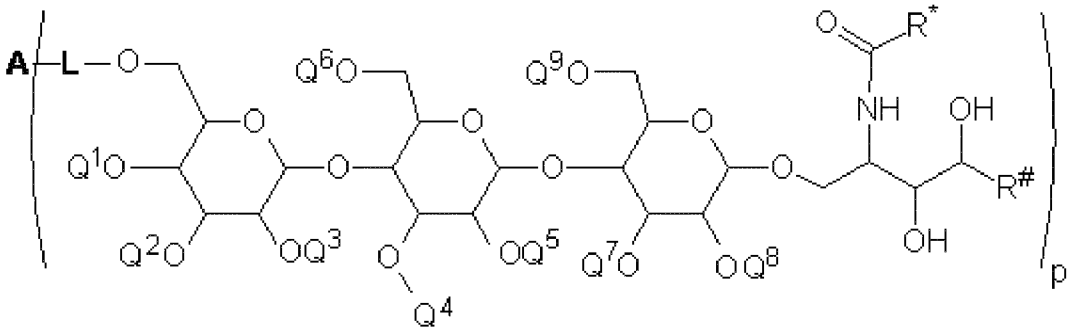
Furthermore, the following subformulas (VI, VII, VIII, IX) of the general formula (I) are preferred:



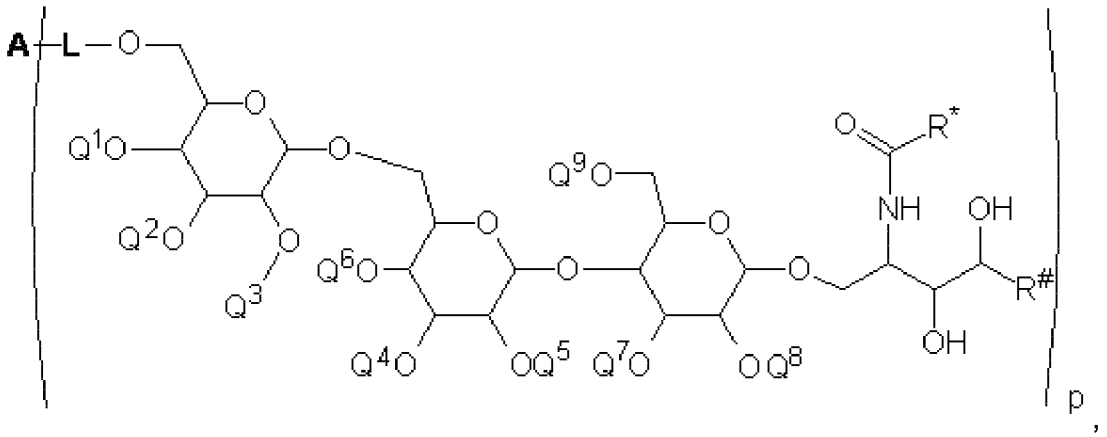
(VIc)



(VIIc)



(VIIIc)



(IXc)

wherein

A, **L**, **R*** , **R#** and **p** have the meanings as defined herein.

Q1, **Q2**, **Q3**, **Q4**, **Q5**, **Q6**, **Q7**, **Q8**, **Q9** represent independently of each other: –H, –CH_3, –C_2H_5, –C_3H_7, –SO_2–CH_3, –SO_2–C_2H_5, –SO_2–C_3H_7, –$COCH_3$,

All embodiments of this invention comprise the enantiomers, stereoisomeric forms, mixtures of enantiomers, anomers, deoxy-forms, diastereomers, mixtures of diastereomers, prodrugs, tautomers, hydrates, solvates and racemates of the above mentioned compounds and pharmaceutically acceptable salts thereof.

The expression prodrug is defined as a pharmacological substance, a drug, which is administered in an inactive or significantly less active form. Once administered, the prodrug is metabolized in the body in vivo into the active compound.

The expression tautomer is defined as an organic compound that is interconvertible by a chemical reaction called tautomerization. Tautomerization can be catalyzed preferably by bases or acids or other suitable compounds.

The extraction and isolation of carbohydrate antigens from a pathogen may be accomplished by a variety of means (MICROBIOLOGICAL REVIEWS, Vo. 42, Nr.1, 84-113, 1978; JOURNAL OF IMMUNOLOGICAL METHODS Vo. 44, Nr. 3, 249-270, 1981). One common method is described as follows:
The isolation and purification usually involve alkaline extraction of cell walls or cells that first had been delipidated with organic solvents, followed by precipitation with organic solvents. Further purification is achieved with ion-exchange chromatography.

Proteolytic enzymes are used to remove remaining peptide or protein components followed by affinity chromatography as a final purification step.

The synthesis of synthetic carbohydrate antigens may be accomplished by a variety of means (Nature Reviews Drug Discovery 4, 751-763, September 2005). The automated solid-phase method is described as follows:
Automated solid-phase oligosaccharide synthesis has been developed from insights gained from oligopeptide and oligonucleotide assembly. The first building block is added to a polystyrene resin equipped with an easily cleavable linker containing a free hydroxyl group. An activating agent induces couplings involving glycosyl phosphate and glycosyl trichloroacetimidate building blocks. Unlike oligonucleotide and peptide couplings, glycosidic bond formation occurs mostly at low temperatures and requires a reaction chamber that can be cooled. Excess building blocks (that is, a 5–10-fold molar excess, sometimes applied twice) are added to the chamber for each coupling.

Washing and filtration remove any side products or remaining reagents before selective removal of a temporary protective group readies the next hydroxyl group for subsequent coupling. Coupling efficiencies can be assessed by spectrometric read-out after protecting-group removal when temporary protecting groups that absorb ultraviolet radiation, such as 9-fluorenylmethyloxycarbonyl (Fmoc), are used. Originally, this coupling–deprotection cycle was automated using a converted peptide synthesizer.

After completion of the oligosaccharide sequence, the fully protected product is cleaved from solid support. After global deprotection, the oligosaccharide is purified and its structure verified. A series of increasingly complex oligosaccharides has been assembled, each within 1 day or less, using the automated oligosaccharide synthesizer. This compares favourably with the weeks to months taken using solution-phase methods.

Another aspect of the present invention comprises the synthesis of the compounds of the general formula (I)

$$A[L—CH—CA]_p$$

(I)

In one embodiment the synthesis of the compounds of the present invention are proceeds as follows:

L + CH → L–CH

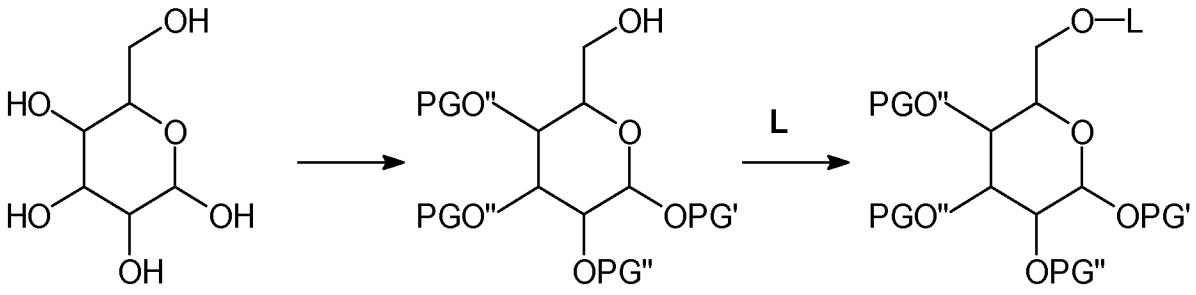
L–CH + CA → L–CH–CA

L–CH–CA + A → A[L—CH—CA]$_p$

Specifically, in a particular preferred embodiment of the present invention the CH moiety is reacted with a linker molecule L after being protected with appropriate protection groups (PGs). Therein, the PGs may either be the same PGs or may also be different PGs such as PG' and PG'' depending on the hydroxyl group on the CH moiety. In a preferred embodiment of the present invention the protections groups PG' and PG'' are different from each. In another preferred embodiment the protection groups PG' and PG'' are the same.

As used herein protecting groups are preferably useful for secondary alcohols. In one embodiment silyl protecting groups such as trimethylsilyl (TMS), tert-butyldiphenylsilyl (TBDPS), tert-butyldimethylsilyl (TBS or TBDMS), triisopropylsilyl (TIPS) and [2-(trimethylsilyl)ethoxy]methyl (SEM) are used. In another preferred embodiment carbon ether protection groups are used such as methyl, *n*-butyl, *tert*.-butyl, p-methoxybenzyl, methoxy-methyl, trityl, vinyl, allyl, benzyloxymethyl, acetyl, pivolyl, 2-trichlor-1-imidoacetyl, 2-trichlor-1-N-phenylimioacetyl and tetrahydropyranyl. Yet, in another preferred embodiment of the present invention at least one silyl group for PG' and at least one carbon ether for PG'' are used in one of the molecules (XXIX), (XXX) and (XXXII). Still in another preferred embodiment of the present invention in the molecules (XXIX), (XXX) and (XXXII) two different carbon ether protection groups are used for the protection groups PG' and PG'', preferably at least one benzyl for PG'' and at least one allyl group for PG', more preferably three benzyl groups for PG'' in each 3, 4 and 5 four position in molecules (XXIX), (XXX) and (XXXII) and one allyl group for PG' in 2 position in molecules (XXIX) and (XXX).

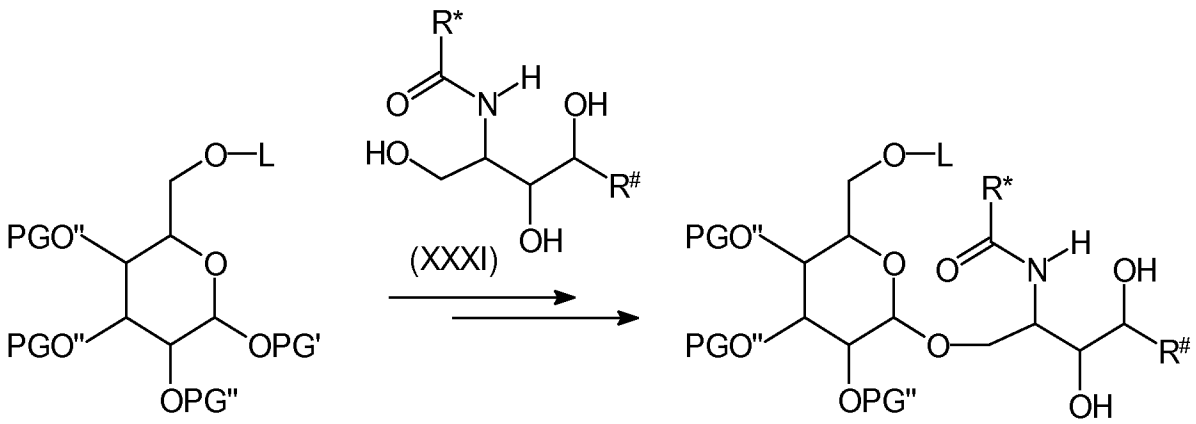
Therefore, in a particular preferred embodiment a reaction sequence is conducted as follows:



(XXVIII) (XXIX) (XXX)

wherein L, PG' and PG'' are as defined herein.

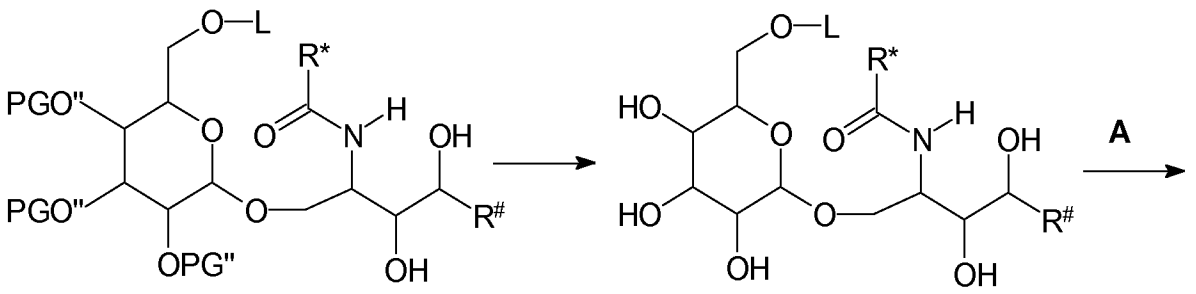
Subsequently in this embodiment the L–CH molecule (XXX) is converted in at least one reaction step, preferably in two reaction steps to intermediate L–CH–CA with a suitable precursor (XXXI) for the molecule CA:



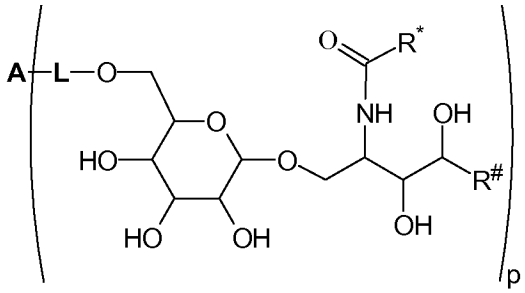
(XXX) (XXXII)

wherein A, L, PG', PG'', R* and $R^{\#}$ are as defined herein.

In the next reaction sequence of this particular embodiment intermediate (XXXII) is then deprotected from the protection groups PG'' to intermediate (XXXIII) and reacted with any suitable antigen A to yield the compound (X) as one representative of the inventive compounds of the general formula (I):



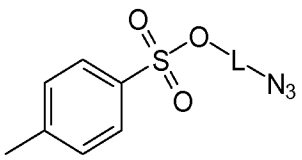
(XXXII) (XXXIII)



(X)

wherein A, L, PG'', R* and $R^{\#}$ are as defined herein.

In the synthesis of the compounds of the general formula (I) A[L—CH—CA]$_p$, and in particular as shown above in the synthesis of compounds of the general formula (X) it is preferred that the linker molecule L is introduced via a precursor which originates from diol (glycol) compound. Preferred are asymmetric precursor molecules for the linker **L** which have on the one side a nucleophilic group such as a halide or an activated hydroxy group and on the other side a functional group which can be converted into an amino group such as an azide, a protected amino group or nitrile. In a more preferred embodiment of the present invention the precursor molecules for the linker **L** have on the one side an activated alcohol functional group with a leaving group such as tosylate, triflat, or mesylate, and on the other side preferably a protected amino group or an azide. In a particularly preferred embodiment of the present invention the precursor molecule for the linker **L** has the general formula (XXXV)



(XXXV) which can be generally synthesized from diol (glycol) compounds of the general formuly (XXXVI)

HO—L—OH (XXXVI).

Also, preferred are linker being a linear or branched carbon chain with 2 to 30 carbon atoms and 0 to 6 hetero atoms selcted from the group of –O–, –S– and –N(R^N)– and/or with one or more aromatic and/or carbocyclic and/or heterocyclic ring systems, wherein the linker is bond through a carbon atom to an oxygen atom of the carbonhydrate moiety (CH), preferably to the oxygen atom at the C6 carbon atom of the carbonhydrate moiety, and is directly or indirectly bond through a carbon atom to the antigen. This carbon chain is preferably bond through a methylen group of the carbon chain to the oxygen and preferably the C6-oxygen of the carbonhydrate moiety. Moreover this

carbon chain is preferably bond through a methylen group or a carbonyl group of the carbon chain to a heteroatom and preferably a nitrogen atom of the antigen (A) and more preferably to a nitrogen atom of an amino group of the antigen. As used herein "directly bond" means that the carbon chain is attached to a functional group of the antigen, preferably an amino group of the antigen while the term "indirectly bond" refers to an attachment of the carbon chain to a spacer attached to the antigen so that the carbon chain is attached to the spacer which is connected to the antigen. Thus, such a spacer is interposed between the linker or respectively carbon chain and the antigen and can for example arise from the cleavage of an anhydride or a succinimide. Preferably the carbon chain has 2 to 25, more preferably 2 to 20, still more preferably 2 to 15 or 2 to 12 carbon atoms. It is also preferred that the carbon chain has up to 4 oxygen atoms and more preferably 1, 2 or 3 oxygen atoms and/or up to 4 sulfur atoms, preferably 1 or 2 sulfur atoms. Furthermore one or two substituted or unsubstituted phenylen rings can be present within the carbon chain.

In all described embodiments above a residue $-R^N$ represents –H, $-CH_3$, $-C_2H_5$, $-C_3H_7$, $-C_4H_9$, $-C_5H_{11}$, $-C_6H_{13}$, $-C_7H_{15}$, $-C_8H_{17}$, –OH, $-OCH_3$, $-OC_2H_5$, $-OC_3H_7$, –O–cyclo-C_3H_5, $-OCH(CH_3)_2$, $-OC(CH_3)_3$, $-OC_4H_9$, –OPh, $-OCH_2$–Ph, $-OCPh_3$, $-CH_2-OCH_3$, $-C_2H_4-OCH_3$, $-C_3H_6-OCH_3$, $-CH_2-OC_2H_5$, $-C_2H_4-OC_2H_5$, $-C_3H_6-OC_2H_5$, $-CH_2-OC_3H_7$, $-C_2H_4-OC_3H_7$, $-C_3H_6-OC_3H_7$, $-CH_2$–O–cyclo-C_3H_5, $-C_2H_4$–O–cyclo-C_3H_5, $-C_3H_6$–O–cyclo-C_3H_5, $-CH_2-OCH(CH_3)_2$, $-C_2H_4-OCH(CH_3)_2$, $-C_3H_6-OCH(CH_3)_2$, $-CH_2-OC(CH_3)_3$, $-C_2H_4-OC(CH_3)_3$, $-C_3H_6-OC(CH_3)_3$, $-CH_2-OC_4H_9$, $-C_2H_4-OC_4H_9$, $-C_3H_6-OC_4H_9$, $-CH_2$–OPh, $-C_2H_4$–OPh, $-C_3H_6$–OPh, $-CH_2-OCH_2$–Ph, $-C_2H_4-OCH_2$–Ph, $-C_3H_6-OCH_2$–Ph, $-NO_2$, –F, –Cl, –Br, $-COCH_3$, $-COC_2H_5$, $-COC_3H_7$, –CO–cyclo-C_3H_5, $-COCH(CH_3)_2$, $-COC(CH_3)_3$, –COOH, $-COOCH_3$, $-COOC_2H_5$, $-COOC_3H_7$, –COO–cyclo-C_3H_5, $-COOCH(CH_3)_2$, $-COOC(CH_3)_3$, –OOC–CH_3, –OOC–C_2H_5, –OOC–C_3H_7, –OOC–cyclo-C_3H_5, –OOC–$CH(CH_3)_2$, –OOC–$C(CH_3)_3$, $-CONH_2$, $-CONHCH_3$, $-CONHC_2H_5$, $-CONHC_3H_7$, –CONH–cyclo-C_3H_5, $-CONH[CH(CH_3)_2]$, $-CONH[C(CH_3)_3]$, $-CON(CH_3)_2$, $-CON(C_2H_5)_2$, $-CON(C_3H_7)_2$, $-CON(\text{cyclo-}C_3H_5)_2$, $-CON[CH(CH_3)_2]_2$, $-CON[C(CH_3)_3]_2$.

In another embodiment of the present invention the order of connecting the respective moieties of the compounds of the present invention may be varied.

In another particular embodiment of the present invention first the moieties CH and CA are connected via suitable chemical reaction or reactions to yield intermediate CH–CA, and subsequently a linker molecule L is added to yield intermediate L–CH–CA which is

then further reacted to furnish the compounds of the present invention of the general formula (I).

In another embodiment of the present invention antigen A is modified with linker molecule L to yield intermediate $[L–]_qA$. Intermediate $[L–]_qA$ can then further be reacted with intermediate CA–CH yielding the compounds of the present invention of the general formula (I).

L + A → $[L–]_qA$

CH + CA → CH–CA

$[L–]_qA$ + CH–CA → $A[L—CH—CA]_p$

All reaction approaches may be modified to use or yield the respective preferred compounds of the subformulas (II) to (XXVII).

In that, according to the reaction sequence

L + CH → L–CH

L–CH + CA → L–CH–CA

L–CH–CA + A → $A[L—CH—CA]_p$

CH moieties with different connectivity as exemplified in subformulas (II) to (V) may be used. Similarly, CH moieties being monosaccarides, disaccarides or trisaccarides as exemplified in subformulas (VI) to (XIII), also with respect to stereochemical aspects as exemplified in subformulas (XIV) to (XVII) are suitable for the above reaction sequence. Further, the synthetic approach is also suitable to be applied to specific ceramid moieties as exemplified in subformulas (XVIII) to (XXIV), which also holds true for specific linker molecules as exemplified for the subsformulas (XXV) and (XXVII).

Therefore, the reaction sequence

L + CH → L–CH

L–CH + CA → L–CH–CA

L–CH–CA + A → $A[L—CH—CA]_p$

is suitable also for the synthesis of intermediates (II) to (XXVII) by choosing the respective moieties L, CH, and CA.

In a further preferred embodiment of the present invention the carbohydrate moiety and the ceramide are first joined together prior to introduction of the linker molecule. Therefore, a reaction sequent could also be as follows:

CH + CA → CH–CA

CH–CA + L → L–CH–CA

L–CH–CA + A → A[L—CH—CA]$_p$

The present invention also comprises pharmaceutically acceptable salts of the compounds according to the general formula (I), all stereoisomeric forms of the compounds according to the general formula (I) as well as solvates, especially hydrates or prodrugs thereof.

In case, the inventive compounds bear basic and/or acidic substituents, they may form salts with organic or inorganic acids or bases. Examples of suitable acids for such acid addition salt formation are hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, acetic acid, citric acid, oxalic acid, malonic acid, salicylic acid, p-aminosalicylic acid, malic acid, fumaric acid, succinic acid, ascorbic acid, maleic acid, sulfonic acid, phosphonic acid, perchloric acid, nitric acid, formic acid, propionic acid, gluconic acid, lactic acid, tartaric acid, hydroxymaleic acid, pyruvic acid, phenylacetic acid, benzoic acid, p-aminobenzoic acid, p-hydroxybenzoic acid, methanesulfonic acid, ethanesulfonic acid, nitrous acid, hydroxyethanesulfonic acid, ethylenesulfonic acid, p-toluenesulfonic acid, naphthylsulfonic acid, sulfanilic acid, camphorsulfonic acid, china acid, mandelic acid, o-methylmandelic acid, hydrogen-benzenesulfonic acid, picric acid, adipic acid, d-o-tolyltartaric acid, tartronic acid, (o, m, p)-toluic acid, naphthylamine sulfonic acid, and other mineral or carboxylic acids well known to those skilled in the art. The salts are prepared by contacting the free base form with a sufficient amount of the desired acid to produce a salt in the conventional manner.

Examples for suitable inorganic or organic bases are, for example, NaOH, KOH, NH_4OH, tetraalkylammonium hydroxide, lysine or arginine and the like. Salts may be prepared in a conventional manner using methods well known in the art, for example by treatment of a solution of the compound of the general formula (I) with a solution of an acid, selected out of the group mentioned above.

Some of the compounds of the present invention may be crystallised or recrystallised from solvents such as aqueous and organic solvents. In such cases solvates may be formed. This invention includes within its scope stoichiometric solvates including hydrates as well as compounds containing variable amounts of water that may be produced by processes such as lyophilisation.

Certain compounds of the general formula (I) may exist in the form of optical isomers if substituents with at least one asymmetric center are present, e.g. diastereoisomers and mixtures of isomers in all ratios, e.g. racemic mixtures. The invention includes all such forms, in particular the pure isomeric forms. The different isomeric forms may be separated or resolved one from the other by conventional methods, or any given isomer may be obtained by conventional synthetic methods or by stereospecific or asymmetric syntheses. Where a compound according to the general formula (I) contains an alkene moiety, the alkene can be presented as a cis or trans isomer or a mixture thereof. When an isomeric form of a compound of the invention is provided substantially free of other isomers, it will preferably contain less than 5% w/w, more preferably less than 2% w/w and especially less than 1% w/w of the other isomers.

Another aspect of the present invention relates to the use of the inventive carbohydrate-glycolipid conjugate derivatives as drugs, i.e. as pharmaceutically active agents applicable in medicine.

Surprisingly it was found that the novel carbohydrate-glycolipid conjugates of the present invention are also suitable to raise an immune response in an animal and are suitable for vaccination against infectious diseases which are caused by pathogens selected from the group of bacteria, viruses, sporozoa, parasites or fungi. Moreover if the saccharide antigen is specific to cancer cells, the novel carbohydrate-glycolipid conjugates are suitable for the treatment and prophylaxis of cancers.
Both isolated and synthetic carbohydrate antigens are suitable for the formation of the described conjugate. Moreover it was found, that the treatment of an animal with the novel carbohydrate-glycolipid conjugates of the current invention lead to the formation of immunoglobuline IgG-isotypes, which prove the development of memory

B-cells in the living organism. The presence of memory B-cells demonstrates immunological memory. Thus it has been shown, that the carbohydrate-glycolipid conjugates of the current invention are capable to induce a long term protection in an animal against a pathogen. The described vaccination is moreover independent on further adjuvants, does not need any protein-carrier and refrigeration of the vaccine.

Therefore, compounds according to the general formula (I - XXVII) are suitable for the use as a pharmaceutically active agent applicable in medicine, especially for use in vaccination against infectious diseases.

The infectious diseases for which vaccines can be provided by the compounds according to the present invention are selected from the group of bacterial, sporozoal, parasitic, fungal or viral infectious diseases.

The bacterial infectious disease for which vaccines can be provided by the compounds according to the invention is caused by a pathogen selected from the group comprising:
Allochromatium vinosum, Acinetobacter baumanii, Bacillus anthracis, Campylobacter jejuni, Clostridium spp., Citrobacter spp., Escherichia coli, Enterobacter spp., Enterococcus faecalis., Enterococcus faecium, Francisella tularensis, Haemophilus influenzae, Helicobacter pylori, Klebsiella spp., Listeria monocytogenes, Moraxella catharralis, Mycobacterium tuberculosis, Neisseria meningitidis, Neisseria gonorrhoeae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella spp., Serratia spp., Shigella spp., Stenotrophomonas maltophilia, Staphyloccocus aureus, Staphyloccocus epidermidis, Streptococcus pneunmoniae, Streptococcus pyogenes, Streptococcus agalactiae, Yersina pestis, und Yersina enterocolitica.

The parasitic infectious disease for which vaccines can be provided by the compounds according to the invention is caused by a pathogen selected from the group comprising:
Babesia, Balantidium, Besnoitia, Blastocystis, Coccidia, Cryptosporidium, Cytauxzoon, Cyclospora, Dientamoeba, Eimeria, Entamoeba, Enterocytozoon, Enzephalitozoon, Eperythrozoon, Giardia, Hammondia, Isospora, Leishmania, Microsporidia, Naegleria, Plasmodium, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium knowlesi, Pneumocystis, Schistosoma, Sarcocystis, Theileria, Trichinella, Toxoplasma, Trichomonas, Trypanosoma, Unicaria, Cestoda, Dipylidium, Dranunculus, Echinococcus, Fasciola,

Fasciolopsis, Taenia, Ancylostoma, Ascaris, Brugia, Enterobius, Loa loa, Mansonella, Necator, Oncocerca, Strongyloides, Strongylus, Toxocara, Toxascaris, Trichuris oder Wucheria.

The fungal infectious disease for which vaccines can be provided by the compounds according to the invention is caused by a pathogen selected from the group comprising:
Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton interdigitale, T. schönleinii, T. verrucosum, T. violaceum, T. tonsurans, Trichophyton spp., M. canis, Candida albicans, C. guillermondii, C. krusei, C. parapsilosis, C. tropicalis, C. glabrata, Candida spp., Microsporum spp., Microsporum canis, Microsporum audonii, Microsporum gypseum, M. ferrugineum, Trichosporum beigelii, Trichosporum inkiin, Aspergillus niger, Alternaria, Acremonium, Fusarium, or Scopulariopsis.

The viral infectious disease for which vaccines can be provided by the compounds according to the invention is caused by a pathogen selected from the group comprising:
Adenoviruses, Ebolavirus, Epstein-Barr-virus, Flavivirus, FSME-virus, Influenza virus, Hanta-virus, human immunodeficiency virus ("HIV"), herpes simplex virus ("HSV", type 1 or 2), human herpes virus 6 (HHV-6), human Papilloma virus ("HPV", type 16 or 18), human Cytomegalovirus ("HCMV"), human hepatitis B or C virus (“HBV”, Type B; "HCV", type C), Lassavirus, Lyssavirus (EBL 1 or EBL 2), Marburgvirus, Norovirus, Parvovirus B19, Pestvirus, Poliovirus, Rhinovirus, Rotaviruses, SARS-assciated Coronavirus, Varicella-Zoster virus.

Among the cancers the novel carbohydrate-glycolipid conjugates are suitable for, the attention has been given to Bladder Cancer, Breast Cancer, Colon and Rectal Cancer, Endometrial Cancer, Kidney (Renal Cell) Cancer, Leukemia, Lung Cancer Melanoma, Non-Hodgkin Lymphoma, Pancreatic Cancer, Prostate Cancer, Thyroid Cancer.

Among the infectious diseases, the attention has been given to Haemophilus influenzae and Streptococcus pneunmoniae.

Therefore, another aspect of the present invention is directed to pharmaceutical compositions comprising at least one compound of the present invention as active ingredient, together with at least one pharmaceutically acceptable carrier, excipient and/or diluents. The pharmaceutical compositions of the present invention can be

prepared in a conventional solid or liquid carrier or diluent at suitable dosage level in a known way. The preferred preparations are adapted for oral application. These administration forms include, for example, pills, tablets, film tablets, coated tablets, capsules, powders and deposits.

Furthermore, the present invention also includes pharmaceutical preparations for parenteral application, including dermal, intradermal, intragastral, intracutan, intravasal, intravenous, intramuscular, intraperitoneal, intranasal, intravaginal, intrabuccal, percutan, rectal, subcutaneous, sublingual, topical, or transdermal application, which preparations in addition to typical vehicles and/or diluents contain at least one compound according to the present invention and/or a pharmaceutical acceptable salt thereof as active ingredient.

The **pharmaceutical compositions** according to the present invention containing at least one compound according to the present invention, and/or a pharmaceutical acceptable salt thereof as active ingredient will typically be administered together with suitable carrier materials selected with respect to the intended form of administration, i.e. for oral administration in the form of tablets, capsules (either solid filled, semi-solid filled or liquid filled), powders for constitution, extrudates, deposits, gels, elixirs, dispersable granules, syrups, suspensions, and the like, and consistent with conventional pharmaceutical practices. For example, for oral administration in the form of tablets or capsules, the active drug component may be combined with any oral non-toxic pharmaceutically acceptable carrier, preferably with an inert carrier like lactose, starch, sucrose, cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, talc, mannitol, ethyl alcohol (liquid filled capsules) and the like. Moreover, suitable binders, lubricants, disintegrating agents and coloring agents may also be incorporated into the tablet or capsule. Powders and tablets may contain about 5 to about 95 weight % of the benzothiophene-1,1-dioxide derived compound and/or the respective pharmaceutically active salt as active ingredient.

Suitable binders include starch, gelatin, natural carbohydrates, corn sweeteners, natural and synthetic gums such as acacia, sodium alginate, carboxymethylcellulose, polyethylene glycol and waxes. Among suitable lubricants there may be mentioned boric acid, sodium benzoate, sodium acetate, sodium chloride, and the like. Suitable disintegrants include starch, methylcellulose, guar gum, and the like. Sweetening and flavoring agents as well as preservatives may also be included, where appropriate. The disintegrants, diluents, lubricants, binders etc. are discussed in more detail below.

Moreover, the pharmaceutical compositions of the present invention may be formulated in sustained release form to provide the rate controlled release of any one or more of the components or active ingredients to optimise the therapeutic effect(s), e.g. antihistaminic activity and the like. Suitable dosage forms for sustained release include tablets having layers of varying disintegration rates or controlled release polymeric matrices impregnated with the active components and shaped in tablet form or capsules containing such impregnated or encapsulated porous polymeric matrices.

Liquid form preparations include solutions, suspensions, and emulsions. As an example, there may be mentioned water or water/propylene glycol solutions for parenteral injections or addition of sweeteners and opacifiers for oral solutions, suspensions, and emulsions. Liquid form preparations may also include solutions for intranasal administration. Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be present in combination with a pharmaceutically acceptable carrier such as an inert, compressed gas, e.g. nitrogen. For preparing suppositories, a low melting fat or wax, such as a mixture of fatty acid glycerides like cocoa butter is melted first, and the active ingredient is then dispersed homogeneously therein e.g. by stirring. The molten, homogeneous mixture is then poured into conveniently sized moulds, allowed to cool, and thereby solidified.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions, and emulsions.

The compounds according to the present invention may also be delivered transdermally. The transdermal compositions may have the form of a cream, a lotion, an aerosol and/or an emulsion and may be included in a transdermal patch of the matrix or reservoir type as is known in the art for this purpose.

The term capsule as recited herein refers to a specific container or enclosure made e.g. of methyl cellulose, polyvinyl alcohols, or denatured gelatins or starch for holding or containing compositions comprising the active ingredient(s). Capsules with hard shells are typically made of blended of relatively high gel strength gelatins from bones or pork skin. The capsule itself may contain small amounts of dyes, opaquing agents, plasticisers and/or preservatives. Under tablet a compressed or moulded solid dosage form is understood which comprises the active ingredients with suitable

diluents. The tablet may be prepared by compression of mixtures or granulations obtained by wet granulation, dry granulation, or by compaction well known to a person of ordinary skill in the art.

Oral gels refer to the active ingredients dispersed or solubilised in a hydrophilic semi-solid matrix. Powders for constitution refers to powder blends containing the active ingredients and suitable diluents which can be suspended e.g. in water or in juice.

Suitable diluents are substances that usually make up the major portion of the composition or dosage form. Suitable diluents include carbohydrates such as lactose, sucrose, mannitol, and sorbitol, starches derived from wheat, corn rice, and potato, and celluloses such as microcrystalline cellulose. The amount of diluent in the composition can range from about 5 to about 95 % by weight of the total composition, preferably from about 25 to about 75 weight %, and more preferably from about 30 to about 60 weight %.

The term disintegrants refers to materials added to the composition to support break apart (disintegrate) and release the pharmaceutically active ingredients of a medicament. Suitable disintegrants include starches, “cold water soluble” modified starches such as sodium carboxymethyl starch, natural and synthetic gums such as locust bean, karaya, guar, tragacanth and agar, cellulose derivatives such as methylcellulose and sodium carboxymethylcellulose, microcrystalline celluloses, and cross-linked microcrystalline celluloses such as sodium croscaramellose, alginates such as alginic acid and sodium alginate, clays such as bentonites, and effervescent mixtures. The amount of disintegrant in the composition may range from about 2 to about 20 weight % of the composition, more preferably from about 5 to about 10 weight %.

Binders are substances which bind or “glue” together powder particles and make them cohesive by forming granules, thus serving as the “adhesive” in the formulation. Binders add cohesive strength already available in the diluent or bulking agent. Suitable binders include carbohydrates such as sucrose, starches derived from wheat corn rice and potato, natural gums such as acacia, gelatin and tragacanth, derivatives of seaweed such as alginic acid, sodium alginate and ammonium calcium alginate, cellulose materials such as methylcellulose, sodium carboxymethylcellulose and hydroxypropylmethylcellulose, polyvinylpyrrolidone, and inorganic compounds such as magnesium aluminum silicate. The amount of binder in the composition

may range from about 2 to about 20 weight % of the composition, preferably from about 3 to about 10 weight %, and more preferably from about 3 to about 6 weight %.

Lubricants refer to a class of substances which are added to the dosage form to enable the tablet granules etc. after being compressed to release from the mould or die by reducing friction or wear. Suitable lubricants include metallic stearates such as magnesium stearate, calcium stearate, or potassium stearate, stearic acid, high melting point waxes, and other water soluble lubricants such as sodium chloride, sodium benzoate, sodium acetate, sodium oleate, polyethylene glycols and D,L-leucine. Lubricants are usually added at the very last step before compression, since they must be present at the surface of the granules. The amount of lubricant in the composition may range from about 0.2 to about 5 weight % of the composition, preferably from about 0.5 to about 2 weight %, and more preferably from about 0.3 to about 1.5 weight % of the composition.

Glidents are materials that prevent caking of the components of the pharmaceutical composition and improve the flow characteristics of granulate so that flow is smooth and uniform. Suitable glidents include silicon dioxide and talc. The amount of glident in the composition may range from about 0.1 to about 5 weight % of the final composition, preferably from about 0.5 to about 2 weight %.

Coloring agents are excipients that provide coloration to the composition or the dosage form. Such excipients can include food grade dyes adsorbed onto a suitable adsorbent such as clay or aluminum oxide. The amount of the coloring agent may vary from about 0.1 to about 5 weight % of the composition, preferably from about 0.1 to about 1 weight %.

Said pharmaceutical compositions may further comprise at least one active carbohydrate-glycolipid conjugate of the general formula (I).

The pharmaceutical compositions may further comprise at least one further active agent. It is preferred if this active agent is selected from the group consisting of anti-depressantand other psychotropic drugs. It is further preferred if the anti-depressant is selected from amitriptyline, amioxide clomipramine, doxepine, duloxetine, imipramine trimipramine, mirtazapine, reboxetine, citaloprame, fluoxetine, moclobemide and sertraline.

A further embodiment of the invention comprises the average ratio of the carbohydrate antigen **A** to the glycolipid (**L-CH-CA**) which may vary between 1:4 and 1:100 (n/n).

Another embodiment of the invention comprises the compounds of the invention, according to the general formula (I) which may be used for the preparation of a vaccine formulation for the use in vaccination of an animal. The mentioned vaccine formulation may comprise one or more of the compounds of the present invention or a mixture of different compounds of the invention and preferably of the general formula (I), wherein the mixture of different compounds of the general formula (I) preferably comprises a mixture of different serotypes of the used carbohydrate antigen **A**, and/or the mixture of different compounds of the general formula (I) may comprise a mixture of different carbohydrate antigens **A**, which are used in different compounds of the general formula (I). The mentioned mixture of different compounds of the general formula (I) within the vaccine formulation can therefore constitute a combinantion of vaccines which can be used for a combinated vaccination against more than at least one pathogen.

In a further embodiment of the invention, the vaccine formulation may comprise a mixture of different compounds of the general formula (I).

The mentioned vaccine formulations may further comprise a combination with at least one pharmaceutically acceptable carrier, excipient and/or diluents.

The compounds of the invention of the general formula (I) are present in said vaccine formulation in the range of 10 to 1000 µg/g.

In a preferred embodiment of the invention the compounds of the general formula (I) are present in said vaccine formulation in the range of 10 to 1000 ng/g.

In a more preferred embodiment of the invention the compounds of the general formula (I) are present in said vaccine formulation in the range of 100 to 1000 pg/g.

The mentioned vaccine formulation displayes an extraordinary stability at room temperature due to the modulary constitution of the compounds of the present invention, wherein said vaccine formulation may be maintained at a temperature of at least 25°C for a period of at least 3 months prior to reconstitution.

In a preferred embodiment of the invention the said period is comprises 6 months or at least 12 months.

The surprising advantages of the conjugates of the present invention were found by *in vitro* and *in vivo* application.
Specifically, when applied in an *in vitro* the glycoconjugate vaccine according to the present invention retains the capacity to stimulate iNKT cells when presented by CD1d-positive antigen-presenting cells (APC). Additionally it was found that the compounds of the present invention fail to stimulate the same iNKT cells when loaded onto plate-bound recombinant CD1d. Without being bound to theory it appears that the saccharidic moiety is properly coupled and hinders T cell recognition.

Further, when applied *in vivo* the conjugates of the present invention were found of being capale of effectively and continuously immunizing against a pathogen. This is rather advantageous since thereby the conjugates of the present invention cannot only stimulate the generation of antibodies of high titers and long lasting resistance in *in vivo* conditions, moreover the compounds of the present invention themselves exhibit a long-term stability at room temperature. Therefore, the conjugates of the present invention are particular heat stable and thus no refrigeration is required.

Examples

General methods:

Cells. The APC lines MOLT-4 (ATCC CRL 1582), which expresses only negligible CD1d, and human CD1d-transfected C1R and HeLa cells (C1R-hCD1d and HeLa-hCD1d, respectively) [6] were maintained in RPMI-1640 medium containing 10% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 µM non-essential aminoacids, and 100 µg/ml kanamycin. The same maintenance conditions were used for RAW (mouse leukemic monocyte macrophage cell line), J774A.1 (mouse, BALB/c, monocyte-macrophage, not defined tumor), HL60 and NB4 (both human promyelocytic leukemia) cells. Isolation of iNKT cell clones from PBMC of healthy donors has been described before [7]. iNKT cells were maintained in RPMI-1640 medium containing 5% HS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 µM non-essential aminoacids, 100 µg/ml kanamycin, and 100 U/ml recombinant IL-2.

Mice. C57BL/6, BALB/c and B6;129-CD1<tm1Gru> (CD1KO) [8] mice were bred at our institute (Versuchsstation Departement Biomedizin, Basel, Switzerland) or C57BL/6 were also bought from Charles River Laboratories (Sulzfeld, Germany). This study was reviewed and approved by the "Kantonales Veterinäramt Basel-Stadt" in Basel, Switzerland.

Bacteria. *Streptococcus pneumoniae* serotype 4 reference strain (Statens Serum Institute, Denmark) was grown in Todd-Hewitt broth supplemented with 0.5% yeast extract at 37°C.

Infections. Non-/ and vaccinated mice were challenged with *S. pneumoniae* serotype 4 and mortality, weight loss and clinical score were recorded over time.

Opsonizations. 5mM 5-chloromethylfluorescein diacetate (CMFDA, Invitrogen, Switzerland) labeled and non-fixed or fixed bacteria were coated with 10% rabbit complement (HD supplies, UK) and/or purified CPS-specific m**Abs** for up to 1 h. Mixed bacteria with dimethylformamide- (Sigma-Aldrich, Switzerland) or non-induced cells at a ratio of 10-100:1 for up to 2 h at 37°C. Samples were acquired on a CyAn ADP flow cytometer (Beckman Coulter, Switzerland). Data were gated to exclude non-viable cells on the basis of light scatter, pulse width, and incorporation of propidium iodide and futher analyzed using Summit software (Beckman Coulter).

Activation assays. *In vitro* antigen presentation assays by living APC or plate-bound antigen-presenting molecules were performed as previously described [9]. Briefly, living APC were plated at 2.5 x 10^4/well in 96-well plates and incubated during the whole assay at 37°C with vehicle or titrating doses of αGalCer or conjugate vaccine. After 1 h human iNKT cells (0.5-1 x 10^5/well) were added. Cell culture supernatants

were harvested after 24-48 h and release of cytokines was measured by ELISA. For plate-bound activation, purified recombinant soluble human CD1d (rshCD1d) was obtained by IEF and added to Bir1.4 mAb-coated (10 µg/ml, specific for the BirA tag of rshCD1d) MaxiSorp Plates (Nunc) overnight. Bound rshCD1d was pulsed with 2 µg/ml αGalCer or different doses of conjugate vaccine. Human iNKT cell clones (1.5 x 10^5/well) were added to the plate and after 24-48 h released cytokines were measured by ELISA.

ELISA. For detection of human cytokines, the following purified capture and biotinylated detection monoclonal antibody (mAb) pairs (all BioLegend, San Diego, USA) were used: hTNFα (MAb1 1 µg/ml and MAb11 0.5 µg/ml), hIFNγ (MD-1 2 µg/ml and 4S.B3 0.5 µg/ml), hIL-4 (8D4-8 1 µg/ml and MP4-25D2 0.5 µg/ml), hGM-CSF (BVD2-23B6 3.33 µg/ml and BVD2-21C11 0.5 µg/ml), hIL-8 (JK8-1 1.25 µg/ml and JK8-2 1 µg/ml). For detection of mouse cytokines, the following mAb pairs (all Becton Dickinson (BD), Allschwil, Switzerland) were used: mIL-2 (JES6-1A12 2 µg/ml and JES6-5H4 1 µg/ml), mIL-4 (11B11 1 µg/ml and BVD6-24G2 1 µg/ml), mIFNγ (R4-6A2 2 µg/ml and XMG1.2 1 µg/ml). For detection of **Abs**, plates were coated with 1 µg/ml biotin goat anti-mouse (GAM) Ig (BD, 553999) and revealed with 1:10'000 HRP-labeled GAM-IgG (Sigma-Aldrich, Buchs, Switzerland, A0168) or with 1:1'000 (all SouthernBiotech, Birmingham, USA) HRP-labeled GAM-IgM (1020-05), GAM-IgG1 (1070-05), GAM-IgG2a (1080-05), GAM-IgG2b (1090-05), GAM-IgG3 (1100-05) or coated with 2.5 µg/ml CPS and revealed with (all Biolegend) biotinylated rat anti-mouse (RAM)-IgG1 (clone RMG1-1, 1 µg/ml), -IgG2a (clone RMG2a-62, 1 µg/ml), -IgG2b (clone RMG2b-1, 0.5 µg/ml), -IgG3 (clone RMG3-1, 0.5 µg/ml) or donkey anti-mouse IgM (Jackson ImmunoResearch, Suffolk, UK, 0.95 µg/ml) or GAM F(ab')2 IgG (abcam, 0.1 µg/ml) GAM Ig (BD, 2 µg/ml).

Statistical analysis. Survival data were compared with the Mantel-Cox and Gehan-Breslow-Wilcoxon test. All analyses were performed using GraphPad Prism software (version 5.03). Differences were considered significant at $P<0.05$.

Chemicals and Structure Analysis. All chemicals used were reagent grade and used as supplied except where noted. Dimethylformamide (DMF), tetrahydrofuran (THF), toluene, dichloromethane (CH_2Cl_2) and diethyl ether (Et_2O) were purchased from *JT Baker* or *VWR International* and purified by a Cycle-Tainer Solvent Delivery System. Pyridine, triethylamine (NEt_3) and acetonitrile (MeCN) were refluxed over calcium hydride and distilled. Solvents for chromatography and workup procedures were distilled. Reactions were performed under an argon or nitrogen atmosphere except where noted. Analytical thin-layer chromatography was performed on *E. Merck* silica gel 60 F_{254} plates (0.25 mm). Compounds were visualized by UV-light at 254 nm and by dipping the plates in a cerium sulfate ammonium molybdate (CAM)

solution or a sulfuric acid/methanol solution followed by heating. Liquid chromatography was performed using forced flow of the indicated solvent on *Fluka* silica gel 60 (230-400 mesh). ^{1}H NMR spectra were obtained on a *Varian* VXR-300 (300 MHz), Varian VXR-400 (400MHz), *Bruker* DRX500 (500 MHz), and *Bruker* AV600 (600 MHz) and are reported in parts per million (δ) relative to the resonance of the solvent or to TMS (0.00 ppm). Coupling constants (*J*) are reported in Hertz (Hz). ^{13}C NMR spectra were obtained on a *Varian* VXR-300 (75 MHz), Varian VXR-400 (101MHz), *Bruker* DRX500 (125 MHz), and *Bruker* AV600 (150 MHz) and are reported in δ relative to the resonance of the solvent or to TMS (0.00 ppm). IR Spectra: Measured as 1-2% $CHCl_3$ solution on a *Perkin-Elmer*-782 spectrophotometer or neat on a *Perkin-Elmer*-100 FT-IR spectrometer. Recycling preparative size exclusion HPLC (LC-9101, *Japan Analytical Industry Co.*); flow rate: 3.5 mL/min; solvent: $CHCl_3$. Optical rotations $[\alpha]^{rt}_{D}$ were measured on a *Jasco* DIP-370 polarimeter (10 cm, 1 mL cell); the solvents and concentrations (in g/100 mL) are indicated. High-resolution mass spectra were performed by the MS service FU Berlin and are given in *m/z*.

Example 1

In vitro activity of the conjugate vaccine

The glycoconjugate vaccine (*S. pneumoniae* serotype 4 CPS coupled to αGalCer) retains the capacity to stimulate iNKT cells when presented by CD1d-positive antigen-presenting cells (APC) but fails to stimulate the same iNKT cells when loaded onto plate-bound recombinant CD1d (**Figure 3A** and **3B**, respectively). These findings indicate that the saccharidic moiety is properly coupled and hinders T cell recognition but can be cleaved off from the stimulatory αGalCer glycolipid by living APC.

Example 2

In vivo activity of the conjugate vaccine

The glyconjugate consisting of CPS type 4 coupled to αGalCer was used to immunize wild-type (WT) C57BL/6 mice. Three immunizations were performed with intervals of 14 days. These mice showed high titers of anti-polysaccharide **Abs** compared to naïive or CPS only immunized mice (**Figure 4A**) up to 3 months after the last immunization. This argues in favor of a long-lasting **Abs** response by B cells only when helped by αGalCer-responsive iNKT cells.

The glyconjugate vaccine was used to immunize WT C57BL/6 and CD1d-deficient (CD1d-/-, CD1KO) mice. Two immunizations were performed with an interval of 7 days. WT mice showed high titers of anti-polysaccharide antibodies (**Abs**), which instead were not observed in CD1d-deficient mice (**Figure 4B**), indicating that expression of CD1d is necessary for the adjuvant-like effect of αGalCer.
Conclusively, the glycoconjugate vaccine-induced antibody response is dependent on the presence of iNKT cells and of CD1d as CD1d KO mice fail to generate high titers of CPS-specific antibodies after immunization.

Example 3

Analysis of the in vivo antibody response after vaccination

When CPS-specific **Abs** were investigated by ELISA using isotype-specifc secondary reagents, the presence of IgG1 CPS-specific **Abs** was detected only in WT mice whereas CD1KO mice were unable to induce IgG1 (**Figure 5A**). The same finding was confirmed with other IgG subtypes. These experiments prove that immunization with CPS type 4 coupled to αGalCer glyconjugate facilitates the class switch of polysaccharide-specific antibodies to all IgG isotypes.
The generated **Abs** partially cross-reacted with CPS of type 2 *S. pneumoniae* (**Figure 5B**). They might also recognize common epitopes on CPS of other serotypes as very high titers of total immunoglobulin were detected assessing reactivity to a CPS mix of several *S. pneumoniae* serotypes (data not shown).

Several hybridomas expressing CPS-specific **Abs** were established from mice immunized twice and sacrificed 1.5 months after the last boost. We could isolate hybridomas expressing IgM and all IgG subclasses, with the exception of IgG2b. The IgM-positive hybridomas were affinity matured (**Figure 6**).
These preliminary experiments demonstrate that immunization with CPS type 4 coupled to αGalCer glycoconjugate facilitates switching of polysaccharide-specific B cells to IgG isotypes and/or affinity maturation of the CPS-specific **Abs**.
All hybridomas derived from glycoconjugate immunized mice showed class switching and affinity maturation. Somatic mutation seems a frequent event as two of the IgG1 hybridomas used the same VDJ rearrangement. Moreover several IgM hybridomas were identical except for junctional diversity by P- and N-nucleotides.
These m**Abs** were assessed for their ability to fix complement and enhance opsonization by phagocytic cells. Using CMD-labelled bacteria, we found that CPS-specific **Abs** upregulated bacterial phagocytosis (**Figure 7**).

Example 4

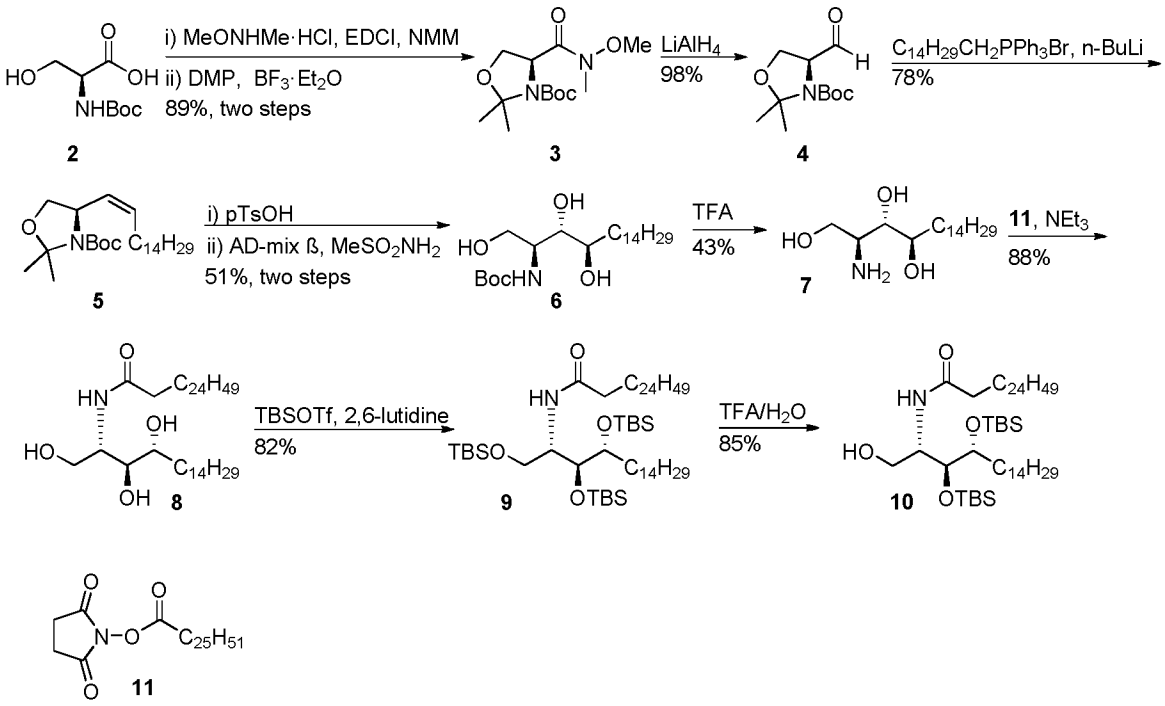
Protection from infection with *S. pneumoniae* in a mouse model

Immunization with the glycoconjugate vaccine protects C57BL/6 mice from infection with *S. pneumoniae*. αGalCer-CPS type 4 vaccinated mice show short- and long-term protection to challenge with *S. pneumoniae* (**Figure 8B**). Furthermore, mice vaccinated with αGalCer-CPS type 4 suffered a less severe disease than CPS type 4 only immunized mice as shown by no weight loss upon infection (**Figure 8A**, **3** and **3** representative animals).

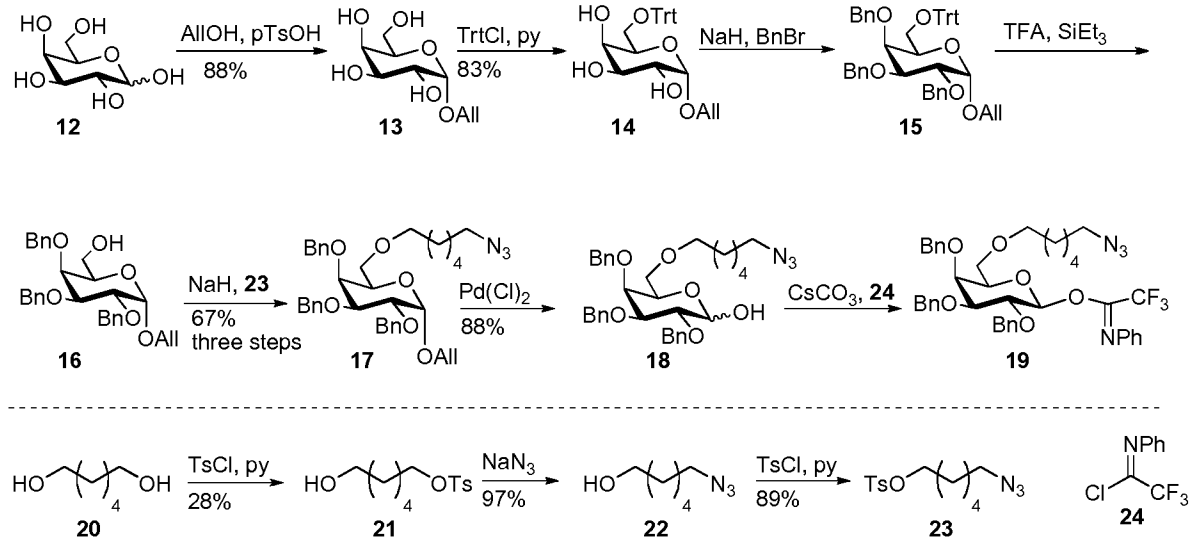
Example 5

Synthesis of the carbohydrate-glycolipid conjugate vaccine

Synthesis of the lipid portion of the conjugate vaccine started using Weinreb amide of *N*-Boc-L-serine **2** (**Scheme 1**) which was formed using EDCI as coupling reagent, *N*-methyl morpholine as base, and *N,O*-dimethyl hydroxylamine. Mixed *N,O*-acetal formation with 2,2-dimethoxypropane and catalytic amounts of $BF_3{\cdot}OEt_2$ yielded amide **3**. Reduction of the latter with lithium aluminium hydride at 0 C yielded *Garner's* aldehyde **4**. *Z*-Selective Wittig olefination using pentadecyltriphenylphosphonium ylide furnished alkene **5**. Removal of the acetal group on olefin **5** was followed by *Sharpless'* asymmetric dihydroxylation with AD-mix β and methylsulfonamide, furnishing *N*-Boc protected diol **6** in good yield and selectivity. Subsequent removal of the carbamate group furnished phytosphingosine **7**. Amide bond formation was performed with hexacosanoic *N*-hydroxy succinimidyl ester **11** and triethylamine as base, to yield **8**. Addition of TBSOTf and 2,6-lutidine yielded trisilyl ether **9**. The silyl ether on the primary hydroxyl group was then selectively removed with aq. TFA to give ceramide acceptor **10**. [2]



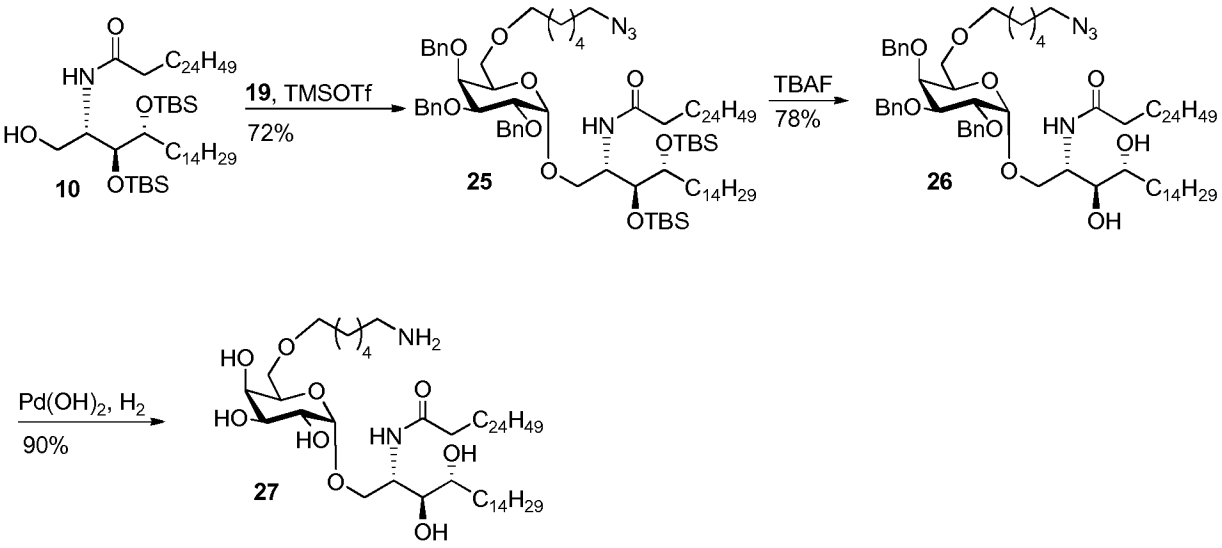
Scheme 1. Synthesis of lipid **10**. [1,2,3,4]



Scheme 2. Synthesis of galactosylating agent **19**.

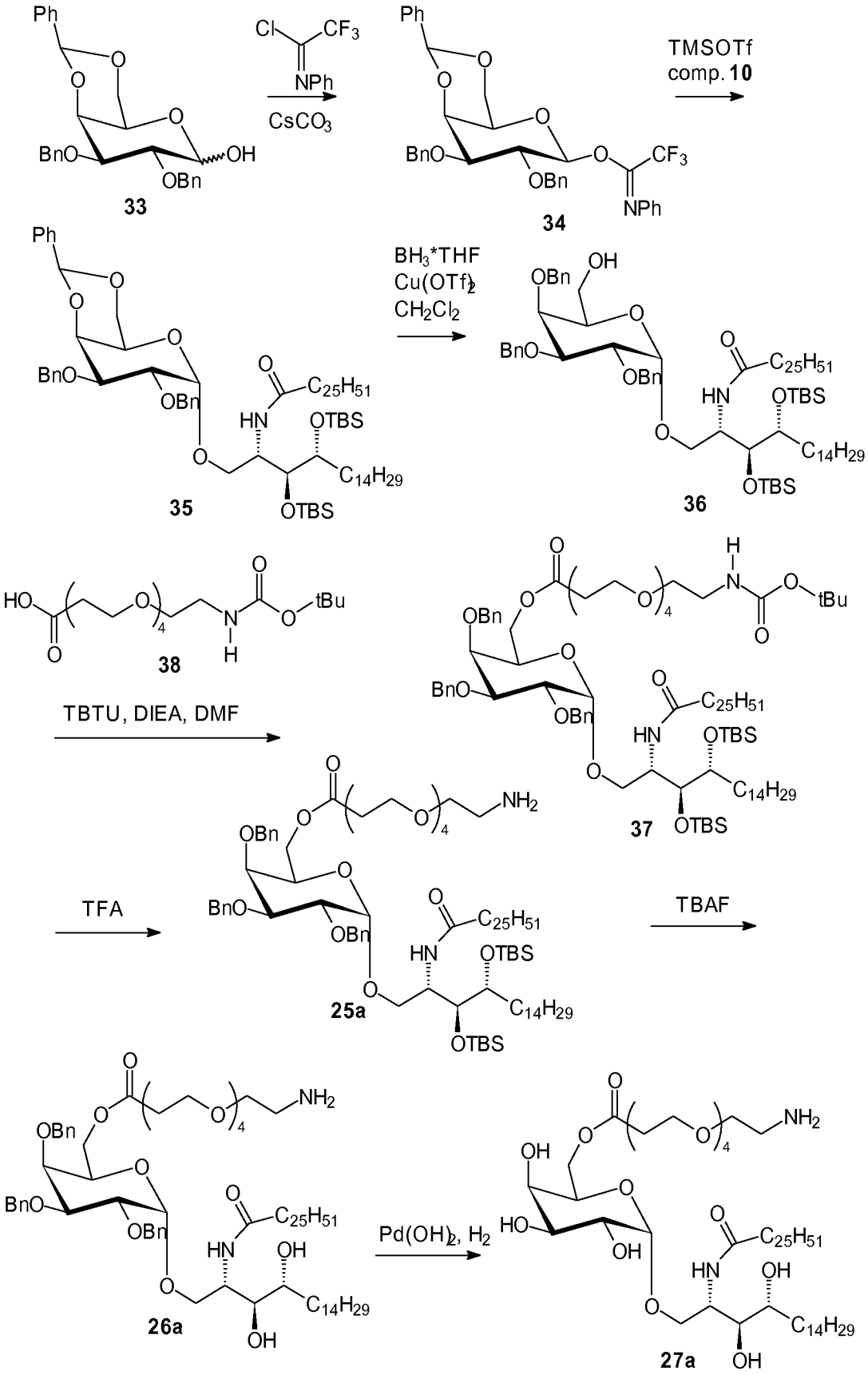
p-Toluensulfonic acid catalyzed *Fischer* glycosidation of galactose **12** with allyl alcohol yielded glycoside **13** (Scheme 2). [5] Subsequent tritylation of the primary C6 hydroxyl group yielded triol **14**. Benzyl ether formation with sodium hydride and benzylbromide furnished the fully protected galactose **15**. The trityl group was subsequently removed with trifluoroacetic acid and triethyl silane to free the C6 hydroxyl group for further functionalization. Williamson etherification of alcohol **16** and azide **23** using sodium hydroxide furnished galactose derivative **17**. Catalytic isomerization of the anomeric allyl protecting group to the corresponding enol ether with palladium(II) chloride and subsequent hydrolysis yielded lactol **18** which was converted into glycosyl imidate **19** with cesium carbonate and *N*-phenyltrifluoroacetimidazoyl chloride **24**.

Linker **23** was prepared starting from 1,6-hexanediol **20** which was reacted with tosyl chloride to yield a mixture of the corresponding mono- and di-tosylated product along with the starting material. After separation, the tosyl group of **21** was displaced by sodium azide to yield azide **22**. Subsequent tosylation of the hydroxyl group on **22** gave the tosylate **23**.



Scheme 3. Synthesis of linker-equipped glycolipid **27**.

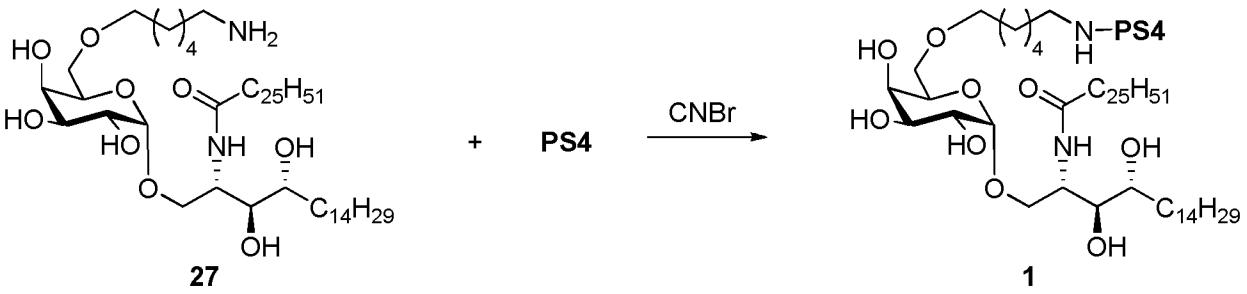
Linker-equipped glycolipid **25** (**Scheme 3**) was obtained via TMSOTf-catalyzed glycosylation of galactose building block **19** and ceramide **10**. The reaction proceeded in 72% yield and with complete α-selectivity. Removal of the silylether protecting groups with TBAF yielded diol **26** that was converted to **27** by hydrogenolysis with *Perlman's* catalyst.


Ph
Cl
CF3
NPh
CsCO3
BnO
OBn
OH
33
TMSOTf
comp. 10
34
BH3*THF
Cu(OTf)2
CH2Cl2
C25H51
HN
OTBS
C14H29
35
36
HO
tBu
38
TBTU, DIEA, DMF
NH2
37
TFA
25a
TBAF
26a
Pd(OH)2, H2
27a

Scheme 3. Alternative synthesis of linker-equipped glycolipid **27a**.

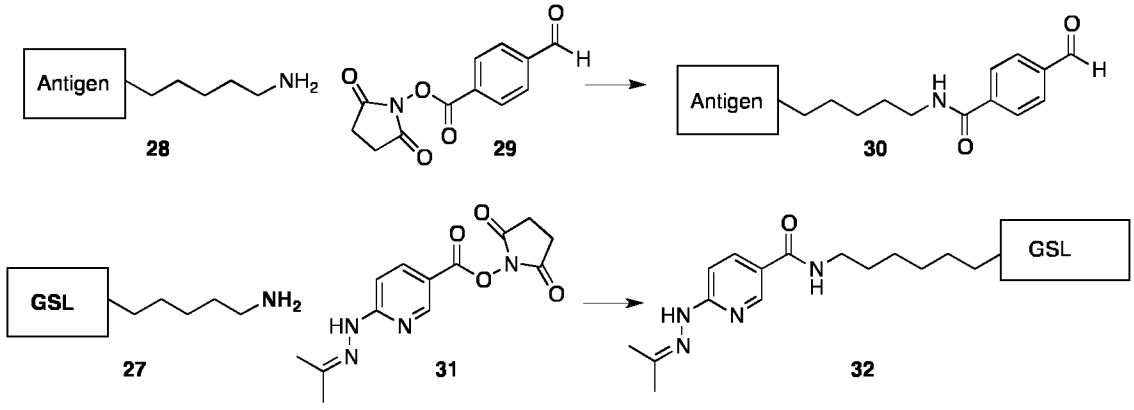
The glycolipid **36** was prepared in three steps from the known compound **33** by reacting the activated compound **34** with derivatives of compounds **10** by the above TMSOTf-catalyzed glycosylation of galactose building block. After deprotection of compound **35** the linker was introduced by condensation reaction with compound **38** in moderate yields. The linker-equipped glycolipid **27a** was subsequently prepared via intermediates **25a** and **26a** by complete deprotection of linker-epuipped compound **37**.

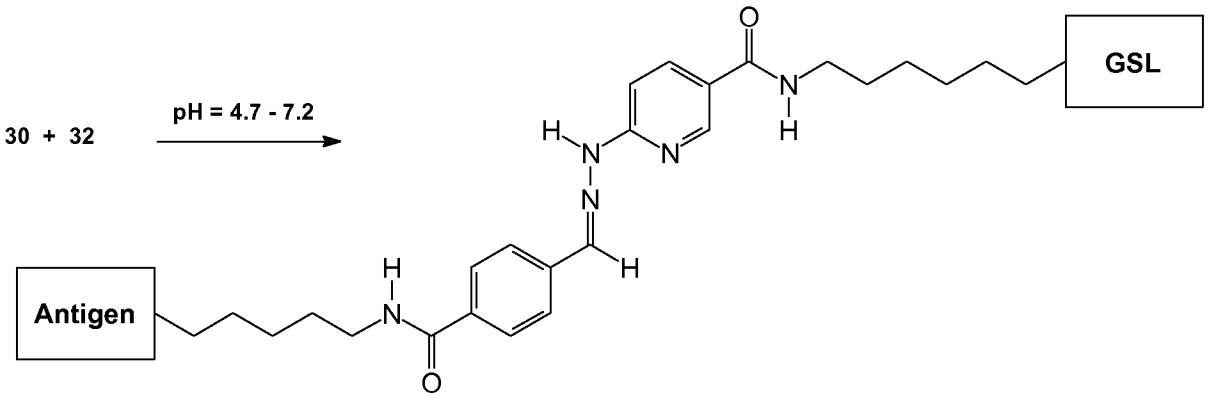
Conjugation of the polysaccharide to the glycolipid **27** was accomplished *via* a covalent linkage. To this end PS4 was activated with cyanogen bromide to which **27** was added in order to give conjugate **1** (**Scheme 4**).



Scheme 4. Conjugation of glycolipid 27 to PS4.

A hydrazone linkage provides an alternative conjugation method to link the epitope moiety with the glycolipid. To this end a hydrazone linkage can be used (**Scheme 5**). Antigen **28** has to be modified to aromatic aldehyde **30** using NHS-ester **29** and GSL **27** will be converted to hydrazone **32** using NHS-ester **31**. Coupling of aldehyde **30** and hydrazone **32** occurs at a pH of 4.7 to 7.2. The linker system is commercially available from Novabiochem (HydraLinK TM).





Scheme 5. Conjugation of amine linker-equipped antigen **28** with glycolipid **27** *via* hydrazone linkage.

Experimental procedures:

(*S*)-3-(*tert*-Butoxycarbonyl)-*N*-methoxy-2,2,*N*-trimethyloxazolidine-4-carboxamide (3)

To a solution of L-Boc-serine **2** (12.33 g, 60.1 mmol) in CH_2Cl_2 (240 mL) were added *N*,*O*-dimethylhydroxylamine hydrochloride (6.04 g, 61.9 mmol) and *N*-methylmorpholine (6.8 mL, 61.9 mmol) at 0 °C. To this solution was added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (11.86 g, 61.9 mmol) portionwise over a period of 20 min. and the solution was stirred for another 1 h. Then, aq. HCl solution (1.0 M, 30 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ solution (30 mL) and the aqueous layer was again extracted with CH_2Cl_2 (100 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed *in vacuo* to obtain the corresponding Weinreb amide (14.07 g, 94%) as white solid. R_f = 0.3 (EtOAc); ^{1}H NMR (250 MHz, *CDCl*$_3$) δ 5.60 (d, *J* = 6.0 Hz, 1 H), 4.77 (br s, 1 H), 1.42 (s, 9 H), 3.80 (d, *J* = 3.3 Hz, 2 H), 3.76 (s, 3 H), 3.21 (s, 3 H), 2.66 (br s, 1 H). The crude product was dissolved in acetone (180 mL) to which 2,2-dimethoxypropane (57 mL) and $BF_3{\cdot}Et_2O$ (0.5 mL) were added. The orange solution was stirred for 90 min. at r.t. and then quenched with Et_3N (1.2 mL) and solvents removed *in vacuo.* The crude product was purified by flash column chromatography on silica gel (gradient EtOAc/cyclohexane = 1:2 → 1:1) to yield isopropylidene-protected Weinreb amide **3** (15.32 g, 89% over two steps) as a white solid. The NMR spectra consist of two sets of signals due to the presence of rotamers. $[\alpha]_D^{r.t.}$ = –30.9 (*c* = 1, $CHCl_3$); R_f = 0.45 (Hexanes/EtOAc = 1:1); IR (film)

ν_{max} 2976, 2938, 1702, 1682, 1364, 1167, 1098, 998, 848, 768, 716; ^{1}H NMR (250 MHz, *CDCl*$_3$) δ 4.77 (dd, *J* = 9.8, 2.8 Hz, 1 H), 4.70 (dd, 7.5, 3.8, Hz, 1 H), 4.18 (dd, *J* = 7.5, 4.0 Hz, 1 H), 4.15 (dd, *J* = 7.8, 3.8 Hz, 1 H), 3.95 (dd, *J* = 9.3, 3.0 Hz, 1 H), 3.91 (dd, *J* = 9.0, 3.5 Hz), 3.72 (s, 3 H), 3.68 (s, 3 H), 3.19 (s, 6 H), 1.68 (s, 3 H), 1.66 (s, 3 H), 1.54 (s, 3 H), 1.50 (s, 3 H), 1.47 (s, 9 H), 1.39 (s, 9 H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ 171.4, 170.7, 152.2, 151.4, 95.1, 94.5, 80.6, 80.0, 66.2, 66.0, 61.3, 61.3, 57.9, 57.8, 28.5, 28.4, 25.8, 25.5, 24.8, 24.6; HR ESI Calcd for $C_{13}H_{24}N_2O_5$ [M+Na$^+$]: 311.1577 found: 311.1582.

tert-Butyl (*S*)-4-formyl-2,2-dimethyloxazolidine-3-carboxylate (4)

To a solution of Weinreb amide **3** (8.00 g, 27.7 mmol) in THF (100 mL) at 0 °C were added $LiAlH_4$ (1.0 M in THF, 13.9 mL, 13.9 mmol) dropwise and the solution was stirred for 1 h at 0 °C. After 1 h, the solution was cooled to –10 °C and $KHSO_4$ (1M, 70 mL) was added carefully and the solution was diluted with Et_2O (170 mL). The mixture was allowed to warm to r.t. and stirred for 30 min. The organic layer was separated, dried over $MgSO_4$, filtered and the solvent was removed *in vacuo* to yield *Garner's* aldehyde **4** as a pale yellow oil (6.24 g, > 95% purity by ^{1}H NMR). The NMR spectra consist of two sets of signals due to the presence of rotamers. ^{1}H NMR (250 MHz, *CDCl*$_3$) δ 9.58 (d, *J* = 0.8 Hz, 1H), 9.52 (d, *J* = 2.5 Hz, 1 H), 4.32 (m, 1 H), 4.16 (m, 1 H), 4.06 (m, 4 H), 1.53–1.63 (m, 12 H), 1.49 (s, 9 H), 1.40 (s, 9 H). The crude product was used in the subsequent reaction without further purification.

(4*R*,1'*Z*)-3-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-(1'-hexadecenyl)oxazolidine (5)

n-BuLi (1.6 M in hexane, 25.2 mL, 40.3 mmol) was added dropwise to pentadecyltriphenylphosphonium bromide (24.03 g, 43.4 mmol) in anhydrous THF (220 mL) at –78 °C. The resulting orange solution was allowed to warm to 0 °C and stirred for another 30 min. The solution was then cooled to –78 °C and *Garner's* aldehyde **4** (6.23 g, 27.2 mmol) in anhydrous THF (30 mL) was added slowly. After being stirred for 2 h at r.t., the reaction was diluted with sat. aq. NH_4Cl solution (35 mL) and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 35 mL) and the combined organic extracts were washed with sat. aq. NaCl solution (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash column chromatography on silica (EtOAc/Hexanes = 1:2) gel gave (*Z*)-olefin **5** as a pale yellow oil (11.27 g, 78%). $[\alpha]_D^{r.t.}$ = +45.2 (*c* = 1, $CHCl_3$); R_f = 0.40 (EtOAc/Hexanes = 1:2); IR (film) ν_{max} 2923, 2854, 1699, 1457, 1382, 1251, 1175, 1093, 1056, 850, 768 cm^{-1}; ^{1}H NMR (250 MHz, *CDCl*$_3$) δ 5.27–5.40 (m, 2 H), 4.58 (br s, 1 H), 4.02 (dd, *J* = 6.3, 8.8 Hz, 1 H), 3.61 (dd, *J* = 3.3, 8.5 Hz, 1 H), 1.96 (br s, 2 H), 1.23–1.56 (m, 39 H), 0.85 (t, *J* = 7 Hz, 3 H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ 152.1, 130.9, 130.4, 94.1,

79.8, 69.2, 54.7, 32.1, 29.9, 29.8, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 28.6, 28.6, 27.6, 22.8, 14.2; HR ESI Calcd for $C_{26}H_{49}NO_3$ [M+Na+]: 446.3605 found: 446.3614. All spectral data were in good accordance with reported data. [4]

The desired (*Z*)-olefin can easily be distinguished from the undesired (*E*)-olefin by-product, when considering the olefinic protons in the ^{1}H NMR spectrum: *Z*-**5** ^{1}H NMR (250 MHz, *CDCl*$_3$) δ 4.05 (dd, *J* = 6.3, 8.6 Hz, l H), 3.64 (dd, *J* = 3.3, 8.6 Hz, 1 H) cf. *E*-**5** ^{1}H NMR (250 MHz, *CDCl*$_3$) δ4.01 (dd, *J* = 6.1, 8.7 Hz, 1 H), 3.71 (dd, *J* = 2.1, 8.7 Hz, 1 H).

Pentadecyltriphenylphosphonium bromide

A solution of 1-bromopentadecane (30.0 g, 103 mmol) and triphenylphosphine (27.02 g, 103 mmol) in MeCN (200 mL) was refluxed at 80 °C for five days. After removal of the solvent *in vacuo*, Et_2O (30 mL) was added and the resulting white precipitate was filtered off, washed with Et_2O and dried on high vacuum for 24 h to give pentadecyltriphenylphosphonium bromide (49.66 g, 87%) as a white powder.

(2*R*,3*Z*)-2-(*tert*-Butoxycarbonyl)amino-3-octadecen-1-ol (5b)

Para-Toluensulfonic acid (371 mg, 1.95 mmol) was added to a stirred solution of (*Z*)-olefin **5** (5.00 g, 12.2 mmol) in MeOH/water (50 mL total, ratio = 9:1 v/v) and the mixture was stirred for 68 h. The reaction mixture was concentrated *in vacuo* to yield a white solid, which was re-dissolved in CH_2Cl_2 (100 mL). The solution was washed with brine (30 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo*. Purification by flash column chromatography on silica gel (gradient cyclohexane/EtOAc = 4:1 → 2:1) afforded alcohol **5b** as a white solid (2.71 g, 59%). All spectral data were in good accordance with reported data.

(2*S*,3*S*,4*R*)-2-(*tert*-Butoxycarbonyl)amino-1,3,4-octadecanetriol (6)

Alcohol **5b** (1.50 g, 3.91 mmol) was dissolved in *t*-BuOH/water (38 mL total, ratio 1:1) and methanesulfonamide (371 mg, 3.91 mmol) was added. The reaction mixture was cooled to 0 C and AD-mix-β (5.48 g) was added. The resulting mixture was stirred at 0°C for 41 h and another 7 h at r.t., then it was quenched by the addition of solid Na_2SO_3 (6.0 g) and left to stir for 30 min. Extraction with EtOAc (3 x 40 mL) followed. The organic extracts were washed with NaOH (1 M, 20 mL), water (20 mL) and sat. aq. NaCl solution (20 mL), dried over $MgSO_4$ and solvents were removed *in vacuo*. Purification by flash column chromatography on silica gel (gradient EtOAc/cyclohexane = 1:1 → 2:1) provided triol **6** as a white solid (1.05 g, 64%).

Phytosphingosine (7)

Triol **6** (60 mg, 0.14 mmol) was dissolved in TFA (0.6 mL) and stirred at r.t. for 30 min. The solution was diluted with CH_2Cl_2 (1.5 mL) and then carefully neutralized (to pH ~ 8) with sat. aq. $NaHCO_3$ solution (10 mL) upon which precipitation of a white solid occured. The white solid removed by filtration, washed with water (3 x 10 mL) and dried under reduced pressure. Recrystallization from MeCN yielded phytosphingosine **7** as a white powder (20 mg, 43%).

Ceramide (8)

To a solution of phytosphingosine **7** (15 mg, 0.047 mmol) in anhydrous THF (1 mL) was added hexacosanoic acid succinimidyl ester **11** (34 mg, 0.071 mmol) and Et_3N (24 µL, 0.14 mmol). The solution was heated to 50 °C and stirred for 20 h. EtOAc (5 mL) was added and the resulting suspension was centrifuged (30 min., 3000 rpm). The white precipitate was removed by filtration and dried under reduced pressure to yield amide **8** (29 mg, 88%).

Hexacosanoic *N*-hydroxysuccinimidyl ester (11)

To a solution of hexacosanoic acid (121 mg, 0.304 mmol) in CH_2Cl_2 (4 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.058 mL, 0.33 mmol) and *N*-hydroxysuccinimide (42 mg, 0.37 mmol). The reaction mixture was heated to 40 °C, stirred for 3 h and then quenched with water (4 mL). The solution was diluted with Et_2O (8 mL) and the two layers were separated. The aqueous phase was extracted with Et_2O (8 mL) and the combined organic layers were washed with sat aq. NaCl solution (5 mL), dried over $MgSO_4$ and filtered. After removal of the solvent *in vacuo*, *N*-hydroxysuccinimidyl ester **11** was obtained as a white solid (85 mg, 57%).

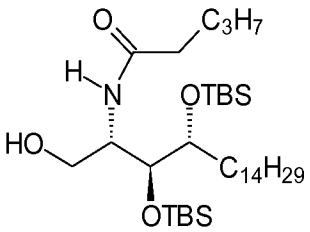
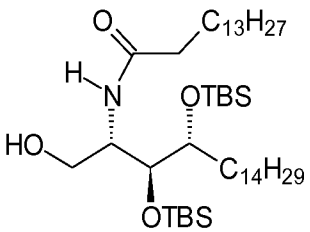
(2*S*,3*S*,4*R*)-1,3,4-Tri-*t*-butyl-dimethylsilyloxy-2-hexacosanoylamino-1-octadecane (9)

To a stirred suspension of amide **8** (25 mg, 0.036 mmol) in CH_2Cl_2 (1.2 mL) was added TBSOTf (43 µL, 0.18 mmol) and 2,6-lutidine (65 µL, 0.054 mmol) at 0 °C. The reaction mixture was stirred at r.t. for 2 h. The reaction was quenched with MeOH (0.2 mL). The mixture was diluted with Et_2O (2 mL) and washed with sat. aq. $NaHCO_3$ solution (1 mL) and sat. aq. NaCl solution (1 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (cyclohexane/Et_2O = 15:1) to give TBS protected ceramide **9** as a colorless oil (27 mg, 71%).

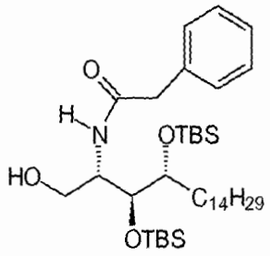
(2*S*,3*S*,4*R*)-3,4-Bis-*tert*-butyldimethylsilyloxy-2-hexacosanoylamino-4-octadecanol (10)

To a solution of ceramide **9** (90 mg, 0,087 mmol) in THF (2 mL) was added TFA (40 µL, 0.519 mmol) in water (0.5 mL, 27.8 mmol) at –10 °C. The reaction mixture was left to warm to 10 °C over a period of 2 h. Then, the reaction mixture was quenched by the addition of sat. aq. $NaHCO_3$ solution until neutral pH was reached. The resulting mixture was diluted with Et_2O (10 mL), washed with water (10 mL), sat. aq. $NaHCO_3$ (10 mL), sat. aq. NaCl solution (10 mL), and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography on silica gel (gradient EtOAc/cyclohexane = 10:1 → 5:1) to yield alcohol **10** (68 mg, 85%) as a colorless oil. $[\alpha]_D^{r.t.}$ = –11.6 (*c* = 1, $CHCl_3$); R_f = 0.3 (cyclohexane/EtOAc = 4:1); IR (film) ν_{max} 3285, 2920, 2851, 1645, 1465, 1253, 1034, 835, 776, 721, 680 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.27 (d, *J* = 7.8 Hz, 1H), 4.21 (dd, *J* = 11.3, 3.0 Hz, 1H), 4.06 (td, *J* = 6.5, 3.2 Hz, 1H), 3.91 (t, *J* = 2.8 Hz, 1H), 3.76 (td, *J* = 6.4, 2.6 Hz, 1H), 3.59 (dd, *J* = 11.3, 3.7 Hz, 1H), 2.24 – 2.14 (m, 2H), 1.69 – 1.45 (m, 4H), 1.45 – 1.16 (m, 68H), 0.92 (s, 9H), 0.90 (s, 9H), 0.87 (t, *J* = 6.9 Hz, 6H), 0.11 (s, 6H), 0.08 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.8, 77.6, 76.6, 63.8, 51.4, 37.1, 34.6, 32.1, 30.0, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 26.2, 26.1, 26.0, 25.8, 22.8, 18.3, 18.3, 14.3, -3.6, -3.9, -4.4, -4.8; HR ESI Calcd for $C_{56}H_{117}NO_4Si_2$ [M+Na^+]: 924.8594 found: 924.8604.

According to the synthetic procedure for compound **10** starting from compound **2** derivatives **10a** to **10o** were prepared accordingly using the respective triphenylphosphonium bromides in the reaction of compound **4** to compound **5** and the corresponding compounds **11** in the conversion of compounds **7** to compounds **8**:

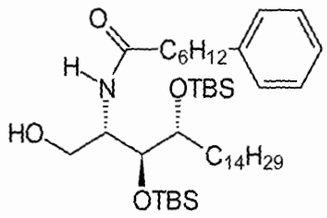
comp.	structure	mass spec
10a		$C_{35}H_{75}NO_4Si_2$ Calc.: 631.1544 [M+H^+] Found: 631.1521
10b		$C_{45}H_{95}NO_4Si_2$ Calc.: 771.4206 [M+H^+] Found: 771.4181

10c



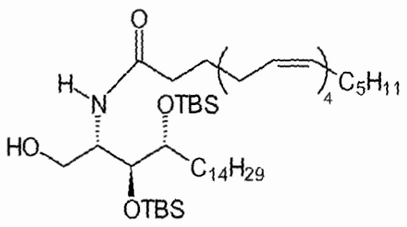
$C_{38}H_{73}NO_4Si_2$
Calc.: 665.1707 [M+H$^+$]
Found: 665.1733

10d



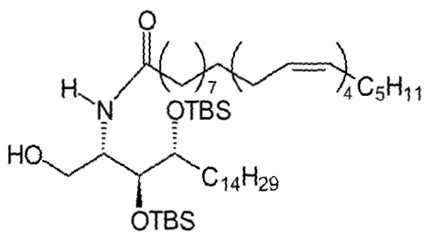
$C_{43}H_{83}NO_4Si_2$
Calc.: 735.3038 [M+H$^+$]
Found: 735.3001

10e



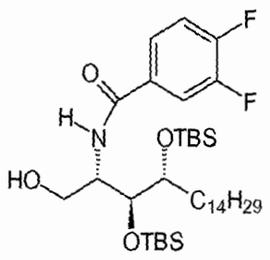
$C_{50}H_{97}NO_4Si_2$
Calc.: 833.4901 [M+H$^+$]
Found: 833.4887

10f



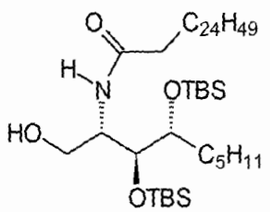
$C_{56}H_{109}NO_4Si_2$
Calc.: 917.6498 [M+H$^+$]
Found: 917.6528

10g



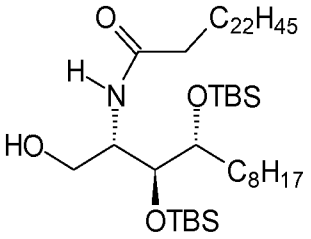
$C_{37}H_{69}F_2NO_4Si_2$
Calc.: 687.1250 [M+H$^+$]
Found: 687.1212

10h



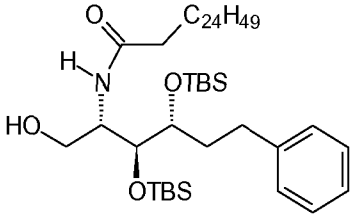
$C_{47}H_{99}NO_4Si_2$
Calc.: 799.4738 [M+H$^+$]
Found: 799.4791

10i



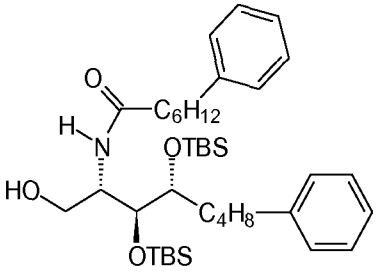
$C_{48}H_{101}NO_4Si_2$
Calc.: 813.5004 [M+H$^+$]
Found: 813.4962

10j



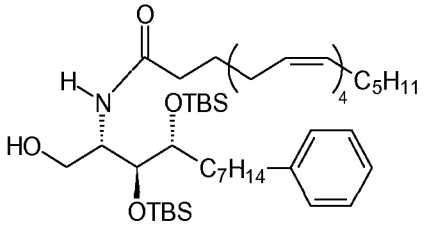
$C_{50}H_{97}NO_4Si_2$
Calc.: 833.4901 [M+H$^+$]
Found: 833.4913

10k



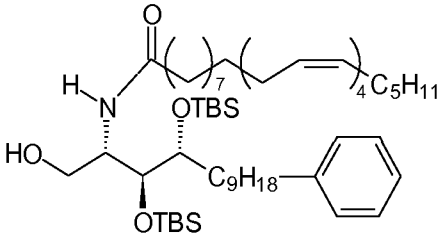
$C_{39}H_{67}NO_4Si_2$
Calc.: 671.1338 [M+H$^+$]
Found: 671.1306

10l



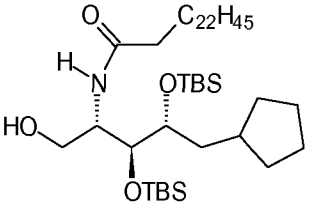
$C_{49}H_{87}NO_4Si_2$
Calc.: 811.4000 [M+H$^+$]
Found: 811.4063

10m



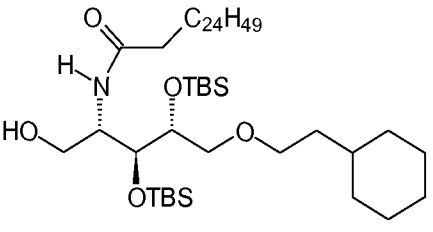
$C_{57}H_{103}NO_4Si_2$
Calc.: 923.6129 [M+H$^+$]
Found: 923.6097

10n



$C_{46}H_{95}NO_4Si_2$
Calc.: 783.4313 [M+H$^+$]
Found: 783.4281

10o



$C_{51}H_{105}NO_5Si_2$
Calc.: 869.5638 [M+H$^+$]
Found: 869.5604

1-*O*-Allyl α-D-galactopyranoside (13)

To a stirred suspension of D-galactose **12** (22.2 g, 123 mmol) in allyl alcohol (250 mL) was added *para*-toluenesulfonic acid (2.3 g, 12.09 mmol). The mixture was heated to 100 °C and stirred for 24 h after which it was cooled to r.t. and quenched by the addition of NEt_3. The solvent was removed *in vacuo* and the crude product was co-evaporated twice with toluene and purified by flash column chromatography on silica gel (gradient CH_2Cl_2/MeOH = 9:1 → 4:1). Recrystalization from EtOAc yielded galactoside **13** (22.2 g, 88%) as a white solid.

1-*O*-Allyl 6-*O*-trityl-α-D-galactopyranoside (14)

1-*O*-Allyl-galactoside **13** (4 g, 18.2 mmol) was dissolved in pyridine (18 mL). To the solution was added trityl chloride (6.58 g, 23.6 mmol) and the mixture was stirred at r.t. for 18 h after which the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH = 10:1) to yield pyranoside **14** (7.0 g, 83%) as colorless oil. $[\alpha]_D^{r.t.}$ = +60.0 (*c* = 1, $CHCl_3$); R_f = 0.8 (CH_2Cl_2/MeOH = 5:1); IR (film) ν_{max} 3402, 2929, 1491, 1449, 1218, 1152, 1070, 1032, 746, 703 cm^{-1}; ^1H NMR (400 MHz, *CDCl*$_3$) δ 7.51–7.18 (m, 15H), 5.99–5.88 (m, 1H), 5.25 (ddq, *J* = 35.9, 10.4, 1.4 Hz, 2H), 4.95 (d, *J* = 3.8 Hz, 1H), 4.25 (ddt, *J* = 12.8, 5.4, 1.4 Hz, 1H), 4.05 (ddt, *J* = 12.8, 6.3, 1.3 Hz, 1H), 3.96 (s, 1H), 3.89 (t, *J* = 5.8 Hz, 1H), 3.81 (d, *J* = 5.7 Hz, 1H), 3.75 (d, *J* = 9.8 Hz, 1H), 3.47 (s, 1H), 3.43 (dd, *J* = 9.8, 6.1 Hz, 1H), 3.32 (dd, *J* = 9.8, 5.3 Hz, 1H), 2.86 (d, *J* = 2.1 Hz, 1H), 2.71 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, *CDCl*$_3$) δ 143.8, 133.7, 128.6, 127.8, 127.1, 117.8, 97.5, 86.9, 71.2, 69.8, 69.5, 69.5, 68.5, 63.3; HR ESI Calcd for $C_{25}H_{25}O_5$ [M+Na^+]: 485.1935 found: 485.1941.

1-*O*-Allyl 2,3,4-*tri*-*O*-benzyl-6-*O*-trityl-α-D-galactopyranoside (15)

To a solution of allyl 6-*O*-trityl-α/β-D-galactopyranoside **14** (3.7 g, 8.0 mmol) in DMF (32 mL) was added sodium hydride (60% in mineral oil, 1.50 g, 36.0 mmol) portionwise at r.t. After 1 h benzyl bromide (4.2 mL, 35.2 mmol) was added. The reaction mixture was left to stir for 48 h after which it was quenched by the addition of MeOH (5 mL). The mixture was diluted with Et_2O and extracted twice from sat. aq. $NaHCO_3$. The combined organic layer was washed with water (3 x 100 mL) and sat. aq. NaCl solution and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was over a plug of silica gel (hexanes/EtOAc = 2:1, silica gel was neutralized with 1% NEt_3) to yield the benzyl ether **15** (5.5 g) as a pale yellow oil which was used in the subsequent step without further purification.

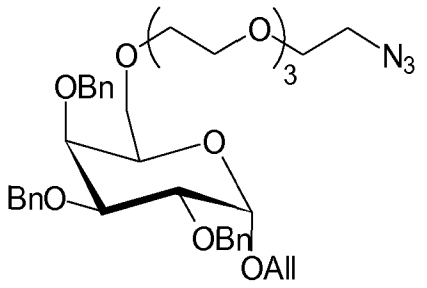
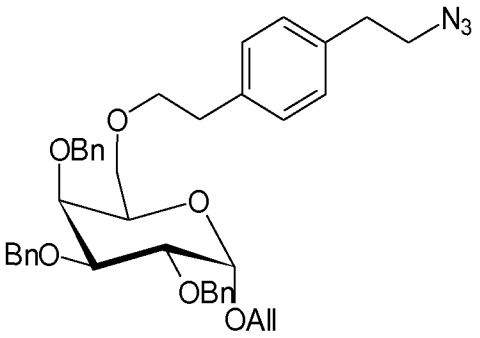
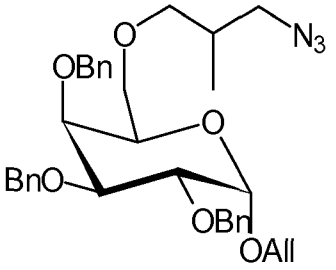
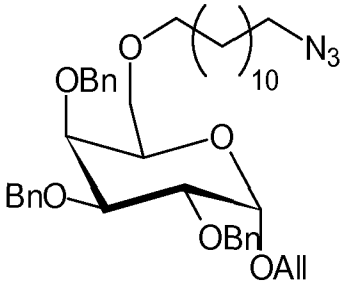
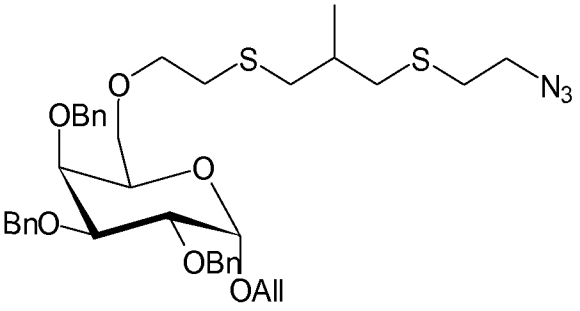
1-*O*-Allyl 2,3,4-*tri*-*O*-benzyl-α-D-galactopyranoside (16)

A solution of allyl 2,3,4-*tri*-*O*-benzyl-6-*O*-trityl-α-D-galactopyranoside **15** (5.00 g, 6.82 mmol) and triethyl silane (5.45 mL, 34.1 mmol) in CH_2Cl_2 (68 mL) was cooled to 0 °C. To the stirred solution was added trifluoroacetic acid (2.6 mL, 34.1 mmol) dropwise. The mixture was quenched after 15 min. with sat. aq. $NaHCO_3$ solution and extracted with CH_2Cl_2. The crude product was filtered over a plug of silica gel. All silane and trityl residues were removed with 10:1 hexanes/EtOAc and the product was eluted with EtOAc to yield **16** (3.0 g) as a pale yellow oil which was used without further purification in the subsequent reaction.

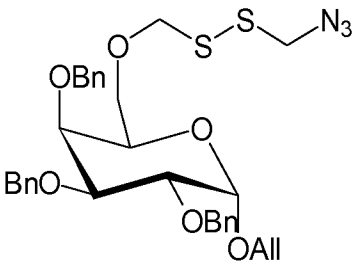
1-*O*-Allyl 6-(6'-azidohexyl)-2,3,4-*tri*-*O*-benzyl-α-D-galactopyranoside (17)

To a solution of allyl 2,3,4-*tri*-*O*-benzyl-α-D-galactopyranoside **16** (1.0 g, 2.04 mmol) in DMF (10 mL) was added sodium hydride (60% in mineral oil, 0.12 g, 3.1 mmol) at 0 °C. After 15 min, the mixture was warmed to r.t. and stirred for another 1 h. Then, 6-azidohexyl 4-methylbenzenesulfonate **23** (0.9 g, 3.1 mmol) was added and the reaction mixture was stirred at r.t. for a further 8 h after which the mixture was quenched by the addition of MeOH (2 mL). After dilution with CH_2Cl_2, sat. aq. NH_4Cl solution was added and the mixture was extracted with CH_2Cl_2 (3 x). The combined organic layer was washed with water and sat. aq. NaCl solution. The organic layer was dried over $MgSO_4$, the solvent was removed *in vacuo* and the crude product was purified by flash column chromatography on silica gel (gradient hexanes/EtOAc = 1:0 → 1:1) to yield azide **17** (1.0 g, 68% over three steps) as a colorless oil. $[\alpha]_D^{r.t.}$ = +25.4 (*c* = 1, $CHCl_3$); R_f = 0.65 (Hexanes/EtOAc = 4:1); IR (film) ν_{max} 2933, 2863, 2094, 1497, 1454, 1358, 1177, 1098, 1059, 926, 816, 736, 697 cm^{-1}; ^1H NMR (400 MHz, *CDCl*$_3$) δ 7.94 – 7.16 (m, 15H), 5.95 (dddd, *J* = 17.1, 10.3, 6.6, 5.2 Hz, 1H), 5.31 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.21 (ddd, *J* = 10.3, 2.8, 1.1 Hz, 1H), 5.01 – 4.58 (m, 7H), 4.17 (ddt, *J* = 13.0, 5.2, 1.4 Hz, 1H), 4.09 – 3.99 (m, 3H), 3.98 – 3.90 (m, 2H), 3.50 – 3.18 (m, 6H), 1.72 – 1.47 (m, 4H), 1.44 – 1.30 (m, 4H); ^{13}C NMR (75 MHz, *CDCl*$_3$) δ 138.9, 138.8, 138.6, 134.0, 129.8, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.6, 127.5, 127.4, 117.9, 96.3, 79.1, 76.5, 75.3, 74.7, 73.3, 73.3, 71.3, 70.3, 69.5, 69.4, 68.2, 51.4, 51.2, 29.6, 28.8, 28.7, 28.6, 26.6, 26.1, 25.7, 25.0, 21.6. HR ESI Calcd for $C_{36}H_{45}N_3O_6$ [M+Na^+]: 638.3201 found: 638.3229.

The below compounds were prepared according to the synthetic procedure above with the corresponding compounds **23** in moderate to high yields:

comp.	structure	mass spec
17a		$C_{38}H_{50}N_3O_9$ Calc.: 693.8278 [M+H$^+$] Found: 693.8241
17b		$C_{36}H_{46}N_3O_6$ Calc.: 617.7764 [M+H$^+$] Found: 617.7721
17c		$C_{34}H_{42}N_3O_6$ Calc.: 589.7231 [M+H$^+$] Found: 589.7274
17d		$C_{42}H_{58}N_3O_6$ Calc.: 701.9361 [M+H$^+$] Found: 701.9400
17e		$C_{38}H_{50}N_3S_2O_6$ Calc.: 709.9618 [M+H$^+$] Found: 709.9651

17f

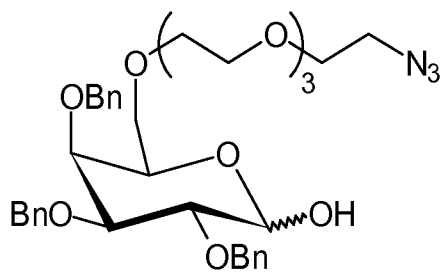


$C_{32}H_{38}N_3S_2O_6$
Calc.: 625.8021 [M+H$^+$]
Found: 625.7996

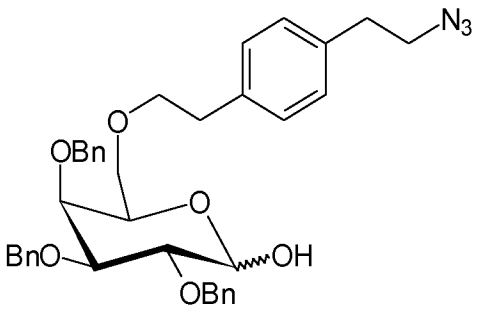
6-(6'-Azidohexyl)-2,3,4-*tri*-*O*-benzyl-α/β-D-galactopyranose (18)

Allyl 6-(6'-azidohexyl)-2,3,4-*tri*-*O*-benzyl-α-D-galactopyranoside **17** (1.4 g, 2.3 mmol) was dissolved in MeOH (16 mL) and $PdCl_2$ (0.21 g, 1.17 mmol) was added to the solution at r.t. The mixture was stirred at for 4 h after which the mixture was filtered over celite and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (gradient hexanes/EtOAc = 1:0 → 1:1) to yield lactol **18** (1.2 g, 88%) as a colorless oil. R_f = 0.50 (Hexanes/EtOAc = 2:1); IR (film) ν_{max} 3414, 2933, 2862, 2093, 1454, 1255, 1060, 910, 733, 696 cm^{-1}; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ 7.45 – 7.20 (m, 30H), 5.33 – 5.27 (m, 1H), 5.01 – 4.90 (m, 3H), 4.85 – 4.71 (m, 7H), 4.66 (ddd, *J* = 16.7, 11.5, 6.0 Hz, 3H), 4.18 – 4.09 (m, 1H), 4.05 (dd, *J* = 9.2, 3.6 Hz, 1H), 3.96 (s, 2H), 3.93 (d, *J* = 2.8 Hz, 1H), 3.88 (d, *J* = 2.8 Hz, 1H), 3.78 (dd, *J* = 9.6, 7.5 Hz, 1H), 3.63 – 3.52 (m, 3H), 3.52 – 3.37 (m, 5H), 3.37 – 3.28 (m, 2H), 3.28 – 3.21 (m, 5H), 1.65 – 1.49 (m, 8H), 1.42 – 1.24 (m, 8H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ 138.8, 138.7, 138.5, 138.4, 128.5, 128.5, 128.4, 128.3, 128.3, 128.3, 128.3, 128.1, 127.9, 127.7, 127.7, 127.7, 127.6, 127.6, 97.9, 92.0, 82.3, 80.9, 78.8, 76.7, 75.2, 74.9, 74.8, 74.7, 73.8, 73.7, 73.6, 73.1, 73.1, 71.5, 71.4, 69.6, 69.6, 69.5, 51.5, 29.5, 28.9, 26.6, 25.8; HR ESI Calcd for $C_{33}H_{41}N_3O_6$ [M+Na$^+$]: 598.2883 found: 598.2869.

The below compounds were prepared according to the synthetic procedure above with the corresponding compounds **17** in average good yields:

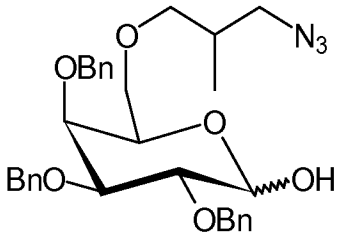
comp.	structure	mass spec
18a		$C_{35}H_{46}N_3O_9$ Calc.: 653.7638 [M+H$^+$] Found: 653.7601

18b



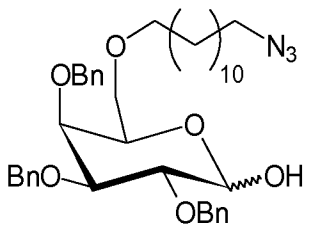
$C_{33}H_{42}N_3O_6$
Calc.: 577.7124 [M+H$^+$]
Found: 577.7193

18c



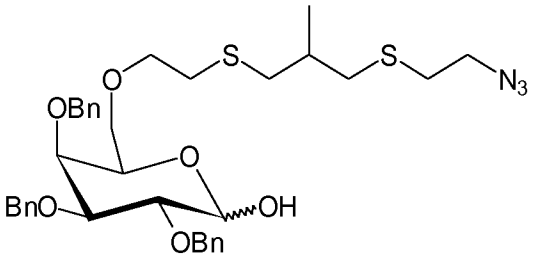
$C_{31}H_{38}N_3O_6$
Calc.: 549.6592 [M+H$^+$]
Found: 549.6556

18d



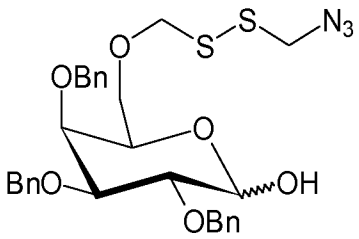
$C_{39}H_{54}N_3O_6$
Calc.: 661.8721 [M+H$^+$]
Found: 661.8791

18e



$C_{35}H_{46}N_3S_2O_6$
Calc.: 669.8978 [M+H$^+$]
Found: 669.9003

18f



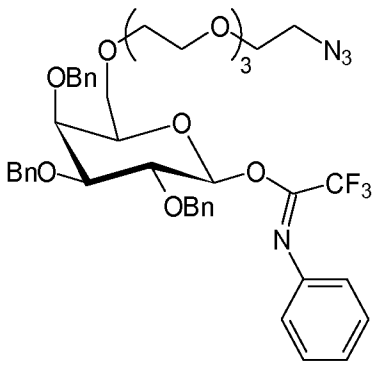
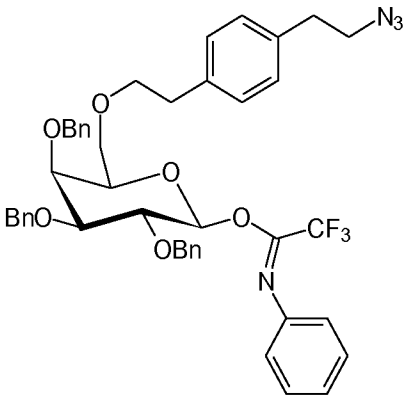
$C_{29}H_{34}N_3S_2O_6$
Calc.: 585.7381 [M+H$^+$]
Found: 585.7323

6-(6'-Azidohexyl)-2,3,4-tri-*O*-benzyl -β-D-galactopyranosyl *N*-phenyl trifluoro-acetimidate (19)

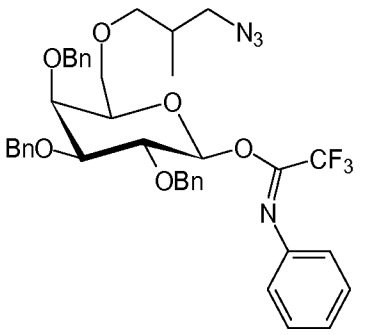
To a solution of 6-(6'-azidohexyl)-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranose **18** (400 mg, 0.70 mmol) in CH_2Cl_2 (7 mL) was added cesium carbonate (340 mg, 1.04 mmol). To the mixture was added 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride **24** (216 mg, 1.04 mmol) and the reaction mixture was stirred at r.t. for 3.5 h after which it was filtered over celite and washed with CH_2Cl_2. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography on silica gel

(gradient hexanes/EtOAc = 10:1 → 1:1) to yield the imidate **19** (490 mg, 94%) as a colorless oil. $[\alpha]_D^{r.t.}$ = +60.8 (*c* = 0.4, $CHCl_3$); R_f = 0.80 (Hexanes/EtOAc = 2:1); IR (film) ν_{max} 3064, 2934, 2865, 2094, 1717, 1598, 1454, 1321, 1207, 1099, 1027, 910, 734, 696 cm^{-1}; ^1H NMR (400 MHz, *CDCl*$_3$) δ 7.45 – 6.60 (m, 20H), 5.56 (s, 1H), 4.90 (d, *J* = 11.5 Hz, 1H), 4.75 (s, *J* = 1.5 Hz, 2H), 4.68 (s, *J* = 12.4 Hz, 2H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.00 (t, *J* = 8.7 Hz, 1H), 3.84 (d, *J* = 2.4 Hz, 1H), 3.58 – 3.39 (m, 4H), 3.34 (dt, *J* = 9.3, 6.5 Hz, 1H), 3.23 (dt, *J* = 9.3, 6.5 Hz, 1H), 3.14 (t, *J* = 6.9 Hz, 2H), 1.52 – 1.38 (m, 4H), 1.32 – 1.16 (m, 4H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ 138.6, 138.3, 138.2, 128.8, 128.6, 128.5, 128.4, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 124.3, 119.4, 82.3, 78.3, 77.4, 77.2, 76.8, 75.7, 74.9, 74.6, 73.4, 73.2, 71.4, 68.7, 51.5, 29.7, 28.9, 26.7, 25.8; HR ESI Calcd for $C_{41}H_{45}F_3N_4O_6$ [M+Na$^+$]: 769.3183 found: 769.3239.

The below compounds were prepared according to the synthetic procedure above with the corresponding compounds **18** in average moderate to good yields:

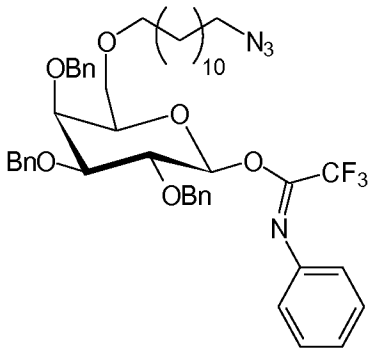
comp.	structure	mass spec
19a		$C_{43}H_{50}F_3N_4O_9$ Calc.: 824.8834 [M+H$^+$] Found: 824.8804
19b		$C_{41}H_{46}F_3N_4O_6$ Calc.: 748.8320 [M+H$^+$] Found: 748.8299

19c



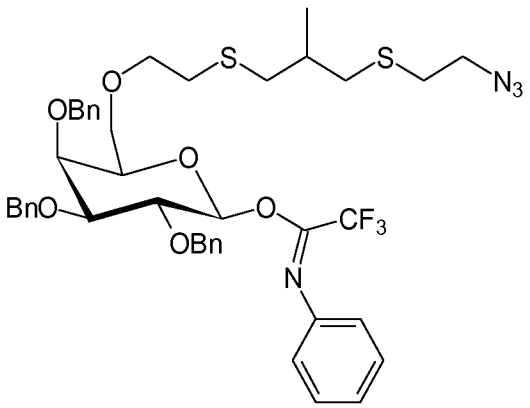
$C_{39}H_{42}F_3N_4O_6$
Calc.: 720.7788 [M+H$^+$]
Found: 720.7712

19d



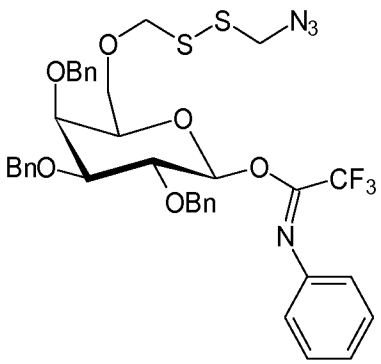
$C_{47}H_{58}F_3N_4O_6$
Calc.: 832.9917 [M+H$^+$]
Found: 832.9977

19e



$C_{43}H_{50}F_3N_4S_2O_6$
Calc.: 841.0174 [M+H$^+$]
Found: 841.0108

19f



$C_{37}H_{38}F_3N_4S_2O_6$
Calc.: 756.8577 [M+H$^+$]
Found: 756.8506

6-Hydroxyhexyl 4-methylbenzenesulfonate (21)

To a solution of hexane-1,6-diol **20** (10.0 g, 85 mmol) in CH_2Cl_2 (200 mL) was added 4-methylbenzene-1-sulfonyl chloride (17.8 g, 93 mmol) dissolved in pyridine (100 mL) at 5° C dropwise over 15 min. The reaction mixture was warmed to r.t. over the period of 5 h. Solvents were removed *in vacuo* and the crude was purified by silica

flash column chromatography (gradient hexanes/EtOAc = 1:0 → 1:1) to afford monotosylated hexanediol **21** (6.5 g, 28%) as a colorless oil. R_f = 0.55 (Hexanes/EtOAc = 1:1); IR (film) ν_{max} 3381, 2935, 2862, 1598, 1461, 1352, 1172, 959, 921, 813, 661 cm^{-1}; ^1H NMR (400 MHz, *CDCl*$_3$) δ 7.76 – 7.71 (m, 2H), 7.29 (dt, *J* = 4.3, 1.2 Hz, 2H), 3.97 (t, *J* = 6.5 Hz, 2H), 3.55 (t, *J* = 6.5 Hz, 2H), 2.40 (s, 3H), 1.65 – 1.56 (m, 2H), 1.55 (s, 1H), 1.52 – 1.41 (m, 2H), 1.36–1.18 (m, 4H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ 144.7, 133.1, 129.8, 127.8, 70.5, 62.6, 32.4, 28.7, 25.1, 25.0, 21.6; HR ESI Calcd for $C_{13}H_{20}O_4S$ [M+Na^+]: 295.0975 found: 295.0968.

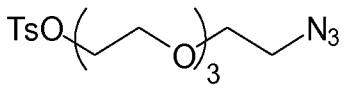
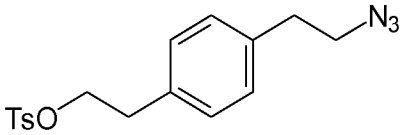
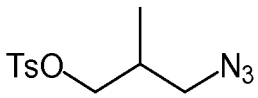
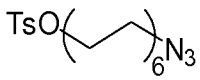
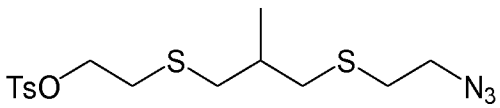
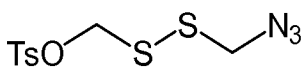
6-Azidohexan-1-ol (22)

6-Hydroxyhexyl 4-methylbenzenesulfonate **21** (4.3 g, 15.79 mmol) was dissolved in DMF (23 mL) and sodium azide (1.75 g, 26.8 mmol) was added. The mixture was heated to 55 °C and after 16 h it was cooled to r.t. and diluted with water (150 mL). The mixture was extracted three times with CH_2Cl_2 and washed with sat. aq. NaCl solution. The organic layer was dried over $MgSO_4$ and solvents were removed *in vacuo*. The crude product was purified by silica flash column chromatography on silica gel (gradient hexanes/EtOAc = 1:0 → 1:1) to afford 6-azidohexan-1-ol **22** (2.2 g, 97%) as a colorless oil. R_f = 0.50 (Hexanes/EtOAc = 2:1); IR (film) ν_{max} 3329, 2935, 2891, 2090, 1256, 1349, 1258, 1055, 910, 731 cm^{-1}; ^1H NMR (400 MHz, *CDCl*$_3$) δ 3.63 (t, *J* = 6.5 Hz, 2H), 3.25 (t, *J* = 6.9 Hz, 2H), 1.64 – 1.51 (m, 4H), 1.43 – 1.32 (m, 4H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ 62.8, 51.5, 32.6, 28.9, 26.6, 25.4; HR ESI Calcd for $C_6H_{13}N_3O$ [M+Na^+]: 166.0951 found: 166.0945.

6-Azidohexyl 4-methylbenzenesulfonate (23)

To a solution of 6-azidohexan-1-ol **22** (2.7 g, 18.9 mmol) in pyridine (70 mL) was added 4-methylbenzene-1-sulfonyl chloride (4.0 g, 21.0 mmol). The reaction mixture was left to stir for 5 h at r.t. after which the solvent was removed *in vacuo* and the crude product was dissolved in CH_2Cl_2, washed with water and dried over $MgSO_4$. Solvents were removed *in vacuo* and the crude product was purified by silica flash column chromatography on silica gel (gradient hexanes/EtOAc = 1:0 → 1:1) to afford azide **23** (5.0 g, 89%) as a colorless oil. R_f = 0.50 (Hexanes/EtOAc = 3:1); IR (film) ν_{max} 2938, 2863, 2092, 1598, 1455, 1356, 1258, 1174, 1097, 956, 919, 813, 724, 662 cm^{-1}; ^1H NMR (400 MHz, *CDCl*$_3$) δ; 7.85 – 7.67 (m, 2H), 7.33 (dd, *J* = 8.5, 0.6 Hz, 2H), 4.01 (t, *J* = 6.4 Hz, 2H), 3.21 (t, *J* = 6.9 Hz, 2H), 2.43 (s, 3H), 1.71 – 1.57 (m, 2H), 1.52 (dd, *J* = 9.1, 4.9 Hz, 2H), 1.38 - 1.12 (m, 4H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ 144.8, 133.2, 129.9, 127.9, 70.4, 51.3, 28.7, 28.7, 26.1, 25.0, 21.7; HR ESI Calcd for $C_{13}H_{19}N_3O_3S$ [M+Na^+]: 320.1045 found: 320.1057.

According to the synthetic route set forth above for compounds **20** to compounds **23** various starting materials have been tried out and successfully converted to corresponding compounds **23**. For these syntheses Tetraethylenglycol was pruchased at Merck, Germany; 2-(4-(2-hydroxyeth-1-yl)phenyl)ethanol was purchased at Sigma Aldrich; 2-methyl-1,3-propanol was purchased at Sigma Aldrich; dodecandiol was purchased by Sigma Aldrich; 2-Methypropane-1,3-bis(2-hydroxyethysulfide) was prepared according to the procedure disclosed in US2012/0295228.

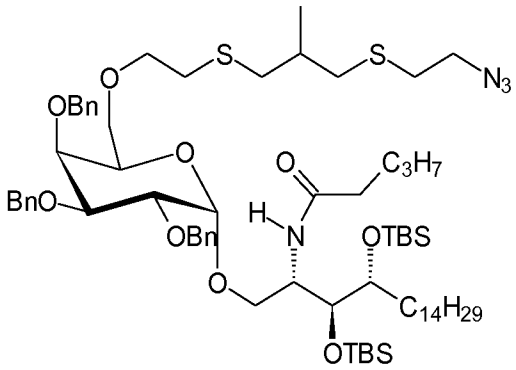
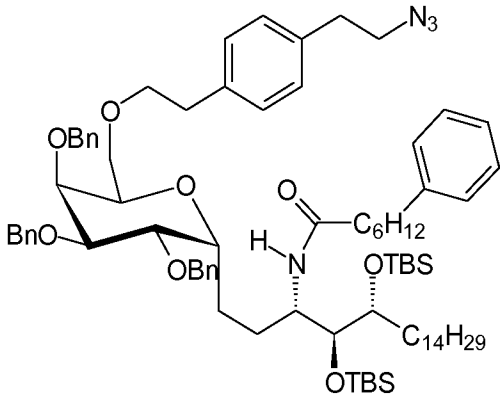
comp.	structure	mass spec
23a		$C_{15}H_{23}N_3SO_6$ Calc.: 374.4344 [M+H$^+$] Found: 374.4388
23b		$C_{17}H_{19}N_3SO_3$ Calc.: 346.4259 [M+H$^+$] Found: 346.4212
23c		$C_{11}H_{15}N_3SO_3$ Calc.: 270.3297 [M+H$^+$] Found: 270.3229
23d		$C_{19}H_{31}N_3SO_3$ Calc.: 382.5426 [M+H$^+$] Found: 382.5461
23e		$C_{15}H_{23}N_3S_3O_3$ Calc.: 390.5683 [M+H$^+$] Found: 390.5662
23f		$C_9H_{11}N_3S_3O_3$ Calc.: 306.4086 [M+H$^+$] Found: 306.4041

(2*S*,3*S*,4*R*)-3,4-*Bis*-*tert*-butyldimethylsilyloxy-2-hexacosanoylamino-1-(6-(6'-azidohexyl)-2,3,4-tri-*O*-benzyl)-α-D-galactopyranosyl)octadecane (25)

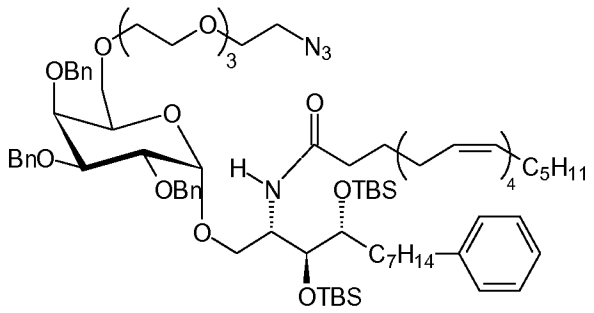
Nucleophile **10** (156 mg, 0.169 mmol) and glycosylating agent **19** (189 mg, 0.253 mmol) were co-evaporated with toluene three times and dried on high vacuum for 3 h after which they were dissolved in Et_2O (2 mL) and THF (0.4 mL) and cooled to –40 °C. To the mixture was added TMSOTf (9.0 µL, 0.051 mmol) and the solution was warmed to –10 °C over the period of 3 h. The reaction was quenched by the addition of NEt_3 (0.05 mL) and solvents were removed *in vacuo* and the crude product was purified by silica flash column chromatography (gradient hexanes/EtOAc = 10:1 →

4:1) to afford glycoside **25** (180 mg, 72% α-anomer) as a white foam. $[\alpha]_D^{r.t.}$ = +18.9 (*c* = 1, $CHCl_3$); R_f = 0.46 (Hexanes/EtOAc = 6.5:1); IR (film) ν_{max} 3328, 2925, 2854, 2096, 1731, 1656, 1452, 1348, 1246, 1156, 1099, 1058, 835, 777, 696 cm^{-1}; ^1H NMR (400 MHz, *CDCl*$_3$) δ 7.64 – 7.09 (m, 15H), 6.07 (d, *J* = 7.1 Hz, 1H), 4.94 (d, *J* = 11.5 Hz, 1H), 4.82 (d, *J* = 3.7 Hz, 1H), 4.80 – 4.56 (m, 5H), 4.09 (td, *J* = 7.6, 4.2 Hz, 1H), 4.03 (dd, *J* = 10.1, 3.6 Hz, 1H), 3.97 – 3.85 (m, 5H), 3.82 (dd, *J* = 10.9, 8.2 Hz, 1H), 3.66 – 3.61 (m, 1H), 3.50 – 3.42 (m, 1H), 3.38 (ddd, *J* = 13.6, 8.1, 6.2 Hz, 2H), 3.29 (dt, *J* = 9.4, 6.8 Hz, 1H), 3.22 (t, *J* = 6.9 Hz, 2H), 1.99 (dd, *J* = 16.6, 9.2 Hz, 2H), 1.60 – 1.45 (m, 8H), 1.39 – 1.15 (m, 70H), 0.91 – 0.84 (m, 26H), 0.06 (s, 3H), 0.05 (s, 3H), 0.02 (s, 6H). ^{13}C NMR (101 MHz, *CDCl*$_3$) δ 173.2, 138.6, 138.5, 138.0, 128.6, 128.6, 128.4, 128.3, 128.3, 128.1, 127.8, 127.8, 127.6, 99.3, 79.5, 76.4, 76.2, 74.9, 74.6, 74.4, 73.5, 72.9, 71.56, 70.1, 70.0, 69.4, 51.5, 49.6, 36.9, 33.5, 32.1, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.5, 28.9, 26.7, 26.1, 25.9, 25.9, 22.8, 14.3; HR ESI Calcd for $C_{89}H_{156}N_4O_9Si_2$ [M+Na^+]: 1505.1333 found: 1505.1388.

The below compounds **25c-h** were prepared according to the synthetic procedure above with the corresponding compounds **10** and **19** in average moderate to good yields:

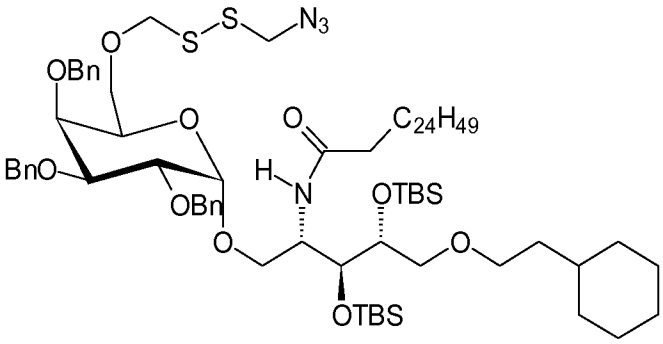
comp.	structure	mass spec
25c		$C_{70}H_{119}N_4O_9S_2Si_2$ Calc.: 1282.0290 [M+H^+] Found: 1282.0317
25d		$C_{76}H_{123}N_4O_9Si_2$ Calc.: 1293.9930 [M+H^+] Found: 1293.9903

25e



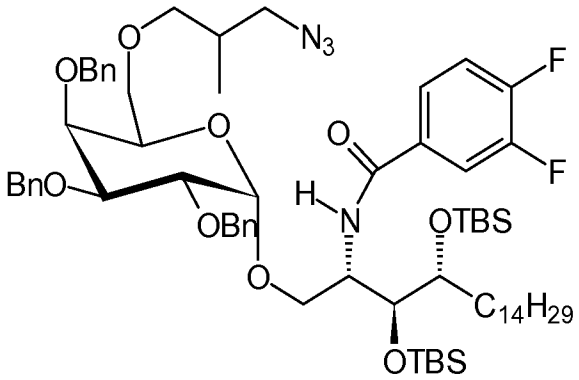
$C_{84}H_{131}N_4O_{12}Si_2$
Calc.: 1446.1406 [M+H$^+$]
Found: 1446.1458

25f



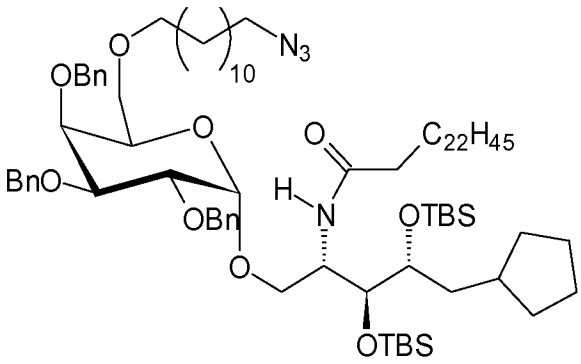
$C_{80}H_{137}N_4O_{10}S_2Si_2$
Calc.: 1436.2787 [M+H$^+$]
Found: 1436.2744

25g



$C_{68}H_{105}F_2N_4O_9Si_2$
Calc.: 1217.7610 [M+H$^+$]
Found: 1217.7588

25h



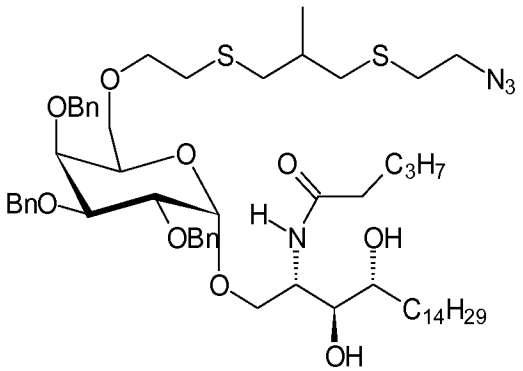
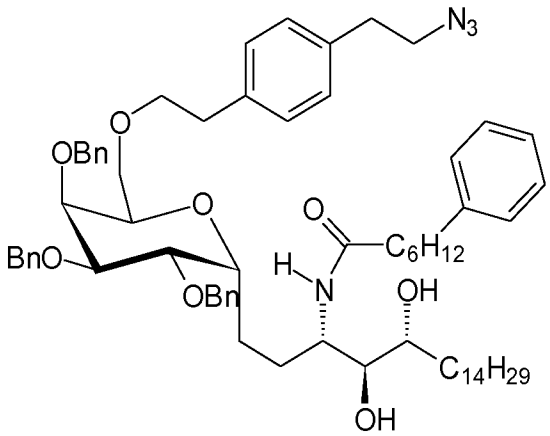
$C_{85}H_{147}N_4O_9Si_2$
Calc.: 1426.2802 [M+H$^+$]
Found: 1426.2826

(2*S*,3*S*,4*R*)-2-Hexacosanoylamino-1-(6-(6'-azidohexyl)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)octadecane-3,4-diol (26)

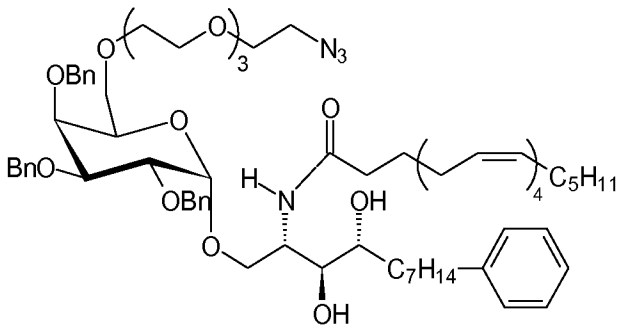
To a solution of *bis*-TBS ether **25** (16.0 mg, 10.8 µmol) in THF (1 mL) was added a solution of TBAF (1 M in THF, 0.150 mL, 0.15 mmol) slowly. After 3.5 h the reaction mixture was diluted with CH_2Cl_2 (10 mL). Solvents were removed *in vacuo* and crude product was purified by silica flash column chromatography (gradient hexanes/EtOAc

= 1:0 → 1:1) to afford diol **26** (10.5 mg, 78%) as a clear oil. $[\alpha]_D^{r.t.}$ = +121.9 (*c* = 0.2, $CHCl_3$); R_f = 0.40 (Hexanes/EtOAc = 2:1); IR (film) ν_{max} 3329, 2919, 2851, 2096, 1640, 1543, 1467, 1455, 1350, 1094, 1046, 907, 730, 696 cm^{-1}; ^1H NMR (400 MHz, *CDCl*$_3$) δ 7.58 – 7.08 (m, 15H), 6.37 (d, *J* = 8.4 Hz, 1H), 4.94 (d, *J* = 11.4 Hz, 1H), 4.88 (d, *J* = 11.6 Hz, 1H), 4.85 (d, *J* = 3.8 Hz, 1H), 4.82 – 4.73 (m, 2H), 4.68 (d, *J* = 11.6 Hz, 1H), 4.60 (d, *J* = 11.5 Hz, 1H), 4.22 (dq, *J* = 6.8, 3.3 Hz, 1H), 4.05 (dd, *J* = 10.0, 3.8 Hz, 1H), 3.95 (d, *J* = 1.8 Hz, 1H), 3.88 (d, *J* = 2.7 Hz, 2H), 3.87 – 3.75 (m, 2H), 3.55 – 3.36 (m, 5H), 3.31 (dt, *J* = 9.4, 6.7 Hz, 1H), 3.25 (t, *J* = 6.9 Hz, 2H), 2.20 – 2.11 (m, 3H), 1.70 – 1.44 (m, 8H), 1.41 – 1.17 (m, 73H), 0.88 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ 173.2, 138.6, 138.5, 130.0, 128.6, 128.6, 128.4, 128.3, 128.2, 128.1, 127.8, 127.8, 127.6, 99.3, 79.5, 76.4, 76.2, 74.9, 74.6, 74.4, 73.5, 72.9, 71.6, 70.1, 70.0, 69.4, 51.5, 49.6, 36.9, 33.5, 32.1, 29.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.5, 28.9, 26.7, 26.1, 25.9, 25.9, 22.8, 14.3; HR ESI Calcd for $C_{77}H_{128}N_4O_9$ [M+Na^+]: 1275.9574 found: 1275.9536.

The below compounds **26c-h** were prepared according to the synthetic procedure above for compound **26** in average moderate to good yields:

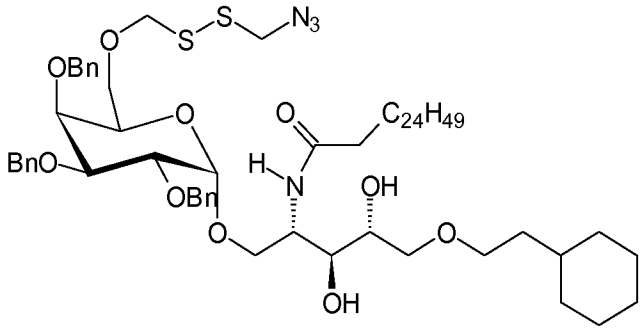
comp.	structure	mass spec
26c		$C_{58}H_{91}N_4O_9S_2$ Calc.: 1053.5070 [M+H^+] Found: 1053.5046
26d		$C_{64}H_{95}N_4O_9$ Calc.: 1065.4710 [M+H^+] Found: 1065.4677

26e



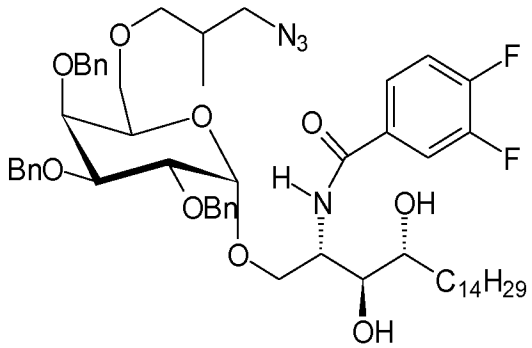
$C_{72}H_{103}N_4O_{12}$
Calc.: 1217.6187 [M+H$^+$]
Found: 1217.6203

26f



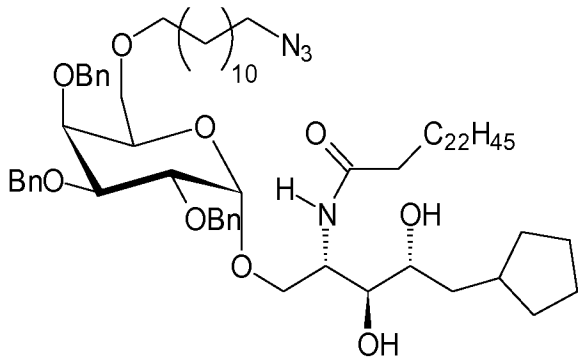
$C_{68}H_{109}N_4O_{10}S_2$
Calc.: 1207.7567 [M+H$^+$]
Found: 1207.7532

26g



$C_{56}H_{77}F_2N_4O_9$
Calc.: 989.2390 [M+H$^+$]
Found: 989.2371

26h



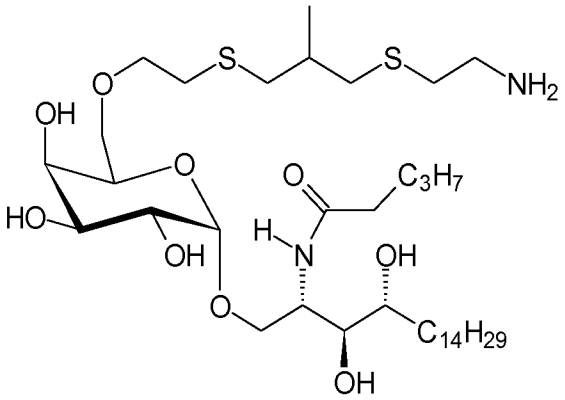
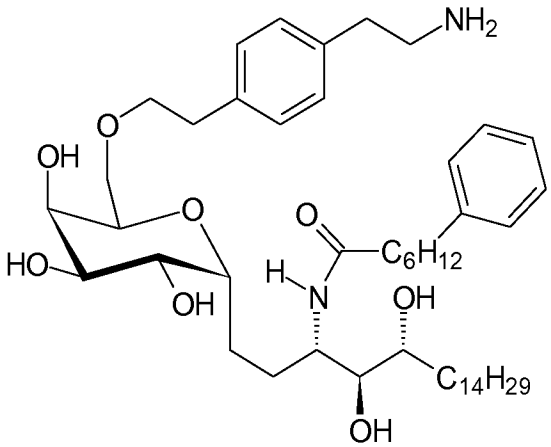
$C_{73}H_{119}N_4O_9$
Calc.: 1197.7582 [M+H$^+$]
Found: 1197.7614

(2*S*,3*S*,4*R*)-1-(6-(6'-Aminohexyl)-α-D-galactopyranosyl)-2-hexacosanoylaminooctadecane-3,4-diol (27)

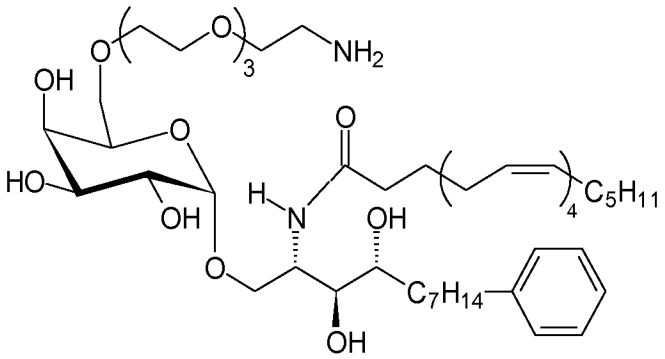
To a solution diol **26** (55 mg, 0.044 mmol) in EtOH (0.5 mL) and chloroform (0.15 mL) was added $Pd(OH)_2$ on charcoal (10% w/w, wet 38 mg). The solution was stirred at r.t. under an atmosphere of Ar for 15 min. after which H_2 gas was inserted into the suspension and the mixture was hydrogenated for 12 h. The mixture was filtered over celite and thoroughly washed with CH_2Cl_2, THF and MeOH. Solvents were removed

in vacuo and the crude was purified by silica flash column chromatography on silica gel (CH_2Cl_2/MeOH = 4:1) to afford linker equipped GSL **27** (38 mg, 90%) as a pale yellow powder. $[\alpha]_D^{r.t.}$ = +66.1 (*c* = 1.0, Pyridine); R_f = 0.44 (CH_2Cl_2/MeOH = 4:1); IR (film) ν_{max} 3292, 2918, 2850, 1640, 1539, 1468, 1304, 1073, 1038, 970, 721cm^{-1}; ^{1}H NMR (400 MHz, *d-pyr*) δ 8.66 (d, *J* = 8.6 Hz, 1H), 5.48 (d, *J* = 3.8 Hz, 1H), 4.59 (dd, *J* = 10.6, 5.9 Hz, 1H), 4.49 (dd, *J* = 9.7, 3.8 Hz, 1H), 4.39 – 4.15 (m, 1H), 3.91 (ddd, *J* = 15.3, 10.4, 5.9 Hz, 1H), 3.74 (q, *J* = 7.0 Hz, 1H), 3.44 – 3.31 (m, 2H), 3.17 (dd, *J* = 13.1, 5.2 Hz, 2H), 2.42 (t, *J* = 6.6 Hz, 2H), 2.17 (s, 1H), 1.89 (s, 2H), 1.84 – 1.65 (m, 4H), 1.65 – 0.97 (m, 75H), 0.75 (t, *J* = 6.7 Hz, 6H); ^{13}C NMR (101 MHz, *d-pyr*) δ 171.9, 99.7, 75.5, 70.9, 70.1, 70.0, 69.6, 68.7, 66.7, 55.9, 49.9, 38.4, 35.4, 33.1, 30.7, 30.7, 29.0, 28.8, 28.6, 28.6, 28.6, 28.6, 28.5, 28.5, 28.4, 28.4, 28.2, 28.2, 26.8, 25.3, 25.1, 25.1, 24.7, 21.5, 17.8, 12.9; HR ESI Calcd for $C_{56}H_{112}N_2O_9$ [M+H$^+$]: 957.8441 found: 957.8468.

The below compounds **26c-h** were prepared according to the synthetic procedure above for compound **27** in average moderate to good yields:

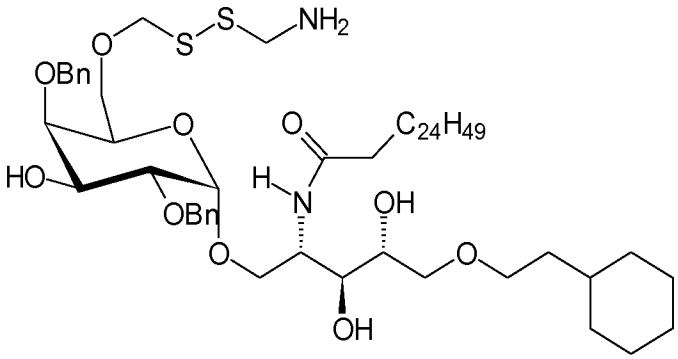
comp.	structure	mass spec
27c		$C_{37}H_{75}N_2O_9S_2$ Calc.: 757.1410 [M+H$^+$] Found: 757.1437
27d		$C_{43}H_{79}N_2O_9$ Calc.: 769.1050 [M+H$^+$] Found: 769.1078

27e



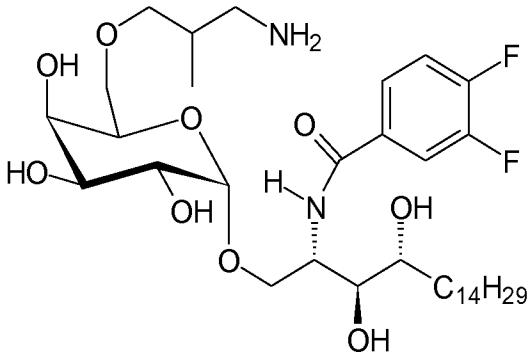
$C_{51}H_{87}N_2O_{12}$
Calc.: 921.2527 [M+H$^+$]
Found: 921.2500

27f



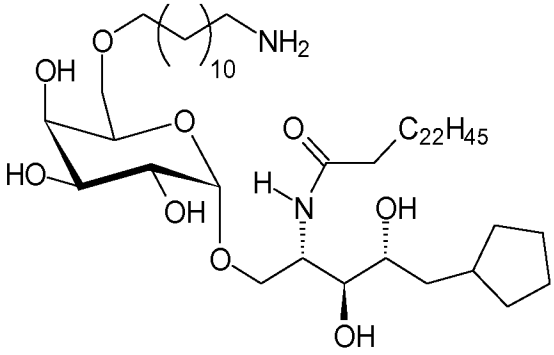
$C_{47}H_{93}N_2O_{10}S_2$
Calc.: 911.3907 [M+H$^+$]
Found: 911.3934

27g



$C_{35}H_{61}F_2N_2O_9$
Calc.: 692.8730 [M+H$^+$]
Found: 692.8707

27h



$C_{52}H_{103}N_2O_9$
Calc.: 901.3922 [M+H$^+$]
Found: 901.3958

2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-D-galactose (**33**) was prepared according to *ChemBioChem* **2012**, 1349.

2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactosyl trifluoroacetimidate (34)

To a solution of 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-D-galactose (800 mg, 1.786 mmol, coevaporated 3 times with dry toluene) **33** in CH_2Cl_2 (7 mL) was added cesium

carbonate (867 mg, 2.65 mmol). To the mixture was added 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride **24** (551 mg, 2.65 mmol) and the reaction mixture was stirred at r.t. overnight after which it was filtered over celite and washed with CH_2Cl_2. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography on silica gel (gradient hexanes/EtOAc = 8:1 → 1:1) to yield the imidate **34** (1.02 g, 92%) as a colorless oil. HR ESI Calcd for $C_{35}H_{32}F_3NO_6$ [M+H$^+$]: 620.6362 found: 620.6327.

(2*S*,3*S*,4*R*)-3,4-*Bis*-*tert*-butyldimethylsilyloxy-2-hexacosanoylamino-1-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)octadecane (35)

Nucleophile **10** (150 mg, 0.162 mmol) and glycosylating agent **34** (151 mg, 0.243 mmol) were co-evaporated with toluene three times and dried on high vacuum for 3 h after which they were dissolved in Et_2O (2 mL) and THF (0.4 mL) and cooled to –40 °C. To the mixture was added TMSOTf (8.0 µL, 0.043 mmol) and the solution was warmed to –10 °C over the period of 3 h. The reaction was quenched by the addition of NEt_3 (0.05 mL) and solvents were removed *in vacuo* and the crude product was purified by silica flash column chromatography (gradient hexanes/EtOAc = 10:1 → 4:1) to afford glycoside **35** (140 mg, 64% α-anomer) as a white oil. HR ESI Calcd for $C_{83}H_{143}NO_9Si_2$ [M+H$^+$]: 1356.2067 found: 1356.2098.

(2*S*,3*S*,4*R*)-3,4-*Bis*-*tert*-butyldimethylsilyloxy-2-hexacosanoylamino-1-(2,3,4-tri-*O*-benzyl-6-hydroxy-α-D-galactopyranosyl)octadecane (36)

To a solution of **35** (80 mg, 0.06 mmol) in anhydrous CH_2Cl_2 (2 mL) under argon atmosphere were added copper(II) triflate (2 mg, 0.006 mmol) and BH_3·THF (0.30 mL, 0.30 mmol). After stirring for 2 h at room temperature, the yellow reaction mixture was quenched with methanol. Subsequently the mixture was diluted with EtOAc and washed with sat. $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo* and the crude product was purified by silica flash column chromatography (gradient hexanes/EtOAc: 8.5/1.5) to afford glycoside **36** (62 mg, 78%) as a yellowish foam. HR ESI Calcd for $C_{83}H_{145}NO_9Si_2$ [M+H$^+$]: 1358.2226 found: 1358.2196.

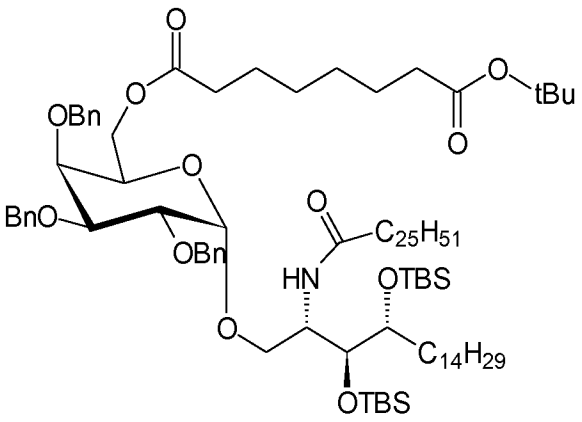
The Boc-protected PEG derivative **38** was purchased at Creative PEGWorks, Winston Salen, NC, USA.

(2*S*,3*S*,4*R*)-3,4-*Bis*-*tert*-butyldimethylsilyloxy-2-hexacosanoylamino-1-(2,3,4-tri-*O*-benzyl-6-(carbonyl-1-ethyl-2-(tri(1-ethanoyl)1-ethanoyl-2-(*tert*-butoxy-carbonyl)amino)-α-D-galactopyranosyl)octadecane (37)

To a solution of **38** (18 mg, 0.05 mmol) in DMF (5 mL) was added O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate TBTU (16.1 mg, 0.05) and diisopropylethylamine (12.9 mg, 17 µl, 0.1 mmol). The mixture was stirred for 30 min at r.t.. Then a mixture of **36** (50 mg, 0.04 mmol) in DMF (1 ml) was added to the reaction mixture and stirred for 5 hours. Subsequently, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and the resulting mixture was washed with 5% HCl (2 x 3 mL), 1M $NaHCO_3$ (3 x 3 mL) and water (2 x 3 mL). The organic layer was collected, dried ($MgSO_4$), filtered and concentrated to give the crude ester product which was purified by flash column chromatography on silica gel (gradient hexanes/EtOAc = 8:1 → 1:1) to yield the linker-equipped glycolipid **37** (40 mg, 63%) as a colorless oil. HR ESI Calcd for $C_{99}H_{174}N_2O_{16}Si_2$ [M+H$^+$]: 1705.6272 found: 1705.6231.

Mono-*tert*.-butyl suberic acid was prepared according to *Chem*. *Commun*. **1999**, 823.

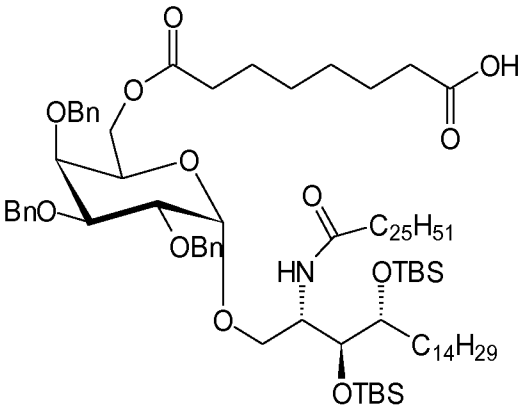
Compound 37a was prepared according to the above reaction procedure in 53% yield.

comp.	structure	mass spec
39		$C_{95}H_{167}NO_{12}Si_2$ Calc.: 1572.5243 [M+H$^+$] Found: 1572.5216

(2*S*,3*S*,4*R*)-3,4-*Bis*-*tert*-butyldimethylsilyloxy-2-hexacosanoylamino-1-(2,3,4-tri-*O*-benzyl-6-(carbonyl-1-ethyl-2-(tri(1-ethanoyl)1-ethanoyl-2-amino)-α-D-galactopyranosyl)octadecane (25a)

37 (40 mg, 0,02 mmol) was dissolved in TFA (1 mL) and stirred at r.t. for 30 min. The solution was diluted with CH_2Cl_2 (2 mL) and then carefully neutralized (to pH ~ 8) with sat. aq. $NaHCO_3$ solution (8 mL). Additional CH_2Cl_2. was added and the organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo* and the crude product was purified by silica flash column chromatography (gradient hexanes/EtOAc: 10:1 → 1:1) to afford the linker-equipped glycolipid **25a** (33 mg, 89%) as a yellowish oil. HR ESI Calcd for $C_{94}H_{166}N_2O_{14}Si_2$ [M+H$^+$]: 1605.5112 found: 1605.5088.

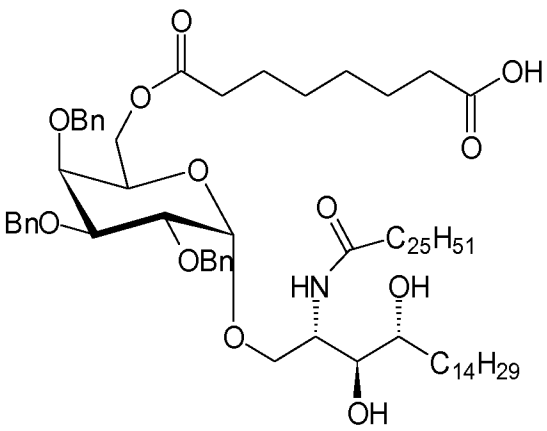
Compound **25b** was prepared accordingly from compound **39**:

comp.	structure	mass spec
25b		$C_{91}H_{159}NO_{12}Si_2$ Calc.: 1516.4179 [M+H$^+$] Found: 1516.4223

(2*S*,3*S*,4*R*)-2-Hexacosanoylamino-1-(2,3,4-tri-*O*-benzyl-6-(carbonyl-1-ethyl-2-(tri(1-ethanoyl)1-ethanoyl-2-amino)-α-D-galactopyranosyl)octadecane-3,4-diol (26a)

To a solution of *bis*-TBS ether **25a** (33.0 mg, 20.7 µmol) in THF (1 mL) was added a solution of TBAF (1 M in THF, 0.150 mL, 0.15 mmol) slowly. After 3.5 h the reaction mixture was diluted with CH_2Cl_2 (10 mL). Solvents were removed *in vacuo* and crude product was purified by silica flash column chromatography (gradient hexanes/EtOAc = 1:0 → 1:1) to afford diol **26a** (24.5 mg, 86%) as a clear oil. HR ESI Calcd for $C_{82}H_{138}N_2O_{14}$ [M+H$^+$]: 1376.9893 found: 1376.9876.

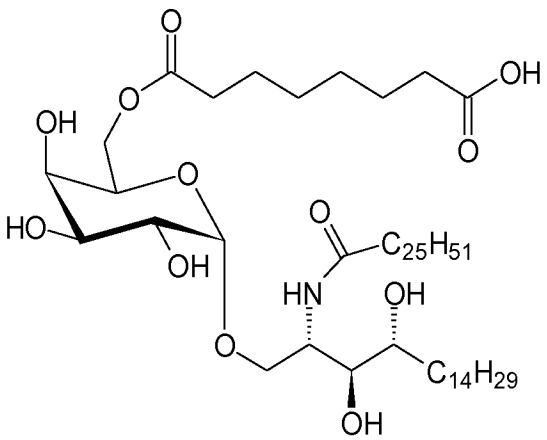
Compound **26b** was prepared accordingly from compound **25b**:

comp.	structure	mass spec
26b		$C_{79}H_{131}NO_{12}$ Calc.: 1287.8959 [M+H$^+$] Found: 1287.8914

(2*S*,3*S*,4*R*)-1-(6-(Carbonyl-1-ethyl-2-(tri(1-ethanoyl)1-ethanoyl-2-amino)-α-D-galactopyranosyl)-2-hexacosanoylaminooctadecane-3,4-diol (27a)

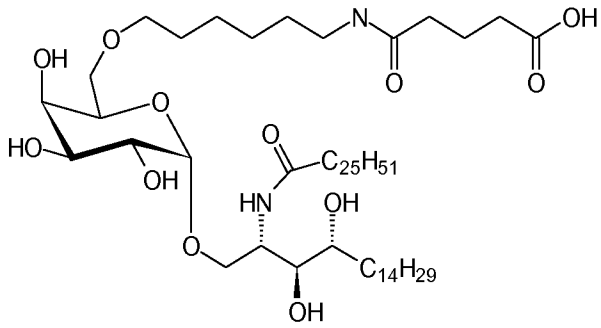
To a solution diol **26a** (25 mg, 17.7 µmol) in EtOH (0.5 mL) and chloroform (0.15 mL) was added $Pd(OH)_2$ on charcoal (10% w/w, wet 35 mg). The solution was stirred at r.t. under an atmosphere of Ar for 15 min. after which H_2 gas was inserted into the suspension and the mixture was hydrogenated for 12 h. The mixture was filtered over celite and thoroughly washed with CH_2Cl_2, THF and MeOH. Solvents were removed *in vacuo* and the crude was purified by silica flash column chromatography on silica gel (CH_2Cl_2/MeOH = 4:1) to afford linker equipped GSL **27a** (18 mg, 92%) as a colorless oil. HR ESI Calcd for $C_{61}H_{120}N_2O_{14}$ [M+H$^+$]: 1106.6209 found: 1106.6177.

Compound **27b** was prepared accordingly from compound **26b**:

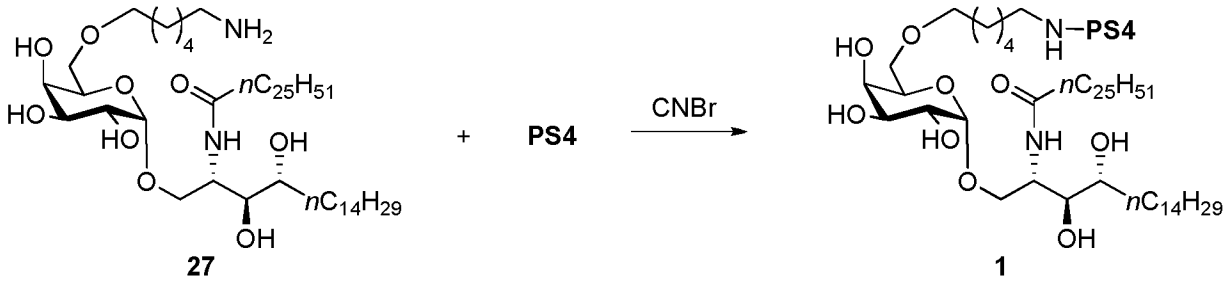
comp.	structure	mass spec
27b		$C_{58}H_{113}NO_{12}$ Calc.: 1017.5275 [M+H$^+$] Found: 1017.5231

5-((6-(((2R,3R,4S,5R,6S)-6-(((2S,3S4R)-2-hexacosanamido-3,4-dihydroxyoctadecyl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)methoxy)hexyl)amino)-5-oxopentanoic acid (40)

To gylocolipid 27 (10 mg, 10.44 µmol) in chloroform:methanol:triethylamine mixture (1:1:0.1, 7 ml) wass added excess glutaric anhydride (14.9 mg, 131 µmol) in on eportion and left to stir at room temperature. Aftert three days the completion of the reaction was indicated by the disappereance of tthe starting material mass on LCMS. The reaction was then evaporated to dryness and the resultant residue was triturated with dichloromethane to give the desired product **40** (8 mg, 72%) as a white solid.

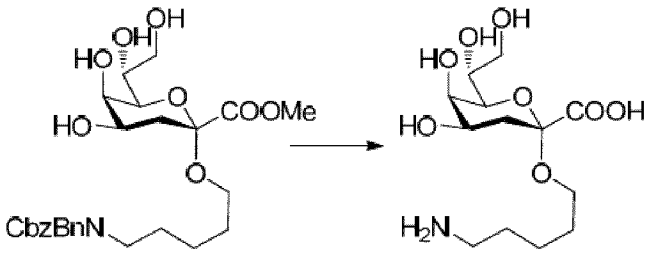
comp.	structure	mass spec
40		$C_{61}H_{118}N_2O_{12}$ Calc.: 1069.861 [M-2H$^+$] Found: 1069.642

Synthesis of the antigen-carbohydrate glycolipid conjugate:



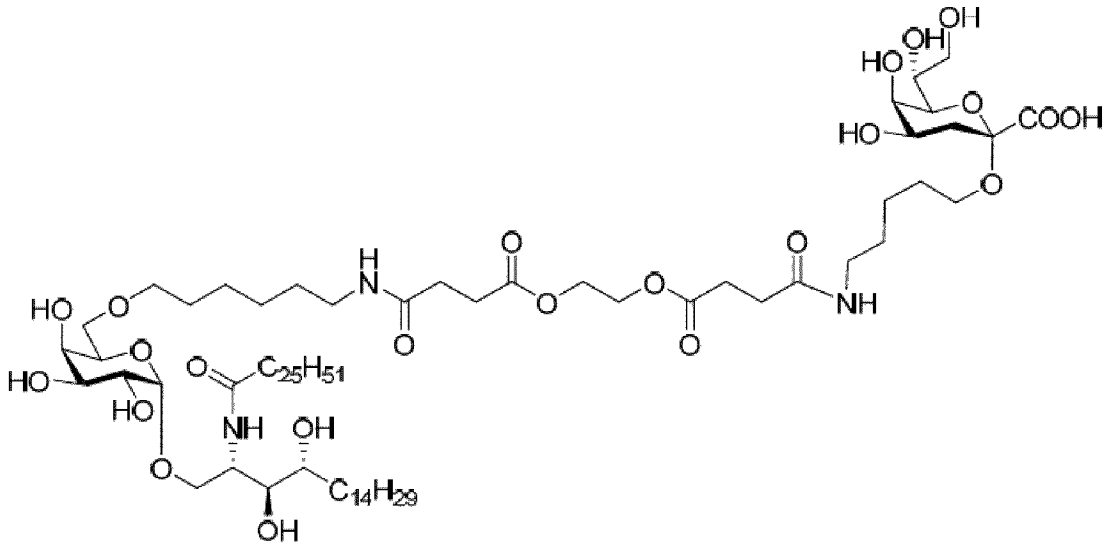
PS4 (1 mg) was dissolved in aq. NaOH solution (pH 10.95) to a final concentration of 10 mg/mL. The PS4 was activated with 15 µL of cyanogen bromide (10 mg/mL in acetonitrile) and left to stir at the room temperature for 10 min. To the activated PS4, 20 µL of **27** was added (10 mg/2 mL in DMSO:THF, 1:1) and the mixture was incubated for 18 h at room temperature. After adjusting the pH to 6 with 0.1M aq. HCl, the mixture was dialyzed (12-14k MWCO) against double distilled water, concentrated *via* ultrafiltration (10k MWCO) then lyophilized.

Compounds **27a** and **27c-h** have been conjugated to PS4 accordingly and also showed immunogenic activity.



41 **42**

Methyl ester 4.57 (provided by *Dr. M. Oberli*) (10 mg, 0,018 mmol) was dissolved in a mixture of THF (1.0 mL) and NaOH (0.1 M, 1 mL). The reaction mixture was stirred at r.t. for 1 h after which it was neutralized by the addition of Amberlite IR-120 (H+) resin. The resin was removed by filtration and solvents were removed *in vacuo*. The crude product was purified by silica gel chromatography (20% MeOH in CH2Cl2) to yield a white powder which was dissolved in THF (1.0 ml), water (1.0 mL) and MeOH (1.0 mL). To the mixture was added Pd on charcoal (20 mg). A stream of hydrogen was passed throug the suspension for 20 min., after which the suspension was stirred for another 18 h under an H2 atmosphere. The suspension was filtered over celite and washed with MeOH and water (2 x). Solvents were removed *in vacuo* and the crude product was purified by Sephadex G25 size-exclusion chromatography (eluent: 5% EtOH in water) to yield acid 4.13 (5.0 mg, 85% over two steps) a white powder. [α]D r.t. = –14.2 (*c* = 1.0, water); Rf = 0.67 (Isopropanol/1M aq. NH4OAc = 2:1); IR (film) vmax 3256, 2938, 1571, 1410, 1050, 830 cm-1; 1H NMR (400 MHz, *D2O*) δ 4.00 – 3.84 (m, 3H), 3.74 (dd, *J* = 19.7, 9.9 Hz, 3H), 3.63 (d, *J* = 8.9 Hz, 1H), 3.46 (dd, *J* = 15.8, 6.5 Hz, 1H), 3.01 (t, *J* = 7.5 Hz, 2H), 2.43 (dd, *J* = 12.1, 4.6 Hz, 1H), 1.79 (t, *J* = 12.3 Hz, 1H), 1.67 (dd, *J* = 14.0, 6.5 Hz, 2H), 1.63 – 1.57 (m, 2H), 1.44 (dd, *J* = 15.2, 7.9 Hz, 2H); 13C NMR (101 MHz, *D2O*) δ 181.4, 173.9, 101.1, 73.3, 69.0, 67.4, 65.2, 64.1, 39.3, 34.7, 28.2, 26.3, 23.2, 22.0; HR ESI Calcd for C13H25NO8 [M-H+]: 322.1507 found: 322.1502.



(2*S*,3*S*,4*R*)-1-(6-(6'-Hexanyl succinamido ethyleneglycol succinimidamido 5''-pentanyl α-3'''- deoxy-D-*manno*-oct-2'''-ulosonic acid pyranoside)-α-D-galacto-pyranosyl)-2-hexacosanoylaminooctadecane-3,4-diol (Glycoconjugate 43)

To a solution of linker-equipped KDO **42** (1.5 mg, 4.6 μmol) and glycolipid **27** (4.4 mg, 4.6 μmol) in DMSO/pyridine (0.1 mL, ratio = 1:1 v/v) was added ethyleneglycol bissuccinimidyl succinate (EGS) (2.1 mg, 4.6 μmol) dissolved in DMF (0.1 mL). The reaction mixture was stirred at r.t. for 24 h after which solvents were removed by lyophilization. The crude product was purified by LH-20 size exclusion chromatography (eluent: MeOH/CH2Cl2 = 1:1) to yield conjugate **43** (3.0 mg, 42%) as a pale yellow powder. [α]D r.t. = +43.9 (*c* = 0.2, Pyridine); Rf = 0.54 (CH2Cl2/MeOH = 85:15); IR (film) vmax 3308, 2918, 2850, 1781, 1709, 1645, 1548, 1467, 1378, 1211, 1157, 1071, 1020, 952, 816, 719 cm-1; 1H NMR (400 MHz, *d-pyr*) 8.52 (m, 2H), 8.44 (d, *J* = 8.7 Hz, 1H), 5.56 (d, *J* = 3.9 Hz, 1H), 5.26 (s, 1H), 4.88 (s, 1H), 4.66 (ddd, *J* = 13.1, 9.9, 4.4 Hz, 2H), 4.55 (d, *J* = 4.6 Hz, 1H), 4.52 – 4.39 (m, 5H), 4.39 – 4.31 (m, 7H), 4.20 – 3.93 (m, 2H), 3.85 (d, *J* = 7.3 Hz, 1H), 3.79 – 3.72 (m, 1H), 3.47 (ddd, *J* = 20.0, 14.8, 8.3 Hz, 3H), 3.39 – 3.32 (m, 1H), 3.22 (dd, *J* = 11.9, 4.5 Hz, 1H), 3.08 (ddd, *J* = 6.7, 5.8, 2.5 Hz, 1H), 2.94 – 2.84 (m, 4H), 2.79 (dd, *J* = 8.5, 5.0 Hz, 3H), 2.73 (t, *J* = 4.8 Hz, 2H), 2.53 – 2.49 (m, 18H), 2.33 (t, *J* = 6.9 Hz, 1H), 1.99 – 1.66 (m, 4H), 1.66 – 1.47 (m, 6H), 1.42 – 1.20 (m, 71H), 0.89 (t, *J* = 6.3 Hz, 6H).δ; 13C NMR (151 MHz, *d-pyr*) δ 173.6, 171.7, 170.5, 169.3, 101.9, 101.0, 77.1, 76.8, 72.9, 71.9, 71.9, 71.6, 71.4, 71.2, 71.1, 70.6, 69.5, 69.1, 67.8, 66.5, 64.4, 63.4, 63.3, 62.9, 62.8, 61.9, 51.7, 43.5, 41.5, 40.2, 40.1, 37.2, 37.2, 34.8, 32.6, 32.5, 31.3, 30.8, 30.6, 30.5, 30.5, 30.5, 30.4, 30.4, 30.4, 30.4, 30.3, 30.3, 30.3, 30.2, 30.0, 30.0, 29.3, 27.6, 27.0, 26.5, 26.5, 24.3, 23.4, 14.7; HR ESI Calcd for C79H147N3O23 [M+Na+]: 1529.0318 found: 1529.0363

Description of the Figures:

Figure 1. Model of glycoconjugate vaccine action.
The mode of action is illustrated by the antigen of invasive pneumococcal disease: the pneumococcal capsule polysaccharide (CPS) is covalently attached to a glycolipid. B cells specific for CPS will internalize the conjugate by receptor-mediated endocytosis and the conjugate will be cleaved in late endosomes, generating free αGalCer. In the late endosomal compartment, αGalCer will be complexed with CD1d antigen-presenting molecules and upon plasmamembrane recycling of CD1d be presented to invariant natural killer T (iNKT) cells.
Stimulation of iNKT cells by the αGalCer:CD1d complex on the surface of the antigen-presenting B cell will induce the release of soluble cytokines necessary for B cell help and memory generation. By this strategy a final long term immunological memory is induced, leading to the production of memory B-cells and the supply of high affinity IgG antibodies.

Figure 2. Glycoconjugate vaccine **1** containing the antigenic capsular polysaccharide portion PS4.

Figure 3. *In vitro* activity of the conjugate vaccine. αGalCer-CPS-pulsed CD1d-positive APC stimulate iNKT cells. Different batches of αGalCer-CPS type 4 conjugate vaccine (diamonds) are active *in vitro* when αGalCer is freed from CPS in living cells (A). αGalCer is entirely conjugated to CPS as remaining activity is not found when activating iNKT cells in a cell-free system (B). Unconjugated CPS type 4 (open circles) or αGalCer (closed circles) alone as control.

Figure 4. *In vivo* activity of the conjugate vaccine. Only αGalCer-CPS increases **Abs** response in C57BL/6 mice and the **Abs** response is dependent on NKT cells / CD1d. (A) WT C57BL/6 mice vaccinated with αGalCer-CPS (closed symbols) or CPS alone (open symbols) are bled after immunization and the CPS-specific **Abs** are assessed by ELISA. (B) WT C57BL/6 (WT, closed symbols) or CD1d-deficient (CD1d-/-, open symbols) mice immunized with αGalCer-CPS are bled after vaccination and the CPS-specific **Abs** are measured by ELISA.

Figure 5. *In vivo* antibody response after vaccination. The **Abs** response inlcudes IgG subclasses and shows reactivity to common epitopes on different *S. pneumoniae* CPS. (A) WT C57BL/6 (WT, closed symbols) or CD1d-deficient (CD1d-/-, open symbols) mice immunized with αGalCer-CPS are bled after vaccination and the CPS-specific **Abs** subclasses are measured by ELISA (IgG1 given as representative example). (B) C57BL/6 mice vaccinated with αGalCer-CPS are bled after immunization and the CPS type 4 (closed symbols) or CPS type 2 (open symbols)-specific **Abs** are assessed by ELISA.

Figure 6. CPS-specific hybridomas express affinity matured IgM and all IgG subclasses with some preferential V,D,J segment usage. Hybridomas from αGalCer-CPS-immunized mice were established and classified by ELISA and sequencing. * aminoacid (aa) or nucleotide (nuc) substitutions in comparison to germ-line sequence.

Figure 7. Protection from infection with *S. pneumoniae* in a mouse model. CSP-specific m**Abs** promote bacterial opsonization. Uptake of fluorescently labeled *S. pneumoniae* serotype 4 into APC alone, in the presence of complement (C') and/or m**Abs** 12F10 (CPS-specific hybridoma purified) or C15 (anti-human TCRAV24). Percent of positive cells according to background (OPA marker) is given in a table.

Figure 8. αGalCer-CPS-vaccinated C57BL/6 mice show long-term protection to challenge with *S. pneumoniae*. Mice vaccinated with αGalCer-CPS (A: closed symbols; B: line) or CPS alone (A: open symbols; B: dashed line) are infected with *S. pneumoniae* one week (A) or up to 3 months (B) after the last immunization. Mice are scored for disease, weight and survival over several days (given in hours). All αGalCer-CPS injected mice survived (B) without disease symptoms. Severe weight loss (A) is just observed for the CPS alone condition independently of the animal's survival (B).

Figure 9 and 10. Isotype and specificity of anti-polysaccharide Abs (IgG, Figure 9; IgM, Figure 10).

Claims

1. Compound of the general formula (I):

$$\mathbf{A[L{-}{-}CH{-}{-}CA]_p}$$
$$\mathbf{(\ I\ )}$$

wherein

A represents a carbohydrate antigen of 1 to 10.000 carbohydrate monomers and the carbohydrate antigen belongs to the class of polysaccharides, wherein the carbohydrate monomers of the carbohydrate antigen are optionally modified to carry amide, carbonate, carbamate, carbonyl, thiocarbonyl, carboxy, thiocarboxy, ester, thioester, ether, epoxy, hydroxyalkyl, alkylenyl, phenylene, alkenyl, imino, imide, isourea, thiocarbamate, thiourea and/or urea moieties,

p is the number of residues **–L–CH–CA** which are bound to the carbohydrate antigen **A**, and

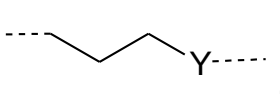
p is an integer defined as follows:

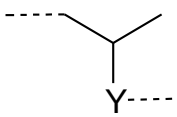
p is 1 or 2		if u is 1
p is 1, 2, 3 or 4		if u is 2
p is 1, 2, 3, 4, 5 or 6		if u is 3
p is 1, 2, 3, 4, 5, 6, 7 or 8		if u is 4
$1 \leq p \leq 10$	if	$5 \leq u \leq 10$
$2 \leq p \leq 50$	if	$11. \leq u \leq 100$
$20 \leq p \leq 200$	if	$101 \leq u \leq 1000$
$50 \leq p \leq 400$	if	$1001 \leq u \leq 10000$

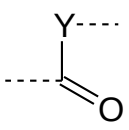
u is the number of carbohydrate monomers of the carbohydrate antigen **A**

L represents **–L^1–L^2–**, **–L^2–**, **–L^2–L^3–** or **–L^1–L^2–L^3–**;

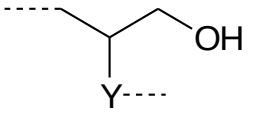
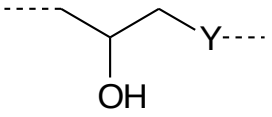
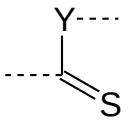
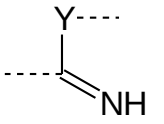
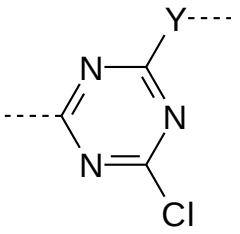
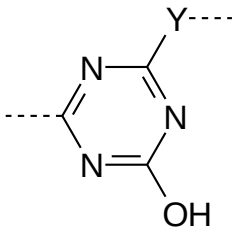
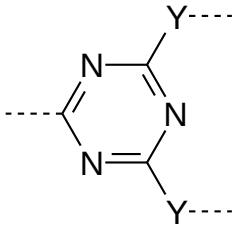
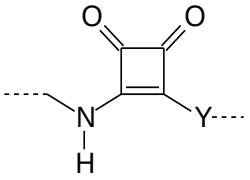
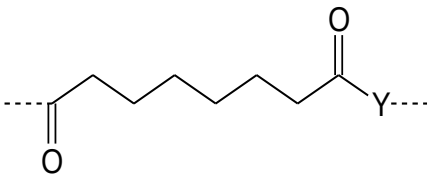
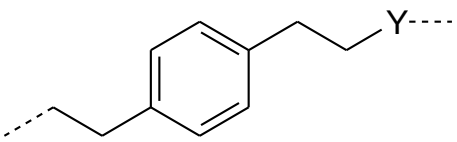
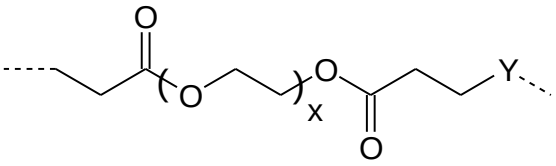
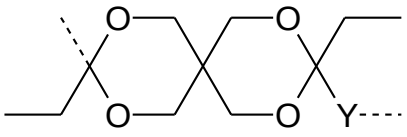
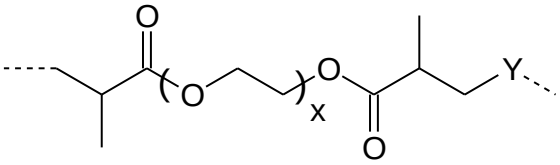
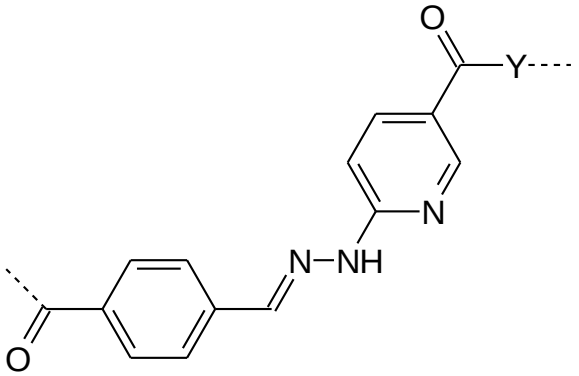
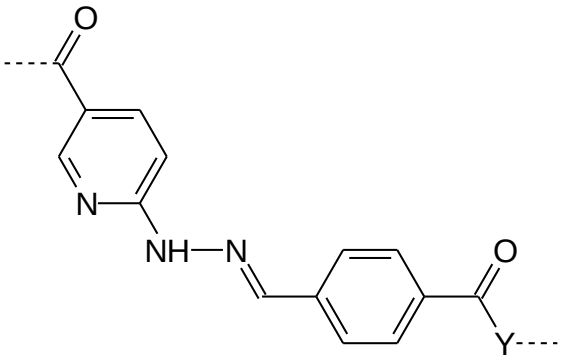
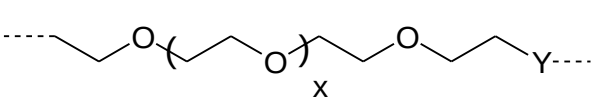
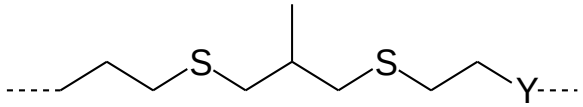
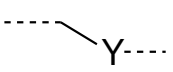
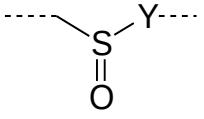
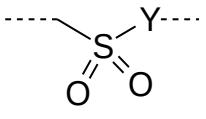
L^1 represents one of the following residues:

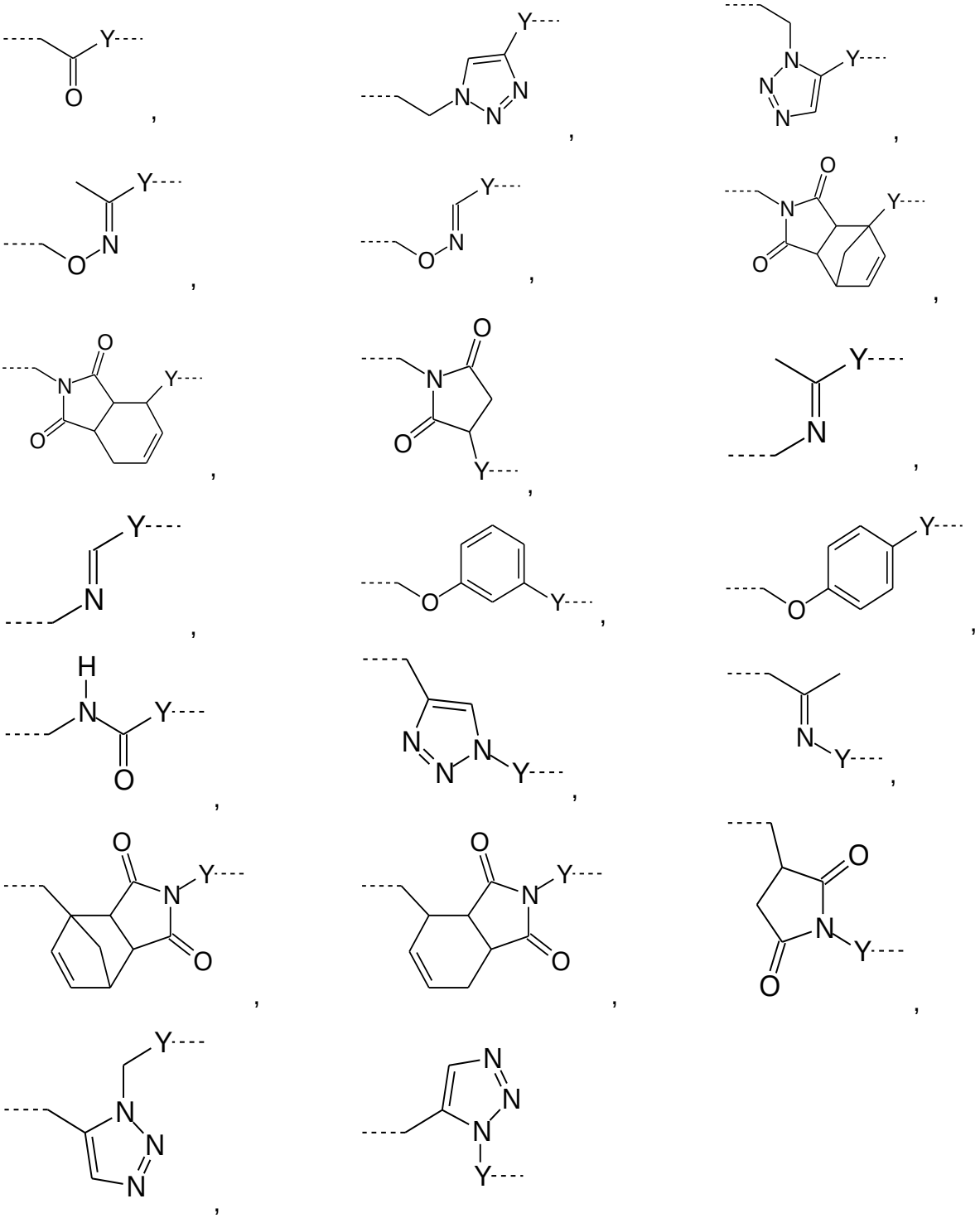


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wherein x is in integer from 1 to 60;

Y represents a bond, –NH–, –O–, –S–; –S–S–;

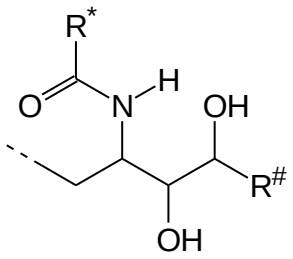
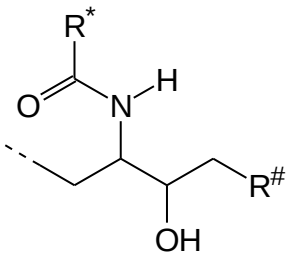
L² represents –CH_2–, –C_2H_4–, –C_3H_6–, –C_4H_8–, –C_5H_{10}–, –C_6H_{12}–, –C_7H_{14}–, –C_8H_{16}–, –C_9H_{18}–, –$C_{10}H_{20}$–, –$CH(CH_3)$–, –$C[(CH_3)_2]$–, –CH_2–$CH(CH_3)$–, –$CH(CH_3)$–CH_2–, –$CH(CH_3)$–C_2H_4–, –CH_2–$CH(CH_3)$–CH_2–, –C_2H_4–$CH(CH_3)$–, –CH_2–$C[(CH_3)_2]$–, –$C[(CH_3)_2]$–CH_2–, –$CH(CH_3)$–$CH(CH_3)$–,

–C[(C_2H_5)(CH_3)]–, –CH(C_3H_7)–, –(CH_2–CH_2–O)$_n$–CH_2–CH_2–, –CO–CH_2–, –CO–C_2H_4–, –CO–C_3H_6–, –CO–C_4H_8–, –CO–C_5H_{10}–, –CO–C_6H_{12}–, –CO–C_7H_{14}–, –CO–C_8H_{16}–, –CO–C_9H_{18}–, –CO–$C_{10}H_{20}$–, –CO–CH(CH_3)–, –CO–C[(CH_3)$_2$]–, –CO–CH_2–CH(CH_3)–, –CO–CH(CH_3)–CH_2–, –CO–CH(CH_3)–C_2H_4–, –CO–CH_2–CH(CH_3)–CH_2–, –CO–C_2H_4–CH(CH_3)–, –CO–CH_2–C[(CH_3)$_2$]–, –CO–C[(CH_3)$_2$]–CH_2–, –CO–CH(CH_3)–CH(CH_3)–, –CO–C[(C_2H_5)(CH_3)]–, –CO–CH(C_3H_7)–, –CO–(CH_2–CH_2–O)$_n$–CH_2–CH_2–;

n represents an integer from 1 to 60;

L^3 represents –CO–, –O–CO–, –NH–CO–, –NH(C=NH)–, –SO_2–, –O–SO_2–, –NH–, –NH–CO–CH_2–;

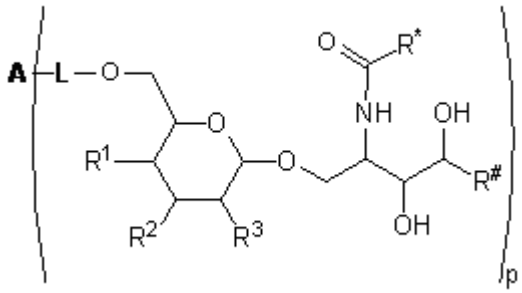
CH represents a monosaccharide, a disaccharide or a trisaccharide;

CA represents  or 

R* and **R**$^{\#}$ represent independently of each other a linear or branched or cyclic, substituted or unsubstituted, saturated or unsaturated carbon residue consisting of 1 to 30 carbon atoms;

and tautomers and pharmaceutically acceptable salts thereof.

2. Compound according to claim 1 of the general formula (II):



(II)

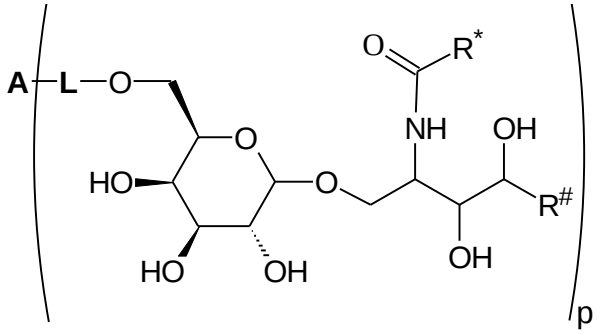
wherein

A, **L** and **p** have the meanings as defined in claim 1;

R^1, **R^2**, **R^3** represent independently of each other:
–H, –OH, –OCH_3, –OC_2H_5, –OC_3H_7, –O–SO_2–CH_3, –O–SO_2–C_2H_5, –O–SO_2–C_3H_7, –O–COOCH_3, –NHCOCH_3, –NH_2;

R* and **R$^\#$** represent independently of each other:
–CH_3, –$(CH_2)_r$–CH_3, –CH(OH)–$(CH_2)_s$–CH_3, –CH=CH–CH_3, –CH=CH–$(CH_2)_t$–CH_3, –CH(OH)–$(CH_2)_v$–$CH(CH_3)_2$, –CH(OH)–$(CH_2)_w$–$CH(CH_3)$–CH_2–CH_3, –$(CH_2)_a$–CH=CH–$(CH_2)_b$–CH_3, –$(CH_2)_c$–CH=CH–$(CH_2)_d$–CH=CH–$(CH_2)_e$–CH_3, –$(CH_2)_f$–CH=CH–$(CH_2)_g$–CH=CH–$(CH_2)_h$–CH=CH–$(CH_2)_i$–CH_3, –$(CH_2)_j$–CH=CH–$(CH_2)_k$–CH=CH–$(CH_2)_l$–CH=CH–$(CH_2)_o$–CH=CH–$(CH_2)_q$–CH_3,
wherein a, b, c, d, e, f, g, h, i, j, k, l, o, q are integers from 1 to 26 with the proviso that: (a+b) ≤ 27; (c+d+e) ≤ 25; (f+g+h+i) ≤ 23; (j+k+l+o+q) ≤ 21; and wherein r is an integer from 1 to 29, s is an integer from 1 to 28, t is an integer from 1 to 27, v is an integer from 1 to 26, w is an integer from 1 to 26.

3. Compound according to claim 1 of the general formula (XIV):



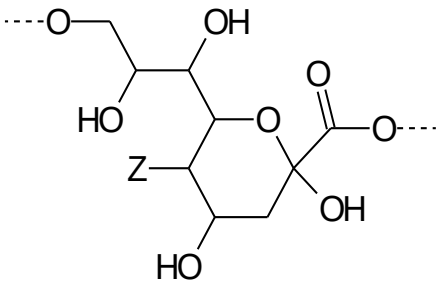
(XIV)

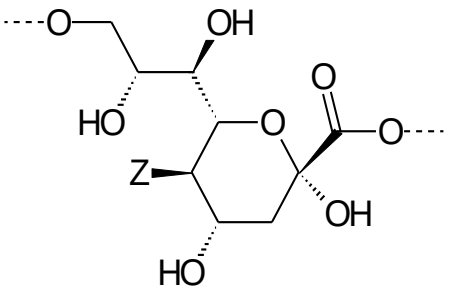
wherein
A, **L**, **R***, **R$^\#$** and **p** have the meanings as defined in claim 1.

4. Compound according to any one of claims 1 – 3, wherein the carbohydrate monomers of the carbohydrate antigen belong to the group of carbohydrates comprising:
α-D-ribopyranose, α-D-arabinopyranose, α-D-xylopyranose, α-D-lyxopyranose, α-D-allopyranose, α-D-altropyranose, α-D-glucopyranose, α-D-mannpyranose, α-D-glucopyranose, α-D-idopyranose, α-D-galactopyranose, α-D-talopyranose,

α-D-psicopyranose, α-D-fructopyranose, α-D-sorbopyranose, α-D-tagatopyranose, α-D-ribofuranose, α-D-arabinofuranose, α-D-xylofuranose, α-D-lyxofuranose, α-D-Allofuranose, α-D-Altrofuranose, α-D-Glucofuranose, α-D-Mannofuranose, α-D-gulofuranose, α-D-idofuranose, α-D-galactofuranose, α-D-talofuranose, α-D-psicofuranose, α-D-fructofuranose, α-D-sorbofuranose, α-D-tagatofuranose, α-D-xylulofuranose, α-D-ribulofuranose, α-D-threofuranose, α-D-rhamnopyranose, α-D-erythrofuranose, α-D-glucosamine, α-D-glucopyranuronic acid, β-D-ribopyranose, β-D-arabinopyranose, β-D-xylopyranose, β-D-lyxopyranose, β-D-allopyranose, β-D-altropyranose, β-D-glucopyranose, β-D-mannpyranose, β-D-glucopyranose, β-D-idopyranose, β-D-galactopyranose, β-D-talopyranose, β-D-psicopyranose, β-D-fructopyranose, β-D-sorbopyranose, β-D-tagatopyranose, β-D-ribofuranose, β-D-arabinofuranose, β-D-xylofuranose, β-D-lyxofuranose, β-D-rhamnopyranose, β-D-allofuranose, β-D-altrofuranose, β-D-glucofuranose, β-D-mannofuranose, β-D-gulofuranose, β-D-idofuranose, β-D-galactofuranose, β-D-talofuranose, β-D-psicofuranose, β-D-fructofuranose, β-D-sorbofuranose, β-D-tagatofuranose, β-D-xylulofuranose, β-D-ribulofuranose, β-D-threofuranose, β-D-erythrofuranose, β-D-glucosamine, β-D-glucopyranuronic acid, α-L-ribopyranose, α-L-arabinopyranose, α-L-xylopyranose, α-L-lyxopyranose, α-L-allopyranose, α-L-altropyranose, α-L-glucopyranose, α-L-mannpyranose, α-L-glucopyranose, α-L-idopyranose, α-L-galactopyranose, α-L-talopyranose, α-L-psicopyranose, α-L-fructopyranose, α-L-sorbopyranose, α-L-tagatopyranose, α-L-rhamnopyranose, α-L-ribofuranose, α-L-arabinofuranose, α-L-xylofuranose, α-L-lyxofuranose, α-L-Allofuranose, α-L-Altrofuranose, α-L-Glucofuranose, α-L-Mannofuranose, α-L-gulofuranose, α-L-idofuranose, α-L-galactofuranose, α-L-talofuranose, α-L-psicofuranose, α-L-fructofuranose, α-L-sorbofuranose, α-L-tagatofuranose, α-L-xylulofuranose, α-L-ribulofuranose, α-L-threofuranose, α-L-erythrofuranose, α-L-glucosamine, α-L-glucopyranuronic acid, β-L-ribopyranose, β-L-arabinopyranose, β-L-xylopyranose, β-L-lyxopyranose, β-L-allopyranose, β-L-altropyranose, β-L-glucopyranose, β-L-mannpyranose, β-L-glucopyranose, β-L-idopyranose, β-L-galactopyranose, β-L-talopyranose, β-L-psicopyranose, β-L-fructopyranose, β-L-sorbopyranose, β-L-tagatopyranose, β-L-ribofuranose, β-L-arabinofuranose, β-L-xylofuranose, β-L-lyxofuranose, β-L-allofuranose, β-L-altrofuranose, β-L-glucofuranose, β-L-mannofuranose, β-L-gulofuranose, β-L-idofuranose, β-L-galactofuranose, β-L-talofuranose, β-L-psicofuranose, β-L-fructofuranose, β-L-sorbofuranose, β-L-tagatofuranose, β-L-xylulofuranose, β-L-ribulofuranose, β-L-threofuranose, β-L-erythrofuranose, β-L-glucosamine, β-L-glucopyranuronic acid, β-L-rhamnopyranose,

or N- or O-substituted derivatives of neuraminic acid of the following formula:





wherein Z represents $–NH_2$, –NHAc, or –OH. ;

5. Compound according to any one of claims 1 – 4, wherein the carbohydrate monomers of the **CH**-moiety belong to the group of carbohydrates comprising:
α-D-ribopyranose, α-D-arabinopyranose, α-D-xylopyranose, α-D-lyxopyranose, α-D-allopyranose, α-D-altropyranose, α-D-glucopyranose, α-D-mannpyranose, α-D-glucopyranose, α-D-idopyranose, α-D-galactopyranose, α-D-talopyranose, α-D-psicopyranose, α-D-fructopyranose, α-D-sorbopyranose, α-D-tagatopyranose, α-D-ribofuranose, α-D-arabinofuranose, α-D-xylofuranose, α-D-lyxofuranose, α-D-Allofuranose, α-D-Altrofuranose, α-D-Glucofuranose, α-D-Mannofuranose, α-D-gulofuranose, α-D-idofuranose, α-D-galactofuranose, α-D-talofuranose, α-D-psicofuranose, α-D-fructofuranose, α-D-sorbofuranose, α-D-tagatofuranose, α-D-xylulofuranose, α-D-ribulofuranose, α-D-threofuranose, α-D-erythrofuranose, α-D-glucosamine, α-D-glucopyranuronic acid, α-D-rhamnopyranose, β-D-ribopyranose,
β-D-arabinopyranose, β-D-xylopyranose, β-D-lyxopyranose, β-D-allopyranose, β-D-altropyranose, β-D-glucopyranose, β-D-mannpyranose, β-D-glucopyranose, β-D-idopyranose, β-D-galactopyranose, β-D-talopyranose, β-D-psicopyranose, β-D-fructopyranose, β-D-sorbopyranose, β-D-tagatopyranose, β-D-ribofuranose, β-D-arabinofuranose, β-D-xylofuranose, β-D-lyxofuranose, β-D-allofuranose, β-D-altrofuranose, β-D-glucofuranose, β-D-mannofuranose, β-D-gulofuranose, β-D-idofuranose, β-D-galactofuranose, β-D-talofuranose, β-D-psicofuranose, β-D-fructofuranose, β-D-sorbofuranose, β-D-tagatofuranose, β-D-xylulofuranose, β-D-ribulofuranose, β-D-threofuranose, β-D-erythrofuranose, β-D-rhamnopyranose, β-D-glucosamine, β-D-glucopyranuronic acid, α-L-ribopyranose, α-L-arabinopyranose, α-L-xylopyranose, α-L-lyxopyranose, α-L-allopyranose, α-L-altropyranose, α-L-glucopyranose, α-L-mannpyranose, α-L-glucopyranose, α-L-idopyranose, α-L-galactopyranose, α-L-talopyranose, α-L-psicopyranose, α-L-fructopyranose, α-L-sorbopyranose, α-L-tagatopyranose, α-L-ribofuranose, α-L-arabinofuranose, α-L-xylofuranose, α-L-lyxofuranose, α-L-Allofuranose, α-L-Altrofuranose, α-L-Glucofuranose, α-L-Mannofuranose, α-L-

gulofuranose, α-L-idofuranose, α-L-galactofuranose, α-L-talofuranose, α-L-psicofuranose, α-L-fructofuranose, α-L-sorbofuranose, α-L-tagatofuranose, α-L-xylulofuranose, α-L-ribulofuranose, α-L-rhamnopyranose α-L-threofuranose, α-L-erythrofuranose, α-L-glucosamine, α-L-glucopyranuronic acid, β-L-ribopyranose, β-L-arabinopyranose, β-L-xylopyranose, β-L-lyxopyranose, β-L-allopyranose, β-L-altropyranose, β-L-glucopyranose, β-L-mannpyranose, β-L-glucopyranose, β-L-idopyranose, β-L-galactopyranose, β-L-talopyranose, β-L-psicopyranose, β-L-fructopyranose, β-L-sorbopyranose, β-L-tagatopyranose, β-L-ribofuranose, β-L-arabinofuranose, β-L-xylofuranose, β-L-lyxofuranose, β-L-allofuranose, β-L-altrofuranose, β-L-glucofuranose, β-L-mannofuranose, β-L-gulofuranose, β-L-idofuranose, β-L-galactofuranose, β-L-talofuranose, β-L-psicofuranose, β-L-fructofuranose, β-L-sorbofuranose, β-L-tagatofuranose, β-L-xylulofuranose, β-L-ribulofuranose, β-L-threofuranose, β-L-erythrofuranose, β-L-glucosamine, β-L-glucopyranuronic acid, β-L-rhamnopyranose.

6. Compound according to any of claims 1 – 5, wherein the saccharide antigen **A** is a bacterial capsular saccharide, a saccharide of a viral glycoprotein, a saccharide antigen of sporozoa or parasites, a saccharide antigen of pathogenic fungi, or a saccharide antigen which is specific to cancer cells.

7. Compound according to any of the claims 1 – 6 for use as pharmaceutically active agent in medicine or for use in vaccination against infectious diseases.

8. Compound according to claim 7, wherein the infectious disease is caused by a pathogen selected from the group comprising:
Allochromatium vinosum, Acinetobacter baumanii, Bacillus anthracis, Campylobacter jejuni, Clostridium spp., Citrobacter spp., Escherichia coli, Enterobacter spp., Enterococcus faecalis., Enterococcus faecium, Francisella tularensis, Haemophilus influenzae, Helicobacter pylori, Klebsiella spp., Listeria monocytogenes, Moraxella catharralis, Mycobacterium tuberculosis, Neisseria meningitidis, Neisseria gonorrhoeae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella spp., Serratia spp., Shigella spp., Stenotrophomonas maltophilia, Staphyloccocus aureus, Staphyloccocus epidermidis, Streptococcus pneunmoniae, Streptococcus pyogenes, Streptococcus agalactiae, Yersina pestis, Yersina enterocolitica,
influenza virus, human immunodeficiency virus ("HIV"), herpes simplex virus ("HSV", type 1 or 2), human papilloma virus ("HPV", type 16 or 18), human cytomegalovirus ("HCMV") or human hepatitis B or C virus (“HBV”, Type B; "HCV", type C).

9. Compound according to claims 6, wherein the bacterial capsular saccharide belongs to bacteria selected from:
Allochromatium vinosum, Acinetobacter baumanii, Bacillus anthracis, Campylobacter jejuni, Clostridium spp., Citrobacter spp., Escherichia coli, Enterobacter spp., Enterococcus faecalis., Enterococcus faecium, Francisella tularensis, Haemophilus influenzae, Helicobacter pylori, Klebsiella spp., Listeria monocytogenes, Moraxella catharralis, Mycobacterium tuberculosis, Neisseria meningitidis, Neisseria gonorrhoeae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella spp., Serratia spp., Shigella spp., Stenotrophomonas maltophilia, Staphyloccocus aureus, Staphyloccocus epidermidis, Streptococcus pneunmoniae, Streptococcus pyogenes, Streptococcus agalactiae, Yersina pestis, und Yersina enterocolitica.

10. Compound according to claims 6, wherein the saccharide of viral glycoproteins belongs to viruses selected from:
Adenoviruses, Ebolavirus, Epstein-Barr-virus, Flavivirus, FSME-virus, Influenza virus, Hanta-virus, human immunodeficiency virus ("HIV"), herpes simplex virus ("HSV", type 1 or 2), human herpes virus 6 (HHV-6), human Papilloma virus ("HPV", type 16 or 18), human Cytomegalovirus ("HCMV"), human hepatitis B or C virus (“HBV”, Type B; "HCV", type C), Lassavirus, Lyssavirus (EBL 1 or EBL 2), Marburgvirus, Norovirus, Parvovirus B19, Pestvirus, Poliovirus, Rhinovirus, Rotaviruses, SARS-assciated Coronavirus, Varicella-Zoster virus.

11. Compound according to claims 6, wherein the saccharide antigen of sporozoa or parasites belongs to sporozoa or parasites selected from:
Babesia, Balantidium, Besnoitia, Blastocystis, Coccidia, Cryptosporidium, Cytauxzoon, Cyclospora, Dientamoeba, Eimeria, Entamoeba, Enterocytozoon, Enzephalitozoon, Eperythrozoon, Giardia, Hammondia, Isospora, Leishmania, Microsporidia, Naegleria, Plasmodium, Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium knowlesi, Pneumocystis, Schistosoma, Sarcocystis, Theileria, Trichinella, Toxoplasma, Trichomonas, Trypanosoma, Unicaria, Cestoda, Dipylidium, Dranunculus, Echinococcus, Fasciola, Fasciolopsis, Taenia, Ancylostoma, Ascaris, Brugia, Enterobius, Loa loa, Mansonella, Necator, Oncocerca, Strongyloides, Strongylus, Toxocara, Toxascaris, Trichuris oder Wucheria.

12. Compound according to claims 6, wherein the saccharide antigen of fungi belongs to fungi selected from:
Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton interdigitale, T. schönleinii, T. verrucosum, T. violaceum, T. tonsurans, Trichophyton spp., M. canis, Candida albicans, C. guillermondii, C. krusei, C. parapsilosis, C. tropicalis, C. glabrata, Candida spp., Microsporum spp., Microsporum canis, Microsporum audonii, Microsporum gypseum, M. ferrugineum, Trichosporum beigelii, Trichosporum inkiin, Aspergillus niger, Alternaria, Acremonium, Fusarium, or Scopulariopsis.

13. Compound according to claims 6, wherein the saccharide antigen which is specific to cancer cells belongs to the group of cancers selected from:
Bladder Cancer, Breast Cancer, Colon and Rectal Cancer, Endometrial Cancer, Kidney (Renal Cell) Cancer, Leukemia, Lung Cancer Melanoma, Non-Hodgkin Lymphoma, Pancreatic Cancer, Prostate Cancer, Thyroid Cancer.

14. Compound according to any of claims 1 – 6, wherein the average ratio of the carbohydrate antigen **A** to the glycolipid (**L-CH-CA**) is between 1:4 and 1:100 (n/n).

15. Vaccine formulation comprising one compound or a mixture of different compounds of any one of claims 1 – 14.

Figure 1

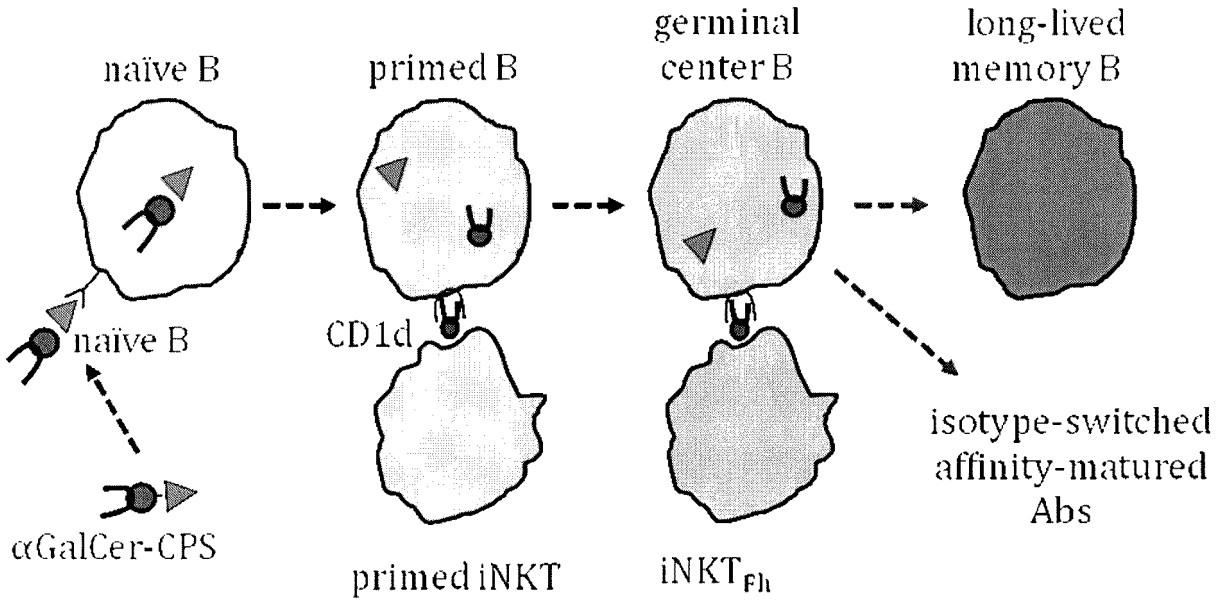
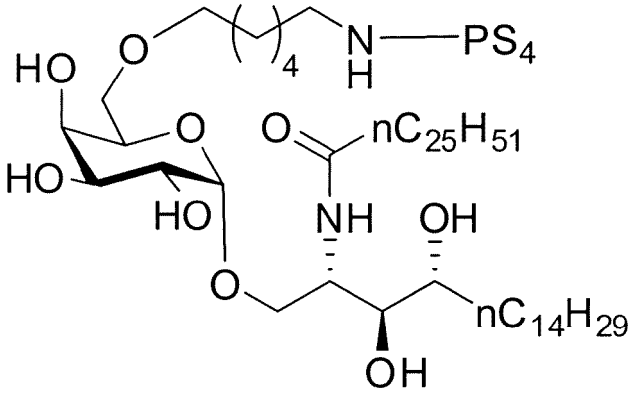


Figure 2



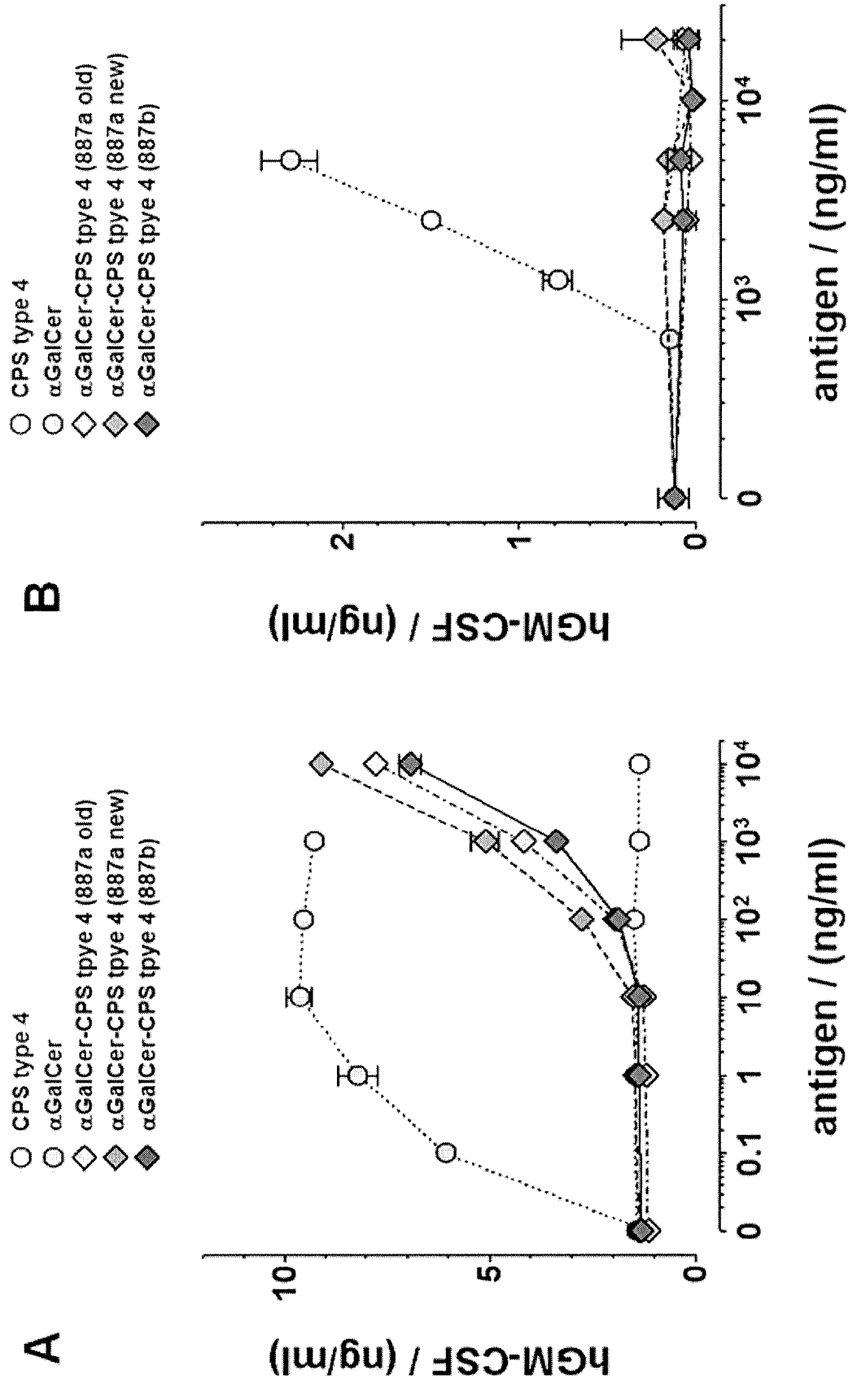
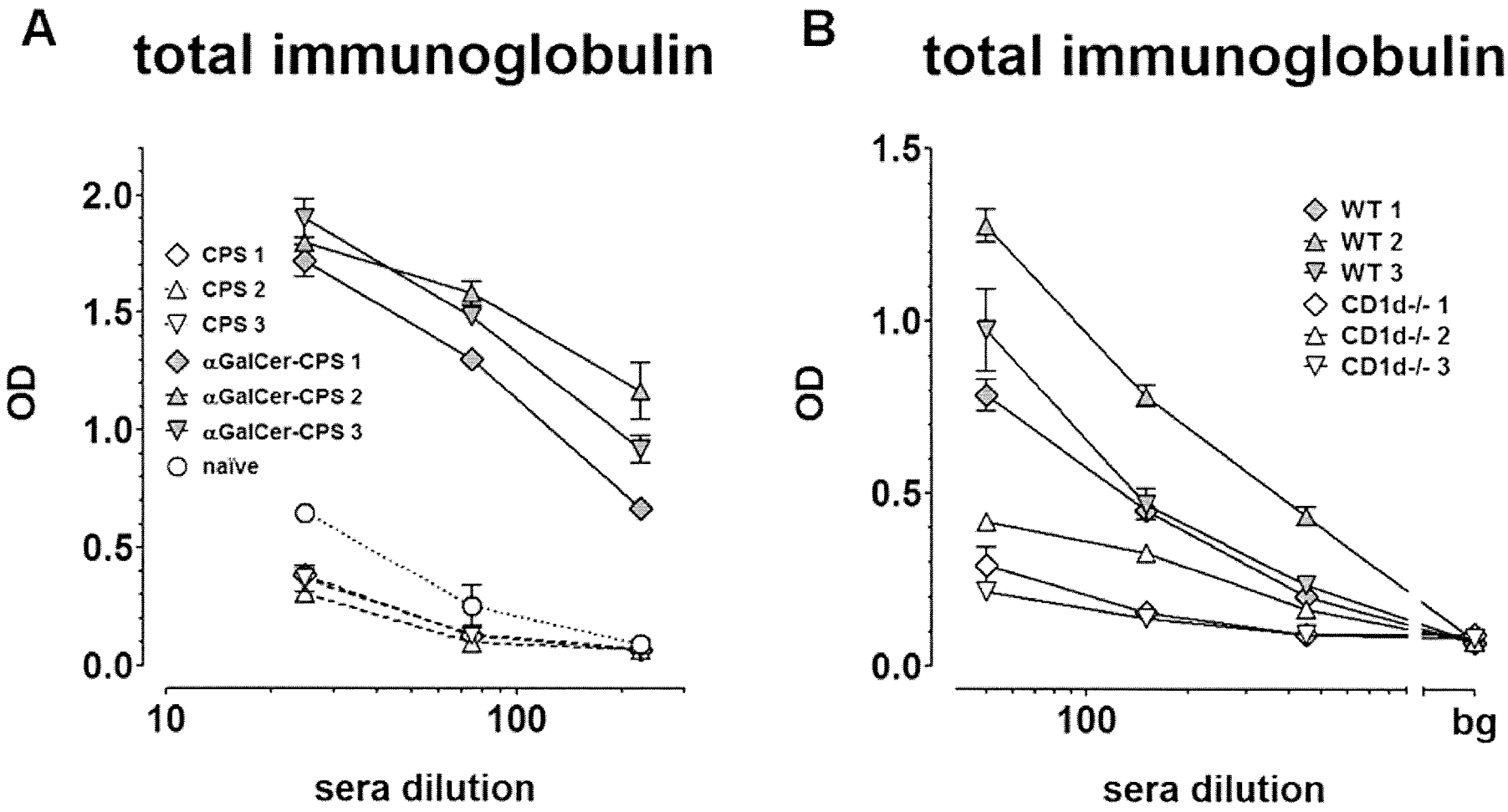

A
hGM-CSF / (ng/ml)
antigen / (ng/ml)
CPS type 4
αGalCer
αGalCer-CPS tpye 4 (887a old)
αGalCer-CPS tpye 4 (887a new)
αGalCer-CPS tpye 4 (887b)
B
hGM-CSF / (ng/ml)
antigen / (ng/ml)
CPS type 4
αGalCer
αGalCer-CPS tpye 4 (887a old)
αGalCer-CPS tpye 4 (887a new)
αGalCer-CPS tpye 4 (887b)

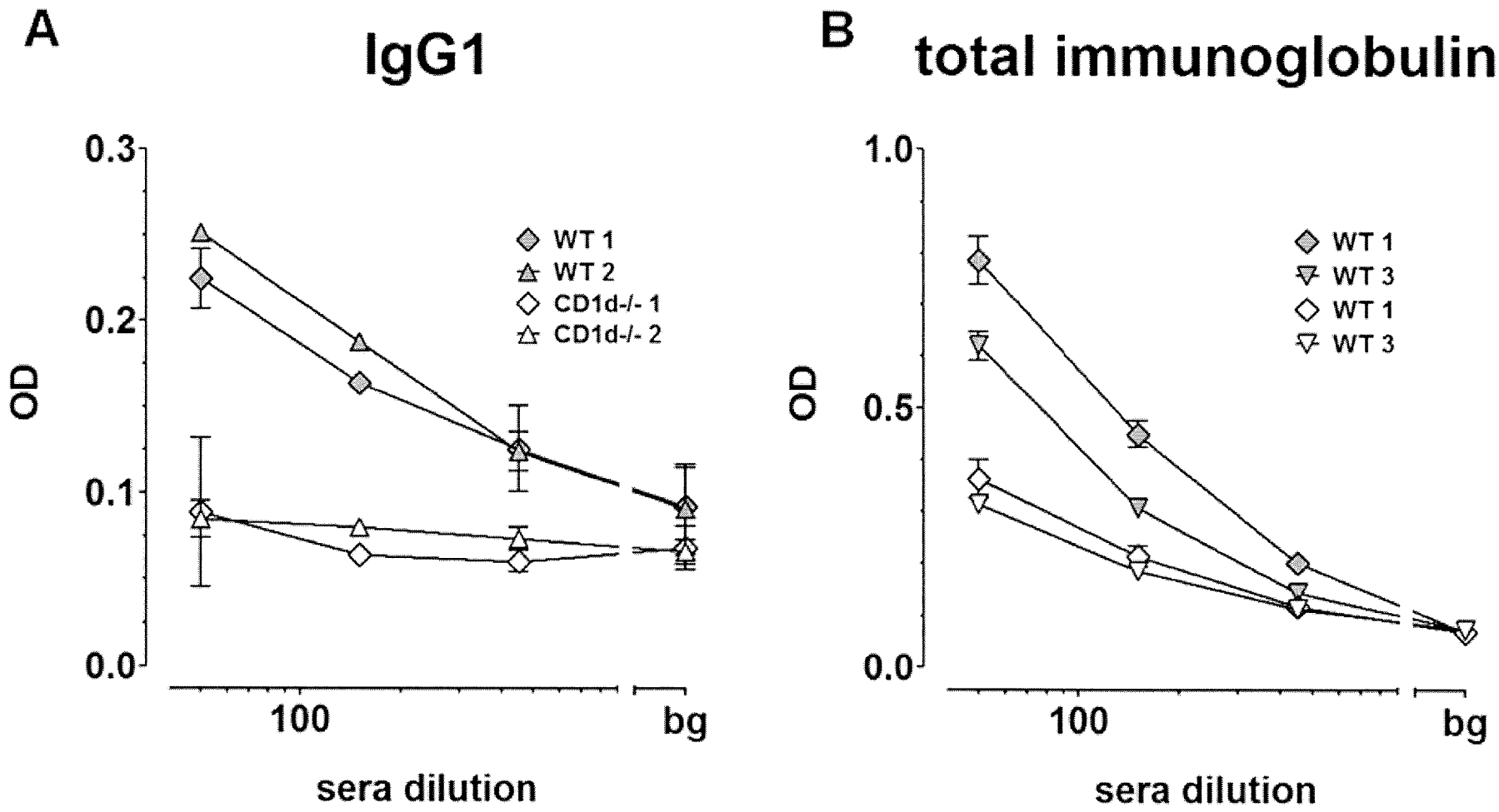
Figure 3

Figure 4



SUBSTITUTE SHEET (RULE 26)

Figure 5



SUBSTITUTE SHEET (RULE 26)

Figure 6

Hybridoma	Heavy chain	CDR1	CDR2	CDR3	Mutations (aa/nuc)*
6C2	γ1	GYSITSGYS	I-HY--SGYT	ARSANYGPM-----DYWGQG	4/6
2F4	γ1	GFSLTS-YG	I--W-SDGST	ARHSKLGQF-----AYWGQG	0/1
1B4	γ1	GYSITSGYS	I-HY--SGYT	ARSANYGPM-----DYWGQG	4/6
12F10	γ1	GYSITSGYS	I-HY--SGST	ARSANYGPM-----DYWGQG	2/5
2B5	γ2a	GFSLTS-YG	I--W-SGGST	VRNGVYRDF-----AYWGQG	0/0
26D2	γ3	GFSLTS-YG	I--W-GDGST	AKIYYYGRN--YAMDYWGQG	1/2
26H11	μ	GYTFTS-YY	I-IYPGNVNT	ARGGNYYYY---AMDYWGQG	0/0
10B4	μ	GYSFTG-YN	IDPY--YGGT	ARSTGTAWF-----AYWGQG	0/0
5H3	μ	GFTFSS-YG	I-S-SGGSYT	ARLYD-GYY-VAWFAYWGQG	0/0
14D3	μ	GFSLTS-YG	I--W-SGGST	ARNSGTGWY----FDVWGAG	0/0
18 E4	μ	GFSLTS-YG	I--W-SGGST	ARNGNRAWF-----AYWGQG	0/0
16D9	μ	GYTFT-DYA	ISTY--YGNT	AR----GDW----EDYWGQG	4/9
3 E10	μ	GFSLTS-YG	I--W-SGGST	ARDGYPAWF-----AYWGQG	0/0
26F3	μ	GYTFTS-YW	I--NPSTGYT	ARGRN--HY----FDYWGQG	0/1
25B7	μ	GFSLTD-YG	I--W-GGGST	AKQGNWADY--YAMDYWGQG	0/0
25A8	μ	GYTFSR-YW	I--LPGSGTT	ARLLR---Y----FDYWGQG	3/5
21B6	μ	GYSFTG-YN	IDPY--YGGT	ARS-NWTYY--YAMDYWGQG	0/0
18G6	μ	GYSFTG-YN	IDPY--YGGT	AR-----GY---AMDYWGQG	0/0
18G6	μ	GYSITSDYA	I-SY--SGST	ARSL-YGNYGDYAMDYWGQG	0/0

SUBSTITUTE SHEET (RULE 26)

Cells	%positive
APCalone	0
APC+C	47
APC+C +12F10	60
APC+C +C15	37
APC+12F10	0
APC+C15	3

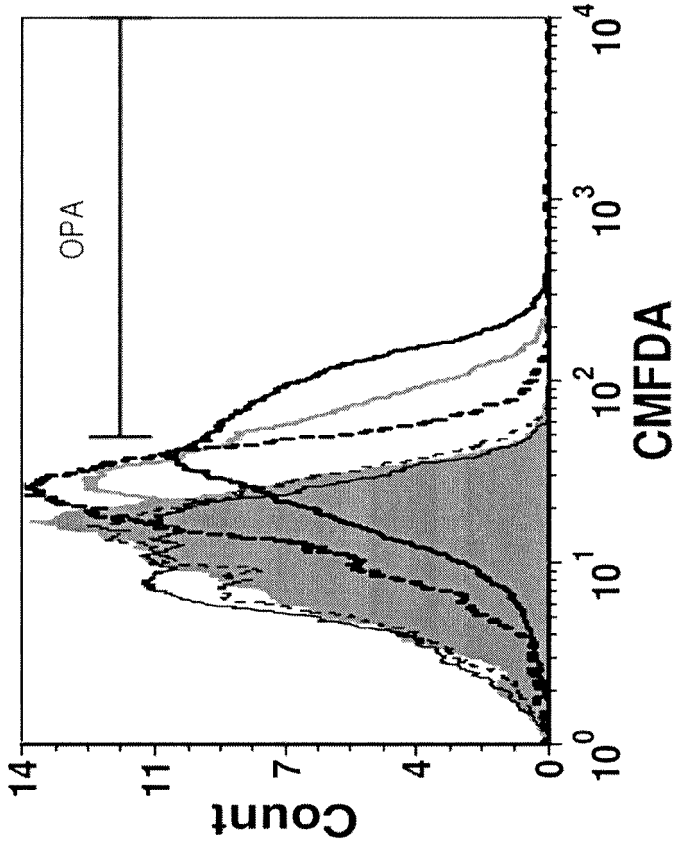
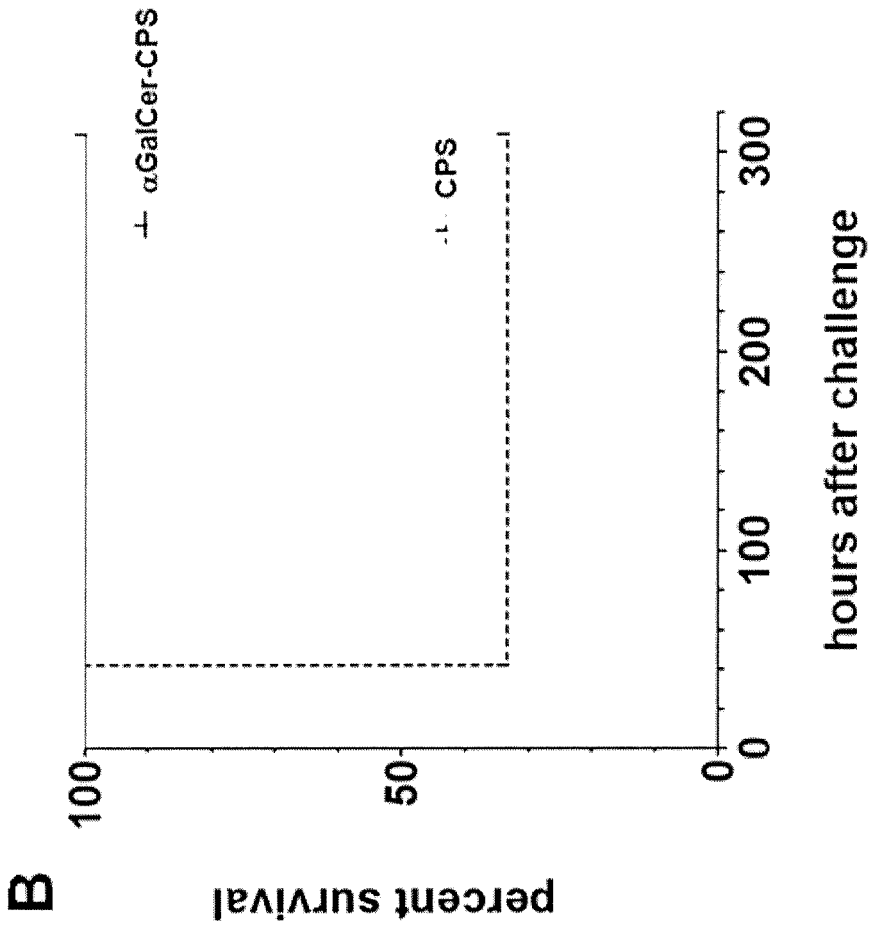


Figure 7


B
percent survival
100
50
0
0
100
200
300
hours after challenge
αGalCer-CPS
CPS

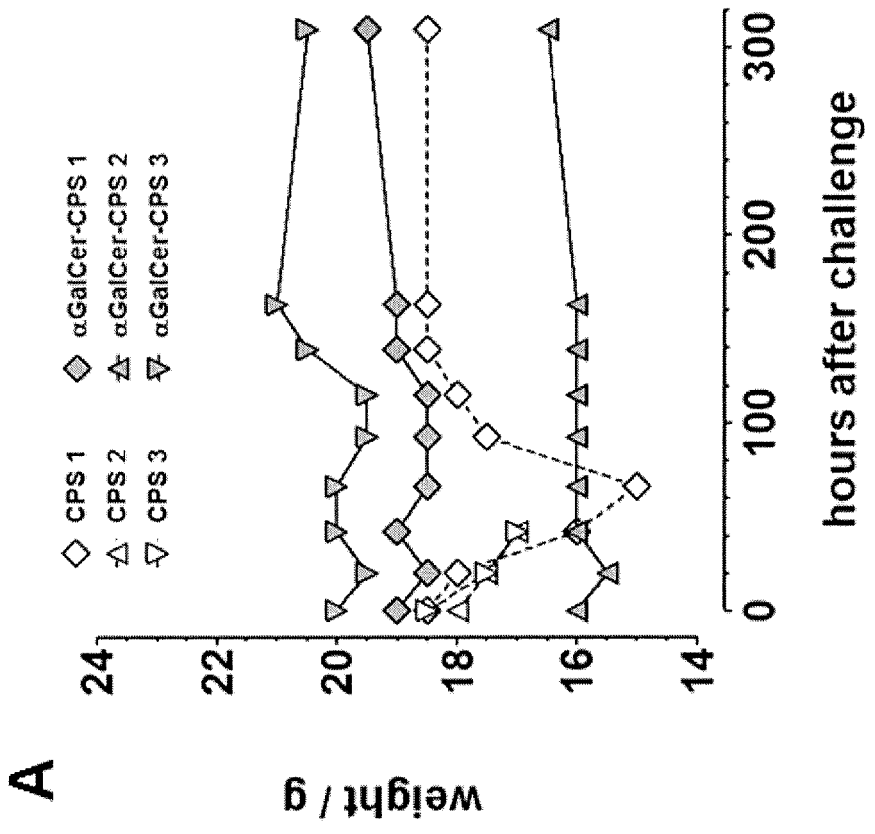

A
weight / g
24
22
20
18
16
14
0
100
200
300
hours after challenge
CPS 1
CPS 2
CPS 3
αGalCer-CPS 1
αGalCer-CPS 2
αGalCer-CPS 3

Figure 8

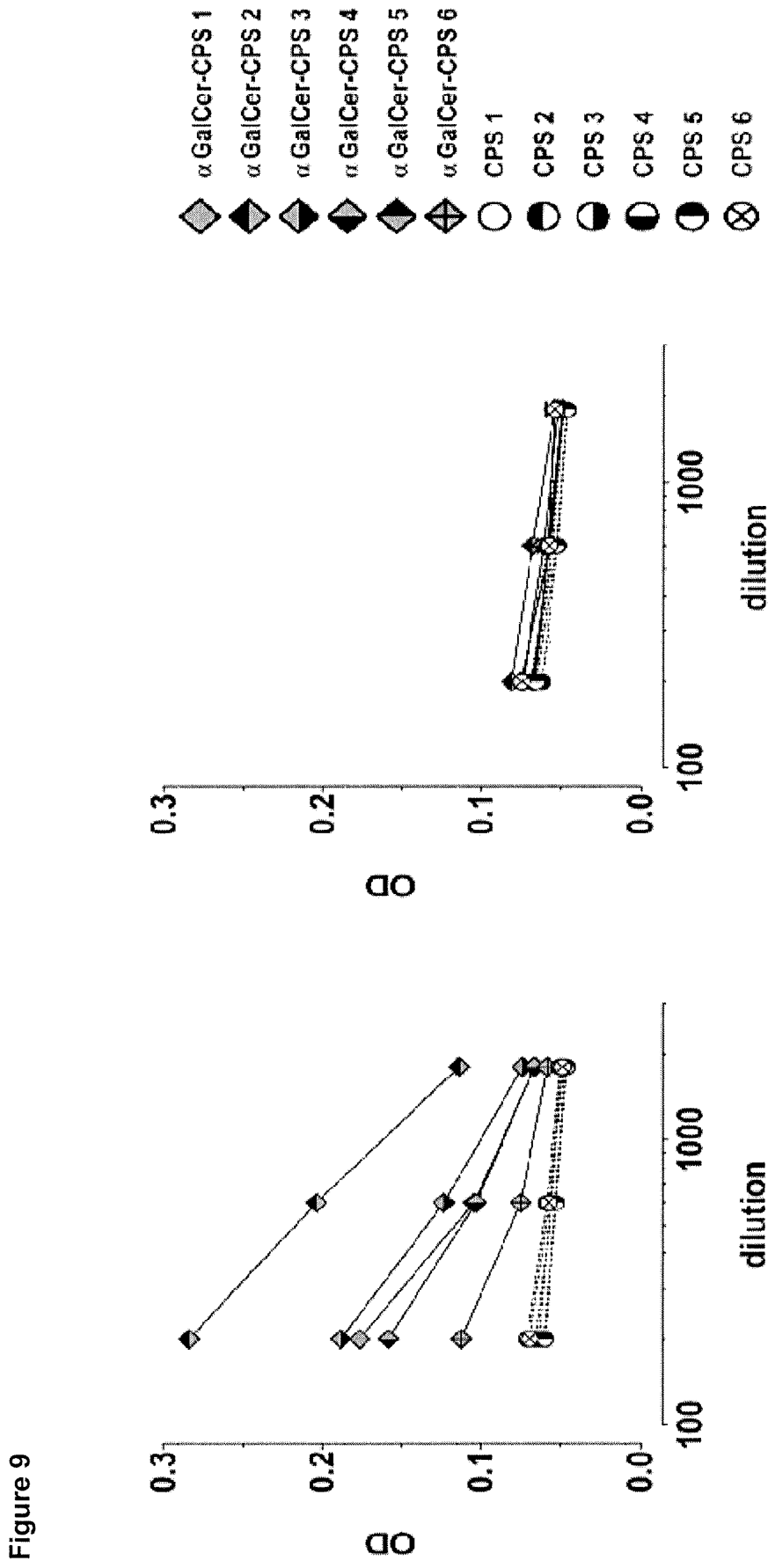

α GalCer-CPS 1
α GalCer-CPS 2
α GalCer-CPS 3
α GalCer-CPS 4
α GalCer-CPS 5
α GalCer-CPS 6
CPS 1
CPS 2
CPS 3
CPS 4
CPS 5
CPS 6
OD
0.3
0.2
0.1
0.0
100
1000
dilution
OD
0.3
0.2
0.1
0.0
100
1000
dilution

Figure 9

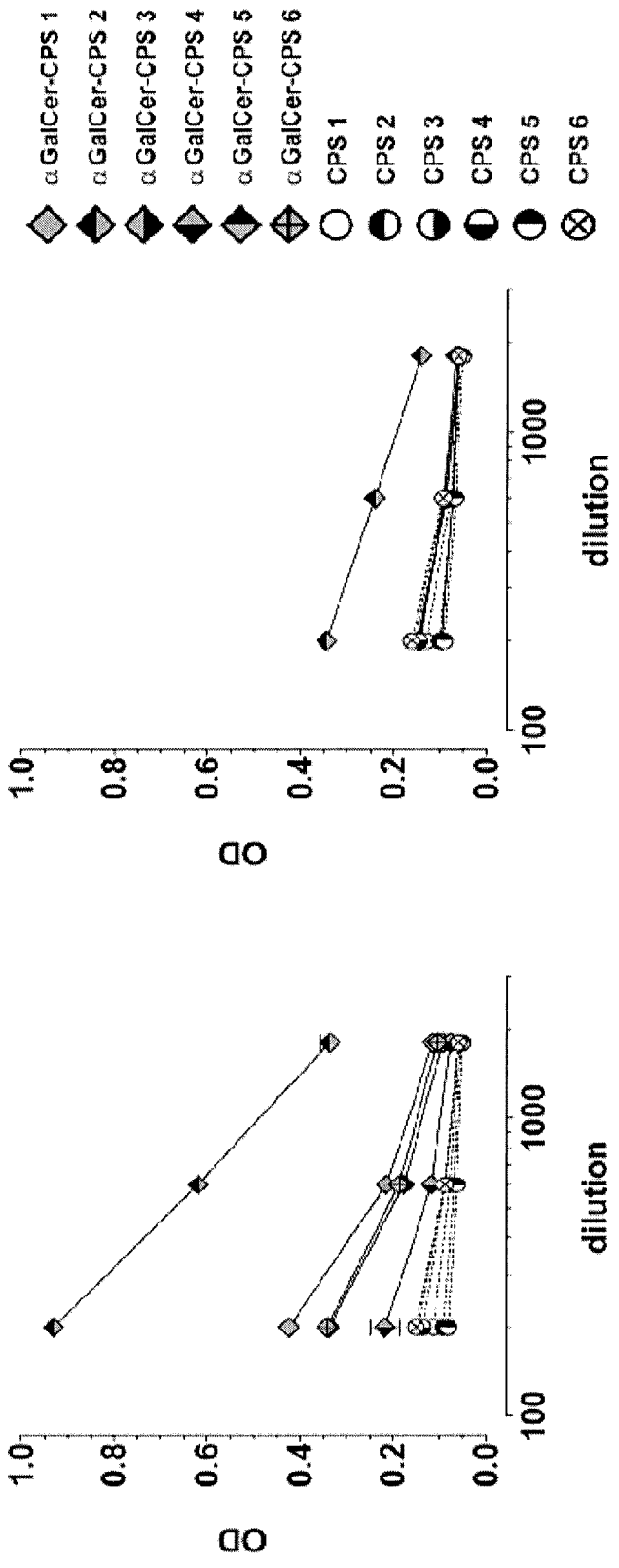

αGalCer-CPS 1
αGalCer-CPS 2
αGalCer-CPS 3
αGalCer-CPS 4
αGalCer-CPS 5
αGalCer-CPS 6
CPS 1
CPS 2
CPS 3
CPS 4
CPS 5
CPS 6
OD
1.0
0.8
0.6
0.4
0.2
0.0
100
1000
dilution

Figure 10