

DESCRIPTION

"New carbohydrate derivatives as mimetics of blood group A and B antigens"

5 Technical Field

The present invention relates to new carbohydrate derivatives with activity as mimetics of blood group A and B antigens.

Technical Background

10 The ABO blood classification is used to denote the presence of group A, group B and group O (H) antigens (ABH antigens) on erythrocytes.

Group A and B antigens are carbohydrate molecules comprising a terminal trisaccharide with the characteristic feature that the nonreducing terminal carbohydrate of said trisaccharide is either a *N*-acetylgalactosamine or a galactose residue, respectively, which is linked by an α (1 $\rightarrow$ 3) glycosidic bond to another galactose residue, which, in turn, bears a fucose residue linked at the
15 2 position through a α (1 $\rightarrow$ 2) glycosidic linkage.

The ABH antigens can be further divided into subgroups, depending on the inner core saccharide chain, for example, they are expressed on type 1 (Gal β 1,3GlcNAc), type 2 (Gal β 1,4GlcNAc), type 3 (Gal β 1,3GalNAc α) and type 4 (Gal β 1,3GalNAc β) chains, the only
20 difference being the linkage between the inner galactose residue and an additional carbohydrate residue, which is either *N*-acetylglucosamine or *N*-acetylgalactosamine. Thus, for example, group A type 2 antigen has the sequence GalNAc α 1,3(Fuc α 1,2)Gal β 1,4GlcNAc and group B type 2 antigen has the sequence Gal α 1,3(Fuc α 1,2)Gal β 1,4GlcNAc.

The ABO blood group antigens are located on the surfaces of red blood cells and epithelial
25 cells and they are medically highly relevant, especially in the field of transfusions and transplantation, due to their associated anti-A and anti-B antibodies. As the blood group antigen that is expressed differs between individuals, preformed antibodies against the blood group A and B epitopes are produced in individuals not expressing these antigens and they therefore represent the first obstacle for donation of blood, organs or tissues between individuals who express
30 incompatible blood groups. These natural antibodies mainly belong to the IgM isotype and are carbohydrate-binding proteins (CBPs), which bind to the carbohydrate epitopes at the cell surface, leading to complement fixation, and causing intravascular haemolysis of red blood cells in blood transfusion and hyperacute rejection of vascularised organs.

Due to the chronic shortage of donors, strategies are being developed to perform blood
35 transfusions and organ transplants across the ABO barrier.

One strategy known in the art for allowing ABO-incompatible transplantations is the use of carbohydrate-based therapeutics to eliminate/inhibit the recipient preformed anti-carbohydrate

antibodies before grafting and for a defined period post-transplantation, until accommodation occurs. For example, extracorporeal anti-carbohydrate removal can be achieved by using carbohydrate-based immunoadsorbents or, alternatively (or complementarily), the anti-carbohydrate antibodies may be inhibited *in vivo* by injectable antigens, as disclosed, for example, 5 in Holgerson *et al.*, Immunol. Cell Biol., 2005, 83, 694-708.

For example, it has been disclosed in the art the use of blood group A and B trisaccharide epitopes linked to crystalline silica and to Sepharose® for removal of anti-A/B antibodies (Holgerson *et al.*, *op. cit.*)

As for the injection of material to inhibit antibodies *in vivo*, the main difficulties for the use 10 of free oligosaccharides are the low affinity protein-carbohydrate binding and short serum half-life. Therefore, the use of glycoconjugates is advantageous, where multiple antigenic oligosaccharides are bound to a biodegradable backbone, allowing multivalent binding and, therefore, increased affinity and inhibiting activity, as disclosed, for example, in Duthaler *et al.*, Chimia, 2010, 64, 23-28, or in Katopodis *et al.*, J. Clin. Invest., 2002, 110, 1869-1877, or in Wang 15 *et al.*, J. Am. Chem. Soc., 1999, 121, 8174-8181.

So far, it has been always disclosed in the art that, either for *in vitro*, extracorporeal or for *in vivo* inhibition/adsorption of natural antibodies, it is important to use inhibitors or adsorbents bearing active epitopes that correspond as far as possible to the natural carbohydrate antigens.

Thus, for example, in the European patent EP-B-2010920 it is disclosed the preparation 20 of a solid support, in particular, in the form of microbeads, to which several A and B antigens are attached, and it is also disclosed their use for determining the presence of blood group reactive antibodies in serum samples, or for removing blood group reactive antibodies in serum. The carbohydrate antigens are specifically selected from either type 1, 2, 3 or 4 antigens A or B.

Analogously, in the international patent application WO-A-2013/062479 it is disclosed a 25 method for extracorporeal elimination of anti-A and/or anti-B antibodies from blood with an absorbent substance comprising a matrix linked to a saccharide which is a blood group determinant A or a blood group determinant B, namely, a group A-trisaccharide determinant (GalNAc α 1,3(Fuc α 1,2)Gal β 1) or a group B-trisaccharide determinant (Gal α 1,3(Fuc α 1,2)Gal β 1) further linked to inner saccharide as subtype 1, 2, 3 or 4. The matrix is, for example, agarose, 30 dextran, starch or starch derivatives, amylose, amylopectin or polyacrylamide.

The international patent application WO-A-2018/167230 discloses polymers comprising a multitude of carbohydrate ligands, which bind to different types of carbohydrate-binding proteins, including agglutinins, and their use for diagnosis and treatment of diseases, including those associated with CBP-mediated agglutination. Specific examples 9, 32 and 35 correspond to a 35 polylysine glycoconjugate bearing the blood group A antigen type 5 tetrasaccharide, the blood group A trisaccharide antigen and the blood group B trisaccharide antigen, respectively, wherein such antigens are bound to the polylysine backbone through a linker chain group.

The group A and B glycopolymers disclosed so far in the art, comprising the natural group A and B determinants, have still some drawbacks derived from the low affinity binding between these natural antigens and the antibodies.

Therefore, there is still the need to provide new glycoconjugates having improved affinity
5 towards anti-A and/or anti-B antibodies to be used in different biological and medical applications, for example, for effectively removing/inhibiting those antibodies before ABO-incompatible transplantation.

Object of the invention

10 The object of the present invention is a compound of formula (I).

Another aspect of the present invention is a process for the preparation of the compound of formula (I).

Another aspect of the invention is a glycoconjugate of the compound of formula (I).

Another aspect of the invention is the use of said glycoconjugate for inhibiting and/or
15 removing anti-A and/or anti-B antibodies from samples of blood or other blood derivatives and by-products.

Another aspect of the invention is said glycoconjugate for use in medicine.

Another aspect of the invention is a pharmaceutical composition comprising said glycoconjugate and at least one pharmaceutically acceptable excipient.

20

Description of the drawings

Figure 1 shows Scheme I, depicting a synthetic route for the compounds of formula (I).

Figure 2 and Figure 3 show Schemes Ia and Ib, respectively, depicting a synthetic route for the compounds of formula (I) wherein R₂ is a group of formula (II) and R₃ is a group of formula
25 (III).

Figure 4 shows Scheme Ic, depicting a synthetic route for the compounds of formula (I) wherein R₂ is OH and R₃ is a group of formula (III).

Figure 5 shows Scheme II, depicting the preparation of a glycoconjugate of the compound of formula (I) with polyacrylic acid.

Figure 6 and Figure 7 show Schemes III and IV, respectively, depicting the preparation of a glycoconjugate of the compound of formula (I) with poly-L-lysine.

Figure 8 shows the synthetic scheme for the glycoconjugate with poly-L-lysine (a=25) disclosed in Example 4.

Figure 9 represents the binding of purified human anti-B (graphs 9-1 and 9-2) and anti-A
35 (graphs 9-3 and 9-4) antibodies, either IgG or IgM, to different glycan antigens, measured in an ELISA assay, as disclosed in Example 6.2. Ordinates represent the optical density (OD) values for the different tested samples (1 to 9).

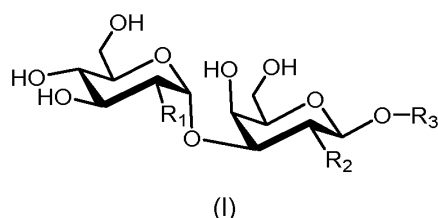
Figure 10 represents the percentage of removal (in the ordinates) of purified anti-A antibodies with different poly-L-Lysine glycoconjugates, as disclosed in Example 6.3. Figures 10-1 and 10-2 represent the removal for IgG and IgM, respectively. Black bars correspond to the Comparative-2 glycoconjugate (comprising natural A type II antigen) and the grey bars correspond to Example 4-8 glycoconjugate (comprising a mimetic according to the present invention), at increasing concentrations (10, 100 and 400 µg/mL).

Figure 11 represents the percentage of removal (in the ordinates) of purified anti-B antibodies with different poly-L-Lysine glycoconjugates, as disclosed in Example 6.3. Figures 11-1 and 11-2 represent the removal for IgG and IgM, respectively. Black bars correspond to the Comparative-1 glycoconjugate (comprising natural B type II antigen) and the grey bars correspond to the glycoconjugate of Example 4-1 (comprising a mimetic according to the present invention), at increasing concentrations (10, 100 and 400 µg/mL).

Figure 12 represents the inhibition of the binding of purified anti-B antibodies to red blood cells (Group B) with poly-L-Lysine glycoconjugates, measured by Flow Cytometry, as disclosed in Example 6.4. The inhibitory capacity is expressed as a percentage of removal referred to the baseline condition (in ordinates). The glycoconjugates assayed were Comparative-1 (black bar, containing natural B type-II antigen), Example 4.1 (light grey bar, containing a B-mimetic according to the present invention), and the combination of Comparative-1 and Example 4.1 glycoconjugates (dark grey bar).

Detailed description of the invention

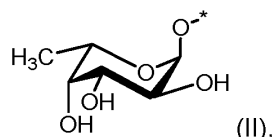
The object of the present invention is a carbohydrate derivative of formula (I):



wherein:

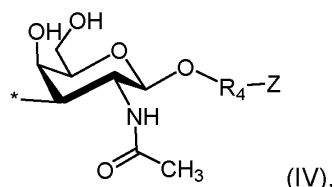
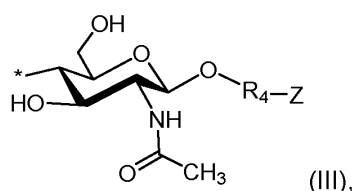
R_1 is selected from OH and $-NH-CO-R_5$, wherein R_5 is C_1-C_3 alkyl and wherein one or more hydrogen atoms in R_5 may be replaced by a hydroxyl group;

R_2 is selected from OH and a group of formula (II):



wherein * indicates the point of attachment of said group with the rest of the molecule;

R_3 is selected from R_4-Z , a group of formula (III) and a group of formula (IV):



wherein

* indicates the point of attachment of said group with the rest of the molecule,

5 R_4 is a spacer group, and

Z is selected from $-NH_2$, $-COOH$, and $-SH$.

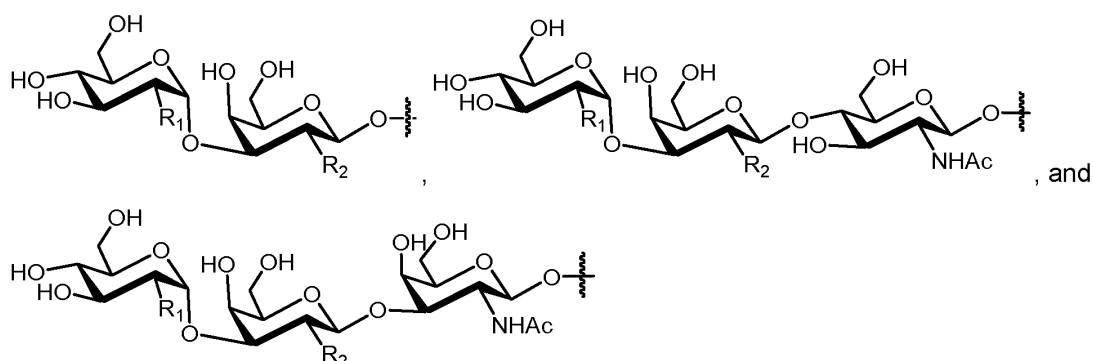
The authors of the present invention have developed new carbohydrate derivatives of formula (I), which, surprisingly, have high affinity towards natural anti-A/B antibodies despite the fact that they have a glucose or a glucosamine derivative at the nonreducing end, instead of a galactose or a N-acetylgalactosamine, as in natural A and B antigens.

Definitions

The term “glycan”, as is commonly used in the art, is a general term to designate carbohydrates, sugars, monosaccharides, oligosaccharides and polysaccharides. In particular, the term “glycan” is used herein to designate the carbohydrate portion of the carbohydrate derivative of formula (I) of the invention; in this regard, the terms “glycan” and “carbohydrate moiety” or “oligosaccharide moiety” are used interchangeably and are also used to designate the carbohydrate portion of the glycoconjugates.

The carbohydrate moiety in the compound of formula (I) is also occasionally referred to herein as “epitope”, or “glycoepitope”, or “glycotope” to denote that it is the active site which is recognized and bound to by the anti-A and/or anti-B antibodies. These carbohydrate moieties are also referred to as “mimetics”, or “glycomimetics”, or “Glc-analogues”, to denote that they have different structure relative to the natural A/B antigens but are also recognized by the natural A/B antibodies.

The carbohydrate derivative of formula (I) is also referred to herein as “functionalized glycan”, or “ligand” and is also generally represented as Glyc-sp-Z, wherein Glyc means the glycan or carbohydrate moiety, sp represents the R_4 spacer group, and Z is the functional group as defined above; i.e., the glycan “Glyc” in Glyc-sp-Z jointly represents the following glycans:



wherein R₁ and R₂ are as defined above, as well as the specific examples of each group.

In a particular embodiment, when -Z is -NH₂, the carbohydrate derivative of formula (I) may be represented as Glyc-sp-NH₂. In another particular embodiment, when -Z is -SH, the carbohydrate derivative of formula (I) may be represented as Glyc-sp-SH.

The term “glycoconjugate”, as is conventionally understood, relates to carbohydrates covalently linked to other chemical species, in particular, to a polymeric substance, which is also referred to as polymeric backbone or support, for example, a protein, a peptide, a lipid, or a polysaccharide, among others. In particular, the glycoconjugates of the present invention comprise the functionalized glycan of formula (I) attached to a polymeric backbone. The glycoconjugate of the present invention is also interchangeably referred to herein as glycopolymer.

C_n-C_m alkyl means a linear or branched alkyl group of n to m carbon atoms. In particular, C₁-C₃ alkyl in the definition of the compound of formula (I) means an alkyl group of 1 to 3 carbon atoms, and includes therefore methyl, ethyl, *n*-propyl and isopropyl groups.

A linear alkylene group means a -(CH₂)_n- group, with variable values of n. For example, for n from 2 to 20, the alkylene group may be represented as C₂-C₂₀ alkylene, or for n from 2 to 10, the alkylene group may be represented as C₂-C₁₀ alkylene.

The term “alkylamino” as used herein means monoalkylamino group (-NHalkyl) or dialkylamino group (-N(alkyl)₂), i.e., it denotes an amino group substituted with one or two alkyl groups.

The term “alkoxy” as used herein means a -O-alkyl group.

The term “alkylthio” as used herein means a -S-alkyl group.

The term “hydroxyalkyl” as used herein means an alkyl group which is substituted with one or more hydroxyl groups.

The unspecified “alkyl” as used herein preferably relates to linear or branched C₁-C₆ alkyl, and more preferably to C₁-C₃ alkyl.

In general, the terms as used in this application, unless otherwise stated, shall be understood in their ordinary meaning as known in the art.

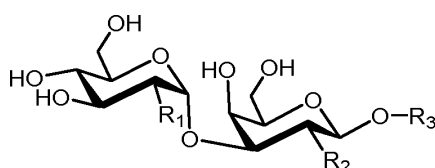
Along the present description, as well as in the claims, singular expressions, generally preceded by the articles “a”, “an” or “the”, are intended to include the plural forms as well, unless the context clearly indicates otherwise.

The terms “about” or “approximately” referred to amounts, as used herein, are meant to include the exact amount and also a certain deviation around the stated amount, namely of $\pm 5\%$.

The numerical ranges disclosed herein are meant to include any number falling within the ranges and also the lower and upper limits.

Compound of formula (I)

The object of the present invention is a carbohydrate derivative of formula (I):



(I)

wherein R_1 , R_2 and R_3 are as defined above.

The compound of formula (I) can also be abbreviatedly represented as $\text{Glc}(R_1)\alpha 1-3(R_2)\text{Gal}\beta-R_3$, where it is shown that the carbohydrate residue at the non-reducing end in the compound of formula (I) is a α -glucose (Glc), which is bound through a α (1 $\rightarrow$ 3) glycosidic linkage to a beta-galactose (Gal) unit.

In the compound of formula (I), as defined above, R_1 is either:

- OH, and then the nonreducing terminal carbohydrate is an α -glucose residue and the carbohydrate derivative of formula (I) is a mimetic of blood group B antigen; or
- $-\text{NH}-\text{CO}-R_5$, wherein R_5 is C_1 - C_3 alkyl and wherein one or more hydrogen atoms in R_5 may be replaced by a hydroxyl group, and then the nonreducing terminal carbohydrate is an α -N-acylglucosamine residue and the carbohydrate derivative of formula (I) is a mimetic of blood group A antigen.

The R_2 group in the β -Gal residue may be either a OH or a fucose residue (of formula (II)).

The $\text{Glc}(R_1)\alpha 1-3(R_2)\text{Gal}\beta-$ group is either directly bound to a linker of formula $-R_4-Z$, or is attached to another carbohydrate residue, which is itself bound to the linker of formula $-R_4-Z$. This optional additional carbohydrate is selected from a β -N-acetylglucosamine, bound at the 4 position (formula III), or a β -N-acetylgalactosamine, bound at the 3 position (formula IV).

In one embodiment, in the compound of formula (I), the group R_1 is selected from OH, $-\text{NH}-\text{CO}-\text{CH}_3$ (N-acetyl or N-Ac), $-\text{NH}-\text{CO}-\text{CH}_2\text{CH}_3$ (N-propionyl) and $-\text{NH}-\text{CO}-\text{CH}_2\text{OH}$ (N-glycolyl).

In one embodiment, in the compound of formula (I), the group R_2 is OH.

In another embodiment, in the compound of formula (I), the group R_2 is a fucosyl residue (group of formula (II)).

The linker group of formula $-R_4-Z$, which is attached at the reducing end of the carbohydrate of formula (I) contains a functional group $-Z$, which is either a $-NH_2$, or $-COOH$, or $-SH$ group, which allows to covalently bind the active carbohydrate moiety to a suitable support, said functional group being linked to the carbohydrate through a spacer group R_4 .

- 5 In one embodiment, Z is $-NH_2$.
 In another embodiment, Z is $-SH$.
 In another embodiment, Z is $-COOH$.

Spacer

- 10 The spacer (R_4) is an arm connecting the oxygen atom at the reducing end of the carbohydrate moiety with the functional group Z . This linking arm maintains the active carbohydrate epitope spatially separated at a suitable distance from the polymeric support in the glycoconjugate of the compound of formula (I). In this regard, therefore, the selection of a particular spacer is not critical insofar as it is suitable for this function.

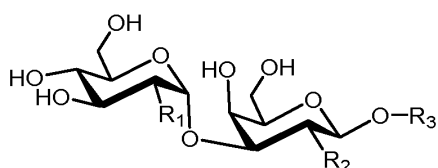
- 15 Typically, the spacer R_4 is a linear chain, for example, of 2 to 20 atoms, which is not immunogenic and also not reactive, typically, under the working conditions of the compound (I), for example, for forming the glycoconjugate with a polymeric support.

- The spacer R_4 is typically a linear alkylene, for example, a C_2-C_{20} linear alkylene, preferably a C_2-C_{10} linear alkylene, which is unsubstituted or substituted, for example, mono-, di-
 20 or trisubstituted, for example, by groups such as alkyl, alkylamino, hydroxyl, alkoxy, hydroxyalkyl, halogen, thiol, alkylthio, or cyano, among others; and wherein some non-consecutive $-CH_2-$ groups in the alkylene chain, for example, up to 5 non-consecutive $-CH_2-$ groups, preferably up to 3 non-consecutive $-CH_2-$ groups may be replaced by other groups, by interrupting the alkylene chain with, for example, ether, thioether, ketone, amine, and/or an amide groups, among others.

- 25 In one embodiment, the spacer R_4 in the compound of formula (I) is a linear C_2-C_{10} alkylene group, preferably unsubstituted, and wherein up to 3 non-consecutive $-CH_2-$ groups may be replaced by a group selected from $-O-$, $-S-$, $-N(H)-$, $-N(R_5)-$, $-N(OR_5)-$, $-N(H)-CO-$, $-CO-N(H)-$, $-N(R_5)-CO-$ and $-CO-N(R_5)-$, wherein R_5 is a C_1-C_3 alkyl group.

- In one particular embodiment, the spacer R_4 is a group of formula $-(CH_2)_{2-8}$, for example,
 30 $-R_4-Z$ is a group of formula $-(CH_2)_3-NH_2$, which is the one used in the preparative examples, but other spacers, as defined above, can be equally suitable.

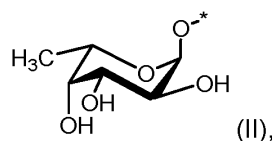
In one embodiment, the object of the present invention is a carbohydrate derivative of formula (I):



(I)

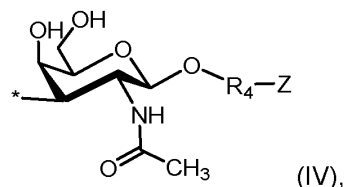
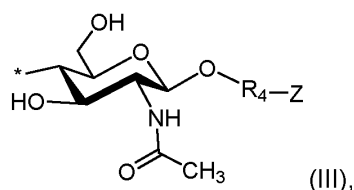
wherein:

- R_1 is selected from OH and -NH-CO- R_5 , wherein R_5 is C_1 - C_3 alkyl and wherein one or more hydrogen atoms in R_5 may be replaced by a hydroxyl group; preferably R_1 is selected from the group consisting of OH, -NH-CO- CH_3 , -NH-CO- CH_2CH_3 and -NH-CO- CH_2OH ; and more preferably R_1 is selected from the group consisting of OH, -NH-CO- CH_3 , and -NH-CO- CH_2CH_3 .
- R_2 is selected from OH and a group of formula (II):



wherein * indicates the point of attachment of said group with the rest of the molecule; and preferably R_2 is a group of formula (II);

- R_3 is selected from R_4 -Z, a group of formula (III) and a group of formula (IV):



wherein

* indicates the point of attachment of said group with the rest of the molecule,

R_4 is a spacer group;

preferably R_4 is a C_2 - C_{20} linear alkylene, more preferably a C_2 - C_{10} linear alkylene, which is unsubstituted or substituted, for example, mono-, di- or trisubstituted, by groups independently selected from alkyl, alkylamino, hydroxyl, alkoxy, hydroxyalkyl, halogen, thiol, alkylthio and cyano, and wherein up to 5 non-consecutive - CH_2 - groups in the alkylene, preferably up to 3 non-consecutive - CH_2 - groups, may be replaced by other groups, interrupting the alkylene chain with ether, thioether, ketone, amine, and/or an amide groups;

more preferably R_4 is a linear C_2 - C_{10} alkylene group, preferably unsubstituted, wherein up to 3 non-consecutive - CH_2 - groups may be replaced by a group selected from -O-, -S-, -N(H)-, -N(R_5)-, -N(OR_5)-, -N(H)-CO-, -CO-N(H)-, -N(R_5)-CO- and -CO-N(R_5)-, wherein R_5 is a C_1 - C_3 alkyl group;

still more preferably, the spacer R_4 is a group of formula $-(CH_2)_{2-8}-$, and still more preferably R_4 is a group of formula $-(CH_2)_3-$; and

Z is selected from $-NH_2$, $-COOH$ and $-SH$, preferably is $-NH_2$

In one particular embodiment of the invention, when R_2 is OH, then R_3 is R_4-Z .

5 Preferred examples of the compound of formula (I) are the following:

Compound:	Structure	Mimetic of:
1	$Glc\alpha 1-3(Fuca 1-2)Gal\beta 1-4GlcNAc\beta -R$	Group B antigen
2	$Glc\alpha 1-3(Fuca 1-2)Gal\beta -R$	Group B antigen
3	$Glc\alpha 1-3Gal\beta -R$	Group B antigen
4	$Glc\alpha 1-3(Fuca 1-2)Gal\beta 1-3GalNAc\beta -R$	Group B antigen
5	$GlcNAc\alpha 1-3(Fuca 1-2)Gal\beta 1-4GlcNAc\beta -R$	Group A antigen
6	$GlcNPropionyl\alpha 1-3(Fuca 1-2)Gal\beta 1-4GlcNAc\beta -R$	Group A antigen
7	$GlcNGlycolyl\alpha 1-3(Fuca 1-2)Gal\beta 1-4GlcNAc\beta -R$	Group A antigen
8	$GlcNAc\alpha 1-3(Fuca 1-2)Gal\beta -R$	Group A antigen
9	$GlcNPropionyl\alpha 1-3(Fuca 1-2)Gal\beta -R$	Group A antigen
10	$GlcNGlycolyl\alpha 1-3(Fuca 1-2)Gal\beta -R$	Group A antigen
11	$GlcNAc\alpha 1-3Gal\beta -R$	Group A antigen
12	$GlcNPropionyl\alpha 1-3Gal\beta -R$	Group A antigen
13	$GlcNGlycolyl\alpha 1-3Gal\beta -R$	Group A antigen
14	$GlcNAc\alpha 1-3(Fuca 1-2)Gal\beta 1-3GalNAc\beta -R$	Group A antigen
15	$GlcNPropionyl\alpha 1-3(Fuca 1-2)Gal\beta 1-3GalNAc\beta -R$	Group A antigen
16	$GlcNGlycolyl\alpha 1-3(Fuca 1-2)Gal\beta 1-3GalNAc\beta -R$	Group A antigen
17	$GlcNAc\alpha 1-3Gal\beta 1-4GlcNAc\beta -R$	Group A antigen
18	$GlcNPropionyl\alpha 1-3Gal\beta 1-4GlcNAc\beta -R$	Group A antigen
19	$GlcNGlycolyl\alpha 1-3Gal\beta 1-4GlcNAc\beta -R$	Group A antigen
20	$Glc\alpha 1-3Gal\beta 1-4GlcNAc\beta -R$	Group B antigen
	(R= $-O-R_4-Z$)	

TABLE 1

The compounds where R_1 is a *N*-acyl group are mimetics of blood group A antigen, while
 10 those where R_1 is OH are mimetics of blood group B antigen.

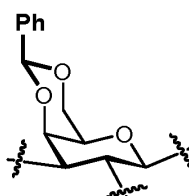
Process for preparing of the compound of formula (I):

The compound of formula (I) can be prepared using conventional synthetic methods, well-known to the skilled in carbohydrate synthesis.

5 A possible general synthetic route for preparing the compounds of formula (I) is depicted in Scheme I (Figure 1).

In Scheme I, PG are conventional protecting groups, for example, each one is typically independently selected from acetyl (Ac, $\text{CH}_3\text{-CO-}$), benzyl ($\text{Ph-CH}_2\text{-}$), or benzylidene (Ph-CH=); and R_3' is selected from $\text{R}_4\text{-Z'}$, a group of formula (III) and a group of formula (IV), wherein the group of formula (III) and the group of formula (IV) are as defined above, but wherein the OH groups in the carbohydrate moiety in formulas (III) and (IV) are protected, for example, with the same protecting groups as defined above, and wherein the Z group is also protected; and wherein Z' is a group of formula Z as defined above which is protected.

Benzylidene protecting group, as is well known in the art, can be used for simultaneously protecting two spatially proximate OH groups, for example, at C-4 and C-6 of a galactose moiety:



15 The Z group, as above defined, is -NH_2 , -SH or -COOH . Any customary protecting group for these functional groups, as are well known in the art, may be used. A suitable protecting group for -NH_2 and -SH is, for example, an acyl group, such as acetyl, or trifluoroacetyl, among others. A suitable protecting group for -COOH is an ester, for example, methyl, *t*-butyl or benzyl ester, among others.

20 In Scheme I, when, in the starting material of formula A, R_a is an azide group (N_3), the compounds of formula (I) wherein R_1 is an acyl group (of formula -NH-CO-R_5 , as defined above) can be obtained. Thus, the azide group is typically reduced to an amine group and then modified with the suitable acyl residue. When, in the starting material of formula A, R_a is a protected hydroxyl (such as, for example, with the benzyl group, i.e., OBn), the compounds of formula (I) wherein R_1 is OH can be obtained.

In Scheme I, the intermediate compounds of formula C wherein the OH group at the position C-2 of the galactose residue is protected ($\text{R}_b=\text{O-PG}$) lead to the compounds of formula (I) wherein $\text{R}_2=\text{OH}$. When this position is left unprotected ($\text{R}_b=\text{OH}$), it allows for linking a fucose residue through a α (1 $\rightarrow$ 2) glycosidic linkage at this position, to provide the compounds of formula I wherein R_2 is a fucose residue (of formula II, as defined above).

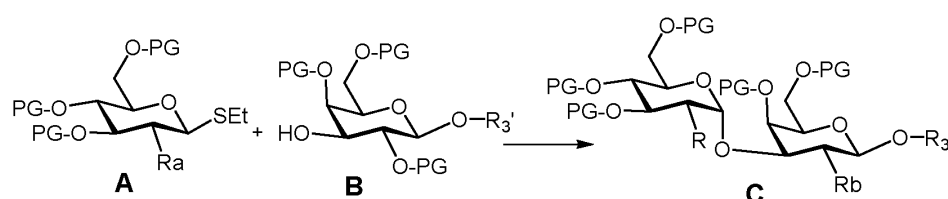
Therefore, it is understood that, depending on the different alternatives, according to the different values of the moieties R_1 , R_2 and R_3 , the skilled in the art will have no difficulty in suitably

adapting the above-described synthetic route to the preparation of a specific compound of formula (I).

In Scheme I, it is to be understood that the compound of Formula (I) directly obtained after the carbohydrate synthesis may have a spacer and linking group attached at the reducing end which is different from the one defined above, and is subsequently converted, using conventional organic synthetic methods, to the linking group $-R_4-Z$, as defined above.

Another aspect of the invention is, therefore, a process for the preparation of a compound of formula (I), characterized in that it comprises the following steps:

- a) Preparing a compound of formula **C** from a compound of formula **A** and a compound of formula **B**:



wherein

PG are protecting groups;

R_a is azide or O-PG;

R₃' is selected from R₄-Z', a group of formula (III) and a group of formula (IV), wherein the group of formula (III) and the group of formula (IV) are as defined above, but wherein the OH groups in the carbohydrate moiety in formulas (III) and (IV) are protected and wherein the Z group is also protected, and wherein Z' is a group of formula Z as defined above which is protected;

R_b is OH or O-PG; and

b)

b1) when R_b=OH, linking a protected fucose residue through a α (1 $\rightarrow$ 2) glycosidic linkage at this position to provide, after deprotection, the compound of formula (I) wherein R₂ is a group of formula II;

b2) when R_b=O-PG, deprotecting the compound of formula C to provide a compound of formula (I) wherein R₂ is OH;

wherein the definitions of protecting groups and different conditions are as discussed above.

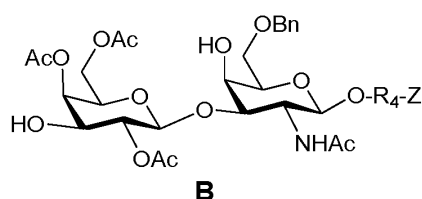
In particular, the preparation of the compounds of formula (I) comprising the fucose residue can be performed, for example, analogously as disclosed in Kunetskiy *et al.*, *Synthesis of blood group A and B (type 2) tetrasaccharides. A strategy with fucosylation at the last stage*, Carbohydrate Research, 2020, Vol. 498, 108192, where the preparation of analogous derivatives of the natural blood group A and B antigens is described, i.e., comprising a α -Gal at the non-reducing end.

Thus, for example, for preparing the compounds of formula (I) wherein R_2 is a group of formula (II), and R_3 is a group of formula (III), the route depicted in Scheme Ia (Figure 2) can be followed.

The compound of **C** is then coupled with the fucose residue at the C-2 position of the galactose residue, to provide, after deprotection, the compound of formula (I), according to the route depicted in Scheme Ib (Figure 3)

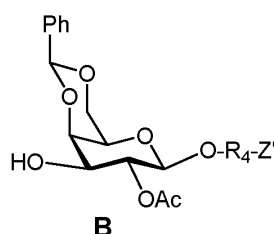
Compounds 1, 5, 6 and 7 of Table 1, for example, can be prepared according to this route.

Analogously, for preparing the compounds of formula (I) wherein R_2 is a group of formula (II), and R_3 is a group of formula (IV), an analogous procedure as depicted in Schemes Ia and Ib can be followed, but using, for example, the following compound of formula **B** as starting material:



In this way, for example, compounds 4, 14, 15 and 16 of Table 1 can be obtained.

Also analogously, for preparing the compounds of formula (I) wherein R_2 is a group of formula (II), and R_3 is a group of formula R_4-Z , an analogous procedure as depicted in Schemes Ia and Ib can be followed, but using, for example, the following compound of formula **B** as starting material:

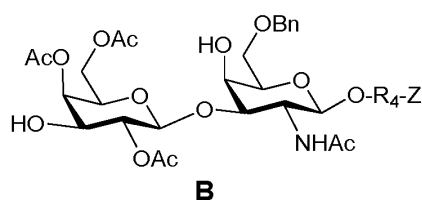


In this way, for example, compounds 2, 8, 9 and 10 of Table 1 can be obtained.

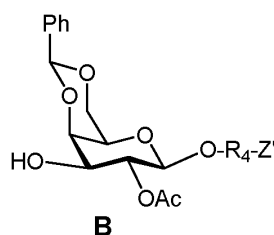
On the other hand, the compounds of formula (I) wherein R_2 is OH and R_3 is a group of formula (III), can be prepared by the route depicted in Scheme Ic (Figure 4).

In this way, for example, compounds 17, 18, 19 and 20 of Table 1 can be obtained.

Analogously, for preparing the compounds of formula (I) wherein R_2 is OH, and R_3 is a group of formula (IV), an analogous procedure as depicted in Scheme Ic can be followed, but using, for example, the following compound of formula **B** as starting material:



Also analogously, for preparing the compounds of formula (I) wherein R_2 is OH, and R_3 is a group of formula R_4-Z , an analogous procedure as depicted in Scheme Ic can be followed, but using, for example, the following compound of formula **B** as starting material:



5

In this way, for example, compounds 3, 11, 12 and 13 of Table 1 may be prepared.

In the Scheme I, the compounds of formula (I) comprising a fucose (i.e., when R_2 is a group of formula II) are prepared by adding the fucose moiety in the final step, to the previously prepared intermediate of formula C. It is to be understood that other alternative synthetic routes may be equally suitable, for example, by first obtaining a fucosylated intermediate B derivative and, subsequently, coupling it to the protected alpha-glucose starting material of formula A. It is well within the capabilities of the skilled in carbohydrate synthesis to identify other alternative routes to synthesise the compound of formula (I).

15 Glycoconjugates of compounds of formula (I)

Another aspect of the invention is a glycoconjugate of the compound of formula (I). The glycoconjugate of the invention comprises the compound of formula (I), more particularly, it comprises the compound of formula (I) and a polymeric backbone or support. In the glycoconjugate, the compound of formula (I) is covalently bound to a polymeric backbone or support, more specifically, the compound of formula (I) is bound through the -Z functional group to the polymer.

It is understood that the $-NH_2$, $-SH$ and $-COOH$ functional groups in the compound of formula (I) are typically converted to $-NH-$, $-S-$, and $-CO-$ or $-CO-O-$ groups, respectively, in the glycoconjugate, when the covalent bond is formed between said functionalized glycan and the polymer. Therefore, when it is defined that the glycoconjugate “comprises” the compound of formula (I), it is to be understood that the -Z group has been modified in this way.

As is well known in the art, such covalent binding can be performed directly between the ligand and the polymer or they can be linked through a suitable additional linking agent (or

crosslinker) or by including a reactive group on either the polymer or the ligand or both. Said reactive groups have therefore the purpose of activating either the polymer or the compound of formula (I) in order to allow or facilitate the reaction between both moieties.

It is to be understood, therefore, that the final glycoconjugate may comprise an additional
5 linking fragment between both parts, as is illustrated below.

Therefore, the glycoconjugate of the present invention can also be defined as comprising the carbohydrate moiety of the compound of formula (I) linked to a polymeric support or polymeric backbone through any suitable spacer.

The glycoconjugate of the present invention can be prepared by linking the carbohydrate
10 derivative of formula (I) to a suitable polymeric backbone through a covalent bond. More particularly, said glycoconjugate is formed with a multitude of compounds of formula (I) linked through a covalent bond to a multitude of functional groups on the polymer backbone.

Suitable polymers to be used as backbone or support for the glycoconjugate of the invention are, for example, homologous and heterologous α -amino acid polymers (homo and
15 heteropolypeptides), proteins, lipids, nucleic acids, acrylic acid polymers, methacrylic acid polymers, acrylic/methacrylic acid copolymers, polysaccharides such as chitosan or agarose, polyethylene, polypropylene, polystyrene, polyvinyl butyrate, poly (vinyl chloride), or dendrimers, for example, among others. Suitable homologous α -amino acid polymers are, for example, polylysine, polyarginine, polyornithine, polyglutamine, polyasparagine, polyglutamic acid or
20 polyaspartic acid. Said poly α -amino acids may be linear or branched.

The polymer may be used as such or may be combined with an additional material, such as glass, ceramics, or metals, for example. In one embodiment, the polymer is included in magnetic nanoparticles (MNPs), which, as is well known in the art, are characterized in that they
25 comprise a magnetic material in the core, such as iron, nickel or cobalt, and a polymeric shell, for example, of polyacrylic acid or other polymers, which bears suitable functional groups to be bound to the ligand.

The selection of the particular polymer or the particular form of it for preparing the glycoconjugate of the invention will be based on the desired end use of the glycoconjugate.

In one embodiment, the polymer in the glycoconjugate of the invention is selected from
30 polyacrylic acid, a polysaccharide resin and an α -amino acid homopolymer, preferably the polymer is selected from polyacrylic acid, agarose and polylysine.

The polylysine is either linear or branched poly-L-lysine, preferably linear poly-L-lysine.

In one embodiment, the polymer in the glycoconjugate is included in a magnetic nanoparticle.

35 For a glycoconjugate prepared using a given polymer, it is possible to bind a single compound of formula (I) to the multiple reactive sites on the polymer or, alternatively, different

compounds of formula (I) may be bound to the polymer to obtain a polyfunctional glycopolymer. Preferably, a single compound of formula (I) is comprised in a given glycopolymer.

Furthermore, for a particular glycoconjugate, comprising a given polymer and a given compound of formula (I), different specific products can be obtained depending on the polymerization degree of the polymer, i.e., its molecular weight.

As is well known in the art, said polymers suitable to prepare glycoconjugates are characterized in that they comprise functional groups in their repetitive polymeric structure, such as hydroxyl, carboxyl or amino groups, among others, through which a covalent bond is formed with the functional group on carbohydrate ligand of the invention, in particular, with the amine, the carboxyl, or the thiol functional group on the ligand. Usually, the polymer needs to be activated with a compound that is reactive towards said functional group on the ligand. The activated complex can then generate a covalent linkage with said compound, resulting in immobilization of the carbohydrate epitope on the polymer, to form the glycoconjugate.

There are different suitable methods for activating the polymeric support, which vary depending on the specific polymer, as is well known in the art. Different activation methods, for example, are described in the chapter *Immobilization of Ligands on Chromatography Supports*, in: Bioconjugate Techniques, G.T. Hermanson, Ed., Chapter 15, p: 589-740.

For example, some well-known activation strategies for linking the polymer with an amine group on the glycan are, for example, activation of carboxylate groups on the polymer as *N*-hydroxysuccinimide (NHS) ester groups, which afford amide bonds with the ligand; aldehyde activated polymers which afford secondary amine bonds with the ligand; azlactone activated polymers, which afford amide bonds with the ligand; activation of hydroxyl groups with 1,1'-carbonyl diimidazole (CDI) to form reactive imidazole carbamates, which afford carbamate bonds with the ligand; or activation of carboxylate groups on the polymer as 4-nitrophenyl ester groups, to afford amide bonds with the ligand, among many other options.

Another well-known strategy for linking the ligand of formula (I) to the polymer support is by using a linker, which is a bifunctional moiety, either homobifunctional or heterobifunctional that makes possible to link both moieties. For example, when both the support and the ligand bear an amine functional group, a common homobifunctional linker is a succinimidyl diester, for example, adipic acid succinimidyl diester or disuccinimidyl suberate, among others, which can afford two amide bonds at the two ends of the molecule, to form the glycoconjugate. In this case, either the ligand can be first activated and then reacted with the polymer, or the polymer can be first activated and then reacted with the ligand.

Another well-known option for linking a glycan bearing a thiol functional group with a polymeric support bearing an amine group is by first activating the amine groups on the support by chloroacetylation, and subsequent reaction between the chloroacetylated polymer and the thiol functional groups on the ligand to produce the glycoconjugate. This strategy is disclosed, for

example, in Duthaler *et al.*, Chimia, 2010, 64(1-2), 23-28. Analogously, -OH functional groups on the polymer may also be activated by chloroacetylation, to be linked to a thiol-functionalized glycan.

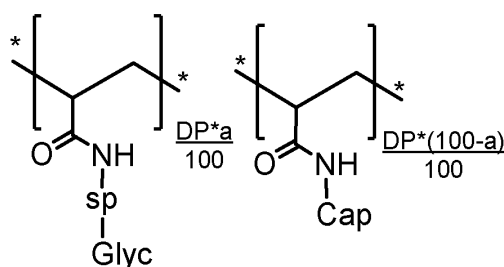
Other suitable activating methods for preparing glycoconjugates are also disclosed, for example, in the international patent application WO-A-2018/167320.

The different strategies for the preparation of glycopolymers are well known in the art and it is well within the capabilities of the skilled in the art to choose the most suitable method and conditions for each case.

The load degree of the ligand on the polymer means the percentage of the functional groups on the polymer, which are attached to the active saccharide of the invention. In general, loads of from 2% to 90% are used, preferably from 5% to 50%, and more preferably from 10% to 40%. The remaining not bound functional groups on the polymer are usually capped, i.e., they are reacted with a suitable capping substance, with the purpose of suppressing the reactivity of the remaining free functional groups on the polymer. The capping agent to be used depends on the particular functional group on the polymer. The selection of the specific capping agent is not critical, as long as it can be easily bound with high yield to the free reactive sites on the polymer and it produces stable, non-reactive capped ends. The capping molecule is preferably hydrophilic to avoid loss of solubility in water, not antigenic, and not highly charged. It is therefore well within the capabilities of the skilled in the art to choose a suitable capping agent in each case. Commonly used capping agents are, for example, glycolic acid, gluconic acid, succinic acid, ethanol amine or other amine derivatives, thio-compounds such as thioglycerol, among many others.

In one embodiment, the glycoconjugate of the invention comprises the compound of formula (I) linked to a polyacrylic acid backbone. The glycoconjugate thus formed is a polyacrylamide.

In one particular embodiment, the glycoconjugate has the formula (V) as shown below:

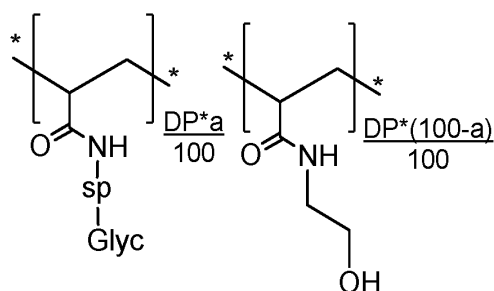


formula (V)

wherein "Cap" means a capping agent, "DP" represents the degree of polymerization of the polymer, expressed as number of monomers, and "a" represents the load percentage of the carbohydrate in the glycoconjugate. The DP is preferably comprised between 50 and 1100, and

more preferably between 100-1000, and the load percentage is preferably comprised between 5 and 80, more preferably between 10 and 70.

Any suitable capping agent, as known in the art, may be used. For example, a suitable capping agent is ethanol amine, and the polyacrylamide glycoconjugate has the following formula:



formula Vb

In one preferred embodiment, the glycoconjugate of formula (V) and (Vb) is prepared from a compound of formula (I) which is selected from compounds 1 to 20 of Table 1.

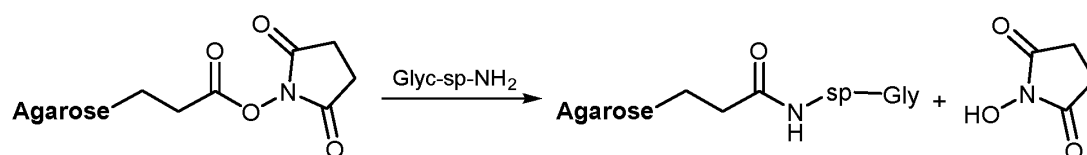
For preparing the glycoconjugate with a polyacrylic acid backbone, the acidic -COOH groups on the polyacrylic acid can be activated, for example, with N-hydroxysuccinimide (NHS), forming *N*-succinimide ester groups, or can be activated as 4-nitrophenyl ester groups, for example, as disclosed in Tuzikov *et al.*, 40 years of glyco-polyacrylamide in glycobiology, Glycoconj. J., 2021, 38, 89-100, or in Bovin *et al.* Synthesis of polymeric neoglycoconjugates based on *N*-substituted polyacrylamides, Glycoconjugate J., 1993, 10, 142-151.

The glycoconjugate with polyacrylic acid may be prepared, for example, as depicted in Scheme II (Figure 5), where the -COOH groups of the polyacrylic acid are activated as 4-nitrophenyl ester groups and the capping agent is ethanolamine.

Other activation methods of polyacrylic acid may also be suitable for preparing the glycoconjugate with the functionalized glycan of the invention.

In another embodiment, the glycoconjugate comprises the compound of formula (I) linked to agarose as polymeric backbone. Agarose, as is well known, is a linear polysaccharide made up of the repeating unit of agarobiose and is commercially available as resin beads. Crosslinked forms of agarose are commercially available under the denomination Sepharose®. For preparing the glycoconjugates, typically, activated forms of agarose are used, which are commercially available. These activated forms are available as resin beads of different sizes.

Suitable activated agarose forms are, for example, *N*-hydroxysuccinimide (NHS)-activated agarose, aldehyde-activated agarose, azlactone-activated, or 1,1'-carbonyl diimidazole (CDI) activated agarose, among others. For example, the linking between the ligand and the NHS-activated agarose may be performed as represented in the following scheme:

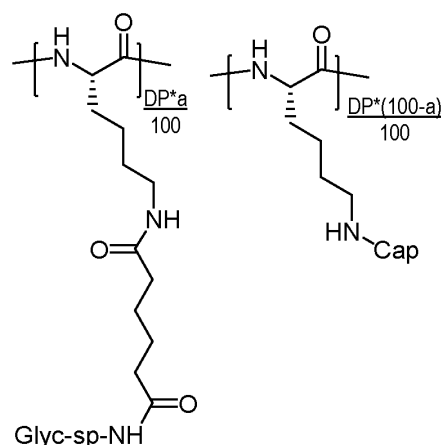


Preferred forms of agarose are those having molecular weight comprised between 25-1000 KDa.

In one preferred embodiment, the glycoconjugate with agarose is prepared from a compound of formula (I) is selected from the compounds 1 to 18 of Table 1.

In another embodiment, the glycoconjugate of the invention comprises the compound of formula (I) linked to a polylysine backbone, in particular to poly-*L*-lysine.

In one particular embodiment, the glycoconjugate with poly-*L*-lysine has the following formula:



formula (VI)

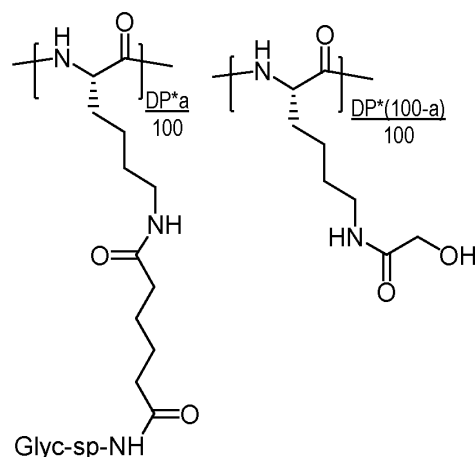
wherein "Cap" means a capping agent, "DP" represents the degree of polymerization of the polymer, expressed as number of lysine units, and "a" represents the load percentage of the carbohydrate in the glycoconjugate.

The polylysine may be linear or branched, and is preferably linear.

Suitable degrees of polymerization of polylysine are comprised between about 50 and 2000, preferably comprised between 100 and 1000. Preferred polylysine of degrees of polymerisation are about 100, about 600 and about 1000, which are commercially available, in the form of the hydrobromide salt. The poly-*L*-lysine hydrobromide may be synthesized, for example, via ring-opening polymerization of *N*-carboxy-anhydrides using N6-trifluoroacetyl-*L*-lysine as precursor, as disclosed in Hadjichristidis *et al.*, Chem. Rev., 2009, 11, 5528-5578.

The ligand loading on the polylysine is typically comprised between 2% and 90%, preferably comprised between 5% and 50%, and more preferably comprised between 10% and 40%.

Any suitable capping agent, as known in the art, may be used for the glycoconjugate with polylysine. For example, a suitable capping agent is glycolic acid, forming glycolic amide terminal groups, and the glycoconjugate has the following formula:



formula (VIb)

In one preferred embodiment, the glycoconjugate of formula (VI) and (VIb) is prepared from a compound of formula (I) is selected from the compounds 1 to 18 of Table 1.

For preparing the glycoconjugates of formula (VI) and (VIb), the ligand of formula Glyc-sp-NH₂ is bound to the amine groups on the side chain of polylysine using a *N*-hydroxysuccinimide diester linker, in particular, with adipic acid diester. Preferably, the ligand is first activated by reaction with the diester, and then, the activated compound is reacted with the polylysine (as hydrobromide), as shown in Scheme III (Figure 6). The preparation of the glycoconjugate is represented in Scheme IV (Figure 7).

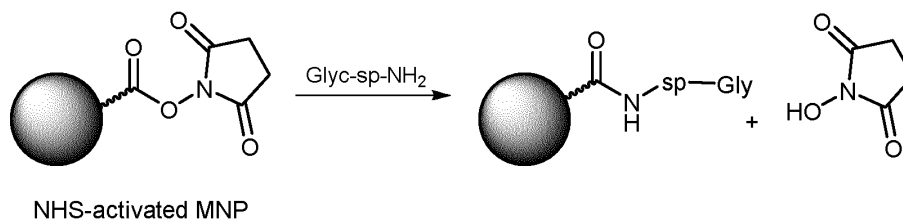
The capping with glycolic acid may be performed, for example, in two steps, by first reacting the free amino groups on the glycoconjugate with an activated and protected glycolic acid (glycolic acid acetate *N*-hydroxysuccinimide ester) and then deprotecting the acetate protected glycolic amide groups, to obtain the final capped product.

In another embodiment, the glycoconjugate of the invention comprises the compound of formula (I) linked to a magnetic nanoparticle. Magnetic nanoparticles (MNPs) are well known in the art, and they are characterized in that they comprise a magnetic material in the core, such as iron, nickel or cobalt, and typically a polymeric shell.

In this particular case, therefore, the glycoconjugate of the invention comprises the compound of formula (I) and a polymeric backbone or support, as disclosed above, and said polymeric support constitutes the shell of the magnetic nanoparticle.

The polymeric shell of the magnetic nanoparticle bears suitable functional groups to be bound to the ligand, using common linking methods as described above. For example, *N*-

hydroxysuccinimide activated magnetic nanoparticles may be linked to the functionalized glycan of the invention, according to the following reaction:



NHS-activated magnetic nanoparticles are commercially available, for example, from the company CD Bioparticles. Other type of activated magnetic nanoparticles and other linking mechanisms with the glycomimetic of the invention may be also suitable, and adapting the process to the specific activated magnetic nanoparticle is well within the capabilities of the skilled in the art.

Furthermore, said glycoconjugates or glycopolymers also are meant to include any functionalized surface, bearing the active mimetics of formula (I), which allow for identification of the antibodies from blood samples.

Uses of the glycoconjugate

The glycoconjugates of the invention, comprising the glycan of formula (I), surprisingly, have high affinity towards natural anti-A/B antibodies, despite the fact that they carry active epitopes which differ from the natural A/B antigens, in particular, despite the fact that they comprise a glucose or a glucosamine derivative at the nonreducing end, instead of a galactose or a *N*-acetylgalactosamine, as in natural A and B antigens, contrary to what was disclosed so far in the art.

Furthermore, not only the new epitopes are effective for binding natural anti-A/B antibodies, but they are occasionally even more active than the natural antigens.

Additionally, they can also be useful to be used in combination with natural antigens.

This is shown in the assays disclosed in Examples 5 to 8, where their efficacy for inhibition and removal of anti-A/B antibodies was tested, both in normal human serum from blood group A or B and in pure fractions of human anti-A/B antibodies, as well as their use for purification of human polyclonal anti-A/B antibodies.

Examples 6.2 and 6.3, in particular, show that the glycoconjugates of the present invention, in particular with poly-*L*-lysine, comprising the new epitopes, were effective in removing purified human anti-A and anti-B antibodies.

This outstanding affinity towards anti-A/B antibodies may be used for inhibiting and/or removing anti-A and/or anti-B antibodies from samples of blood and blood derivatives. This is useful for many applications, for example, to allow transfusion and organ transplants between

incompatible blood donors including living-donor organ transplantation or for inhibition and elimination of anti-A and anti-B antibodies during the process of purification and fractionation of blood products, among other applications.

5 The use of the glycoconjugates for avoiding rejection in organ transplantation is suitable, for example, for kidney, liver, skin, pancreas, cornea, bone marrow or heart transplants. To this end, the anti-A and/or anti-B antibodies can be inhibited/removed either *ex vivo* or *in vivo*, or complementarily *ex vivo* and *in vivo*. For *ex vivo* removal, the blood from an organ transplant recipient is withdrawn from the subject and then contacted with the glycoconjugate of the invention to remove the anti-A and/or anti-B antibodies, and then the treated blood is reinfused into the
10 subject. In the *in vivo* alternative, the inhibition/removal of the anti-A and/or anti-B antibodies is performed intracorporeally, by administering a suitable glycoconjugate which is able to inhibit/remove the circulating antibodies in the subject.

For this intracorporeal use, the polymer in the glycoconjugate is typically a biodegradable, biocompatible polymer. Suitable polymers for this use are poly amino acids, for example,
15 polylysine or polyarginine, among others. One particularly preferred glycoconjugate for intracorporeal use is poly-L-lysine glycoconjugate, as poly-L-lysine has proved to be safe and not immunogenic after *in vivo* administration (Katopodis *et al.*, J. Clin. Invest., 2002, 110, 1869-1877).

On the other hand, the applications related to the purification and fractionation of blood and blood derivatives samples, or the identification of the antibodies from blood or blood
20 derivatives samples are performed *in vitro* or *ex vivo*.

A suitable glycoconjugate may be chosen for each particular purpose, comprising a suitable glycan, either mimetic of blood group A antigen or mimetic of blood B antigen, depending on the antibodies to be removed or detected.

Another aspect of the invention is, therefore, the use of the glycoconjugate of the invention
25 for inhibiting and/or removing anti-A and/or anti-B antibodies from samples of blood or other blood derivatives and by-products. Such blood derivatives and by-products are, for example, serum, plasma, fractioned plasma, or immunoglobulin preparations, among others. According to this aspect, it is understood that the samples of blood and/or blood derivatives/by-products are treated *in vitro* or *ex vivo* with the glycoconjugate, and no treatment of living being is performed.

30 Typically, a method for removing the anti-A and/or anti-B antibodies from samples of blood or other blood derivatives and by-products comprises contacting the sample with the glycoconjugate during sufficient time to allow the antibodies to be bound by the active epitopes on the glycoconjugate, and then separating the glycoconjugate from the sample. The glycoconjugate is typically made of a solid polymeric support, which is, for example, in the form
35 of solid microbeads for facilitating the contact with and subsequent separation from the treated sample.

A method for inhibiting the anti-A and/or anti-B antibodies from said samples is analogous, but the sample may not be separated from the inactivated antibodies, which remain in the sample bound to the glycoconjugate.

5 The high-affinity binding of the anti-A/B antibodies by the glycoconjugate of the invention can also be used for detecting those antibodies in test samples. For several *in vitro* applications, the glycomimetic of the invention may be attached to any suitable surface or device adapted to antibody detection.

10 Another aspect of the invention is the glycoconjugate of the invention for use in medicine, in particular, for use in the treatment and/or prevention of rejection or haemolytic reactions in subjects undergoing organ transplantation or blood transfusion, respectively, from incompatible donors.

15 Another aspect of the invention is a method for treating and/or preventing rejection or haemolytic reactions in a subject in need thereof undergoing organ transplantation or blood transfusion, respectively, from incompatible donors, comprising the step of administering a therapeutically effective amount of the glycoconjugate of the invention.

Another aspect of the invention is the use of the glycoconjugate of the invention for the preparation of a medicament for the treatment and/or prevention of rejection or haemolytic reactions in subjects undergoing organ transplantation or blood transfusion, respectively, from incompatible donors.

20 Incompatible donors are meant to be organ donors or blood donors.

According to this aspect, related to the treatment or prevention of rejection or haemolytic reactions in a human being, the glycoconjugate of the invention comprises a biodegradable and biocompatible polymer. Preferred polymers are homo poly α -amino acids, for example, selected from polylysine, polyarginine, polyornithine, polyglutamine, polyasparagine, polyglutamic acid and polyaspartic acid. Particularly preferred is polylysine, particularly poly-L-lysine, as defined
25 above.

Typically, the glycoconjugate as therapeutic agent is administered by intravenous, subcutaneous, or intramuscular injection.

30 "Biodegradable" as used herein means metabolic biodegradability so that the polymer, after being administered, is biofragmented and removed from the organism. "Biocompatible" as used herein means that the polymer is safe and nontoxic and therefore suitable for being administered to living organisms.

The terms "inhibition" and "removal" may be used interchangeably herein. Inhibition refers to the fact that the antibodies, once bound to the active epitopes on the glycoconjugate of the invention, are inactivated and are not able to further bind to the natural anti-A/B antigens, even if
35 they remain present in the sample. Removal refers to the fact the antibodies are eliminated from the treated sample, either after *in vitro* or *ex vivo* processes, where the treated sample is collected

and the antibodies are retained on the solid glycopolymer, or after *in vivo* intracorporeal inhibition, when immunocomplexes are then metabolized and also removed from blood stream.

As used herein, the subjects undergoing organ transplantation or blood transfusion which are to be treated with the glycoconjugate of the invention are meant to be human beings.

5 Incompatible donors, as used herein, means subjects having ABH-incompatible antigens.

The glycoconjugate of the invention may be administered prior to the subject receiving an ABO-incompatible transplant or blood transfusion, or alternatively or complementarily, the treatment may be administered after a subject receives an ABO incompatible transplant or blood transfusion.

10

Pharmaceutical compositions

Another aspect of the invention is a pharmaceutical composition comprising the glycoconjugate of the invention and at least one pharmaceutically acceptable excipient.

15 It is understood that the glycoconjugate suitable for preparing a pharmaceutical composition is as defined above for the medical treatment, i.e., the polymer of the glycoconjugate is typically biocompatible and biodegradable. Preferably, the polymer is a poly α amino acid, more preferably is a polylysine, still more preferably a poly-L-lysine and still more preferably is a poly-L-lysine as defined above under the Glycoconjugate section.

20 The pharmaceutical composition of the invention is prepared according common procedures, well known in the art, and using common and widely available excipients, as described, for example, in the reference book R.C. Rowe, P.J. Sheskey and P.J. Weller, *Handbook of Pharmaceutical Excipients*, Sixth Edition, Pharmaceutical Press, 2009. Also, common excipients and procedures for preparing the compositions are described in the book J.P Remington and A. R. Genaro, *Remington The Science and Practice of Pharmacy*, 20th edition, Lippincott, Williams & Wilkins, Philadelphia, 2000 [ISBN: 0-683-306472] or in the book M.E. Aulton and K.M.G. Taylor, *Aulton's Pharmaceuticals, the design and manufacture of medicines*, 4th edition, Churchill Livingstone Elsevier, 2013 [ISBN: 978-0-7020-4290-4].

25 The pharmaceutical compositions comprising the glycoconjugate of the invention are preferably for parenteral use.

30 The compositions typically comprise a vehicle, such as water or a saline solution. Nonaqueous solvents such as ethanol, glycerol or propylene glycol may be added as cosolvents to improve the solubility. The compositions may also comprise preservatives, antioxidants, pH regulating agents, tonicity adjusting agents, suspending agents, or combinations thereof, as are well known in the art.

35

Examples

Example 1: Preparation of the compound of formula (I)

The specific compounds of formula (I) listed in Table 2 were synthesised following the synthetic methods described above, according to Schemes Ia, Ib and Ic.

Example:	Structure
1.1	Glc α 1-3(Fuca1-2)Gal β 1-4GlcNAc β -R
1.2	Glc α 1-3(Fuca1-2)Gal β -R
1.3	Glc α 1-3Gal β -R
1.4	Glc α 1-3(Fuca1-2)Gal β 1-3GalNAc β -R
1.5	GlcNAc α 1-3(Fuca1-2)Gal β 1-4GlcNAc β -R
1.6	GlcNPropionyl α 1-3(Fuca1-2)Gal β 1-4GlcNAc β -R
1.7	GlcNGlycolyl α 1-3(Fuca1-2)Gal β 1-4GlcNAc β -R
1.8	GlcNAc α 1-3(Fuca1-2)Gal β -R
1.9	GlcNPropionyl α 1-3(Fuca1-2)Gal β -R
1.10	GlcNGlycolyl α 1-3(Fuca1-2)Gal β -R
1.11	GlcNAc α 1-3Gal β -R
1.12	GlcNPropionyl α 1-3Gal β -R
1.13	GlcNGlycolyl α 1-3Gal β -R
1.14	GlcNAc α 1-3(Fuca1-2)Gal β 1-3GalNAc β -R
1.15	GlcNPropionyl α 1-3(Fuca1-2)Gal β 1-3GalNAc β -R
1.16	GlcNGlycolyl α 1-3(Fuca1-2)Gal β 1-3GalNAc β -R
1.17	GlcNAc α 1-3Gal β 1-4GlcNAc β -R
1.18	GlcNPropionyl α 1-3Gal β 1-4GlcNAc β -R
1.19	GlcNGlycolyl α 1-3Gal β 1-4GlcNAc β -R
1.20	Glc α 1-3Gal β 1-4GlcNAc β -R
R= -O-(CH ₂) ₃ -NH ₂	

TABLE 2

- 5 For further illustrate the preparation methods, the synthesis of some of the above compounds (Examples 1.1, 1.5, 1.8, 1.9, 1.11 and 1.12) is disclosed in more detail below. The rest of compounds were obtained using analogous procedures.

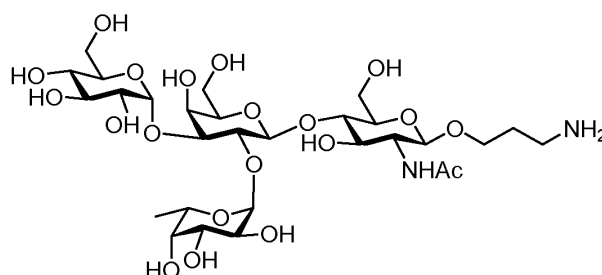
General Procedures

The reactions were performed with the use of commercial reagents (Carbosynth Ltd, Acros, Aldrich, and Fluka); anhydrous solvents were purified according to the standard procedures.

Column chromatography was performed on silica gel 60 0.040–0.063 mm (Merck). Solvents were removed in vacuum at 30–40 °C. Thin layer chromatography (TLC) was performed on silica gel 60 F254 aluminum-backed plates (Merck). The spots of compounds were visualized by dipping a TLC plate into aqueous solution of H₃PO₄ (8%) and subsequent heating (>150 °C).

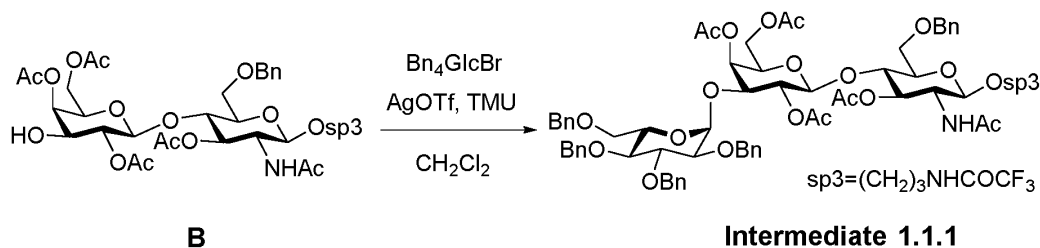
¹H NMR spectra were recorded on a Bruker BioSpin GmbH (700 MHz) spectrometer at 30 °C; chemical shifts (δ, ppm) were referred to solvent residual peak of internal D₂O (δ 4.79), CD₃OD (δ 3.31), CDCl₃ (δ 7.27) and (CD₃)₂SO (δ 2.50); coupling constants (J) were measured in Hz. ¹³C NMR spectra were recorded at 150 MHz. Chemical shifts (δ, ppm) were referred to the peak of internal CD₃OD (δ 49.0), CDCl₃ (δ 77.16) and (CD₃)₂SO (δ 39.62). Signals of ¹H NMR and ¹³C NMR spectra were assigned to the corresponding protons using 2D spectroscopy (COSY, HSQC).

Example 1.1: 3-Aminopropyl α-D-glucopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (Glcα1-3(Fuca1-2)Galβ1-4GlcNAcβ-O-(CH₂)₃-NH₂)



Title compound was prepared following the route depicted in Schemes Ia and Ib, as detailed below.

Ia, step 1: 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-O-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranoside (Intermediate 1.1.1)

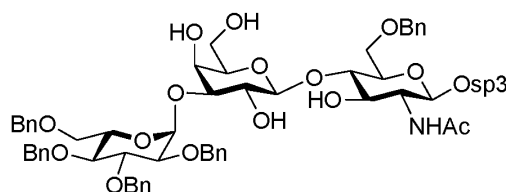


To β -Bn₄GlcSEt (compound of formula A, 553 mg, 0.95 mmol) solution in abs. dichloromethane (5 ml) was added bromine (55 μ L, 1.0 mmol) at 0°C. The reaction was left at 0°C for 1 h. The solvent and bromine was evaporated, residue was co-evaporated with toluene (3×5 mL) to remove traces of bromine, almost colourless oil of Bn₄GlcBr was dried in vacuum at r.t.

To disaccharide B (500 mg, 0.63 mmol) solution in abs. dichloromethane (6 ml) were added freshly dried MS-4Å (2 mL) and tetramethylurea (0.137 ml, 1.15 mmol), the mixture stirred for 30 min, then AgOTf (0.27 g, 1.05 mmol) was added and the mixture was stirred for additional 15 min. To reaction mixture Bn₄GalBr (see above) solution in abs. dichloromethane (3 mL) was added drop-wise under intensive stirring. The reaction was left for 24 h with stirring. Reaction mixture was neutralized with pyridine (0.5 ml), filtered through Celite on glass filter, solid material was washed with portion of dichloromethane and mixture of dichloromethane/methanol 10:1. Resulting solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. Intermediate 1.1.1 was purified with column chromatography on silica gel (hexane/ethyl acetate from 1:1 to 1:5), the fractions containing major Intermediate 1.1.1 were collected, concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum and used in next stage without additional purification.

Spectroscopic data (¹H NMR) was in agreement with the proposed structure.

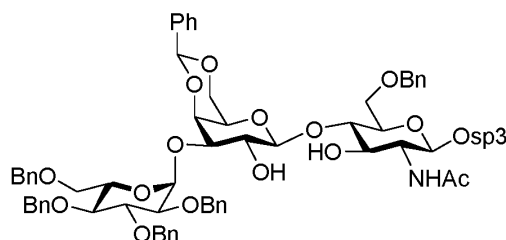
1a, step 2: 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-6-O-benzyl-2-deoxy- β -D-glucopyranoside (Intermediate 1.1.2)



Intermediate 1.1.2

The Intermediate 1.1.1 was dissolved in abs. methanol (30 mL), treated with 2M NaOMe (3 mL, 6 mmol) and reaction mixture was left for 4 h at r.t. Reaction mixture was neutralized with Dowex 50WX4 (6.0 g, PyH⁺-form) and stirred for 15 min and filtered. Obtained solution was concentrated on rotary evaporator, co-evaporated with ethyl acetate, dried in vacuum. The product Intermediate 1.1.2 was purified with column chromatography on silica gel (chloroform/methanol 10:1), the fractions containing major product Intermediate 1.1.2 were collected, concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. Intermediate 1.1.2 was used in next stage without additional purification. Spectroscopic data (¹H NMR) was in agreement with the proposed structure.

la, step 3: 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-4,6-O-benzylidene- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-6-O-benzyl-2-deoxy- β -D-glucopyranoside (Intermediate 1.1.3)



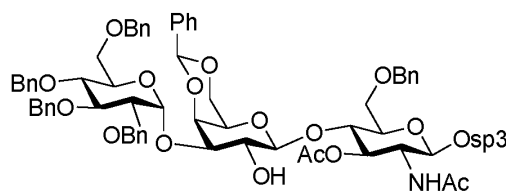
Intermediate 1.1.3

5

Intermediate 1.1.2 trisaccharide was suspended in dry acetonitrile (5 mL), then PhCH(OMe)_2 (0.16 mL, 1.0 mmol) and solution of $\text{TsOH} \cdot \text{H}_2\text{O}$ (6.0 mg, 0.034 mmol) in dry acetonitrile (0.1 mL) were added. Reaction mixture was stirred overnight at r.t. The reaction was neutralised with pyridine (0.2 mL), 30 ml diethyl ether and 30 ml hexane were added under stirring. Solid material was collected on glass filter, washed with ether/hexane 1:1, pure hexane and dried in vacuum. Crude Intermediate 1.1.3 was dissolved in 25 ml DMF and precipitated with 75 ml water, pure solid Intermediate 1.1.3 was collected on glass filter, washed with water, dried under fume hood. Total yield of Intermediate 1.1.3 for three stages: 48% (380 mg, 0.3 mmol). No NMR spectral data due to low solubility of Intermediate 1.1.3.

15

la, step 4: 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-4,6-O-benzylidene- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy- β -D-glucopyranoside (Compound C, $R_a = \text{OBn}$, $R_4\text{-Z}' = (\text{CH}_2)_3\text{NHCOCF}_3$)



C ($R_a = \text{OBn}$)

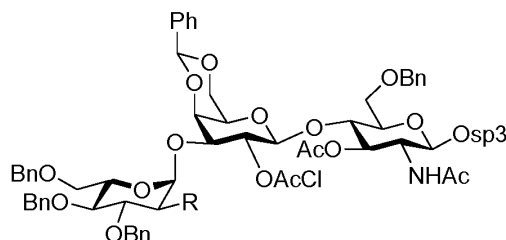
20

To powder of Intermediate 1.1.3 trisaccharide (380 mg, 0.3 mmol) was added solution of chloro acetic anhydride (150 mg, 0.9 mmol) and sym-Collidine (0.8 mL, 5.8 mmol) in abs. dichloromethane (2 ml) under intensive stirring. The mixture was stirred for 3 h at r.t. The reaction was quenched with methanol (1 mL), concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

25

Resulting oil was dissolved in abs. dichloromethane (4 ml) followed by addition of sym-Collidine (0.9 mL, 6.7 mmol) and acetyl chloride (0.35 mL, 4.9 mmol). Reaction mixture was left

for 24 h at r.t. Reaction was quenched with methanol (1.4 mL), concentrated on rotary evaporator, co-evaporated with toluene. The residue was diluted with toluene (7 mL), stirred for 15 min, collidinium salt was collected on glass filter, washed few times with toluene. The filtered solution of Intermediate 1.1.4 was concentrated on rotary evaporator, dried in vacuum.



Intermediate 1.1.4

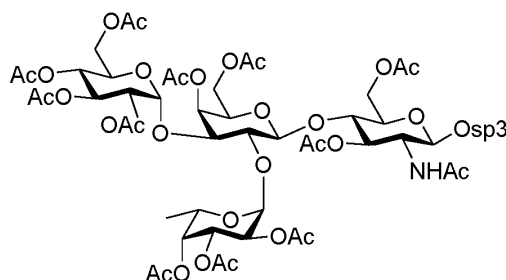
Crude Intermediate 1.1.4 was dissolved in pyridine (7 mL), water (3.5 mL) was added and mixture warmed at 60 °C for 24 h. The mixture was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

The product was purified with column chromatography on silica gel (chloroform/acetone, from 5:1 to 3:1, 0.5% pyridine), the fractions containing major product were collected, concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

White crystalline material. Yield of C: 70% (270 mg, 0.21 mmol).

Spectroscopic data (¹H NMR) was in agreement with the proposed structure.

1b, steps 1 and 2: 3-Trifluoroacetamidopropyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl-(1→3)-[2,3,4-tri-O-acetyl-α-L-fucopyranosyl-(1→2)]-4,6-di-O-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (Intermediate 1.1.7)



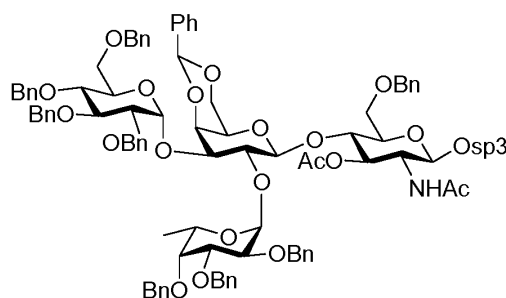
Intermediate 1.1.7

To β-Bn₃FucSEt (0.20 g, 0.42 mmol) solution in abs. dichloromethane (2.63 mL) was added bromine (24 μL, 0.47 mmol) at 0°C. The reaction was left at 0°C for 1 h. The solvent and bromine was evaporated, residue was co-evaporated with toluene (3×2 mL) to remove traces of bromine, almost colourless oil of Bn₃FucBr was dried in vacuum at r.t.

To the compound C (270 mg, 0.21 mmol) solution in abs. dichloromethane (5.25 mL) were added freshly dried MS-4Å (2.6 mL) and tetramethylurea (56 μL, 0.47 mmol), the mixture stirred

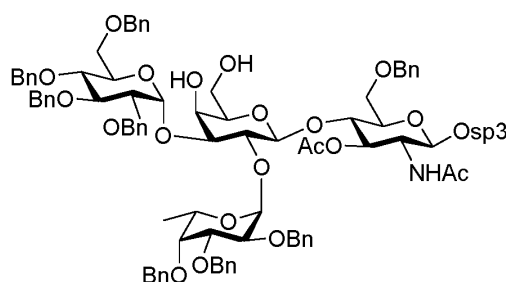
for 30 min, then AgOTf (0.11 g, 0.42 mmol) was added and the mixture was stirred for additional 15 min. To reaction mixture Bn_3FucBr (see above) solution in abs. dichloromethane (2.6 mL) was added drop-wise under intensive stirring. Reaction mixture was neutralized with pyridine (0.2 ml), filtered through Celite on glass filter, solid material was washed with portion of dichloromethane and mixture of dichloromethane/methanol 10:1.

Filtered solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum, to obtain crude Intermediate 1.1.5.



Intermediate 1.1.5

Crude Intermediate 1.1.5 was dissolved in acetic acid (7 mL), water (1.75 mL) was added under stirring and the mixture was warmed at 80 °C for 3 h. The mixture was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum, to obtain Intermediate 1.1.6.



Intermediate 1.1.6

The Intermediate 1.1.6 was purified with column chromatography on silica gel (from toluene/ethyl acetate 1:1 to pure ethyl acetate), the fractions containing major product were collected (TLC control in pure ethyl acetate), concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum and used in next stage without additional purification.

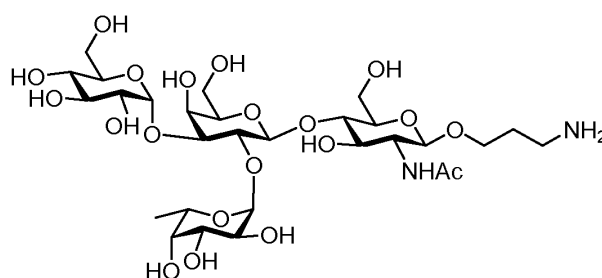
The Intermediate 1.1.6 was dissolved in methanol (10.0 mL), then 10% Pd/C (0.1 g) was added and reaction mixture was degased in vacuum further by filling with H_2 (1 atm) three times. The reaction was left under H_2 (1 atm) with stirring for 48 h at r.t. Conversion was controlled by TLC (propan-2-ol/ethyl acetate/water 2:3:1). Reaction mixture was filtered through Celite on glass filter, solid material was washed with methanol, portion of 50% aq. methanol and resulting solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The residue

was dissolved in pyridine (3.5 mL) and Ac₂O (1.75 mL), reaction mixture was left at 40 °C for 24 h. Reaction mixture was concentrated on rotary evaporator, co-evaporated with toluene.

The Intermediate 1.1.7 thus obtained was purified with column chromatography on silica gel (from pure ethyl acetate to ethyl acetate/ propan-2-ol 50:1), the fractions containing major product were collected (TLC control in ethyl acetate/ propan-2-ol 50:1 and chloroform/ propan-2-ol 9:1), concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

Yield of Intermediate 1.1.7 for last four steps: 35% (97 mg, 74 μmol).

Ib, step 3: 3-Aminopropyl α-D-glucopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (Example 1.1)



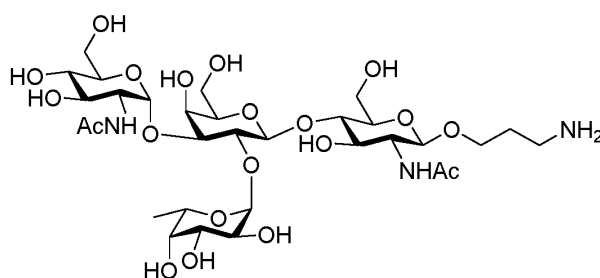
Intermediate 1.1.7 tetrasaccharide (97 mg, 74 μmol) was dissolved in abs. methanol (5.0 mL), treated with 2M NaOMe (0.26 mL, 0.53 mmol) and reaction mixture was left for 2 h. The mixture was concentrated in vacuum (not to dryness) and diluted with water (5.0 mL), reaction mixture was left overnight.

Target deprotected tetrasaccharide was isolated with cation-exchange chromatography on Dowex 50X4-400 (H⁺) by subsequent elution with water, 1M pyridine water solution and 1M aqueous ammonia, fractions containing the carbohydrate were collected, concentrated in vacuum and freeze dried twice, to obtain a white solid material.

Yield of Example 1.1: 90% (50 mg, 63 μmol).

¹H NMR (D₂O+TFA, characteristic signals): δ 1.26 (d, 3H, J_{5,6} 6.6 Hz, H-6 Fuc), 1.93-2.01 (m, 2H, OCH₂CH₂CH₂N), 2.08 (s, 3H, NHC(O)CH₃), 4.51 and 4.63 (2 d, 2×1H, J_{1,2} 8.4 Hz, J_{1,2} 7.7 Hz, H-1 GlcHAcβ and H-1Galβ), 5.23 and 5.37 (2 d, 2×1H, J_{1,2} 3.8 Hz, J_{1,2} 4.2 Hz, H-1 Gala and H-1 Fuca).

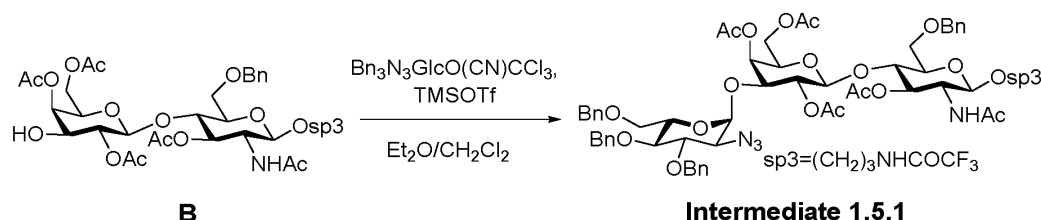
Example 1.5: 3-Aminopropyl 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (GlcNAc1-3(Fuca1-2)Galβ1-4GlcNAcβ-O-(CH₂)₃-NH₂)



This product was prepared analogously as disclosed above for Example 1.1, starting from step 2, using intermediate 1.5.1, whose preparation is described below, instead of intermediate 1.1.1.

5

3-Trifluoroacetamidopropyl 2-azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→3)-2,4,6-tri-O-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3-O-acetyl-6-O-benzyl-2-deoxy-β-D-glucopyranoside (Intermediate 1.5.1)



To disaccharide B (500 mg, 0.63 mmol) and β-Bn₃N₃GlcO(CN)CCl₃ (735 mg, 1.26 mmol) solution in abs. ether/dichloromethane 1:1 (20 ml) were added freshly dried MS-4Å (3 mL) and the mixture stirred for 30 min, then trimethylsilyl trifluoromethanesulfonate (TMSOTf) (12 μL, 63 μmol) was added. The reaction was left for 24 h with stirring. Reaction mixture was neutralized with pyridine (0.5 ml) and MeOH (1 ml), filtered through Celite on glass filter, solid material was washed with portion of dichloromethane and mixture of dichloromethane/methanol 10:1. The filtrate was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The obtained Intermediate 1.5.1 was purified with column chromatography on silica gel (toluene/acetone 2:1 to toluene/acetone 1:1), the fractions containing major Intermediate 1.5.1 were collected, concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum and used in next stage without additional purification.

15

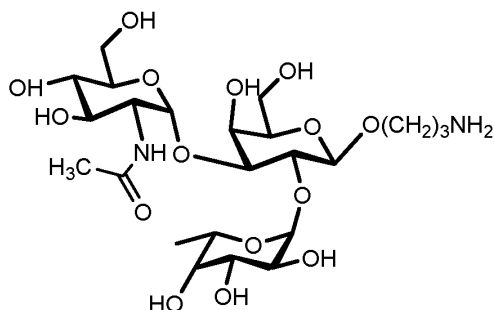
20

Yield of Intermediate 1.5.1: 50% (395 mg, 0.315 mmol).

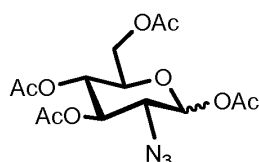
¹H NMR (CDCl₃, characteristic signals): δ 1.76-1.92 (m, 2H, OCH₂CH₂CH₂N), 1.98, 1.98, 2.06, 2.08, 2.20 (5s, 15H, C(O)CH₃), 4.37, 4.45 (2d, 2H, *J*_{1,2} 7.9, *J*_{1,2} 8.0, H-1' and H-1''), 5.12 (d, 1H, *J*_{1,2} 3.5, H-1'''), 5.44 (d, 1H, *J*_{1,2} 3.1, H-4''), ¹³C NMR (CDCl₃, characteristic signals): δ 20.5, 20.7, 20.8, 20.8, 23.2 (C(O)CH₃), 28.5 (OCH₂CH₂CH₂N), 94.0 (C-1'''), 100.8, 102.0 (C-1' and C-1'').

25

Example 1.8: 3-Aminopropyl 2-O-(α -L-fucopyranosyl)-3-O-(2-acetamido-2-deoxy- α -D-glucopyranosyl)- β -D-galactopyranoside (GlcNAc α 1-3(Fuc α 1-2)Gal β -O-(CH₂)₃-NH₂)



1,3,4,6 tetra-O-acetyl-2-azido-2-deoxy- α/β -D-glucose (Intermediate 1.8.1)



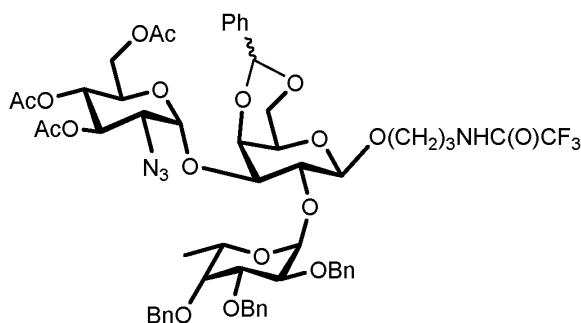
Intermediate 1.8.1

5

To a solution of 2-azido-2-deoxy- α/β -D-glucose (5.0 g, 24.4 mmol) in anhydrous pyridine (5 ml) was added Ac₂O (5 ml), then reaction mixture was kept for 24 h at rt and concentrated *in vacuo*, co-evaporated with toluene (3×20 ml), and then dried *in vacuo* (1 mm Hg) to give 9.0 g of crude product. *R*_f 0.3 (toluene–AcOEt, 3:1).

10

3-Trifluoroacetamidopropyl 4,6-O-benzylidene-2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-3-O-(2,4,6-tri-O-acetyl-2-azido-2-deoxy- α -D-glucopyranosyl)- β -D-galactopyranoside (Intermediate 1.8.2)



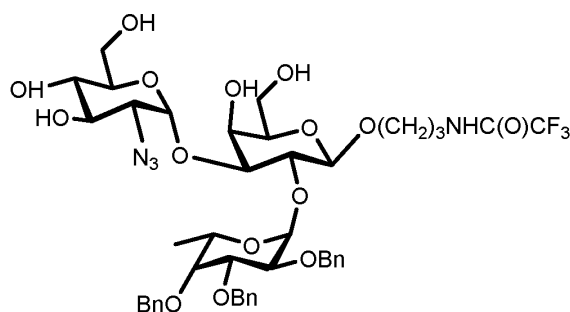
Intermediate 1.8.2

15

To a solution of Intermediate 1.8.1 (2 g, 5.3 mmol) in anhydrous CH₂Cl₂ (20 ml) was added TiBr₄ (2.65 g, 7.9 mmol). The reaction was stirred for 48 h at rt. The mixture was poured into ice and extracted with CHCl₃ (100 ml). The organic extract was washed with H₂O (100 ml), cold saturated aqueous NaHCO₃ (100 ml), concentrated *in vacuo*, co-evaporated with toluene (3×20 ml), and then dried *in vacuo* (1 mm Hg). The resulting 3,4,6 tri-O-acetyl-2-azido-1-bromo-2-deoxy-

α -D-glucose, R_f 0.4 (toluene–AcOEt, 3:1) was dissolved in anhydrous CH_2Cl_2 (20 ml) and added dropwise to a mixture of 3-trifluoroacetamidopropyl 4,6-O-benzylidene-2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)- β -D-galactopyranoside (2.04 g, 2.4 mmol), Ag_2CO_3 (6.61 g, 24.0 mmol), AgOTf (30.8 mg, 0.12 mmol), and molecular sieve 4Å in anhydrous CH_2Cl_2 (70 ml). The reaction mixture was stirred for 24 h at rt in the darkness. The solids were filtered off, washed with CHCl_3 (300 ml), and the combined filtrate was concentrated *in vacuo*. The residue was chromatographed on Silica gel in toluene–AcOEt, 2:1 to give 1.61 g (1.5 mmol) of the title compound in 64% yield. R_f 0.6 (toluene–AcOEt, 1:1).

3-Trifluoroacetamidopropyl 2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-3-O-(2-azido-2-deoxy- α -D-glucopyranosyl)- β -D-galactopyranoside (Intermediate 1.8.3)

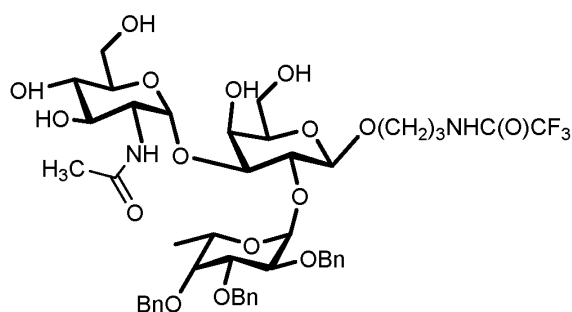


Intermediate 1.8.3

Intermediate 1.8.2 (1.61 g, 1.5 mmol) was dissolved in 80% aqueous AcOH (30 ml), the solution was kept for 2 h at 80 °C, concentrated *in vacuo*, and co-evaporated with toluene (3×50 ml), and then dried *in vacuo* (1 mm Hg). The residue was chromatographed on Silica gel in *n*-hexane– CHCl_3 –2-propanol, 4:2:1 to give 1.18 g (1.22 mmol) of 3-trifluoroacetamidopropyl 2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-3-O-(2,4,6-tri-O-acetyl-2-azido-2-deoxy- α -D-glucopyranosyl)- β -D-galactopyranoside in 80% yield. R_f 0.49 (*n*-hexane– CHCl_3 –2-propanol, 4:2:1).

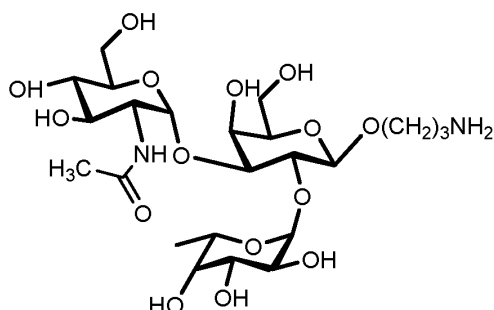
3-Trifluoroacetamidopropyl 2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-3-O-(2,4,6-tri-O-acetyl-2-azido-2-deoxy- α -D-glucopyranosyl)- β -D-galactopyranoside (1.18 g, 1.22 mmol) was dissolved in anhydrous MeOH (40 ml), 2 M NaOMe in MeOH (116 μ l, 0.23 mmol) was added, and the reaction mixture was kept for 2 h at rt. Then cation exchange resin Dowex 50X4-400 (H^+ , 1 ml in MeOH) was added, kept for 10 min and filtered off. The filtrate was concentrated *in vacuo* and the residue was chromatographed on Silica gel in CHCl_3 –2-propanol, 3:1 to give 0.971 g (1.16 mmol) of title compound in 95% yield. R_f 0.32 (CHCl_3 –2-propanol, 4:1).

3-trifluoroacetamidopropyl 2-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)-3-O-(2-acetamido-2-deoxy- α -D-glucopyranosyl)- β -D-galactopyranoside (Intermediate 1.8.4)

**Intermediate 1.8.4**

To a solution of Intermediate 1.8.3 (150 mg, 0.16 mmol) in 2.5 ml of DMF-H₂O (4:1) were added DTT (128 g, 0.8 mmol) and Et₃N (50 μ l). The reaction mixture was kept for 2 h at rt, concentrated *in vacuo*, co-evaporated with toluene (3 $\times$ 30 ml), and then dried *in vacuo* (1 mm Hg).
 5 The residue was dissolved in anhydrous MeOH (3 ml), Ac₂O (500 μ l) and Et₃N (50 μ l) were added, and the reaction mixture was kept overnight at rt. Then the mixture was concentrated *in vacuo*, co-evaporated with toluene (3 $\times$ 20 ml), and then chromatographed on Silica gel in CHCl₃-2-propanol, 2:1 to give 81 mg (0.085 mmol) of title compound in 53% yield. *R*_f 0.40 (CHCl₃-2-propanol, 2:1).

3-Aminopropyl 2-O-(α -L-fucopyranosyl)-3-O-(2-acetamido-2-deoxy- α -D-glucopyranosyl)- β -D-galactopyranoside (Example 1.8)

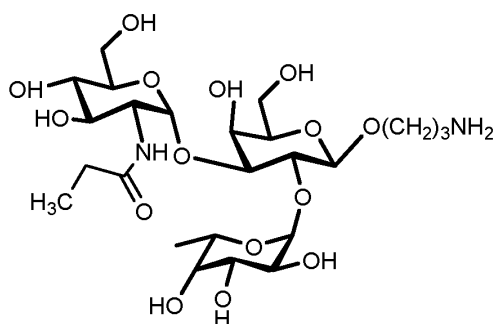


Intermediate 1.8.4 (81 mg, 0.085 mmol) was dissolved in anhydrous MeOH (5 ml), 10% Pd/C (80 mg) was added, and the reaction mixture was stirred for 12 h at rt in the atmosphere of H₂. The catalyst was filtered off and washed with MeOH; the combined filtrate was concentrated *in vacuo*. The residue was purified by HPLC on C18 to give 48 mg of 3-trifluoroacetamidopropyl 2-O-(α -L-fucopyranosyl)-3-O-(2-acetamido-2-deoxy- α -D-glucopyranosyl)- β -D-galactopyranoside, which was treated with 5% aqueous Et₃N (2 ml) (12 h, rt). The reaction mixture was applied on Dowex 50X4-400 (H⁺) cation exchange resin (2 ml). The column was washed with H₂O (5 ml), 1 M aqueous pyridine (5 ml), the title compound was eluted with 1 M aqueous ammonia. Fractions containing Example 1.8 compound were concentrated *in vacuo*, the residue was dissolved in H₂O (2 ml) and freeze-dried.

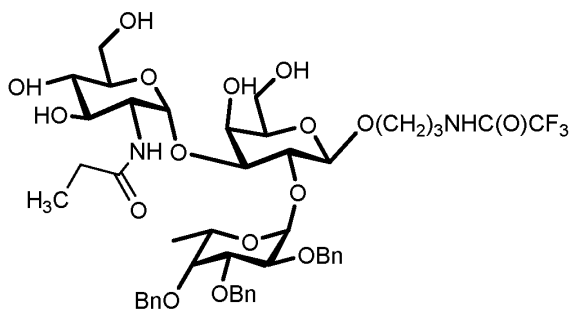
Yield: 40 mg (97%).

¹H NMR (D₂O + TFA): δ 1.25 (d, 2H, *J* 6.6 Hz, H-6 Fuc), 2.02 (m, 2H, OCH₂CH₂CH₂N), 2.05 (s, 3H, NHC(O)CH₃), 3.15 (m, 2H, CH₂N), 3.56 (dd ≈ t, 1H, *J*_{3,4} ≈ *J*_{4,5} 9.5 Hz, H-4 GlcN), 3.68 (ddd ≈ br. dd, 1H, *J*_{5,6a} 8.0 Hz, *J*_{5,6b} 4.3 Hz, *J*_{4,5} < 1.0 Hz, H-5 Gal), 3.76 (dd, 1H, *J*_{6a,6b} 10.7 Hz, *J*_{5,6a} 9.0 Hz, H-3 GlcN), 3.75–3.91 (m, 9H, H-6^a Gal, H-6^b Gal, H-6^a GlcN, H-6^b GlcN, H-2 Gal, H-2 Fuc, H-3 Fuc, H-4 Fuc, OCH), 3.99 (ddd, 1H, *J*_{5,6a} 2.4 Hz, *J*_{5,6b} 4.7 Hz, *J*_{4,5} 10.0 Hz, H-5 GlcN), 3.96–4.05 (m, 3H, H-2 GlcN, H-3 Gal, CHO), 4.22 (dd ≈ br. d, 1H, *J*_{3,4} 3.4 Hz, *J*_{4,5} < 1 Hz, H-4 Gal), 4.38 (br. q, 1H, *J*_{5,6} 6.6 Hz, *J*_{4,5} < 1 Hz H-5 Fuc), 4.57 (d, 1H, *J*_{1,2} 7.8 Hz, H-1 Galβ), 5.16 (d, 1H, *J*_{1,2} 3.8 Hz, H-1 GlcNα), 5.33 (d, 1H, *J*_{1,2} 3.7 Hz, H-1 Fuca).

Example 1.9: 3-Aminopropyl 2-O-(α-L-fucopyranosyl)-3-O-(2-deoxy-2-*n*-propionylamido-α-D-glucopyranosyl)-β-D-galactopyranoside (GlcNPropionylα1-3(Fuca1-2)Galβ-O-(CH₂)₃-NH₂)



3-trifluoroacetamidopropyl 2-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-3-O-(2-deoxy-2-*n*-propionylamido-α-D-glucopyranosyl)-β-D-galactopyranoside (Intermediate 1.9.1)

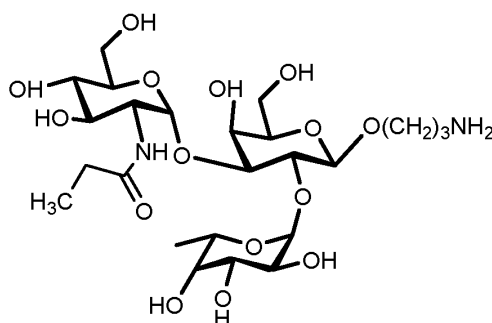


Intermediate 1.9.1

To a solution of Intermediate 1.8.3 (234 mg, 0.25 mmol) in 5 ml of DMF–H₂O (4:1) were added DTT (192 mg, 1.2 mmol) and Et₃N (100 μl). The reaction mixture was kept for 2 h at rt, concentrated *in vacuo*, co-evaporated with toluene (3×50 ml), and then dried *in vacuo* (1 mm Hg). The residue was dissolved in anhydrous MeOH (6 ml), *n*-propionyl anhydride (500 μl) and Et₃N (100 μl) were added, and the reaction mixture was kept overnight at rt. Then the reaction mixture was concentrated *in vacuo*, co-evaporated with toluene (3×20 ml), and then chromatographed on

Silica gel in CHCl_3 -2-propanol, 3:1 to give 144 mg (0.16 mmol) of title compound in 67% yield. R_f 0.30 (CHCl_3 -2-propanol, 3:1).

3-Aminopropyl 2-O-(α -L-fucopyranosyl)-3-O-(2-deoxy-2-*n*-propionylamido- α -D-glucopyranosyl)-
 5 β -D-galactopyranoside (Example 1.9)



Intermediate 1.9.1 (103 mg, 0.106 mmol) was dissolved in anhydrous MeOH (10 ml), 10% Pd/C (120 mg) was added, and the reaction mixture was stirred for 12 h at rt in the atmosphere of H_2 . The catalyst was filtered off, washed with MeOH, the combined filtrate was concentrated *in vacuo*. The residue was dissolved in anhydrous pyridine (1.5 ml), Ac_2O (1.5 ml) was added, and reaction mixture was kept for 24 h at rt and concentrated *in vacuo*, co-evaporated with toluene (3×5 ml), and then dried *in vacuo* (1 mm Hg). The residue was chromatographed on Silica gel in *n*-hexane- CHCl_3 -2-propanol, 4:2:1 to give 105 mg (0.102 mmol) of 3-trifluoroacetamidopropyl 2-O-(2,3,4-tri-O-acetyl- α -L-fucopyranosyl)-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-*n*-propionylamido- α -
 10 D-glucopyranosyl)-4,6-di-O-acetyl- β -D-galactopyranoside in 90% yield. R_f 0.45 (*n*-hexane- CHCl_3 -2-propanol, 4:2:1).

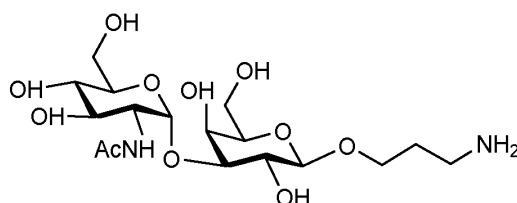
3-Trifluoroacetamidopropyl 2-O-(2,3,4-tri-O-acetyl- α -L-fucopyranosyl)-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-*n*-propionylamido- α -D-glucopyranosyl)-4,6-di-O-acetyl- β -D-galactopyranoside (105 mg, 0.102 mmol) was dissolved in anhydrous MeOH (4 ml), 2 M NaOMe in MeOH (20 μl , 0.04 mmol) was added, and the reaction mixture was kept for 2 h at rt. Then the reaction mixture was concentrated *in vacuo*, the residue was dissolved in H_2O (2 ml), and the mixture was kept for 12 h at rt and then applied on Dowex 50X4-400 (H^+) cation exchange resin (2 ml). The column was washed with H_2O (5 ml), 1 M aqueous pyridine (5 ml), the target product was eluted with 1 M aqueous ammonia. Fractions containing Example 1.9 compound were concentrated *in vacuo*,
 20 the residue was dissolved in H_2O (2 ml) and freeze-dried.

Yield: 55 mg (90%).

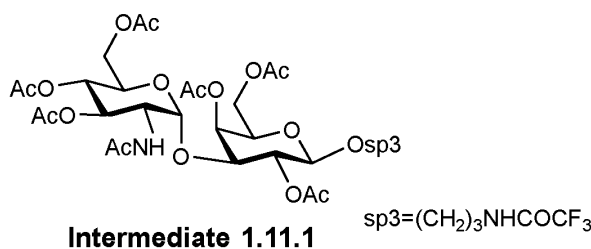
^1H NMR (D_2O + TFA): δ 1.13 (t, 3H, J 7.7 Hz, $\text{NHC(O)CH}_2\text{CH}_3$), 1.25 (d, 3H, J 6.6 Hz, H-6 Fuc), 2.02 (m, 2H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.32 (q, 2H, J 7.6 Hz, $\text{NHC(O)CH}_2\text{CH}_3$), 3.11–3.18 (m, 2H, CH_2N), 3.56 (dd, 1H, $J_{4,5}$ 10.1 Hz, $J_{3,4}$ 9.1 Hz, H-4 GlcN), 3.68 (ddd $\approx$ br. dd, 1H, $J_{5,6a}$ 8.0 Hz, $J_{5,6b}$ 4.3 Hz, $J_{4,5}$ <1.0 Hz, H-5 Gal), 3.76 (dd, 1H, $J_{3,4}$ 9.2 Hz, $J_{2,3}$ 7.7 Hz, H-3 GlcN), 3.75–3.90 (m, 9H, OCH,
 30

H-6^a GlcN, H-6^b GlcN, H-2 Fuc, H-3 Fuc, H-4 Fuc, H-2 Gal, H-6^a Gal, H-6^b Gal), 3.98 (ddd, 1H, $J_{4,5}$ 10.2 Hz, $J_{5,6a}$ 4.8 Hz, $J_{5,6b}$ 2.9 Hz, H-5 GlcN), 4.05–3.95 (m, 3H, CHO, H-2 GlcN, H-3 Gal), 4.21 (dd $\approx$ br. d, 1H, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ <1 Hz, H-4 Gal), 4.37 (br. q, 1H, $J_{5,6}$ 6.6 Hz, $J_{4,5}$ < 1 Hz, H-5 Fuc), 4.56 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1 Gal β), 5.16 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1 GlcN α), 5.33 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1 Fuc α).

Example 1.11: 3-Aminopropyl 2-acetamido-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- β -D-galactopyranoside (GlcNAc1-3Gal β -O-(CH₂)₃-NH₂)

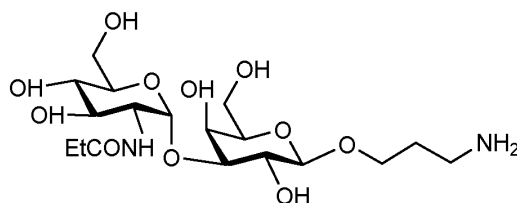


This compound can be prepared analogously to Example 1.12 (preparation disclosed below), through Intermediate 1.11.1, instead of 1.12.1, which is prepared in analogous way as discussed below for intermediate 1.12.1, but using Ac₂O instead of (EtCO)₂O.

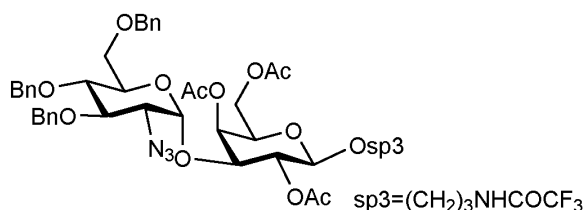


¹H NMR (D₂O + TFA): δ 2.03 (m, 2H, OCH₂CH₂CH₂N), 2.06 (s, 3H, NHC(O)CH₃), 3.19 (m, 2H, CH₂N), 3.56 (dd, 1H, $J_{3,4}$ 10.1 Hz, $J_{4,5}$ 9.0 Hz, H-4 GlcN), 3.64 (dd, 1H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 10.0 Hz, H-2 Gal), 3.68 (ddd $\approx$ br. dd, 1H, $J_{5,6a}$ 4.3 Hz, $J_{5,6b}$ 7.9 Hz, $J_{4,5}$ 1.0 Hz, H-5 Gal), 3.75 (dd 1H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.3 Hz, H-3 Gal), 3.76 (dd, 1H, $J_{6a,6b}$ 11.8 Hz, $J_{5,6a}$ 4.3 Hz, H-6^a Gal), 3.80 (dd, 1H, $J_{6a,6b}$ 11.8 Hz, $J_{5,6b}$ 7.9 Hz, H-6^b Gal), 3.81–3.87 (m, 4H, H-6^a GlcN, H-6^b GlcN, H-3 GlcN, OCH), 3.97 (ddd, 1H, $J_{5,6a}$ 2.8 Hz, $J_{5,6b}$ 4.7 Hz, $J_{4,5}$ 8.9 Hz, H-5 GlcN), 3.98 (dd, 1H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 10.7 Hz, H-2 GlcN), 4.09 (m, 1H, CHO), 4.12 (dd $\approx$ br. d, 1H, $J_{3,4}$ 3.3 Hz, $J_{4,5}$ < 1 Hz, H-4 Gal), 4.47 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1 Gal β), 5.07 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1 GlcN α).

Example 1.12: 3-Aminopropyl 2-propionylamido-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- β -D-galactopyranoside (GlcNPropionyl α 1-3Gal β -O-(CH₂)₃-NH₂)



3-Trifluoroacetamidopropyl 2-azido-3,4,6-tri-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→3)-2,4,6-tri-O-acetyl-β-D-galactopyranoside (Intermediate 1.12.1)



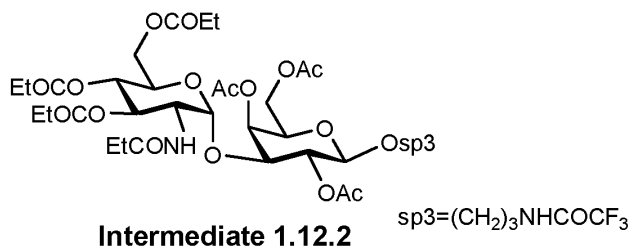
Intermediate 1.12.1

5 To 3-trifluoroacetamidopropyl-2,4,6-tri-O-acetyl-β-D-galactopyranoside obtained from 3-trifluoroacetamidopropyl-2-O-acetyl-3-O-chloroacetyl-4,6-O-benzylidene-β-D-galactopyranoside by sequential debenzylidenation, O-acetylation and removal of chloroacetyl group, (422 mg, 1.0 mmol) and β-Bn₃N₃GlcO(CN)CCl₃ (735 mg, 1.26 mmol) solution in abs. ether/dichloromethane 1:1 (20 ml) were added freshly dried MS-4Å (3 mL) and the mixture stirred for 30 min, then

10 trimethylsilyl trifluoromethanesulfonate (TMSOTf) (12 μL, 63 μmol) was added. The reaction was left for 24 h with stirring. Reaction mixture was neutralized with pyridine (0.5 ml) and MeOH (1 ml), filtered through Celite on glass filter, solid material was washed with portion of dichloromethane and mixture of dichloromethane/methanol 10:1. The filtrate was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The obtained disaccharide

15 was purified with column chromatography on silica gel (toluene/acetone 5:2 to toluene/acetone 1:1), the fractions containing major product were collected, concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum and used in next stage without additional purification, yield 76%.

20 *3-Trifluoroacetamidopropyl 3,4,6-tri-O-propionyl-2-propionylamido-2-deoxy-α-D-glucopyranosyl-(1→3)-2,4,6-tri-O-acetyl-β-D-galactopyranoside (Intermediate 1.12.2)*



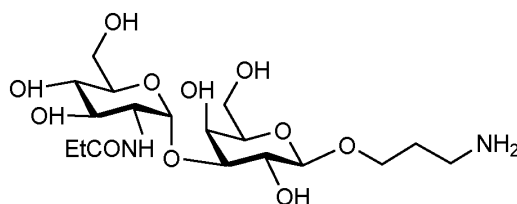
Intermediate 1.12.2

Intermediate 1.12.1 (192 mg, 0.21 mmol) was dissolved in methanol (10.0 mL), then 10% Pd/C (0.1 g) was added and reaction mixture was degassed in vacuum further by filling with H₂ (1 atm) three times. The reaction was left under H₂ (1 atm) with stirring for 1h and (EtCO)₂O (0.1 mL) was added (through septa), stirring was continued for 48 h at r.t. Conversion was controlled by TLC. Reaction mixture was filtered through Celite on glass filter, solid material was washed with methanol, portion of 50% aq. methanol and resulting solution was concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum. The residue was dissolved in pyridine (3.5 mL) and (EtCO)₂O (1.75 mL), reaction mixture was left at 40 °C for 24 h. Reaction mixture was concentrated on rotary evaporator, co-evaporated with toluene.

The product was purified with column chromatography on silica gel, the product was collected, concentrated on rotary evaporator, co-evaporated with toluene, dried in vacuum.

Yield of Intermediate 1.12.2: 91%.

3-Aminopropyl 2-propionylamido-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- β -D-galactopyranoside
(Example 1.12)



Intermediate 1.12.2 was dissolved in absolute methanol (5.0 mL), treated with 2M NaOMe (0.26 mL, 0.53 mmol) and reaction mixture was left for 2 h. The mixture was concentrated in vacuum (not to dryness) and diluted with water (5.0 mL), reaction mixture was left overnight. Target deprotected disaccharide (Example 1.12) was isolated with cation-exchange chromatography on Dowex 50X4-400 (H⁺) by subsequent elution with water, 1M pyridine water solution and 1M aqueous ammonia, fractions containing the carbohydrate were collected, concentrated in vacuum and freeze dried twice.

Yield of Example 1.12: 95%.

¹H NMR (D₂O + TFA): δ 1.13 (t, 3H, *J* 7.7 Hz, NHC(O)CH₂CH₃), 2.03 (m, 2H, OCH₂CH₂CH₂N), 2.32 (q, 2H, *J* 7.6 Hz, NHC(O)CH₂CH₃), 3.16–3.21 (m, 2H, CH₂N), 4.56 (d, 1H, *J*_{1,2} 7.9 Hz, H-1 Gal β), 5.06 (d, 1H, *J*_{1,2} 3.6 Hz, H-1 Glc α).

Example 2: Preparation of glycoconjugates comprising compound of formula (I) with agarose

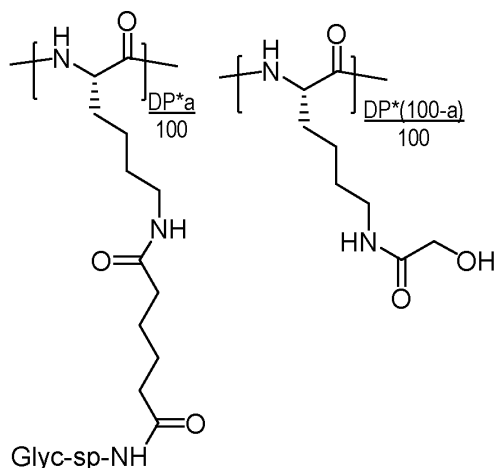
NHS-activated resin (10 mL) was incubated overnight at 4°C (under mild agitation) with 1.25 mL of 2 mg/mL of the ligands prepared in Example 1 in 1.25 mL of coupling solution (0.2 M NaHCO₃ and 0.5 M NaCl, pH 8.3), and 2.5 mL of 1 mM HCl. Non-reacted groups in the resin were capped for 4h at 4°C with 5 mL of 0.5 M ethanol amine in 0.1 M Tris-Cl at pH 8.5.

Example 3: Preparation of glycoconjugates comprising a compound of formula (I) and magnetic nanoparticles

Glycoconjugates with magnetic nanoparticles were prepared using the same procedure disclosed in Example 2, using NHS-activated magnetic nanoparticles.

Example 4: Preparation of glycoconjugates comprising a compound of formula (I) and poly-L-lysine

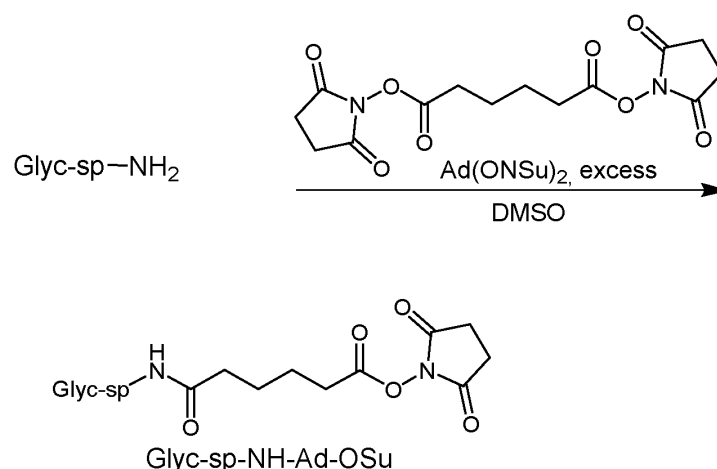
Glycoconjugates of compounds 1 to 16, as disclosed in Example 1, and linear poly-L-lysine of a degree of polymerization of 1000 ± 100 , i.e., having 1000 L-lysine units in the chain on average (DP1000), were prepared. The glycoconjugates had a loading degree of about 25% of glycan. The free amino groups on the lysine were capped with glycolic acid. The glycoconjugates prepared can be represented with the following formula:



where DP=1000 and a = 25.

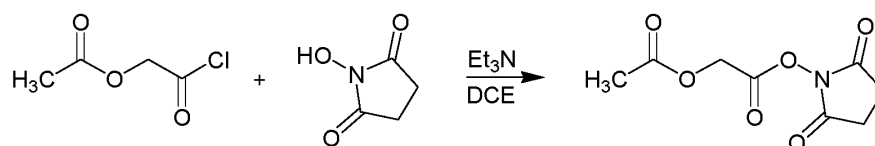
The procedures for preparing the glycoconjugates with polylysine are herein below described. The preparation of the specific glycoconjugates of the glycans of Examples 1.1 and 1.8 are disclosed in detail, but the process is analogous for the preparation of glycoconjugates of all glycans of Examples 1.1 to 1.20.

Activation of the ligands with adipic acid succinimidyl diester



To a stirred solution of adipic acid succinimidyl diester, Ad(ONSu)₂ (452 mg, 1.328 mmol) in DMSO (4 mL) a solution of the compounds of Example 1 (0.166 mmol) in DMSO (1 mL) was added in four portions (~ 0.25 mL) with 10 min interval. The resulting solution was kept for 75 min at room temperature, then acidified with 0.1 mL of AcOH and diluted with 50 ml of cold 1% aqueous AcOH. The precipitate of excessive adipic acid succinimidyl diester was filtered off and washed with 1% aqueous AcOH. The filtrate was evaporated in vacuum (30 °C in a bath) to minimal volume and passed through a Sephadex LH-20 column (180 mL of gel, eluted with 1:1 acetonitrile/water + 1% AcOH). Fractions, containing Glyc-sp-NH-Ad-OSu were combined, evaporated to ~ 2 mL volume, and freeze dried.

Preparation of glycolic acid acetate succinimide ester (AcOCH₂(CO)ONSu) (capping agent)



To a stirred cooled (0° C) solution of *N*-hydroxysuccinimide (992 mg, 8.62 mmol) and Et₃N (1.141 mL, 8.21 mmol) in dry 1,2-dichloroethane (15 mL) a solution of acetoxyacetyl chloride AcOCH₂(CO)Cl (1.121 g, 8.21 mmol) in 1,2-dichloroethane (5 mL) was added in several portions. The reaction mixture (precipitate of Et₃N hydrochloride was formed) was stirred at room temperature for 30 min, then AcOH (0.47 mL) was added. The mixture was diluted with 20 mL of 1% AcOH, stirred by shaking, and organic layer was extracted with 20 mL of 1% AcOH one more time. The organic solution was evaporated, and the residue was dried in vacuum. Crystalline material was rubbed with 20 mL of Et₂O/hexane (1:1) mixture, decanted, rubbed with 20 mL of hexane, decanted, and dried in vacuum. Yield of pure AcOCH₂(CO)ONSu was 1394 mg (79%). TLC: R_f = 0.67 (chloroform/methanol 10:1 by volume).

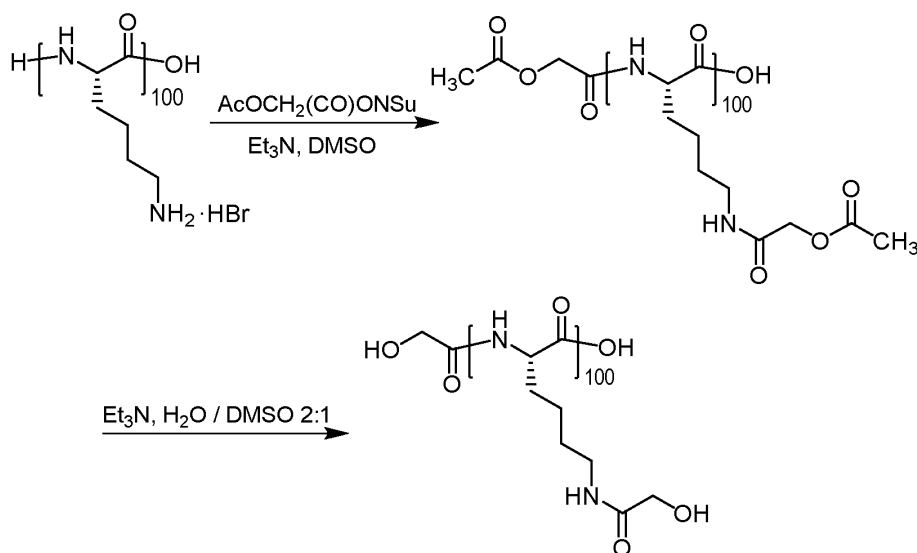
^1H NMR ($\text{CDCl}_3 + 1\% \text{d}_4\text{-AcOH}$, 700 MHz, 30°C) δ 7.258 (s, CHCl_3), 4.943 (s, 2H; OCH_2CO), 2.847 (s, 4H; CH_2CH_2 of succinimide), 2.175 (s, 3H; C(O)CH_3) ppm.

Preparation of the glycoconjugate with poly-L-lysine (a=25)

5 The synthetic scheme is shown in Figure 8. To a stirred solution of polylysine hydrobromide (8.37 mg, 40 micromole of Lys) in DMSO (698 μL , conc. PLys = 12 mg/mL) a solution of Glyc-sp-NH-Ad-ONSu ($40 \cdot a/100$ micromole) in DMSO (conc. Glyc-sp-NH-ONSu = 60 mg/mL) and a solution of Et_3N ($3 \cdot 40 \cdot a/100$ micromole, $3.336 \cdot a \mu\text{L}$ of 5% by volume in DMSO) were added. The solution was kept for 1 h at room temperature, then dry $\text{AcOCH}_2(\text{CO})\text{ONSu}$ (2.5 $\cdot$ 40 $\cdot$ (100-a)/100 micromole 0.2152 $\cdot$ (100-a) mg) and Et_3N (3 $\cdot$ 40 $\cdot$ (100-a)/100 micromole, 0.139 $\cdot$ (120-1.2 $\cdot$ a) μL) were added. After 1 hour at room temperature the solution was diluted with two volumes of water and Et_3N was added (2% of total volume). The reaction mixture was kept at room temperature overnight (~ 16 h), then neutralized with AcOH (equivalent on total Et_3N used). Polylysine conjugate was isolated on Sephadex LH-20 column (100 mL of gel, eluted with 30:70 acetonitrile/water). Fractions contained pure conjugate were evaporated to ~ 2 mL volume and freeze-dried. Yields of Glyc(a%)-PLys conjugates were 92-97%.

The purity and composition of the synthesized glycoconjugates (the percentage of modification of polylysine by Glyc saccharide) was determined by the ^1H NMR spectroscopy by integrating signals of trisaccharide and polylysine fragments.

Preparation of Glycolyl-poly-L-lysine (DP1000) (control)



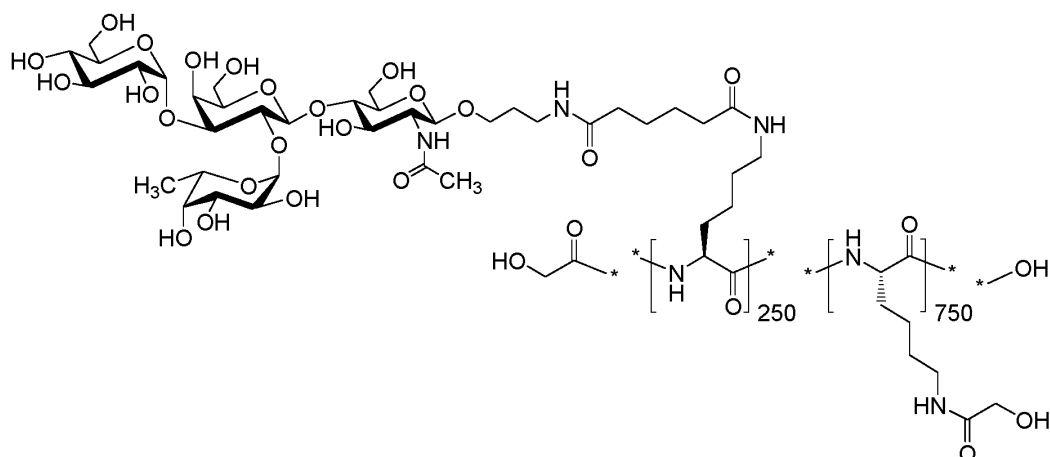
To a stirred solution of polylysine (DP100) hydrobromide (11.43 mg, 54.62 micromole of Lys) in DMSO (750 μL) dry $\text{AcOCH}_2(\text{CO})\text{ONSu}$ (29.4 mg, 136.5 micromole) and Et_3N (22.8 μL , 164 micromole) were added. After 2 hour at room temperature the solution was diluted with two

volumes of water (1500 μ L) and Et₃N was added (102 μ L, corresponds to ~45 μ L of excessive free Et₃N, ~2% of total volume). The reaction mixture was stirred at room temperature 24 h, the small gel-like precipitate was filtered off and washed with water. Combined filtrates were evaporated to ~ 2 mL volume and polylysine conjugate was isolated on Sephadex LH-20 column (100 mL of gel, eluted with 30:70 acetonitrile/water). Fractions contained pure conjugate were evaporated to ~ 2 mL volume and freeze-dried. Yield of Glycolyl_PLys_DP100 conjugate was 8.6 mg (84%).

¹H NMR of polylysine (DP100) hydrobromide (D₂O, 700 MHz, 30°C) δ 4.750 (s, HOD), 4.365 (dd, 100H, J=8.8 Hz, J=5.7 Hz; α -CH), 3.051 (t, 200H, J=7.6 Hz; ϵ -CH₂), 1.770 (m, 400H; β -CH₂ and δ -CH₂), 1.474 (m, 200H; γ -CH₂) ppm.

¹H NMR of Glycolyl_PLys_DP100 conjugate (D₂O + TFA, 14 mM/L, 700 MHz, 30°C) δ 4.750 (s, HOD), 4.107 (m, 100H; α -CH), 4.057 (s, 200H; C(O)CH₂O), 3.253 (m, 200H; ϵ -CH₂), 1.969 (m, 200H; β -CH₂), 1.593 and 1.453 (m, 400H; δ -CH₂ and γ -CH₂) ppm.

Example 4.1: Polylysine DP1000 conjugate with tetrasaccharide Glc α 1-3(Fuc α 1-2)Gal β 1-4GlcNAc β (Example 1.1) (25%)



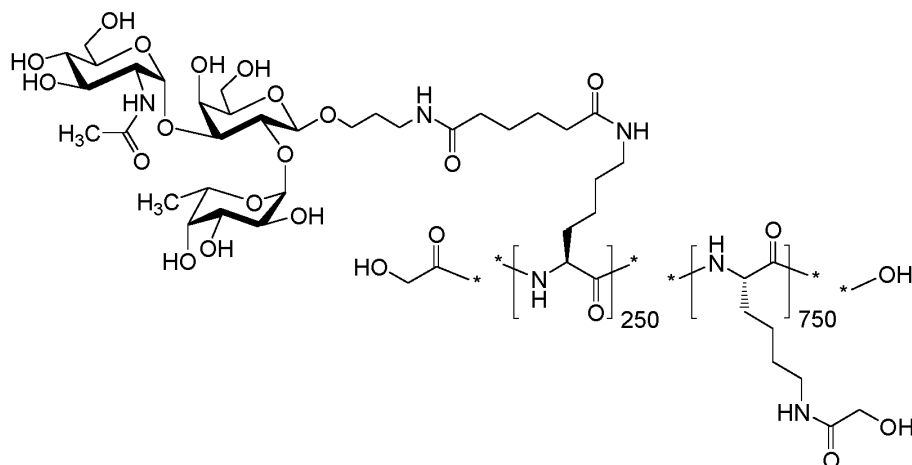
To a stirred solution of polylysine (DP1000) hydrobromide (2.58 mg, 12.34 micromole of Lys) in DMSO (430 μ L) a solution of Glc α 1-3(Fuc α 1-2)Gal β 1-4GlcNAc β -sp-Ad-ONSu (3.01 mg, 3.086 micromole) in DMSO (50 μ L) and a solution of Et₃N (26 μ L of 5% by volume in DMSO, 9.3 micromole) were added. The solution was kept for 1 h at room temperature, then a solution of AcOCH₂(CO)ONSu (4.0 mg, 18.5 micromole) in DMSO (80 μ L) and Et₃N (3.9 μ L, 28 micromole) were added. After 1 hour at room temperature the solution was diluted with ~ two volumes of water (1172 μ L) and Et₃N was added (35 μ L, 2% of total volume). The reaction mixture was kept at room temperature overnight (~17 h), then neutralized with AcOH (16.5 μ L, equivalent on total Et₃N used). Polylysine conjugate was isolated on Sephadex LH-20 column (100 mL of gel, eluted with 30:70 acetonitrile/water). Fractions contained pure conjugate were evaporated to ~ 2 mL

volume and freeze-dried. Yield of Glc α 1-3(Fuc α 1-2)Gal β 1-4GlcNAc β (25%)-PLys1000-Glycolyl conjugate was 4.5 mg (94%).

TLC: $R_f \sim 0$ (2-propanol/acetonitrile/water 4:3:2 by volume), ninhydrin-negative, does not contain free derivatives of Glc α 1-3(Fuc α 1-2)Gal β 1-4GlcNAc β , glycolic acid and salts.

- 5 ^1H NMR selected chemical shifts (D_2O , 700 MHz, 30°C): δ 5.372 (d, 245H, $J = 4.2$ Hz; 245 H-1 of Glc α), 5.236 (d, 245H, $J = 3.9$ Hz; 245 H-1 of Fuc α), 4.750 (s, HOD), 4.632 (d, 245H, $J = 7.7$ Hz; 245 H-1 of Gal β), 4.517 (d, 245H, $J = 8.3$ Hz; 245 H-1 of GlcNAc β), 4.345 (q, 245H, $J = 6.7$ Hz; 245 H-5 of Fuc α), 3.291-3.187 (m, 2490H; 245 CH_2N of glycan spacer and 1000 CH_2N of lysines), 1.261 (d, 735H, $J = 6.5$ Hz; 245 6- CH_3 of Fuc α) ppm.
- 10 Spectrum analysis gives 245 glycans per 1000 lysines, $\sim 25\%$ molar.

Example 4.8: Polylysine DP1000 conjugate with trisaccharide GlcNAc α 1-3(Fuc α 1-2)Gal β (Example 1.8) (25%)



- 15 To a stirred solution of polylysine (DP1000) hydrobromide (3.07 mg, 14.68 micromole of Lys) in DMSO (512 μL) a solution of GlcNAc α 1-3(Fuc α 1-2)Gal β -sp-Ad-ONSu (2.98 mg, 3.67 micromole) in DMSO (50 μL) and a solution of Et_3N (31 μL of 5% by volume in DMSO, 11 micromole) were added. The solution was kept for 1 h at room temperature, then a solution of $\text{AcOCH}_2(\text{CO})\text{ONSu}$ (4.74 mg, 22 micromole) in DMSO (95 μL) and Et_3N (4.6 μL , 33 micromole)
- 20 were added. After 1 hour at room temperature the solution was diluted with $\sim$ two volumes of water (1386 μL) and Et_3N was added (42 μL , 2% of total volume). The reaction mixture was kept at room temperature overnight (~ 16 h), then neutralized with AcOH (19.8 μL , equivalent on total Et_3N used). Polylysine conjugate was isolated on Sephadex LH-20 column (100 mL of gel, eluted with 30:70 acetonitrile/water). Fractions contained pure conjugate were evaporated to ~ 2 mL
- 25 volume and freeze-dried. Yield of GlcNAc α 1-3(Fuc α 1-2)Gal β (25%)-PLys1000-Glycolyl conjugate was 4.7 mg (93%).

TLC: $R_f \sim 0$ (2-propanol/acetonitrile/water 4:3:2 by volume), ninhydrin-negative, does not contain free derivatives of GlcNAc α 1-3(Fuca1-2)Gal β , glycolic acid and salts.

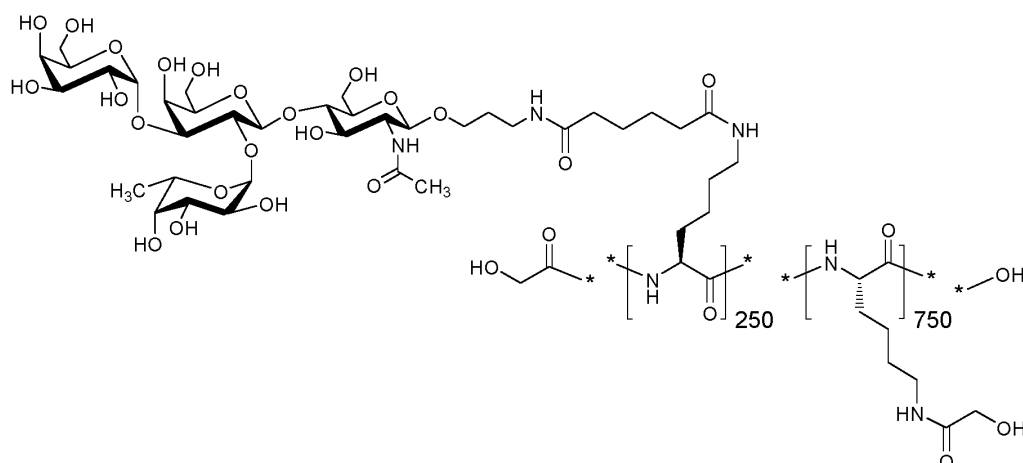
^1H NMR selected chemical shifts (D_2O , 700 MHz, 30°C): δ 5.361 (d, 248H, $J = 3.3$ Hz; 248 H-1 of GlcNAc), 5.171 (d, 248H, $J = 3.9$ Hz; 248 H-1 of Fuca), 4.750 (s, HOD), 4.541 (d, 248H, $J = 7.8$ Hz; 248 H-1 of Gal β), 4.441 (q, 248H, $J = 6.6$ Hz; 248 H-5 of Fuca), 3.306-3.187 (m, 2496H; 248 CH_2N of glycan spacer and 1000 CH_2N of lysines), 1.242 (d, 744H, $J = 6.5$ Hz; 248 6- CH_3 of Fuca) ppm.

Spectrum analysis gives 248 glycans per 1000 lysines, $\sim 25\%$ molar.

10 *Comparative glycoconjugates of natural anti-A and anti-B antigens with poly-L-Lysine*

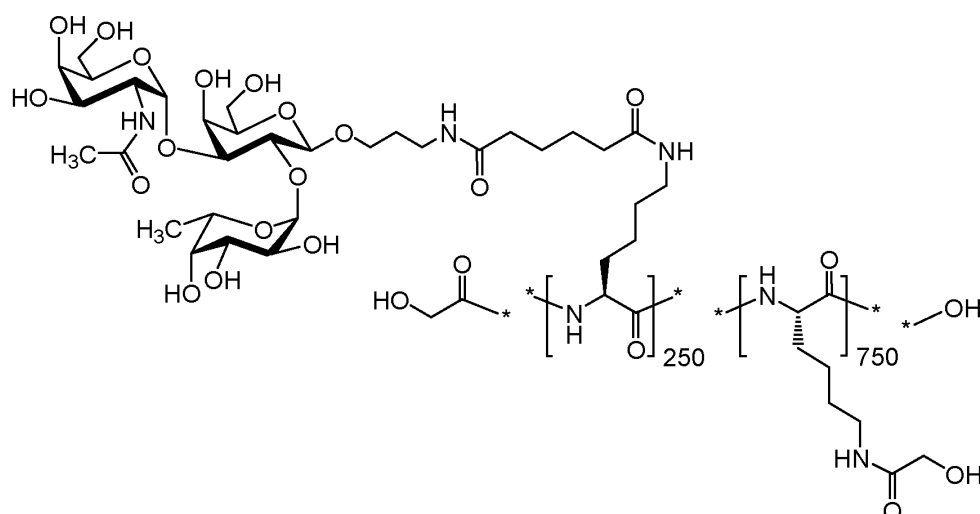
Following analogous process, the following glycoconjugates were prepared with natural antigens, i.e., wherein the carbohydrate has a *N*-acetylgalactosamine or a galactose residue at the nonreducing end

15 *Comparative-1: Polylysine DP1000 conjugate with Gala1-3(Fuca1-2)Gal β 1-4GlcNAc β B (type2) tetrasaccharide*



Content of glycan: 44.7% by weight

20 *Comparative-2: Polylysine DP1000 conjugate with GalNAc α 1-3(Fuca1-2)Gal β A (type2) trisaccharide*



Content of glycan: 38.2% by weight.

Example 5: Inhibitory capacity of poly-L-lysine glycoconjugate of the invention of anti-A/B antibodies using normal human serum from blood group A or B

The glycopolymer assayed was PLys-DP1000 decorated with the glycans of the invention (prepared in Example 4), or with natural A/B antigens (25% of glycan load).

Maxisorp 96-well plates (Nunc®) were coated overnight at 2-8 °C with 10 µg/ml natural antigen A or B conjugated to polyacrylamide (PAA) in coating buffer (0.05 M carbonate--bicarbonate, pH 9.6). Wells coated only with 10 µg/ml of PAA were considered as the background for each serum sample assessed. After coating, plates were washed three times with phosphate buffered saline (PBS) plus 0.1 % (v/v) Tween20 (Merck), and then blocked during 1 h at 2-8 °C with 0.5 % Tween20 (v/v) in PBS + 1% (w/v) of BSA (Sigma-Aldrich). Serum samples were previously incubated for 15-17 h under controlled mild orbital shaking (183rpm) at 2-8 °C at growing concentrations of glycopolymers (0-1000 µg/ml). Washing steps were repeated and serum samples (1:100 for IgG and IgM; 1:50 for IgA) diluted in 0.1 % Tween20 (v/v) in PBS + 0.3% of BSA (w/v) were added to the wells and incubated during 0.5 h at 25 °C with shaking. After washing, plates were incubated 0.5 h at 25 °C (shaking) with Horseradish Peroxidase (HRP)-labeled anti-human IgM, IgA (1:8000) and IgG (1:12000) antibodies diluted in 0.1 % Tween20 (v/v) in PBS + 0.3% of BSA (Invitrogen). After a new washing round, substrate o-phenylenediamine tablet (Sigma-Aldrich) was added to the wells as HRP substrate and incubated 10 min at 25 °C. The reaction was stopped with 3N HCl and the resulting absorbance was registered at 492 nm using a microplate reader (Biotek). For each glycopolymer, the IC₅₀ was calculated considering the inhibition capacity (%) obtained at different polymer concentrations respect to the sample treated only with the vehicle (PBS).

Example 6: Inhibitory capacity of poly-L-lysine glycoconjugate of the invention of anti-A/B antibodies using pure fractions of polyclonal human anti-A/B antibodies

The glycopolymer assayed was PLys-DP1000 decorated with the glycans of the invention (prepared in Example 4), or with natural A/B antigens (25% of glycan load).

- 5 In this Example, pure fractions of human polyclonal anti-A or anti-B antibodies were used, instead of normal human serum. These fractions were isolated using Sepharose® resins functionalized with natural A/ B antigens.

6.1. Purification of human anti-A/B antibodies

- 10 Natural A type-II or natural B type-II conjugated to activated polyacrylamide (PAA), according to the method disclosed in Bovin *et al.*, Glycoconjugate J., 1993, 10, 142-151, and were immobilized on aminated Sepharose 6FF (Pharmacia Biotech, Austria) (column I). Column II was prepared with Sepharose 6FF derivatized with activated PAA.

- 15 Columns I and II (polypropylene, 1,5 x 12 cm, Econo-Pac® Chromatography Columns, BioRad, CA, USA) were packed with 2 mL of affinity adsorbent ligand-Sepharose 6FF.

Ligand density in column I was 0.5 µmol per milliliter of adsorbent to ensure eluted antibody fractions with an isotype proportion similar to that present in pooled normal human serum. The purity of the glycans used was 95–97%, according to NMR and HPLC data.

1. Initial washing of adsorbent ligand-Sepharose 6FF:

- 20 1.1 20 mL of milli-Q grade water
1.2 20 mL 0.1 M NaOH
1.3 20 mL of milli-Q grade water

2. Equilibration adsorbent with PBS + 0.02% NaN₃ (pass 20 mL of buffer through adsorbent)

3. Preparation of pooled sera sample:

- 25 3.1. mix individual healthy donors' sera (n samples) to get a pool of n donors.
3.2. heat pooled serum for 30 minutes at 56 °C to inactivate complement proteins in it.
3.3. after heating, leave pooled serum at the table to reach room temperature.
3.4. centrifuge pooled serum at room temperature for 10 minutes at 7000 g.
3.5. move pooled serum out of the centrifuge vial to a new vial (avoid capture of visible
30 sediment).

- 3.6. dilute pooled serum 1:5 in PBS + 0.02% NaN₃

4. Load diluted pooled serum to adsorbent at speed 0.4 mL/min (passed through column passively by gravity).

5. Washing adsorbent with loaded pooled serum sample:

- 35 5.1. 20 mL PBS + 0.02% NaN₃ + 0.1% Tween 20.
5.2. 100 mL PBS + 0.02% NaN₃. The last portion of buffer should be pipetted carefully to mix buffer and adsorbent for complete washing.

6. Elution of antibodies attached to the ligand of adsorbent:
- 6.1. Elute antibodies (elution I) using 0.2 M Tris-OH buffer + 0.02% NaN₃ (pH 10.4) at a speed 0.2 mL/min. Starting from 1 collected mL of eluate, add 30 µL of neutralizing buffer 0.2 M Gly-HCl + 0.02% NaN₃ (pH 2.5) for each 1 mL of collected eluate.
- 5 6.2. Finally, eluate should be neutralized up to pH 7.4-7.6.
7. Elimination of PAA-cross reacting / non-specific binding antibodies:
- 7.1. Pack column II with 1.5 mL of adsorbent PAA-Sepharose 6FF,
- 7.2. Wash with:
- 10 - 15 mL of milli-Q grade water
- 15 mL 0.1 M NaOH
- 15 mL milli-Q grade water
- 7.3. Equilibrate with 0.2 M TrisOH/Gly-HCl (pH 7.4), passing 15 mL of this buffer.
- 7.4. Load eluate I from Column I at a speed of 0.4 mL/min. Collect eluate II.
8. Concentrate antibodies from eluate II using Microcon-30 kDa centrifugal filter unit with Ultracel-
- 15 30 membrane (Merck Millipore, Burlington, MA, USA) at 14 000 g, for 10 min.

6.2 Determination of specificity of purified anti-A/B antibodies by ELISA

Polyacrylamide-based glycoconjugates were prepared using the method disclosed in Bovin *et al.*, *op. cit.* Thus, a solution of the oligosaccharide in DMF and triethylamine (or diisopropylethylamine) was added to a solution of poly(4-nitrophenylacrylate) in DMF. The

20 resulting mix was incubated with a molar excess of aq. NH₃. PAA-glycoconjugates were separated on Sephadex LH-20 with an acetonitrile-water mixture. In this way, polyacrylamide-based glycoconjugates of the following glycans were prepared:

PAA-glycoconjugate	Glycan	Glycan type
1	Gal α 1-3(Fuc α 1-2)Gal β 1-4GlcNAc β -	B natural type II
2	<u>Glcα1-3(Fucα1-2)Galβ1-4GlcNAcβ-</u>	<u>Example 1.1 (B mimetic)</u>
3	Gal α 1-3(Fuc α 1-2)Gal β -	B-tri
4	Gal α 1-3Gal β 1-4GlcNAc β -	B (Galili-tri)
5	GalNAc α 1-3(Fuc α 1-2)Gal β 1-4GlcNAc β -	A natural type II
6	<u>GlcNAcα1-3(Fucα1-2)Galβ-</u>	<u>Example 1.8 (A mimetic)</u>
7	<u>GlcNPropionylα1-3(Fucα1-2)Galβ-</u>	<u>Example 1.9 (A mimetic)</u>
8	<u>GlcNAcα1-3Galβ-</u>	<u>Example 1.11 (A mimetic)</u>

The PAA-glycoconjugates 2, 6, 7 and 8 are prepared with the mimetics according to the present invention, while the products 1, 3, 4, and 5 are comparative, based on the natural antigens, i.e., all carrying a Gal residue at the nonreducing end.

5 Maxisorp 96-well flat-bottom plates (Thermo Scientific, Waltham, MA, US) were coated for 18 hours at 4°C with 50 µL/well of 10 µg/mL of the above polyacrylamide-based glycan conjugates in coating buffer (0.05 M carbonate-bicarbonate, pH 9.6). Wells coated only with 50 µL/well of 10 µg/mL of PAA were considered the background of each sample assessed.

After coating, the plates were washed three times with 200 µL/well of 0.1% (v/v) PBST (Phosphate Buffered Saline with Tween) using an autowasher and blocked for 1 h at 4°C with 100 µL/well of 0.1% (v/v) PBST + 1% (w/v) BSA (Bovine Serum Albumin). Washing steps were repeated, and 50 µL/well of purified anti-A or anti-B antibodies diluted in 0.1% (v/v) PBST + 0.3% (w/v) BSA were added to the wells and incubated for 0.5 h at 25°C in a rocker.

After washing, plates were incubated 0.5 h at 25°C (shaking) with the corresponding HRP-labeled antibody Goat anti-Human IgG or goat anti-Human IgM (Invitrogen, Waltham, MA, USA) diluted in 0.1% (v/v) PBST + 0.3% (w/v) BSA (1:12000 and 1:8000, respectively). After another round of washing, 50 µL/well of OPD (Sigma-Aldrich, St. Louis, MO, USA) as HRP (Horseradish peroxidase) substrate was added to the wells, and the plates were incubated at 25°C in darkness for 14 minutes. The reaction was stopped with 50 µL/well of 3N HCl, and the resulting absorbance was registered at 490 nm using a microplate reader.

20 Results are shown in Figure 9. Ordinates represent the optical density (OD) values for the different tested samples (1 to 9, in the abscissa, wherein sample 9 corresponds to PAA control).

Graphs 9-1 and 9-2 show the results found for the interaction between purified anti-B antibodies with natural or mimetic B antigens (glycoconjugates 1 to 4 in the above table), and also with the natural A antigen (sample 5) and PAA (sample 9), as controls. Graph 9-1 and 9-2 show the reactivity for IgG (0.36 µg/mL) and IgM (0.217 µg/mL) antibodies, respectively.

Graphs 9-3 and 9-4 show the results found for the interaction between purified anti-A antibodies with natural or mimetic A antigens (glycoconjugates 5 to 8 in the above table), and also with the natural B antigen (sample 1) and PAA (sample 9), as controls. Graph 9-3 and 9-4 show the reactivity for IgG (0.185 µg/mL) and IgM (0.185 µg/mL) antibodies, respectively.

30 These results were unexpected and showed that purified fractions of anti-A/B antibodies bind other glycan structures, including the mimetics of the present invention, with similar or even higher intensity than the natural type-II antigen.

Without wishing to be bound to any theory, all these findings could be explained by the polyclonal and polyreactive nature of human anti-A/B antibodies and support the inhibition of anti-A/B antibodies using glycan structures different from the natural antigen, in particular, using the mimetics of the present invention.

6.3. Inhibition of anti-A or anti-B antibodies with poly-L-Lysine glycoconjugates

ELISA: Maxisorp 96-well flat-bottom plates (Thermo Scientific, Waltham, MA, US) were coated for 18 hours at 4 °C with 50 µL/well of 10 µg/mL of the following PAA-glycoconjugates (as disclosed above in Table 3): 1 (B natural type2) and 5 (A natural type2); in coating buffer (0.05 M carbonate-bicarbonate, pH 9.6). Wells coated only with 50 µL/well of 10 µg/mL of PAA were considered the background of each sample assessed. After coating, the plates were washed three times with 200 µL/well of 0.1% (v/v) PBST using an autowasher and blocked for 1 hour at 4°C with 100 µL/well of 0.1% (v/v) PBST + 1% (w/v) BSA. Washing steps were repeated.

The inhibition samples were prepared in separate tubes using a fixed concentration of anti-A or anti-B antibodies, and an increasing concentration of the following polylysine glycoconjugate inhibitors, prepared in Example 4 (0-400µg/mL):

- Comparative-1 (comprising natural B type-II antigen)
- Example 4.1 (comprising analogue of Example 1.1)
- Comparative-2 (comprising natural A type-II antigen)
- Example 4.8 (comprising analogue of Example 1.8)

As disclosed in Example 4, load of the glycan in the poly-L-lysine conjugates was the same (25%), which guarantees a similar number of exposed carbohydrate residues in each compound.

The tubes were incubated for 18 hours at 4 °C and then a convenient dilution of inhibition mix to the ELISA plate (50 µL/well) was added and incubated for 0.5 hours at 25 °C in a rocker. After washing steps, plates were incubated for 0.5 hours at 25 °C (shaking) with the corresponding HRP-labeled antibody Goat anti-Human IgG or goat anti-Human IgM (Invitrogen, Waltham, MA, USA) diluted in 0.1% (v/v) PBST + 0.3% (w/v) BSA (1:12 000 and 1:8 000, respectively) to detect residual unbound anti-A/B fractions. After another round of washing, 50 µL/well of OPD (Sigma-Aldrich, St. Louis, MO, USA) as HRP substrate was added to the wells, and the plates were incubated at 25 °C in darkness for 14 minutes. The reaction was stopped with 50 µL/well of 3N HCl, and the resulting absorbance was registered at 490 nm using a microplate reader.

To quantify the antibody levels, Maxisorp 96-well plates were coated overnight at 4 °C with decreasing concentrations of pure human IgG or IgM starting from 1000 ng/mL. From this point, the ELISA procedure was the same as for the samples. However, 0.1% (v/v) PBST + 0.3% (w/v) BSA alone was added in the sample-addition step.

The percentage of removal (%) was calculated referred to the baseline condition (without inhibitor compound). The results are represented in Figures 10 and 11, wherein ordinates represent percentage of removal, and are indicative of the inhibitory capacity of the tested glycoconjugates. The abscissa represents the different concentration of the glycoconjugate in the tested samples (in µg/mL).

Figure 10 represents the inhibition of anti-A antibodies. Figures 10-1 and 10-2 show the removal for IgG (0.075 ug/mL) and IgM (0.185 ug/mL) anti-A antibodies, respectively, at increasing concentrations of poly-L-Lysine glycoconjugates.

Black bars correspond to the Comparative-2 (comprising natural A type II antigen) and the grey bars corresponds to Example 4-8 (comprising a mimetic according to the present invention).

Example 4-8, according to the present invention, shower higher capacity to inhibit human purified anti-A antibodies than the comparator, containing the natural antigen at 10, 100 and 400 µg/mL. This result was unexpected and demonstrated the potentiality of using this Glc-analogue for inhibiting the anti-A antibodies.

Figure 11 represents the inhibition of anti-B antibodies. Figures 11-1 and 11-2 show the removal for IgG (0.095 ug/mL) and IgM (0.045 ug/mL) anti-B antibodies, respectively, at increasing concentrations of Poly-L-Lysine glycoconjugates. Black bars correspond to the Comparative-1 (comprising natural B type II antigen) and the grey bars corresponds to Example 4-1 (comprising a mimetic according to the present invention).

In this case, Example 4-1 shows a lower capacity to inhibit human purified anti-B antibodies than the natural comparator. However, when used in combination with the natural antigen (see section 6.4 below) it still provided outstanding activity, Again, without wishing to be bound by theory, this could be due to the polyclonal nature of anti-B antibodies.

6.4. Inhibition of purified antibodies with a poly-L-Lysine glycoconjugate of a mimetic, according to the present invention, combined a poly-L-Lysine glycoconjugate of natural antigen

The combination of antigens (natural type-II plus a mimetic) was assessed using the poly-L-lysine of Example 4.1 (B mimetic) combined with the corresponding poly-L-lysine of the natural B antigen (Comparative-1, prepared in Example-4). The capacity to inhibit the binding of purified anti-B antibodies to red blood cells (Group B), measured by Flow Cytometry was tested.

The Flow Cytometry assay was performed as follows:

50µL of red blood cells (RBC) from healthy donors were diluted at 1.5% (v/v) in PBS. Afterwards, primary antibodies with the corresponding inhibitor were added.

The samples were prepared in separate tubes using 1.0 µg/mL of anti-B antibodies, and the following inhibitors:

- Tube 1: 1.0 µg/mL of anti-B + Comparative-1 (0.25 µg/mL).
- Tube 2: 1.0 µg/mL of anti-B + Example 4.1 (2.5 µg/mL).
- Tube 3: 1.0. µg/mL of anti-B + Comparative-1 (0.25 µg/mL) + Example 4.8 (2.5 µg/mL).

The samples were then incubated for 30 minutes at 37 °C (in the dark, mild horizontal shaking 30-40 rpm). Afterwards the samples were washed twice with PBS (centrifuged at 300g, 3 minutes), and the supernatants were removed by aspiration.

Then, 50 µL of the corresponding fluorochrome secondary anti-Ig antibody (7.5 µg/mL of goat anti-human IgG Alexa Fluor 647, Jackson ImmunoResearch, West Grove, PA, USA, and 20 µg/mL of goat anti-human IgM CF488A, Sigma-Aldrich, St. Louis, MO, USA) diluted in PBS were added to the RBC and incubated for 35 minutes at 37°C (in the dark). The samples were washed
5 twice with PBS (centrifuged at 300g, 3 minutes), and the supernatants were removed by aspiration. Finally, the cells were resuspended in filtered PBS. Cells incubated with only PBS were used as a negative control for the experiment. Cells incubated only with secondary antibodies were considered background signals. Flow Cytometry analysis was performed on a Beckman Coulter Gallios Flow Cytometry analyzer using the blue (488 nm-22mW) and red (635
10 nm-25mW) solid-state laser as exciting source. Every single FACS determination recorded about 20,000 total events. Data was analyzed with KALUZA software (Beckman Coulter, CA, USA).

The results of inhibition of anti-B antibodies (1 µg/mL) binding to red blood cells are shown in Figure 12. The inhibitory capacity was expressed as a percentage of removal referred to the baseline condition (in ordinates).

15 The first bar (black) corresponds to the Comparative-1 (0.25 ug/mL): IgM: 26.4%, IgG: 15.12%

The second bar (light grey) corresponds to Example 4.1 (2.5 ug/mL): IgM: 6.35%, IgG: 5.64%

20 The third bar (dark grey): corresponds to the mixture of Comparative-1 (0.25 ug/mL) and Example 4.1 (2.5 ug/mL): IgM: 63.64%, IgG: 20.24%.

It can be observed that the combination of Example 4.1 (containing the mimetic of Example 1.1, Table 2) with compound Comparative-1 (containing natural B type-II) dramatically increased the inhibitory capacity compared to the use of compound Comparative-1 alone for both isotypes (IgM and IgG). This result was completely unexpected, demonstrating that using the
25 natural type-II antigen in combination with the mimetics of the present invention is a strategy that could provide better inhibition results compared to the use of the natural type-II antigen alone.

Example 7: Removal efficacy of agarose and MNP glycoconjugates of the invention of anti-A/B antibodies from normal human serum

30 The glycopolymers assayed were Sepharose® functionalized with the glycans of the invention (prepared in Example 2) and magnetic nanoparticles functionalized with the glycans of the invention (prepared in Example 3), and were compared to resins and MNPs functionalized the natural A/B antigens.

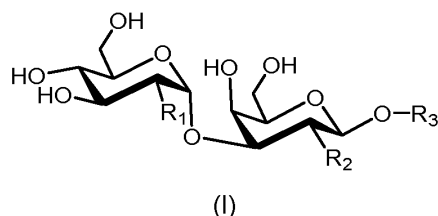
35 The anti A/B antibodies in the normal human serum tested were quantified by ELISA and printed glycan array technology before (baseline) and after passing the serum through the functionalized resin or the functionalized MNPs.

Example 8: Purification of human polyclonal anti-A/B antibodies with agarose and MNP glycoconjugates of the invention

Commercial NHS-Activated Sepharose and MNP were functionalized with the glycans of the invention according to manufacturer instructions. NHS-activated resin (5-10 mL) and MNP (0,5-1mL) was incubated overnight at 4°C (under mild agitation) with 1.25 mL of 2 mg/mL of the ligands prepared in Example 1 in 1.25 mL of coupling solution (0.2 M NaHCO₃ and 0.5 M NaCl, pH 8.3), and 2.5 mL of 1 mM HCl. Non-reacted groups in the support were capped for 4h at 4°C with 5 mL of 0.5 M ethanol amine in 0.1 M Tris-Cl at pH 8.5. After washing steps (PBS-Tween 0,1%, 0,5 M NaCl) the supports were blocked with PBS-Tween 0,01% and BSA 1% (w/v). The blocked supports were incubated (in batch) with pooled human serum (25% v/v, in PBS) of Group A and Group B (gentle shaking, 4-8°C, 2h). Then, in the case of Sepharose, the resin plus serum was introduced in a column (20X2 cm) to separate the unbound serum fraction. For MNP, unbound serum fraction was separated using magnetic separators. After a new round of washing steps, the bound antibodies (anti-A and Anti-B) were eluted from the support with Glycine-HCl Buffer (0.1 M, pH 3.0). Eluted antibodies fractions were immediately neutralized to a pH around 7 and quantified by Bradford (1976). Purity was assessed by SDS-PAGE.

CLAIMS

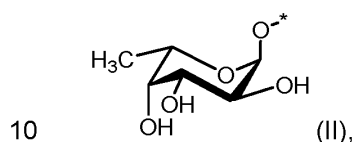
1.- Compound of formula (I):



wherein:

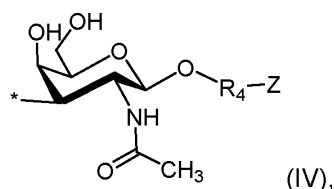
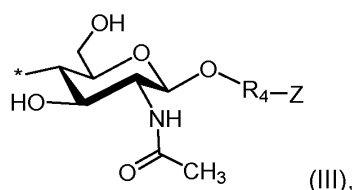
R_1 is selected from OH and -NH-CO- R_5 , wherein R_5 is C_1 - C_3 alkyl and wherein one or more hydrogen atoms in R_5 may be replaced by a hydroxyl group;

R_2 is selected from OH and a group of formula (II):



wherein * indicates the point of attachment of said group with the rest of the molecule;

R_3 is selected from R_4 -Z, a group of formula (III) and a group of formula (IV):



wherein

* indicates the point of attachment of said group with the rest of the molecule,

R_4 is a spacer group, and

Z is selected from -NH₂, -COOH, and -SH.

2.- The compound according to claims 1 or 2, characterized in that R_1 is selected from OH, N-acetyl, N-propionyl and N-glycolyl.

3.- The compound according to claims 1 or 2, characterized in that when R_2 is OH, then R_3 is R_4 -Z.

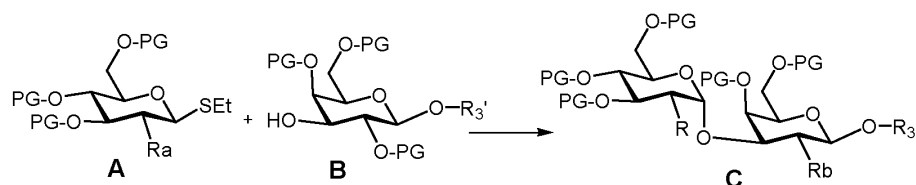
4.- The compound according to claim 1, characterized in that the compound of formula (I) is selected from:

- 1) Glc α 1-3(Fuca1-2)Gal β 1-4GlcNAc β -R
- 5 2) Glc α 1-3(Fuca1-2)Gal β -R
- 3) Glc α 1-3Gal β -R
- 4) Glc α 1-3(Fuca1-2)Gal β 1-3GalNAc β -R
- 5) GlcNAc α 1-3(Fuca1-2)Gal β 1-4GlcNAc β -R
- 6) GlcNPropionyl α 1-3(Fuca1-2)Gal β 1-4GlcNAc β -R
- 10 7) GlcNGlycolyl α 1-3(Fuca1-2)Gal β 1-4GlcNAc β -R
- 8) GlcNAc α 1-3(Fuca1-2)Gal β -R
- 9) GlcNPropionyl α 1-3(Fuca1-2)Gal β -R
- 10) GlcNGlycolyl α 1-3(Fuca1-2)Gal β -R
- 11) GlcNAc α 1-3Gal β -R
- 15 12) GlcNPropionyl α 1-3Gal β -R
- 13) GlcNGlycolyl α 1-3Gal β -R
- 14) GlcNAc α 1-3(Fuca1-2)Gal β 1-3GalNAc β -R
- 15) GlcNPropionyl α 1-3(Fuca1-2)Gal β 1-3GalNAc β -R
- 16) GlcNGlycolyl α 1-3(Fuca1-2)Gal β 1-3GalNAc β -R
- 20 17) GlcNAc α 1-3Gal β 1-4GlcNAc β -R
- 18) GlcNPropionyl α 1-3Gal β 1-4GlcNAc β -R
- 19) GlcNGlycolyl α 1-3Gal β 1-4GlcNAc β -R
- 20) Glc α 1-3Gal β 1-4GlcNAc β -R

wherein R = -O-R₄-Z.

5.- A process for the preparation of the compound of claim 1, characterized in that it comprises the following steps:

- a) Preparing a compound of formula C from a compound of formula A and a compound of formula B:



wherein

PG are protecting groups;

R_a is azide or O-PG;

R_3' is selected from R_4-Z' , a group of formula (III) and a group of formula (IV), wherein the group of formula (III) and the group of formula (IV) are as defined in claim 1, but wherein the OH groups in the carbohydrate moiety in formulas (III) and (IV) are protected and wherein the Z group is also protected, and wherein Z' is a group of formula Z as defined in claim 1 which is protected;

R_b is OH or O-PG; and

b)

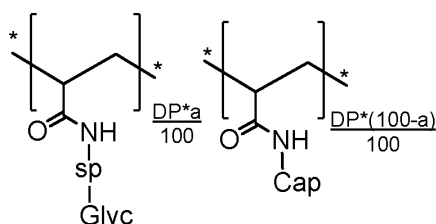
b1) when $R_b=OH$, linking a protected fucose residue through a $\alpha(1\rightarrow2)$ glycosidic linkage at this position to provide, after deprotection, the compound of formula (I) wherein R_2 is a group of formula II;

b2) when $R_b=O-PG$, deprotecting the compound of formula C to provide a compound of formula (I) wherein R_2 is OH;

6.- A glycoconjugate comprising the compound of formula (I) as defined in anyone of claims 1 to 4 and polymeric backbone.

7.- The glycoconjugate of claim 6, characterized in that the polymer is selected from homologous and heterologous α -amino acid polymers (homo and heteropolypeptides), proteins, lipids, nucleic acids, acrylic acid polymers, methacrylic acid polymers, acrylic and methacrylic acid copolymers, polysaccharides such as chitosan or agarose, polyethylene, polypropylene, polystyrene, polyvinyl butyrate, poly (vinyl chloride) and dendrimers, preferably the polymer is selected from polyacrylic acid, a polysaccharide resin and an α -aminoacid homopolymer, and more preferably the polymer is selected from polyacrylic acid, agarose and polylysine.

8.- The glycoconjugate of claim 7, characterized in that it is a polyacrylamide of the following formula:



wherein

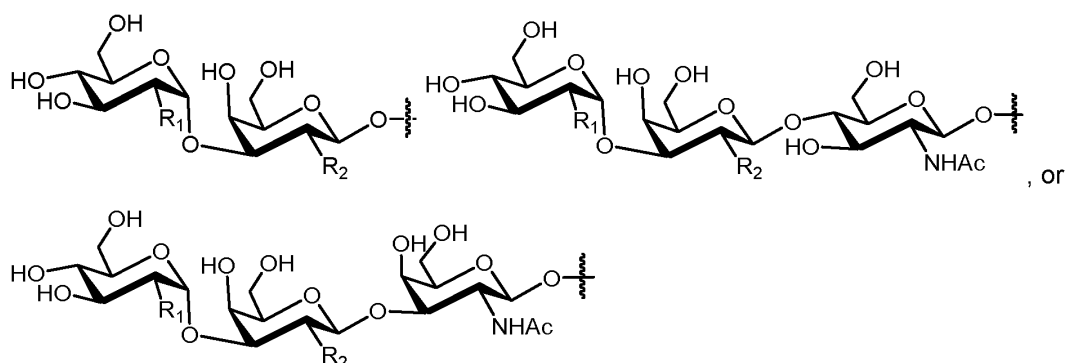
"Cap" means a capping agent,

"DP" is the degree of polymerization of the polymer,

"a" is the load percentage of the carbohydrate in the glycoconjugate,

"sp" represents the spacer R_4 , as defined in claim 1, and

"Glyc" represents anyone of the groups:

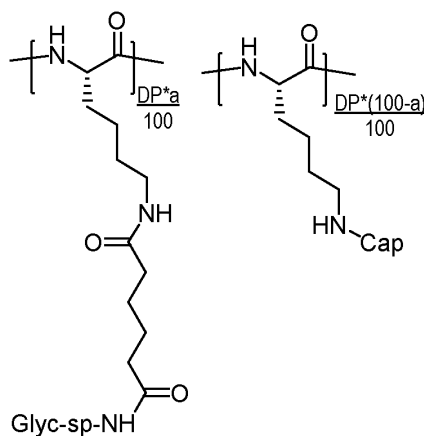


wherein R_1 and R_2 are as defined in anyone of claims 1 to 3;

5 and preferably Glyc is the carbohydrate moiety of the compounds of claim 4.

9.- The glycoconjugate of claim 7, characterized in that it comprises a compound of formula (I) linked to agarose as polymeric backbone.

10 10.- The glycoconjugate of claim 7, characterized in that it is a poly-L-lysine glycoconjugate of the following formula:



wherein

"Cap" means a capping agent;

15 "DP" is the degree of polymerization of the polymer, which is preferably comprised between 50 and 2000, more preferably between 100 and 1000;

"a" is the load percentage of the carbohydrate in the glycoconjugate, which is comprised between 2 and 90, preferably comprised between 5 and 50;

"sp" represents the spacer R_4 , as defined in claim 1, and

20 "Glyc" is a carbohydrate moiety as defined in claim 8.

11.- The glycoconjugate of claims 6 or 7, characterized in that the polymer is in a magnetic nanoparticle.

5 12.- Use of the glycoconjugate according to anyone of claims 6 to 11 for inhibiting and/or removing anti-A and/or anti-B antibodies from samples of blood or other blood derivatives and by-products.

10 13.- Glycoconjugate according to claim 6 for use in medicine, preferably, for use in the treatment and/or prevention of rejection or haemolytic reactions in subjects undergoing organ transplantation or blood transfusion, respectively, from incompatible donors.

14.- Glycoconjugate for use according to claim 13, wherein the glycoconjugate comprises poly-L-lysine as polymeric support and is preferably the glycoconjugate of claim 10.

15 15.- Pharmaceutical composition comprising the glycoconjugate of claim 10 and at least one pharmaceutically acceptable excipient.