Method for producing azido-sugars and compounds obtained thereby

FIELD OF THE INVENTION

[0001] The present invention is in the field of synthesis. More specifically, the present invention relates to a method for preparing a 6-modified azido sugar and applications of said azido sugar.

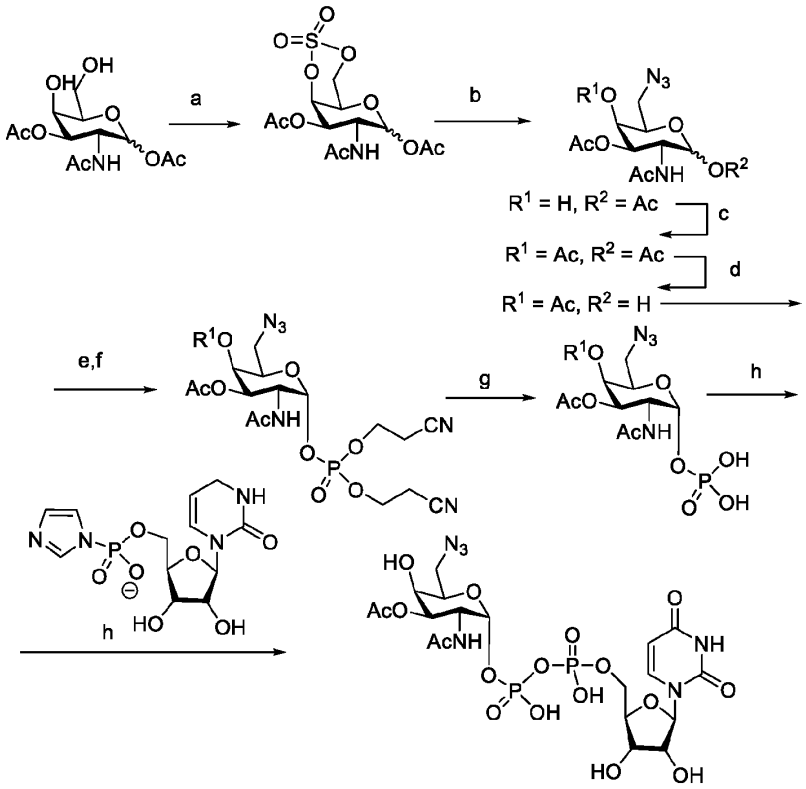
BACKGROUND

[0002] Antibody-drug conjugates (ADC), considered as one of the major classes of targeted therapy, are comprised of an antibody to which is attached a pharmaceutical agent. The antibodies (also known as ligands) can be small protein formats (scFv's, Fab fragments, DARPins, Affibodies, etc.) but are generally monoclonal antibodies (mAbs) which have been selected based on their high selectivity and affinity for a given antigen, their long circulating half-lives, and little to no immunogenicity. Thus, mAbs as protein ligands for a carefully selected biological receptor provide an ideal targeting platform for selective delivery of pharmaceutical drugs. For example, a monoclonal antibody known to bind selectively with a specific cancer-associated antigen can be used for delivery of a chemically conjugated cytotoxic agent to the tumour, via binding, internalization, intracellular processing and finally release of active catabolite. The cytotoxic agent may be small molecule toxin, a protein toxin or other formats, like oligonucleotides. As a result, the tumour cells can be selectively eradicated, while sparing normal cells which have not been targeted by the antibody. Similarly, chemical conjugation of an antibacterial drug (antibiotic) to an antibody can be applied for treatment of bacterial infections, while conjugates of anti-inflammatory drugs are under investigation for the treatment of autoimmune diseases and for example attachment of an oligonucleotide to an antibody is a potential promising approach for the treatment of neuromuscular diseases. Hence, the concept of targeted delivery of an active pharmaceutical drug to a specific cellular location of choice is a powerful approach for the treatment of a wide range of diseases, with many beneficial aspects versus systemic delivery of the same drug.

[0003] ADCs are prepared by bioconjugation, which typically involves the covalent attachment of a linker-drug to an antibody, wherein a reactive moiety linker-drug reacts with complementary reactive moiety present on the antibody. Many technologies are known for bioconjugation, as summarized in G.T. Hermanson, "Bioconjugate Techniques", Elsevier, 3rd Ed. **2013**, incorporated by reference. A popular method of manufacturing ADCs involves attaching a linker-drug to a native glycan of the antibody. WO 2014/065661 describes such a method, wherein the native glycan is trimmed by an endoglycosidase enzyme to afford a core-GlcNAc monosaccharide, and then an azido moiety is enzymatically introduced. The resulting azido modified antibody can be converted into an ADC via metal-free click chemistry with a linker-drug containing an appropriate click probe.

[0004] WO 2016/170186 describes a 6-azido-GalNAc-UDP molecule that is particularly suitable for this modification process. In particular, WO2016/170186 describes a method for synthesizing said 6-azido-

GalNAc-UDP molecule. This synthesis route has also been described in Wijdeven, Marloes A., et al. "Enzymatic glycan remodeling–metal free click (GlycoConnect™). The synthesis route is given below:



[0005] The reaction conditions of this synthesis are as follows : a) 1. $SOCl_2$, Et_3N, CH_2Cl_2, 0°C. 2. RuO_4, $NaIO_4$, CH_2Cl_2, CH_3CN, water, 83% over 2 steps; b) 1. NaN_3, DMF, rt, 2. H_2SO_4, THF, water; c) pyridine, Ac_2O, 80% over two steps; d) 1-propylamine, THF; e) 5-(ethylthio)-1 H-tetrazole, bis (2-cyanoethyl)-N,N-diisopropyl phosphoramidite, CH_2Cl_2, MeCN; f) m-CPBA, 54% over three steps; g) Et_3N, MeOH, H_2O, 50°C, quantitative; h) sodium UMP-imidazolide (13), $MgCl_2$, DMF, 52% yield.

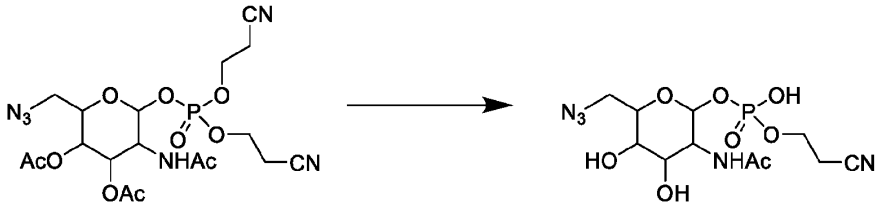
SUMMARY OF THE INVENTION

[0006] Although, the synthesis performs well under laboratory conditions and can be used to prepare 6-modified azido sugars for preparing ADCs, the inventors found that upscaling the synthesis route is hampered by the formation of an impurity. Moreover, the content of the impurity is magnified in the course of the synthesis, meaning that a small amount of impurity may lead to greatly reduced yield of the final ADCs. Purification methods that are available on laboratory scale, such as preparative HPLC, are not feasible on industrial scale or do not remove sufficient amounts of the impurity. Thus, there is a need for an efficient synthesis route that avoids the formation of the impurity and results in a more pure product.

[0007] The inventors surprisingly found that the problem of the formation of the impurity can be solved by deprotecting a phosphate in two steps instead of one, wherein the intermediate product is crystallized. Hence, the first aspect of the invention relates to a method for preparing crystalized compound (**2**). The method according to the invention comprises:

(I) providing compound (**1**);

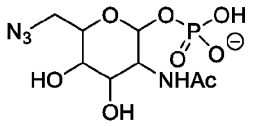
(II) subjecting compound (**1**) to a deprotection step with a base to obtain compound (**2**);



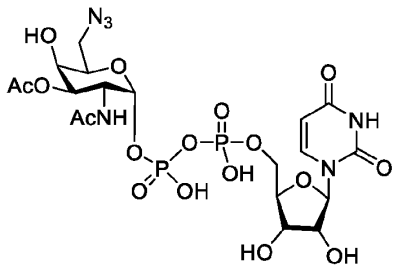
(**1**) (**2**)

(III) crystalizing the product obtained in step (II) to obtain crystalized compound (**2**).

[0008] Also part of the first aspect of the invention are methods for the preparation of further products starting from compound (**2**), wherein compound (**2**) is prepared according to steps (I), (II) and (III) defined above. Such further compounds include compound (**3**), compound (**5**), an azido modified glycoprotein and a functionalized glycoprotein, all as defined herein below.

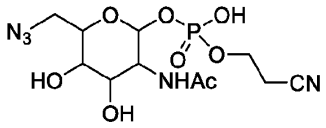


(**3**)



(**5**)

[0009] The invention also relates to the intermediate product that is formed by this method according to the first aspect. Thus, the second aspect of the invention relates to a compound according to structure (**2**).



(**2**)

DETAILED DESCRIPTION

Definitions

[0010] The verb "to comprise", and its conjugations, as used in this description and in the claims is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded.

[0011] In addition, reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there is one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

[0012] The compounds according to the invention may exist in salt form, which are also covered by the present invention. The salt is typically a pharmaceutically acceptable salt, containing a pharmaceutically acceptable anion. The term "salt thereof" means a compound formed when an acidic proton, typically a proton of an acid, is replaced by a cation, such as a metal cation or an organic cation and the like. Where applicable, the salt is a pharmaceutically acceptable salt, although this is not required for salts that are not intended for administration to a patient. For example, in a salt of a compound the compound may be protonated by an inorganic or organic acid to form a cation, with the conjugate base of the inorganic or organic acid as the anionic component of the salt. For instance, if the conjugated acid of phosphate is depicted in the application, it also encompasses the phosphate. In a preferred embodiment, the compounds according to the invention are not in salt form, except for where explicitly indicated in the structure.

[0013] The term "pharmaceutically accepted" salt means a salt that is acceptable for administration to a patient, such as a mammal (salts with counterions having acceptable mammalian safety for a given dosage regime). Such salts may be derived from pharmaceutically acceptable inorganic or organic bases and from pharmaceutically acceptable inorganic or organic acids. "Pharmaceutically acceptable salt" refers to pharmaceutically acceptable salts of a compound, which salts are derived from a variety of organic and inorganic counter ions known in the art and include, for example, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, etc., and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, formate, tartrate, besylate, mesylate, acetate, maleate, oxalate, etc.

[0014] A linker is herein defined as a moiety that connects (covalently links) two or more elements of a compound. A linker may comprise one or more spacer moieties. A spacer-moiety is herein defined as a moiety that spaces (i.e. provides distance between) and covalently links together two (or more) parts of a linker. The linker may be part of e.g. a linker-construct, a linker-conjugate, a linker-payload (e.g. linker-drug) or an antibody-conjugate, as defined below.

[0015] The term "click probe" refers to a functional moiety that is capable of undergoing a click reaction, i.e. two compatible click probes mutually undergo a click reaction such that they are covalently linked in the product. Click reactions and compatible probes for click reactions are known in the art, and preferably

include (cyclic) alkynes, azides and nitrones. In the context of the present invention, one of the click probes is typically the azide as comprised in compound (**5**) or any downstream reaction product thereof.

[0016] A "bioconjugate" is herein defined as a compound wherein a biomolecule is covalently connected to a target molecule via a linker. A bioconjugate may also be referred to as "conjugate". A bioconjugate comprises one or more biomolecules B and one or more payloads D. The linker may comprise one or more spacer moieties.

[0017] A "biomolecule" is herein defined as any molecule that can be isolated from nature or any molecule composed of smaller molecular building blocks that are the constituents of macromolecular structures derived from nature, in particular nucleic acids, proteins, glycans and lipids. Examples of a biomolecule include an enzyme, a (non-catalytic) protein, a polypeptide, a peptide, an amino acid, an oligonucleotide, a monosaccharide, an oligosaccharide, a polysaccharide, a glycan, a lipid and a hormone.

[0018] The term "glycoprotein" is herein used in its normal scientific meaning and refers to a protein comprising one or more monosaccharide or oligosaccharide chains ("glycans") covalently bonded to the protein. A glycan may be attached to a hydroxyl group on the protein (O-linked-glycan), *e.g.* to the hydroxyl group of serine, threonine, tyrosine, hydroxylysine or hydroxyproline, or to an amide function on the protein (*N*-glycoprotein), *e.g.* asparagine or arginine, or to a carbon on the protein (*C*-glycoprotein), *e.g.* tryptophan. A glycoprotein may comprise more than one glycan, may comprise a combination of one or more monosaccharide and one or more oligosaccharide glycans, and may comprise a combination of N-linked, O-linked and C-linked glycans. It is estimated that more than 50% of all proteins have some form of glycosylation and therefore qualify as glycoprotein. Examples of glycoproteins include PSMA (prostate-specific membrane antigen), CAL (candida antartica lipase), gp41, gp120, EPO (erythropoietin), antifreeze protein and antibodies.

[0019] The term "glycan" is herein used in its normal scientific meaning and refers to a monosaccharide or oligosaccharide chain that is linked to a protein. The term glycan thus refers to the carbohydrate-part of a glycoprotein. The glycan is attached to a protein via the C-1 carbon of one sugar, which may be without further substitution (monosaccharide) or may be further substituted at one or more of its hydroxyl groups (oligosaccharide). A naturally occurring glycan typically comprises 1 to about 10 saccharide moieties. However, when a longer saccharide chain is linked to a protein, said saccharide chain is herein also considered a glycan. A glycan of a glycoprotein may be a monosaccharide. Typically, a monosaccharide glycan of a glycoprotein consists of a single N-acetylglucosamine (GlcNAc), glucose (Glc), mannose (Man) or fucose (Fuc) covalently attached to the protein. A glycan may also be an oligosaccharide. An oligosaccharide chain of a glycoprotein may be linear or branched. In an oligosaccharide, the sugar that is directly attached to the protein is called the core sugar. In an oligosaccharide, a sugar that is not directly attached to the protein and is attached to at least two other sugars is called an internal sugar. In an oligosaccharide, a sugar that is not directly attached to the protein but to a single other sugar, *i.e.* carrying no further sugar substituents at one or more of its other hydroxyl groups, is called the terminal sugar. For the avoidance of doubt, there may exist multiple terminal sugars in an oligosaccharide of a glycoprotein,

but only one core sugar. A glycan may be an O-linked glycan, an N-linked glycan or a C-linked glycan. In an O-linked glycan a monosaccharide or oligosaccharide glycan is bonded to an O-atom in an amino acid of the protein, typically via a hydroxyl group of serine (Ser) or threonine (Thr). In an N-linked glycan a monosaccharide or oligosaccharide glycan is bonded to the protein via an N-atom in an amino acid of the protein, typically via an amide nitrogen in the side chain of asparagine (Asn) or arginine (Arg). In a C-linked glycan a monosaccharide or oligosaccharide glycan is bonded to a C-atom in an amino acid of the protein, typically to a C-atom of tryptophan (Trp).

[0020] The term "antibody" (Ab) is herein used in its normal scientific meaning. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. An antibody is an example of a glycoprotein. The term antibody herein is used in its broadest sense and specifically includes monoclonal antibodies, polyclonal antibodies, dimers, multimers, multispecific antibodies (e.g. bispecific antibodies), antibody fragments, and double and single chain antibodies. The term "antibody" is herein also meant to include human antibodies, humanized antibodies, chimeric antibodies and antibodies specifically binding cancer antigen. The term "antibody" is meant to include whole antibodies, but also fragments of an antibody, for example an antibody Fab fragment, $F(ab')_2$, Fv fragment or Fc fragment from a cleaved antibody, a scFv-Fc fragment, a minibody, a diabody or a scFv. Furthermore, the term includes genetically engineered antibodies and derivatives of an antibody. Antibodies, fragments of antibodies and genetically engineered antibodies may be obtained by methods that are known in the art. The antibody may be monoclonal, chimeric and/or humanized.

[0021] An antibody may be a natural or conventional antibody in which two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda (l) and kappa (k). The light chain includes two domains or regions, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CH1, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties, such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The immunoglobulin can be of any type (e.g. IgG, IgE, IgM, IgD, and IgA), class (e.g. IgG1 , IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass, or allotype (e.g. human G1m1 , G1m2, G m3, non-G1m1 [that, is any allotype other than G1m1], G1m17, G2m23, G3m21 , G3m28, G3m1.1, G3m5, G3m13, G3m14, G3m10, G3m15, G3m16, G3m6, G3m24, G3m26, G3m27, A2m1, A2m2, Km1, Km2 and Km3) of immunoglobulin molecule. Preferred allotypes for administration include a non-G1m1 allotype (nG1m1), such as G1m17,1, G1m3, G1m3.1, G1m3.2 or G1m3.1.2. More preferably, the allotype is selected from the group consisting of the G1m17,1 or G1m3 allotype. The antibody may be engineered in the Fc-domain to enhance or nihilate binding to Fc-gamma receptors, as summarized by Saunders et al. *Front. Immunol.* **2019**, *10*, doi: 10.3389/fimmu.2019.01296

and Ward et al., *Mol. Immunol.* **2015**, *67*, 131–141. For example, the combination of Leu234Ala and Leu235Ala (commonly called LALA mutations) eliminate FcγRIIa binding. Elimination of binding to Fc-gamma receptors can also be achieved by mutation of the N297 amino acid to any other amino acid except asparagine, by mutation of the T299 amino acid to any other amino acid except threonine or serine, or by enzymatic Deglycosylation or trimming of the fully glycosylated antibody with for example PNGase F or an endoglycosidase. The immunoglobulins can be derived from any species, including human, murine, or rabbit origin. Each chain contains distinct sequence domains.

[0022] The term "monoclonal antibody" or "mAb" as used herein refers to an antibody molecule of a single amino acid sequence, which is directed against a specific antigen, and is not to be construed as requiring production of the antibody by any particular method. A monoclonal antibody may be produced by a single clone of B cells or hybridoma, but may also be recombinant, i.e. produced by protein engineering.

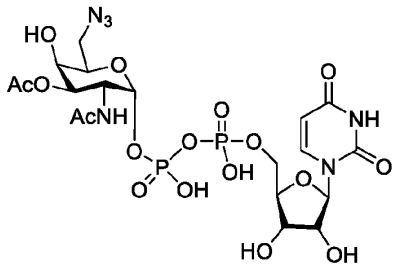
[0023] The term "chimeric antibody" refers to an engineered antibody which, in its broadest sense, contains one or more regions from one antibody and one or more regions from one or more other antibodies. In an embodiment, a chimeric antibody comprises a VH domain and a VL domain of an antibody derived from a non-human animal, in association with a CH domain and a CL domain of another antibody, in an embodiment, a human antibody. As the non-human animal, any animal such as mouse, rat, hamster, rabbit or the like can be used. A chimeric antibody may also denote a multispecific antibody having specificity for at least two different antigens.

[0024] The term "humanised antibody" refers to an antibody which is wholly or partially of non-human origin and which has been modified to replace certain amino acids, for instance in the framework regions of the VH and VL domains, in order to avoid or minimize an immune response in humans. The constant domains of a humanized antibody are most of the time human CH and CL domains. "Fragments" of (conventional) antibodies comprise a portion of an intact antibody, in particular the antigen binding region or variable region of the intact antibody. Examples of antibody fragments include Fv, Fab, F(ab')2, Fab', dsFv, (dsFv)2, scFv, sc(Fv)2, diabodies, bispecific and multispecific antibodies formed from antibody fragments. A fragment of a conventional antibody may also be a single domain antibody, such as a heavy chain antibody or VHH.

[0025] The term "payload" refers to the moiety that is covalently attached to a biomolecule such as an antibody, but also to the molecule that is released from the conjugate upon cleavage of the linker. Payload thus refers to the monovalent moiety having one open end which is covalently attached to the targeting moiety via a linker, which is in the context of the present invention referred to as D, and also to the molecule that is released therefrom.

The invention

[0026] The inventors surprisingly found while upscaling the synthesis of a 6-azido-GalNAc-UDP (**5**), an impurity was consistently present when the conventional synthesis route was used. In particular, the inventors found that the final product contained an impurity in the form of UDP-GalNAc (**S3**):



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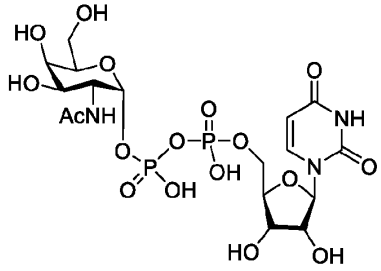
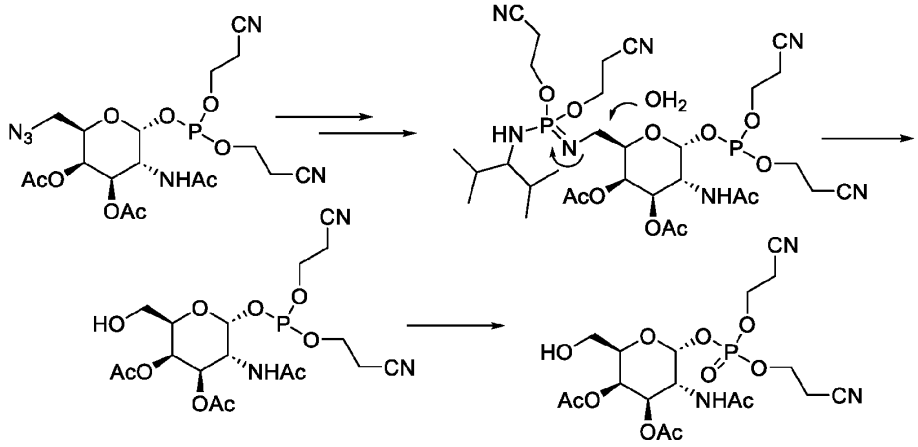


(S3)

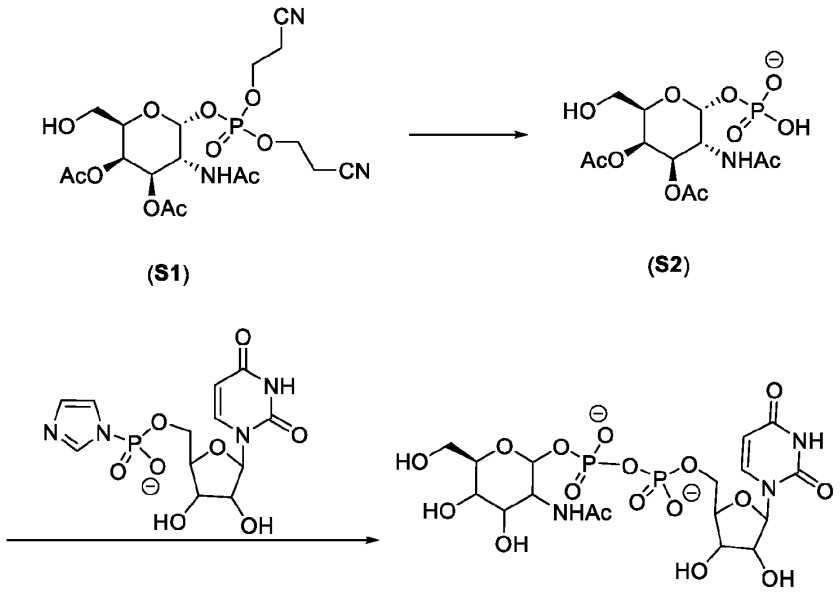
[0027] The compound 6-azido-GalNAc-UDP (**5**) is ideally suitable to introduce an azide in an oligosaccharide or glycoprotein, such as an antibody. UDP-GalNAc (**S3**) is an especially problematic impurity when 6-azido-GalNAc-UDP is enzymatically transferred to the antibody, because UDP-GalNAc can also be enzymatically transferred to a glycan with a galactosyltransferase. In fact, UDP-GalNAc is a better substrate for galactosyltransferase than 6-azido-GalNAc-UDP, meaning that the impurity is built into the antibody at a much higher rate than the 6-azido variant, thus increasing the presence of the impurity. Since the impurity lacks a click probe (the azido group), less payload is attached to the antibody in a bioconjugation reaction, and the resulting ADCs have a significant lower drug-to-antibody ratio (DAR) value than the ADCs without the impurity, as shown in Example 1.

[0028] Although the inventors do not wish to be bound by theory, it is believed that the following cascade reaction occurs during the synthesis of compound (**1**), an intermediate in the synthesis of 6-azido-GalNAc-UDP. A similar cascade reaction is disclosed in J.B. Pawlak, G.P.P. Gential, T.J. Ruckwardt, J.S. Bremmers, N.J. Meeuwenoord, F.A. Ossendorp. H.S. Overkleeft, D.V. Filippov and S.I. van Kasteren; *Angew. Chem. Int. Ed.* **2015**, *54*, 5628 –5631.



(**S1**)

[0029] The GalNAc moiety (**S1**) also reacts with the reagents in the subsequent synthesis steps for producing a 6-azido-GalNAc-UDP, which therefore leads to the formation of (6-OH)GalNAc-UDP (**S3**), as depicted in the scheme below. Therefore, the conventional synthesis route of 6-azido-GalNAc-UDP leads to a small impurity in the form of UDP-GalNAc (**S3**).



(**S1**) (**S2**) (**S3**)

[0030] The method of the present invention avoids the formation of impurity (**S1**), and therefore also impurity (**S3**). When compound (**5**), with impurity (**S3**), is used to incorporate an azido group in a glycoprotein, a significant amount of modified glycoproteins will contain no azido group, when compound (**S3**) is incorporated instead of compound (**5**). The present invention thus maximizes the yield of azido-modified glycoproteins, as no compound (**S3**) is present during the incorporation reaction.

[0031] The present invention in a first aspect concerns a method for preparing crystalized compound (**2**), and possible further synthesis steps therewith. In a second aspect, the present invention concerns compound (**2**), which is conveniently obtained by the method according to the present invention. As the skilled person will appreciate, all what is defined for the method according to the first aspect equally applies to the compound according to the second aspect, and vice versa.

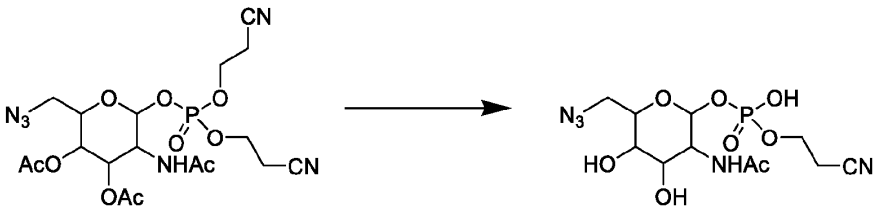
Method according to the invention

[0032] The inventors unexpectedly found that the impurity can be effectively removed by deprotecting the cyanoethyl groups of compound (**1**) in two separate steps, wherein the intermediate product is crystallized.

Thus, the first aspect of the invention relates to a method for preparing crystalized compound (**2**), said method comprising:

(I) providing compound (**1**);

(II) subjecting compound (**1**) to a deprotection step with a base to obtain compound (**2**);



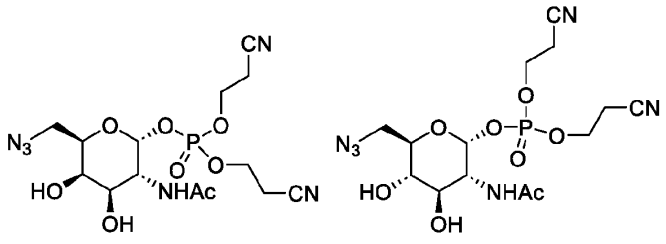
(**1**) (**2**)

(III) crystalizing the product obtained in step (II) to obtain crystalized compound (**2**).

[0033] The inventors found that compound (**2**) is much more suitable for crystallization than compound (**1**) or any one of the later (intermediate) compounds in the synthesis of a 6-modified-UDP sugar. Therefore, crystallization of compound (**2**) is most effective for removing the impurity. In other words, deprotecting one of the cyanoethyl groups and subsequently crystallizing the intermediate compound (**2**) results in a higher yield and a more pure end product 6-azido-GalNAc-UDP.

[0034] The sugar derivatives in the context of the present invention, in particular compounds (**1**), (**2**), (**3**), (**5**), (**a**), (**b**), (**c**), (**d**), (e), (**f**), (f1), (**g**), (**h**) and (**i**) may occur in any stereochemical configuration. Preferably, these compounds are either *N*-acetyl-galactosamine derivatives, denoted in the context of the present with the suffix "**a**" behind the compound numbering, or *N*-acetyl-glucosamine derivatives, denoted in the context of the present with the suffix "**b**" behind the compound numbering. In a preferred embodiment, all sugar derivates in the context of the present invention are *N*-acetyl-galactosamine derivatives.

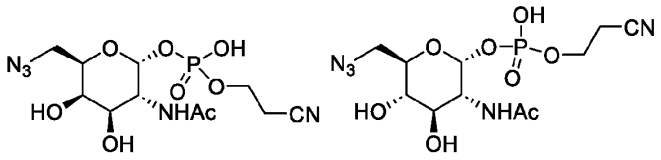
[0035] Thus, compound (**1**) is preferably an *N*-acetyl-galactosamine derivative or an *N*-acetyl-glucosamine derivative, more preferably an *N*-acetyl-galactosamine derivative according to structure (**1a**) or an *N*-acetyl-glucosamine derivative according to structure (**1b**). Most preferably, compound (**1**) is the *N*-acetyl-galactosamine derivative according to structure (**1a**).



(**1a**) (**1b**)

[0036] Likewise, compound (**2**) is preferably an *N*-acetyl-galactosamine derivative or an *N*-acetyl-glucosamine derivative, more preferably an *N*-acetyl-galactosamine derivative according to structure (**2a**)

or an *N*-acetyl-glucosamine derivative according to structure (**2b**). Most preferably, compound (**2**) is the *N*-acetyl-galactosamine derivative according to structure (**2a**).



(**2a**) (**2b**)

Step (II): Mono-deprotection of compound (**1**) to obtain compound (**2**)

[0037] The key aspect of the present invention is the mono-deprotection of compound (**1**), wherein one cyanoethyl moiety is removed from the phosphate group. The resulting phosphate may be in protonated (P–OH) or deprotonated (P–$O^{(-)}$) form, although typically it is in protonated form.

[0038] Preferably, the base in the deprotection step (II) is provided as anhydrous solution, preferably the base is an alkoxy compound dissolved in an alcohol. Excellent results have been obtained with a methoxide dissolved in methanol as base. An equivalent amount of base is chosen in deprotection step (II) such that mono-deprotected compound (**2**) is obtained. Preferably, the amount of base used is in the range of 1.0 – 1.2 equivalent with respect to compound (**1**), more preferably in the range of 1.05 – 1.15 equivalent of base is used, most preferably 1.1 equivalent of base is used. The temperature at which step (II) is performed is preferably below 5 °C, more preferably in the range of –100 to 2 °C, even more preferably in the range of –30 to 0 °C, most preferably in the range of –20 to –5 °C. The inventors obtained excellent results for the mono-deprotection within this temperature range. The reaction of step (II) is normally performed in a polar solvent, preferably a protic solvent. The solvent should be liquid at the reaction conditions. Preferred solvents are alcohol, preferably methanol, ethanol, or a mixture thereof. In one embodiment, the solvent comprises or is ethanol. In an alternative embodiment, the solvent comprises or is methanol, which gave excellent results. The solvent may be a mixture, such as a mixture of ethanol and acetic acid. In one embodiment, an acid such as acetic acid is not present as solvent for step (II), but is added during a quenching step.

[0039] Preferably, deprotection step (II) comprises a quenching step. Such a quenching step typically involves lowering the pH by the addition of an acid. Ideally, the pH after quenching is in the range of 5.0 – 6.8, preferably in the range of 5.5 – 6.5. Any acid known to be suitable can be used for the quenching. In a preferred embodiment, the acid used for quenching is acetic acid.

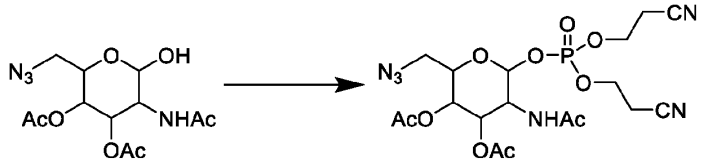
Step (III): Crystallization of compound (**2**)

[0040] The method according to the invention involves the crystallization of the mono-deprotected compound (**2**), which is herein referred to as step (III). The inventors found that the crystallization of compound (**2**) is useful for removing a impurities and thereby avoiding side-reactions in the downstream synthesis route.

[0041] The crystallization of step (III) is preferably performed in a polar solvent, preferably a protic solvent. Excellent results have been obtained with crystallization from an alcohol solvent. Suitable alcohols include MeOH, EtOH, iPrOH and mixtures thereof, preferably MeOH, EtOH or a mixture thereof, most preferably a mixture of MeOH and EtOH. The solvent system may also contain some acid, such as acetic acid, that is beneficial for the deprotection of step (II), such as for a quench. Crystallization may occur at a reduced temperature, but good results have been obtained at ambient temperature. Thus, the crystallisation of step (III) preferably occurs at a temperature in the range of –25 to 50 °C, more preferably in the range of –10 to 35 °C. Step (III) may further involve partially concentrating the obtained solution, for example in order to remove any remaining AcOH. Such concentration typically occurs at reduced pressure.

Step (I): Providing compound (**1**)

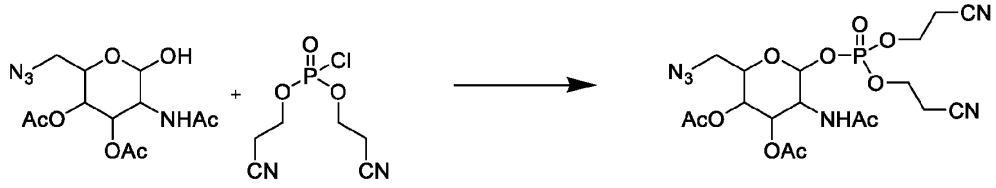
[0042] The di-cyano-ethyl compound (**1**) is known in the art, and can be obtained by any suitable method. For example, compound (**1**) may be obtained by reacting compound (**i**) with X-P($OCH_2CH_2CN)_2$, wherein X is a leaving group, followed by oxidation. Alternatively, the reaction occurs with X-P(O)($OCH_2CH_2CN)_2$, and no oxidation is needed. The conversion of compound (**i**) into compound (**1**) is herein referred to as step (Ii).



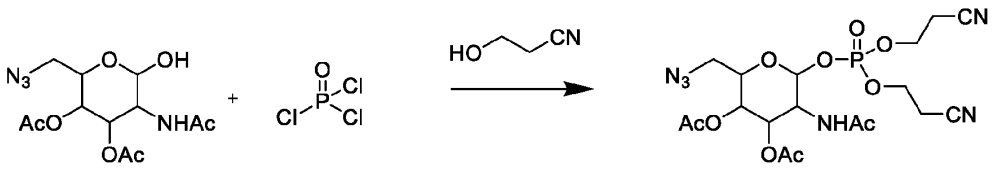
(**i**) (**1**).

[0043] The leaving group X is preferably an amine, more preferably selected from $N(nPr)_2$, $N(Et)_2$ and $N(iPr)_2$, most preferably X is $N(iPr)_2$. The oxidation of a phosphite moiety is well-known in the art, e.g. summarized in Ahmadipour et al., Carbohydr. Res. 2017, 451, 95, which is incorporated herein in its entirety. Typically, the oxidation of the intermediate phosphite is performed by contacting it with an oxidizing agent, such as iodine, m-CPBA, t-BuOOH or H_2O_2 in a dichloromethane or acetonitrile solution.

[0044] Alternatively, compound (**1**) could be obtained through the reaction of compound (**i**) with Cl-P(O)($OCH_2CH_2CN)_2$ or with phosphorus oxychloride and 3-hydroxypropionitrile.

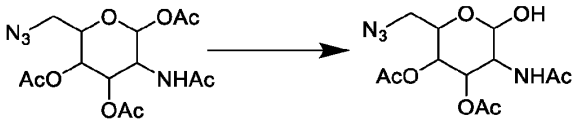


(**i**) (**1**)



(i) (1)

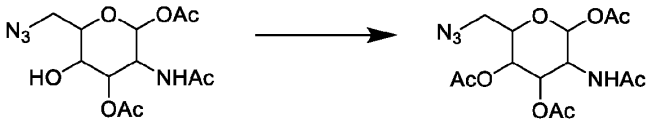
[0045] Compound (**i**) is known in the art, and can be obtained by any suitable method. For example, compound (**i**) is preferably obtained by anomeric deacetylation of compound (**h**). The conversion of compound (**h**) into compound (**i**) is herein referred to as step (Ih).



(**h**) (**i**)

[0046] Preferably, the anomeric deacetylation is performed by an amine, more preferably the amine is selected from propylamine, morpholine, iso-propylamine and benzylamine, most preferably morpholine is used. The solvent used in step (Ih) is preferably an organic solvent, more preferably an ether, such as MTBE or THF, most preferably the solvent is MTBE.

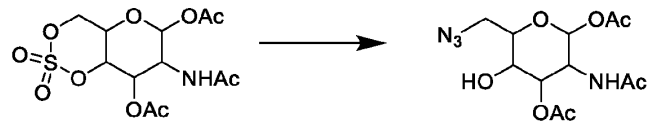
[0047] Compound (**h**) is known in the art, and can be obtained by any suitable method. For example, compound (**h**) may be obtained by acetylating compound (**g**). The conversion of compound (**g**) into compound (**h**) is herein referred to as step (Ig).



(**g**) (**h**)

[0048] The acetylation of compound (**g**) may be achieved by reacting compound (**g**) with acetyl chloride or acetic anhydride. In a preferred embodiment, acetic anhydride is used. The solvent used in step (Ig) is typically an organic solvent, preferably the solvent is a chloromethane or pyridine, most preferably the solvent is DCM. In an especially preferred embodiment, the reaction of step (Ig) includes the use of a catalyst. Suitable catalysts are known in the art, and is suitably DMAP.

[0049] Compound (**g**) is known in the art, and can be obtained by any suitable method. For example, compound (**g**) is obtained by reacting compound (**f**) with a salt comprising an anionic azide. The conversion of compound (**f**) into compound (**g**) is herein referred to as step (If).

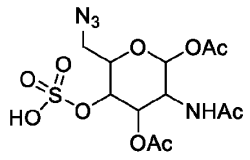


(**f**) (**g**)

[0050] Any azide salt may be used for the conversion of step (If). Preferably, the salt is selected from NaN_3 and NBu_4N_3, more preferably NaN_3 is used. The solvent used for step (If) is typically aprotic and polar. Preferably, the solvent is selected from DMF and DMSO, most preferably the solvent is DMF.

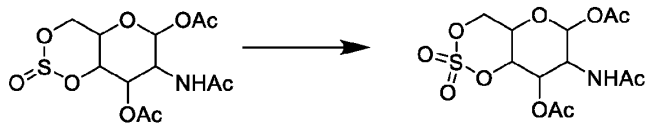
[0051] In a preferred embodiment step (If) comprises the isolation of intermediate sulfate sugar (**f1**), which is advantageously performed by crystallisation. Crystallization of the intermediate sulfate sugar ensures that compound (**g**) is obtained in high purity, which is ideal for a scalable process. Preferably, intermediate sulfate sugar (**f1**) is crystallized from an alcohol, more preferably the alcohol is selected from methanol, ethanol, 2-propanol, 1-propanol, 2-butanol, 1-butanol, most preferably from 2-propanol.

[0052] After further reaction of compound (**f1**), compound (**g**) is obtained in increased purity and higher yields. The intermediate (**f1**) may be converted to compound (**g**) by subjecting compound (**f1**) to acidic conditions. Preferably, an aqueous acid solution is used, more preferably an aqueous sulphuric acid solution.



(**f1**)

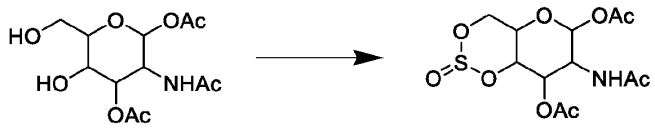
[0053] Compound (**f**) is known in the art, and can be obtained by any suitable method. For example, compound (**f**) is obtained by the oxidation of compound (**e**). The conversion of compound (**e**) into compound (**f**) is herein referred to as step (Ie).



(**e**) (**f**)

[0054] The oxidation of step (Ie) may be performed by any oxidation agent known to be suitable for oxidizing a is preferably achieved with $RuCl_3/NaIO_4$ in water, trichloroisocyanuric acid, hydrogen peroxide, *tert*-butyl sulphoxide into a sulfone. Preferred oxidizing agents include $RuCl_3/NaIO_4$ in water, trichloroisocyanuric acid, hydrogen peroxide, *tert*-butyl hydroperoxide, most preferably $RuCl_3/NaIO_4$ is used.

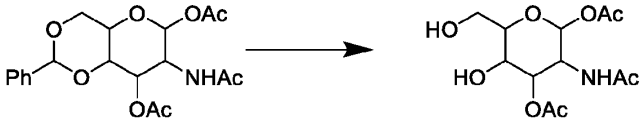
[0055] Compound (**e**) is known in the art, and can be obtained by any suitable method. For example, compound (**e**) is obtained by reacting compound (**d**) with $SOCl_2$ in the presence of a base. The conversion of compound (**d**) into compound (**e**) is herein referred to as step (Id).



(**d**) (**e**)

[0056] Any suitable base can be used, which is preferably selected from Et_3N, Me_3N, iPr_3N, nPr_3N, DIPEA, and morpholine. Preferably, the solvent in step (Id) is an organic solvent, preferably selected from DCM and MeCN, most preferably the solvent is MeCN.

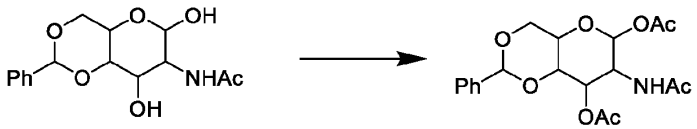
[0057] Compound (**d**) is known in the art, and can be obtained by any suitable method. For example, compound (**d**) is obtained by deprotecting the benzylidene acetal group of compound (**c**). The conversion of compound (**c**) into compound (**d**) is herein referred to as step (Ic).



(**c**) (**d**)

[0058] Preferably, the benzylidene acetal is deprotected by acidic hydrolysis or hydrogenation in the presence of Pd/C, most preferably the benzylidene acetal is deprotected by hydrogenation in the presence of Pd/C in a mixture of MeOH and dioxane.

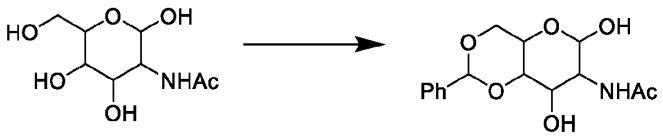
[0059] Compound (**c**) is known in the art, and can be obtained by any suitable method. For example, compound (**c**) is obtained by acetylating compound (**b**). The conversion of compound (**b**) into compound (**c**) is herein referred to as step (Ib).



(**b**) (**c**)

[0060] Preferably, the acetylation is achieved by reacting compound (**b**) with acetyl chloride or acetic anhydride, more preferably acetic anhydride is used.

[0061] Compound (**b**) is known in the art, and can be obtained by any suitable method. For example, compound (**b**) is obtained by protecting the OH groups on the 4 and 6 position of compound (**a**) with a benzylidene acetal group. The conversion of compound (**a**) into compound (**b**) is herein referred to as step (Ia).



(**a**) (**b**)

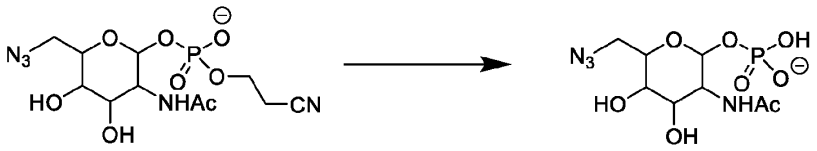
[0062] The benzylidene dimethyl acetal (**b**) may be formed through reaction of compound (**a**) with benzaldehyde dimethylacetal in the presence of an acid, preferably a sulfonic acid, HCl or HNO_3, more preferably camphorsulfonic acid in acetonitrile.

[0063] In an especially preferred embodiment compound (1) is obtained by step (Ii), preferably by steps (Ih) and (Ii), more preferably by steps (Ig) – (Ii), more preferably by steps (Ie) – (Ii), more preferably by steps

(Id) – (Ii), more preferably by steps (Ic) – (Ii), more preferably by steps (Ib) – (Ii), most preferably by performing steps (Ia) - (Ii).

Step (IV) and (V): Deprotection of compound (**2**) to obtain compound (**3**)

[0064] Crystalized compound (**2**) can be used as a precursor for a variety of chemicals. In a preferred embodiment, compound (**2**) is further deprotected to obtain phosphate compound (**3**). Compound (**3**) is obtained in higher purity, in view of the crystallization of step (III). The conversion of compound (**2**) into compound (**3**) is herein referred to as step (IV). Step (IV) preferably entails subjecting the crystallized compound (**2**) to a second deprotection step with a base to obtain compound (**3**).



(**2**) (**3**)

[0065] Although any suitable base and solvent can be used for the reaction of step (IV), excellent results have been obtained with a base dissolved in an anhydrous solution. In a preferred embodiment, the base is an alkoxy compound dissolved in an alcohol, more preferably the base is a methoxide dissolved in methanol. The skilled person us able to tune the amount of base used such that compound (**3**) is obtained. Preferably, the amount of base used is in the range of 1.0 – 1.2 equivalent of with respect to compound (**2**), preferably in the range of 1.05 – 1.15 equivalent, most preferably 1.1 equivalent of base is used.

[0066] The deprotection step (IV) preferably comprises a quenching step. Such a quenching step typically involves lowering the pH by the addition of an acid. Ideally, the pH after quenching is in the range of 5.0 – 6.8, preferably in the range of 5.5 – 6.5. Any acid known to be suitable can be used for the quenching. In a preferred embodiment, the acid used for quenching is acetic acid.

[0067] The temperature at which the deprotection of step (IV) is performed is preferably above 10 °C, more preferably the temperature is in the range of 20 – 100°C, even more preferably in the range of 30 – 90 °C, most preferably in the range of 40 – 80 °C. In an especially preferred embodiment, the deprotection step (II) is performed at a lower temperature than the deprotection step (IV), preferably the temperature in step (II) is at least 10°C lower than in step (IV).

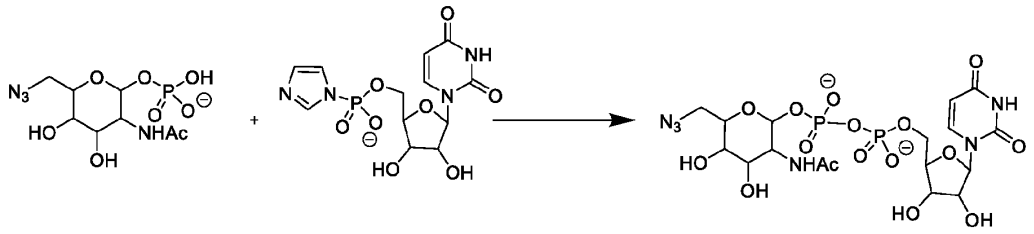
[0068] The method according to the invention preferably further involves the crystallization of compound (**3**), which is herein referred to as step (V). The inventors found that the crystallization of compound (**3**) is useful for removing any impurities originating from the second deprotection step (IV). In other words, performing step (V) further improves the purity of compound (**3**), even beyond the improved purity already obtained by the mono-deprotection and intermediate crystallization of steps (II) and (III).

[0069] The crystallization of step (V) is preferably performed in a polar solvent, preferably from a protic solvent. Especially good results have been obtained with crystallization from an ethanol/acetic acid mixture. Advantageously, the obtained crystals of (**3**) are dissolved in a polar solvent, preferably an alcohol, such as

MeOH, EtOH or a mixture thereof, most preferably the mixture of MeOH and EtOH. Partially concentrating the obtained solution is beneficial to remove any remaining AcOH.

Step (VI): Preparation of 6-azido UDP-sugar (**5**)

[0070] 6-azido sugar compound (**3**) can be used as deemed fit. Compound (**3**) is ideally suited to be incorporated into an antibody. To be able to use 6-azido sugar (**3**) as substrate for an transferase enzyme, it is typically converted into its UDP-sugar variant. Thus, compound (**3**) is preferably reacted with compound (**4**) to obtain the 6-azido UDP-sugar according to compound (**5**). The conversion of compound (**3**) into compound (**5**) is herein referred to as step (VI).



(**3**) (**4**) (**5**)

[0071] Step V is normally performed in an organic solvent, preferably a solvent selected from DMSO and DMF, most preferably the reaction is performed in DMSO. The reaction is typically performed in the presence of catalyst, such as a non-nucleophilic acid, preferably selected from tetrazole, 5-(ethylthio)-1H-tetrazole and Amberlite MAC-3, most preferably the catalyst is Amberlite MAC-3.

[0072] Compound (5) is preferably used for attachment to a glycoprotein, preferably an antibody, to obtain an azido modified glycoprotein.

Step (VII): Providing a glycoprotein

[0073] In step (VII), a glycoprotein is provided. Preferably, the glycoprotein is an antibody, although the method according to the invention is also suitable to modify other glycoproteins. The glycoprotein is preferably trimmed, such that it has a terminal core GlcNAc residue.

[0074] Antibodies are known in the art and include IgA, IgD, IgE, IgG, IgM, Fab, VHH, scFv, diabody, minibody, affibody, affylin, affimers, atrimers, fynomer, Cys-knot, DARPin, adnectin/centryin, knottin, anticalin, FN3, Kunitz domain, OBody, bicyclic peptides and tricyclic peptides. Preferably, the antibody is a monoclonal antibody, more preferably selected from the group consisting of IgA, IgD, IgE, IgG and IgM antibodies. Even more preferably Ab is an IgG antibody. The IgG antibody may be of any IgG isotype. The antibody may be any IgG isotype, e.g. IgG1, IgG2, IgI3 or IgG4. Preferably Ab is a full-length antibody, but Ab may also be a Fc fragment.

[0075] The antibody Ab is typically specific for an extracellular receptor on a tumour cell, preferably wherein the extracellular receptor on the tumour cell is selected from the group consisting of consisting of 5T4, ADAM-9, AMHRII, ASCT2, ASLG659, ASPHD1, av-integrin, Axl, B7-H3, B7-H4, BAFF-R, BCMA,

BMPR1B, brevican, c-KIT, c-Met, C4.4a, CA-IX, cadherin-6, CanAg, CD123, CD13, CD133, CD138/syndecan-1, CD166, CD19, CD20, CD203c, CD205, CD21, CD22, CD228, CD25, CD30, CD324, CD33, CD37, CD38, CD45, CD46, CD48a, CD56, CD70, CD71, CD72, CD74, CD79a, CD79b, CEACAM5, claudin-18.2, claudin-6, CLEC12A, CLL-1, Cripto, CRIPTO, CS1, CXCR5, DLK-1, DLL3, DPEP3, E16, EGFR, ENPP3, EpCAM, EphA2, EphB2R, ETBR, FAP, FcRH1, FcRH2, FcRH5, FGFR2, fibronectin, FLT3, folate receptor alpha (FOL-R1), Gal-3BP, GD3, GDNF-Ra1, GEDA, GFRA1, Globo H, gpNMB, GPR172A, GPR19, GPR54, guanyl cyclase C, HER2, HER3, HLA-DOB, IGF-1R, IL13R, IL20Rα, Lewis Y, LGR5, LIV-1, LRRC15, LY64, Ly6E, Ly6G6D, LY6K, MDP, MFI2, MICA/B, MOSPD2, MPF, MSG783, MUC1, MUC16, NaPi2b, NCA, nectin-4, Notch3, P-cadherin, P2X5, PD-L1, PMEL17, PRLR, PSCA, PSMA, PTK7, RET, RNF43, RON, ROR1, ROR2, SLITRK6, SSTR2, STEAP1, STEAP2, TAG72, TENB2, TF, TIM-1, TM4SF, TMEFF, TMEM118, TMEM46, transferrin, TROP-2, TrpM4, TWEAKR, receptor tyrosine kinases (RTK) and tenascin.

[0076] The antibody may also be specific to an extracellular protein resulting from a viral infection, *e.g.* human polio virus (HPV), human cytomegalovirus (HCMV) or human papillomavirus (HPV). The antibody may also be specific for a tumour-associated carbohydrate antigen (TACA) that is selected from the group of Tn, STn, T-antigen, LDN, Lewis^c (Le^c), Sialyl-Lewis^c (SLe^c), 6-Sialyl-Lewis^c (6SLe^c), LN, alpha-Gal, 3SLN, 6SLN, H-antigen, A-antigen, B-antigen, Lewis^a (Le^a), Sialyl-Lewis^a (SLe^a), 6-Sialyl-Lewis^a (6SLe^a), Lewis^b (Le^b), Sialyl-Lewis^b (SLe^b), 6-Sialyl-Lewis^b (6SLe^b), Lewis^x (Le^x), Sialyl-Lewis^x (SLe^x), 6-Sialyl-Lewis^x (6SLe^x), Lewis^y (Le^y), Sialyl-Lewis^y (SLe^y), 6-Sialyl-Lewis^y (6SLe^y) and or combinations thereof. The antibody may also be specific to both an extracellular protein and a TACA at the same time.

[0077] In another embodiment, the antibody is specific to an immune cell receptor. Preferably, the antibody is specific for an immune cell antigen selected from CTLA-4, PD-1, PD-L1, TIGIT, TIM-3, LAG-3 or VISTA.

[0078] Step (VII) may involve the trimming of the glycoprotein, typically of the antibody. Such trimming is known in the art and involves the deglycosylation of a glycan having a core *N*-acetylglucosamine residue, in the presence of an endoglycosidase, in order to obtain a glycan wherein the core *N*-acetylglucosamine residue, i.e. the *N*-acetylglucosamine that is directly bound to the peptide chain of the glycoprotein, is the terminal saccharide of the glycan. The core *N*-acetylglucosamine may optionally be fucosylated, as is common for antibody glycans. Depending on the nature of the glycan, a suitable endoglycosidase may be selected by the skilled person. The endoglycosidase is preferably selected from the group consisting of EndoS, EndoA, EndoE, EfEndo18A, EndoF, EndoM, EndoD, EndoH, EndoT and EndoSH and/or a combination thereof, the selection of which depends on the nature of the glycan. EndoSH is described in PCT/EP2017/052792, see Examples 1 – 3, and SEQ. ID No: 1, which is incorporated by reference herein.

[0079] Alternatively, the glycoprotein that is provided in step (VII) and used in step (VIII) is not trimmed. In the context of this embodiment, the glycoprotein contains a terminal GlcNAc residue that is not a core GlcNAc residue. Any terminal GlcNAc residue, core or not, can be used for the transfer of step (VIII). Thus, the glycoprotein that is provided in step (VII) should have at least one terminal GlcNAc residue that is available for reaction with a substrate in the presence of a glycosyltransferase.

Step (VIII): Transferring the UDP-sugar (**5**) to the glycoprotein

[0080] In step (VIII), the glycoprotein is contacted with the UDP-sugar (**5**) in the presence of a glycosyltransferase to obtain an azido modified glycoprotein. UDP-sugar (**5**) acts as a substrate for the glycosyltransferase, such that the sugar moiety of (**5**) is transferred to a terminal GlcNAc residue of the glycoprotein. As such, the azide group connected to the sugar moiety of (**5**) is transferred to the glycoprotein, and can be used to further functionalize the glycoprotein.

[0081] Suitable glycosyltransferase catalyst that are capable of transferring the 6-azido GalNAc moiety to the core-GlcNAc moiety are known in the art. A suitable glycosyltransferase is capable of transferring the specific sugar derivative 6-azido sugar-UDP as a substrate. More specifically, the catalyst catalyses the formation of a β(1,4)-glycosidic bond. Preferably, the glycosyltransferase is selected from the group of galactosyltransferases and *N*-acetylgalactosaminyltransferases, more preferably from the group of β(1,4)-N-acetylgalactosaminyltransferases (GalNAcT) and β(1,4)-galactosyltransferases (GalT), most preferably the glycosyltransferase is a β(1,4)-N-acetylgalactosaminyltransferase. Suitable catalysts are disclosed in WO 2014/065661, WO 2016/022027 and WO 2016/170186, all incorporated herein by reference. In one embodiment, the catalyst is a wild-type galactosyltransferase or *N*-acetylgalactosaminyltransferase, preferably an *N*-acetylgalactosaminyltransferase. In an alternative embodiment, the catalyst is a mutant galactosyltransferase or *N*-acetylgalactosaminyltransferases, preferably a mutant *N*-acetylgalactosaminyl-transferase. Mutant enzymes described in WO 2016/022027 and WO 2016/170186 are especially preferred. These galactosyltransferases are able to recognize internal sugars and sugar derivatives as an acceptor. Thus, 6-azido-sugar moiety is attached to the terminal GlcNAc substituent in step (VIII), irrespective of whether said GlcNAc is core or not, and fucosylated or not.

[0082] Step (VIII) is preferably performed in a suitable buffer solution, such as for example phosphate, buffered saline (e.g. phosphate-buffered saline, tris-buffered saline), citrate, HEPES, tris and glycine. Suitable buffers are known in the art. Preferably, the buffer solution is phosphate-buffered saline (PBS) or tris buffer. Step (VIII) is preferably performed at a temperature in the range of 4 – 50 °C, more preferably in the range of 10 – 45 °C, even more preferably in the range of 20 – 40 °C, and most preferably in the range of 30 – 37 °C. Step (VIII) is preferably performed a pH in the range of 5 – 9, preferably in the range of 5.5 – 8.5, more preferably in the range of 6 – 8. Most preferably, step (VIII) is performed at a pH in the range of 7 – 8.

Step (IX): Conjugation

[0083] The azido modified glycoprotein that is obtained in step (VIII) is preferably reacted with a construct containing an azide-reactive click probe Q, to obtain a functionalized glycoprotein. Herein, click probe Q reacts in a click reaction with the azide group on the glycoprotein to form a covalent bond. The construct typically comprises a moiety that is desired to be attached to the glycoprotein, in the art referred to as a payload. Thus, the construct comprising the azide-reactive click probe Q is preferably a payload construct

further comprising a payload D. Preferably, the thus obtained functionalized glycoprotein is an antibody-drug conjugate.

[0084] Q is a moiety capable of reacting with an azide in a click reaction. Click reactions wherein one of the click probes is an azide are known in the art. Preferably, the click reaction is a strain-promoted cycloaddition, such as a strain-promoted alkyne azide cycloaddition (SPAAC). Therefore it is preferred that Q is an alkyne, more preferably a (hetero)cycloalkyne. (Hetero)cycloalkyne group Q may be a heterocycloalkyne group or a cycloalkyne group. Preferably, the (hetero)cycloalkynyl group is a (hetero)cycloheptynyl group, a (hetero)cyclooctynyl group, a (hetero)cyclononynyl group or a (hetero)cyclodecynyl group. Herein, the (hetero)cycloalkynes may optionally be substituted. Preferably, the (hetero)cycloalkynyl group is an optionally substituted (hetero)cycloheptynyl group or an optionally substituted (hetero)cyclooctynyl group. Most preferably, the (hetero)cycloalkynyl group is a (hetero)cyclooctynyl group, wherein the (hetero)cyclooctynyl group is optionally substituted.

[0085] In a preferred embodiment, Q comprises a (hetero)cycloalkynyl according to structure (Q0):



(Q0)

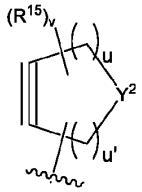
Herein:

- x is an integer in the range of 4-9, preferably 5-7, most preferably 6;
- each Y is individually selected from $C(R^{31})_2$, O, S, $S^{(+)}R^{31}$, $S(O)R^{31}$, $S(O)=NR^{31}$ and NR^{31}, wherein $S^{(+)}$ is a cationic sulphur atom counterbalanced by anion $B^{(-)}$,
- each R^{31} is individually R^{15} or the connection to the remainder of the construct, preferably the connection to the payload D optionally via a linker L;
- each R^{15} is individually selected from the group consisting of hydrogen, halogen, $-OR^{16}$, $-NO_2$, -CN, $-S(O)_2R^{16}$, $-S(O)_3^{(-)}$, C_1 - C_{24} alkyl groups, C_6 - C_{24} (hetero)aryl groups, C_7 - C_{24} alkyl(hetero)aryl groups and C_7 - C_{24} (hetero)arylalkyl groups and wherein the alkyl groups, (hetero)aryl groups, alkyl(hetero)aryl groups and (hetero)arylalkyl groups are optionally substituted, wherein two substituents R_{15} may be linked together to form an optionally substituted annulated cycloalkyl or an optionally substituted annulated (hetero)arene substituent, and wherein R^{16} is independently selected from the group consisting of hydrogen, halogen, C_1 - C_{24} alkyl groups, C_6 - C_{24} (hetero)aryl groups, C_7 - C_{24} alkyl(hetero)aryl groups and C_7 - C_{24} (hetero)arylalkyl groups;
- wherein preferably at least one Y is selected such that it contains at least one R^{31} moiety, and wherein at least one, preferably 1 or 2, occurrences of R^{31} represent the connection to the remainder of the construct.

[0086] Herein, it is preferred that if 2 or more occurrences of Y are selected from O, S, $S^{(+)}R^{31}$, $S(O)R^{31}$ and $S(O)=NR^{31}$, these are not adjacent, with at least one $C(R^{31})_2$ in between. It is further preferred that 0, 1 or 2 occurrences of Y are selected from O, S, $S^{(+)}R^{31}$, $S(O)R^{31}$, $S(O)=NR^{31}$ and NR^{31}, and the remaining occurrences of Y are $C(R^{31})_2$. Preferably, 1 or 2, most preferably 1, occurrences of R^{31} represent a

connection to the remainder of the construct, and the remaining occurrences of R^{31} are R^{15}, preferably wherein R^{15} is H or two occurrences of R^{15} are fused together to form an optionally substituted annulated cycloalkyl or an optionally substituted annulated (hetero)arene substituent.

[0087] In an especially preferred embodiment, Q comprises a (hetero)cycloalkynyl according to structure (Q1):



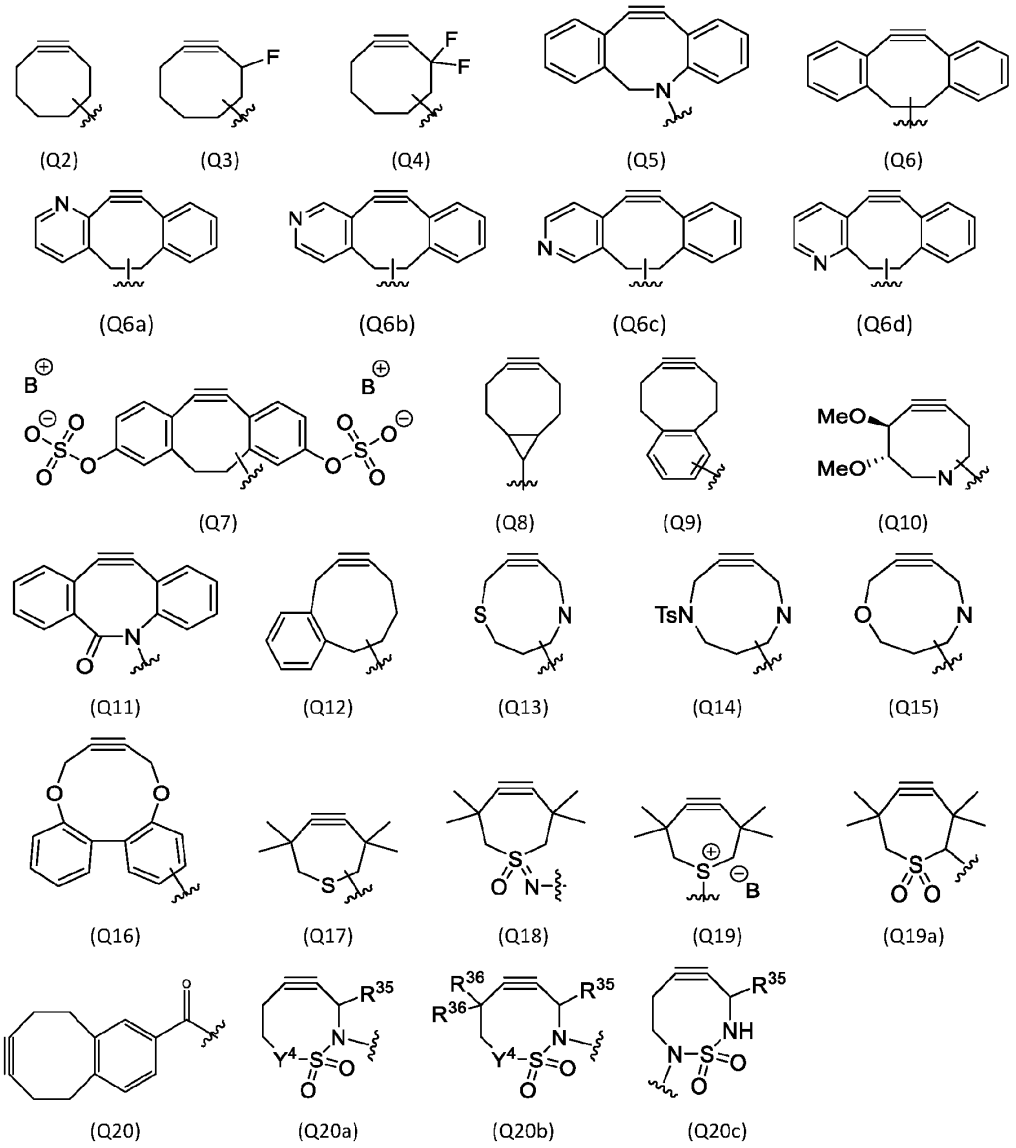
(Q1)

Herein:

- the wavy bond represents the connection to the remainder of the construct, preferably the connection to the payload D optionally via a linker L;
- R^{15} is independently selected from the group consisting of hydrogen, halogen, $-OR^{16}$, $-NO_2$, -CN, $-S(O)_2R^{16}$, $-S(O)_3^{(-)}$, $C_1 - C_{24}$ alkyl groups, $C_6 - C_{24}$ (hetero)aryl groups, $C_7 - C_{24}$ alkyl(hetero)aryl groups and $C_7 - C_{24}$ (hetero)arylalkyl groups and wherein the alkyl groups, (hetero)aryl groups, alkyl(hetero)aryl groups and (hetero)arylalkyl groups are optionally substituted, wherein two substituents R^{15} may be linked together to form an optionally substituted annulated cycloalkyl or an optionally substituted annulated (hetero)arene substituent, and wherein R^{16} is independently selected from the group consisting of hydrogen, halogen, $C_1 - C_{24}$ alkyl groups, $C_6 - C_{24}$ (hetero)aryl groups, $C_7 - C_{24}$ alkyl(hetero)aryl groups and $C_7 - C_{24}$ (hetero)arylalkyl groups;
- Y^2 is $C(R^{31})_2$, O, S, $S^{(+)}R^{31}$, $S(O)R^{31}$, $S(O)=NR^{31}$ or NR^{31}, wherein $S^{(+)}$ is a cationic sulphur atom counterbalanced by $B^{(-)}$, wherein $B^{(-)}$ is an anion, and wherein each R^{31} individually is R^{15} or a connection with D, connected via L;
- u is 0, 1, 2, 3, 4 or 5;
- u' is 0, 1, 2, 3, 4 or 5, wherein u + u' = 4, 5, 6, 7 or 8;
- v is an integer in the range 8 – 16.

[0088] Typically, v = (u + u') x 2 (when the connection to the remainder of the construct, depicted by the wavy bond, is via Y^2) or [(u + u') x 2] – 1 (when the connection to the remainder of the construct, depicted by the wavy bond, is via one of the carbon atoms of u and u'). In a preferred embodiment, u + u' = 4, 5 or 6, more preferably u + u' = 5. In a preferred embodiment, v = 8, 9 or 10, more preferably v = 9 or 10, most preferably v = 10.

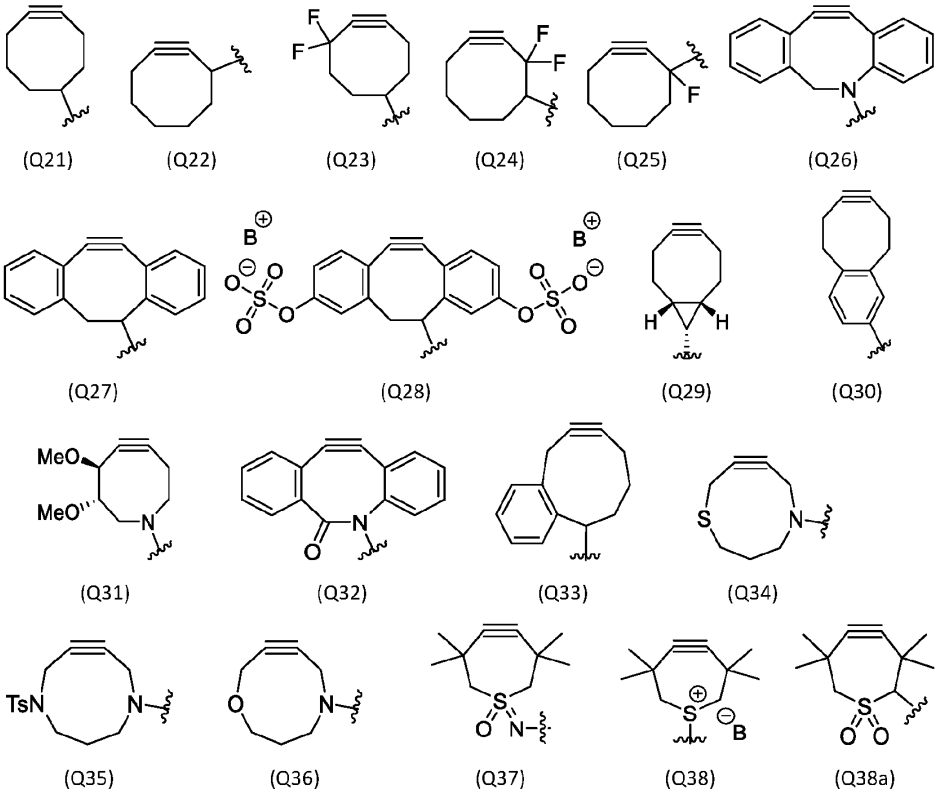
[0089] In a preferred embodiment, Q is a (hetero)cycloalkynyl group selected from the group consisting of (Q2) – (Q20c) depicted here below.

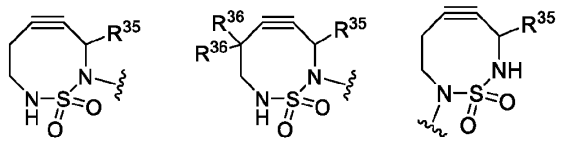


[0090] Herein, the connection to the remainder of the construct, depicted with the wavy bond, may be to any available carbon or nitrogen atom of Q. The nitrogen atom of (Q10), (Q13), (Q14) and (Q15) may bear the connection to the remainder of the construct, or may contain a hydrogen atom or be optionally functionalized. $B^{(-)}$ is an anion, which is preferably selected from $^{(-)}OTf$, $Cl^{(-)}$, $Br^{(-)}$ or $I^{(-)}$, most preferably $B^{(-)}$ is $^{(-)}OTf$. $B^{(+)}$ is a cation, preferably a pharmaceutically acceptable cation. In the conjugation reaction, $B^{(-)}$ does not need to be a pharmaceutically acceptable anion, since $B^{(-)}$ will exchange with the anions

present in the reaction mixture anyway. In case (Q19) is used for Q, the negatively charged counter-ion is preferably pharmaceutically acceptable upon isolation of the conjugate according to the invention, such that the conjugate is readily useable as medicament. R^{36} is an halogen selected from fluor, chlorine, bromine and iodine, preferably R^{36} is fluor. Y^4 is a heteroatom, preferably Y^4 is O or NH. R^{35} is selected from the group consisting of hydrogen, $C_1 – C_{24}$ alkyl groups, $C_3 – C_{24}$ cycloalkyl groups, $C_2 – C_{24}$ (hetero)aryl groups, $C_3 – C_{24}$ alkyl(hetero)aryl groups and $C_3 – C_{24}$ (hetero)arylalkyl groups, the $C_1 – C_{24}$ alkyl groups, $C_3 – C_{24}$ cycloalkyl groups, $C_2 – C_{24}$ (hetero)aryl groups, $C_3 – C_{24}$ alkyl(hetero)aryl groups and $C_3 – C_{24}$ (hetero)arylalkyl groups optionally substituted and optionally interrupted by one or more heteroatoms selected from O, Si, S and NR^{14} wherein R^{14} is independently selected from the group consisting of hydrogen and $C_1 – C_4$ alkyl groups, preferably R^{35} is selected from H, C_5H_{11}, CH_3, CH_2CH_3, CH_2OH or CH_2OTBS.

[0091] In a further preferred embodiment, Q is a (hetero)cycloalkynyl group selected from the group consisting of (Q21) – (Q38d) depicted here below.





(Q38b) (Q38c) (Q38d)

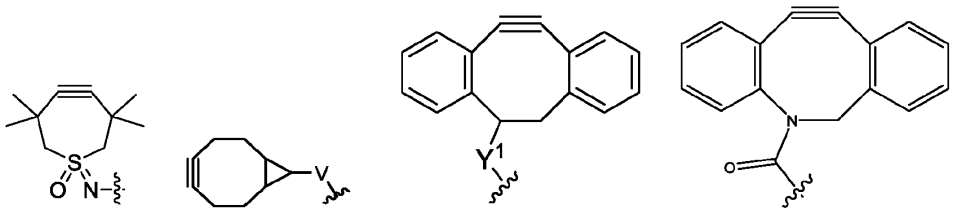
[0092] In structure (Q38), $B^{(-)}$ is an anion, which is preferably selected from $^{(-)}OTf$, $Cl^{(-)}$, $Br^{(-)}$ or $I^{(-)}$, most preferably $B^{(-)}$ is $^{(-)}OTf$. In structure (Q28), $B^{(+)}$ is a cation, preferably a pharmaceutically acceptable cation. Groups R^{35} and R^{36} on (Q38b), (Q38c) and (Q38d) are defined elsewhere and equally apply to the present embodiment.

[0093] In a preferred embodiment, Q comprises a (hetero)cyclooctyne moiety or a (hetero)cycloheptyne moiety, preferably according to structure (Q8), (Q26), (Q27), (Q28), (Q37) or (Q38a), which are optionally substituted. Each of these preferred options for Q are further defined here below.

[0094] Thus, in a preferred embodiment, Q comprises a heterocycloheptyne moiety according to structure (Q37), also referred to as a TMTHSI, which is optionally substituted. Preferably, the heterocycloheptyne moiety according to structure (Q37) is not substituted.

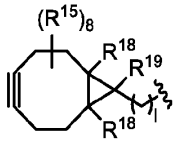
[0095] In an alternative preferred embodiment, Q comprises a cyclooctyne moiety according to structure (Q8), more preferably according to (Q29), also referred to as a bicyclo[6.1.0]non-4-yn-9-yl] group (BCN group), which is optionally substituted. Preferably, the cyclooctyne moiety according to structure (Q8) or (Q29) is not substituted. In the context of the present embodiment, Q preferably is a (hetero)cyclooctyne moiety according to structure (Q39) as shown below, wherein V is $(CH_2)_I$ and I is an integer in the range of 0 to 10, preferably in the range of 0 to 6. More preferably, I is 0, 1, 2, 3 or 4, more preferably I is 0, 1 or 2 and most preferably I is 0 or 1. In the context of group (Q39), I is most preferably 1. Most preferably, Q is according to structure (Q42), defined further below.

[0096] In an alternative preferred embodiment, Q comprises a (hetero)cyclooctyne moiety according to structure (Q26), (Q27) or (Q28), also referred to as a DIBO, DIBAC, DBCO or ADIBO group, which are optionally substituted. In the context of the present embodiment, Q preferably is a (hetero)cyclooctyne moiety according to structure (Q40) or (Q41) as shown below, wherein Y^1 is O or NR^{11}, wherein R^{11} is independently selected from the group consisting of hydrogen, a linear or branched $C_1 - C_{12}$ alkyl group or a $C_4 - C_{12}$ (hetero)aryl group. The aromatic rings in (Q40) are optionally O-sulfonylated at one or more positions, whereas the rings of (Q41) may be halogenated at one or more positions. Preferably, the (hetero)cyclooctyne moiety according to structure (Q40) or (Q41) is not further substituted. Most preferably, Q is according to structure (Q43), defined further below.



(Q37) (Q39) (Q40) (Q41)

[0097] In an especially preferred embodiment, Q comprises a cyclooctynyl group and is according to structure (Q42):



(Q42)

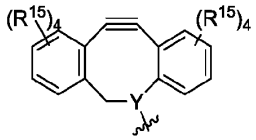
Herein:

- R^{15} is independently selected from the group consisting of hydrogen, halogen, $-OR^{16}$, $-NO_2$, -CN, $-S(O)_2R^{16}$, $-S(O)_3^{(-)}$, $C_1 - C_{24}$ alkyl groups, $C_5 - C_{24}$ (hetero)aryl groups, $C_7 - C_{24}$ alkyl(hetero)aryl groups and $C_7 - C_{24}$ (hetero)arylalkyl groups and wherein the alkyl groups, (hetero)aryl groups, alkyl(hetero)aryl groups and (hetero)arylalkyl groups are optionally substituted, wherein two substituents R^{15} may be linked together to form an optionally substituted annulated cycloalkyl or an optionally substituted annulated (hetero)arene substituent, and wherein R^{16} is independently selected from the group consisting of hydrogen, halogen, $C_1 - C_{24}$ alkyl groups, $C_6 - C_{24}$ (hetero)aryl groups, $C_7 - C_{24}$ alkyl(hetero)aryl groups and $C_7 - C_{24}$ (hetero)arylalkyl groups;
- R^{18} is independently selected from the group consisting of hydrogen, halogen, $C_1 - C_{24}$ alkyl groups, $C_6 - C_{24}$ (hetero)aryl groups, $C_7 - C_{24}$ alkyl(hetero)aryl groups and $C_7 - C_{24}$ (hetero)arylalkyl groups;
- R^{19} is selected from the group consisting of hydrogen, halogen, $C_1 - C_{24}$ alkyl groups, $C_6 - C_{24}$ (hetero)aryl groups, $C_7 - C_{24}$ alkyl(hetero)aryl groups and $C_7 - C_{24}$ (hetero)arylalkyl groups, the alkyl groups optionally being interrupted by one of more hetero-atoms selected from the group consisting of O, N and S, wherein the alkyl groups, (hetero)aryl groups, alkyl(hetero)aryl groups and (hetero)arylalkyl groups are independently optionally substituted, or R^{19} is a second occurrence of D connected via a spacer moiety; and
- l is an integer in the range 0 to 10.

[0098] In a preferred embodiment of the reactive group according to structure (Q42), R^{15} is independently selected from the group consisting of hydrogen, halogen, $-OR^{16}$, $C_1 - C_6$ alkyl groups, $C_5 - C_6$ (hetero)aryl groups, wherein R^{16} is hydrogen or $C_1 - C_6$ alkyl, more preferably R^{15} is independently selected from the group consisting of hydrogen and $C_1 - C_6$ alkyl, most preferably all R^{15} are H. In a preferred embodiment

of the reactive group according to structure (Q42), R^{18} is independently selected from the group consisting of hydrogen, $C_1 – C_6$ alkyl groups, most preferably both R^{18} are H. In a preferred embodiment of the reactive group according to structure (Q42), R^{19} is H. In a preferred embodiment of the reactive group according to structure (Q42), I is 0 or 1, more preferably I is 1.

[0099] In an especially preferred embodiment, Q comprises a (hetero)cyclooctynyl group and is according to structure (Q43):



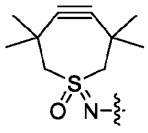
(Q43)

Herein:

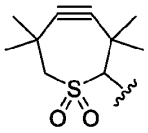
- R^{15} is independently selected from the group consisting of hydrogen, halogen, $-OR^{16}$, $-NO_2$, -CN, $-S(O)_2R^{16}$, $-S(O)_3^{(-)}$, $C_1 – C_{24}$ alkyl groups, $C_5 – C_{24}$ (hetero)aryl groups, $C_7 – C_{24}$ alkyl(hetero)aryl groups and $C_7 – C_{24}$ (hetero)arylalkyl groups and wherein the alkyl groups, (hetero)aryl groups, alkyl(hetero)aryl groups and (hetero)arylalkyl groups are optionally substituted, wherein two substituents R^{15} may be linked together to form an optionally substituted annulated cycloalkyl or an optionally substituted annulated (hetero)arene substituent, and wherein R^{16} is independently selected from the group consisting of hydrogen, halogen, $C_1 – C_{24}$ alkyl groups, $C_6 – C_{24}$ (hetero)aryl groups, $C_7 – C_{24}$ alkyl(hetero)aryl groups and $C_7 – C_{24}$ (hetero)arylalkyl groups;
- Y is N or CR^{15};
- a carbon atom in the fused aromatic rings may be replaced by a nitrogen atom, as in (Q6a) – (Q6d), preferably wherein Y is CR^{15}.

[0100] In a preferred embodiment of the reactive group according to structure (Q43), R^{15} is independently selected from the group consisting of hydrogen, halogen, $-OR^{16}$, $-S(O)_3^{(-)}$, $C_1 – C_6$ alkyl groups, $C_5 – C_6$ (hetero)aryl groups, wherein R^{16} is hydrogen or $C_1 – C_6$ alkyl, more preferably R^{15} is independently selected from the group consisting of hydrogen and $-S(O)_3^{(-)}$. In a preferred embodiment of the reactive group according to structure (Q43), Y is N or CH, more preferably Y = N.

[0101] In an especially preferred embodiment, Q comprises a (hetero)cycloheptynyl group and is according to structure (Q37) or (Q38a).



(Q37)



(Q38a)

[0102] In the preferred embodiments for (hetero)cycloalkynyl group Q defined above, the wavy bond at all times refers to the connection to the remainder of the construct comprising click probe Q, preferably the

remainder of the payload-construct wherein Q is connected to D, optionally via linker L. Even though the exact nature of this remainder of the construct is not relevant in the context of the present invention, it is preferred that the wavy bond represents the connection to $L(D)_r$.

[0103] Payload D, represents the compound that is or is to be connected to the glycoprotein. Payload molecules are well-known in the art, especially in the field of antibody-drug conjugates, as the moiety that is covalently attached to the antibody and that may be released therefrom upon uptake of the conjugate and/or cleavage of the linker. In a preferred embodiment, the payload is selected from the group consisting of an active substance, a reporter molecule, a polymer, a solid surface, a hydrogel, a nanoparticle, a microparticle and a biomolecule. Especially preferred payloads are active substances and reporter molecules, in particular active substances.

[0104] The term "active substance" herein relates to a pharmacological and/or biological substance, i.e. a substance that is biologically and/or pharmaceutically active, for example a drug, a prodrug, a cytotoxin, a diagnostic agent, a protein, a peptide, a polypeptide, a peptide tag, an amino acid, a glycan, a lipid, a vitamin, a steroid, a nucleotide, a nucleoside, a polynucleotide, RNA or DNA. Examples of peptide tags include cell-penetrating peptides like human lactoferrin or polyarginine. An example of a glycan is oligomannose. An example of an amino acid is lysine.

[0105] When the payload is an active substance, the active substance is preferably selected from the group consisting of drugs and prodrugs. More preferably, the active substance is selected from the group consisting of pharmaceutically active compounds, in particular low to medium molecular weight compounds (e.g. about 200 to about 2500 Da, preferably about 300 to about 1750 Da). In a further preferred embodiment, the active substance is selected from the group consisting of cytotoxins, antiviral agents, antibacterial agents, peptides and oligonucleotides.

[0106] Preferred cytotoxins are selected from the groups of nitrogen mustards, nitrosoureas, alkylsulphonates, triazenes, platinum containing compounds, plant alkaloids, DNA topoisomerase inhibitors, anti-metabolites, hormonal therapies, kinase inhibitors, antibiotics, and further cytotoxins defined here below.

[0107] Suitable nitrogen mustards include chlorambucil, chlornaphazine, cyclophosphamide, dacarbazine, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, mannomustine, mitobronitol, melphalan, mitolactol, pipobroman, novembichin, phenesterine, prednimustine, thiotepa, trofosfamide, uracil mustard; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); benzodiazepine monomers or dimers (e.g., pyrrolobenzodiazepine (PBD), tomaymycin, indolinobenzodiazepine, isoindolinebenzodiazepine, imidazobenzothiadiazepine and oxazolidinobenzodiazepine).

[0108] Suitable nitrosoureas include carmustine, lomustine, chlorozotocin, fotemustine, nimustine and ranimustine.

[0109] Suitable alkylsulphonates include busulfan, treosulfan, improsulfan and piposulfan.

[0110] Suitable triazenes include dacarbazine.

[0111] Suitable platinum containing compounds include carboplatin, cisplatin, oxaliplatin; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine.

[0112] Suitable plant alkaloids include vinca alkaloids (e.g. vincristine, vinblastine, vindesine, vinorelbine, navelbin), toxoids (e.g. paclitaxel, docetaxel and their analogs), maytansinoids and their analogs (e.g. DM1, DM2, DM3, DM4, maytansine and ansamitocins), cryptophycins (particularly cryptophycin 1 and cryptophycin 8), epothilones, eleutherobin, discodermolide, bryostatins, dolostatins, auristatins, tubulysins, cephalostatins, pancratistatin, sarcodictyin and spongistatin.

[0113] Suitable DNA topoisomerase inhibitors include any camptothecin, including 9-aminocamptothecin, exatecan, DXd (DX-8951 derivative), crisnatol, daunomycin, etoposide, etoposide phosphate, irinotecan, mitoxantrone, novantrone, retinoic acids (retinols), teniposide, topotecan, 9-nitrocamptothecin (RFS 2000); mitomycins and mitomycin C.

[0114] Suitable anti-metabolites include anti-folate, DHFR inhibitors (e.g. methotrexate, trimetrexate, denopterin, pteropterin, aminopterin (4-aminopteroic acid) or other folic acid analogues), IMP dehydrogenase inhibitors (e.g. mycophenolic acid, tiazofurin, ribavirin, EICAR), ribonucleotide reductase inhibitors (e.g. hydroxyurea, deferoxamine), pyrimidine analogs (e.g. uracil analogs such as ancitabine, azacitidine, 6-azauridine, capecitabine (Xeloda), carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, 5-fluorouracil, floxuridine, ratitrexed (Tomudex); cytosine analogs such as cytarabine, cytosine arabinoside, fludarabine; purine analogs such as azathioprine, fludarabine, mercaptopurine, thiamiprine, thioguanine), folic acid replenisher (e.g. frolinic acid).

[0115] Suitable hormonal therapies include receptor antagonists (e.g. anti-estrogen such as megestrol, raloxifene, tamoxifen, LHRH agonists such as goscrclin, leuprolide acetate; antiandrogens such as bicalutamide, flutamide, calusterone, dromostanolone propionate, epitiostanol, goserelin, leuprolide, mepitiostane, nilutamide, testolactone, trilostane and other androgens inhibitors), retinoids/deltoids (e.g. Vitamin D3 analogs such as CB 1093, EB 1089 KH 1060, cholecalciferol, ergocalciferol), photodynamic therapies (e.g. verteporfin, phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A).

[0116] Suitable kinase inhibitors include BIBW 2992 (anti-EGFR/Erb2), imatinib, gefitinib, pegaptanib, sorafenib, dasatinib, sunitinib, erlotinib, nilotinib, lapatinib, axitinib, pazopanib, vandetanib, E7080 (anti-VEGFR2), mubritinib, ponatinib (AP24534), bafetinib (INNO-406), bosutinib (SKI-606), cabozantinib, vismodegib, iniparib, ruxolitinib, CYT387, axitinib, tivozanib, sorafenib and ispinesib.

[0117] Suitable antibiotics include the enediyne antibiotics (e.g. calicheamicins, especially calicheamicin .γ1, δ1, α1 and β1), dynemicin (e.g. dynemicin A and deoxydynemicin), esperamicin, kedarcidin, C-1027, maduropeptin, as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromomophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin; chromomycins, dactinomycin, daunorubicin, nemorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, morpholino-doxorubicin, cyanomorpholino-

doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, nitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin and zorubicin.

[0118] Suitable polyketides include acetogenins (in particualr bullatacin and bullatacinone), gemcitabine, epoxomicins (e. g. carfilzomib), bortezomib, thalidomide, lenalidomide, pomalidomide, tosedostat, zybrestat, PLX4032, STA-9090, Stimuvax, allovectin-7, Xegeva and Provenge.

[0119] The cytotoxin may further be selected from isoprenylation inhibitors (such as lovastatin), dopaminergic neurotoxins (such as 1-methyl-4-phenylpyridinium ion), cell cycle inhibitors (such as staurosporine), actinomycins (such as actinomycin D and dactinomycin), bleomycins (such as bleomycin A2, bleomycin B2, peplomycin), anthracyclines (e.g. daunorubicin, doxorubicin (adriamycin), idarubicin, epirubicin, pirarubicin, zorubicin, mitoxantrone), MDR inhibitors (e.g. verapamil), Ca2+ ATPase inhibitors (e.g. thapsigargin), histone deacetylase inhibitors (e.g. Vorinostat, Romidepsin, Panobinostat, Valproic acid, Mocetinostat (MGCD0103), Belinostat, PCI-24781, Entinostat, SB939, Resminostat, Givinostat, AR-42, CUDC-101, sulforaphane, Trichostatin A), thapsigargin, Celecoxib, glitazones, epigallocatechin gallate, disulfiram, salinosporamide A.; anti-adrenals (e.g. aminoglutethimide, mitotane, trilostane; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; arabinoside, bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; eflornithine (DFMO), elfomithine; elliptinium acetate, etoglucid; gallium nitrate; gacytosine, hydroxyurea; ibandronate, lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verrucarin A, roridin A and anguidine).

[0120] The term “reporter molecule” herein refers to a molecule whose presence is readily detected, for example a diagnostic agent, a dye, a fluorophore, a radioactive isotope label, a contrast agent, a magnetic resonance imaging agent or a mass label.

[0121] A wide variety of fluorophores, also referred to as fluorescent probes, is known to a person skilled in the art. Several fluorophores are described in more detail in e.g. G.T. Hermanson, *“Bioconjugate Techniques”*, Elsevier, 3rd Ed. **2013**, Chapter 10: *“Fluorescent probes”*, p. 395 - 463, incorporated by reference. Examples of a fluorophore include all kinds of Alexa Fluor (e.g. Alexa Fluor 555), cyanine dyes (e.g. Cy3 or Cy5) and cyanine dye derivatives, coumarin derivatives, fluorescein and fluorescein derivatives, rhodamine and rhodamine derivatives, boron dipyrromethene derivatives, pyrene derivatives, naphthalimide derivatives, phycobiliprotein derivatives (e.g. allophycocyanin), chromomycin, lanthanide chelates and quantum dot nanocrystals.

[0122] Examples of a radioactive isotope label include ^{99m}Tc, ^{111}In, ^{114m}In, ^{115}In, ^{18}F, ^{14}C, ^{64}Cu, ^{131}I, ^{125}I, ^{123}I, ^{212}Bi, ^{88}Y, ^{90}Y, ^{67}Cu, ^{186}Rh, ^{188}Rh, ^{66}Ga, ^{67}Ga and ^{10}B, which is optionally connected via a chelating moiety such as e.g. DTPA (diethylenetriaminepentaacetic anhydride), DOTA (1,4,7,10-tetraazacyclododecane-

N,N',N'',N'''-tetraacetic acid), NOTA (1,4,7-triazacyclononane *N,N',N''*-triacetic acid), TETA (1,4,8,11-tetra-azacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid), DTTA (N^1-(*p*-isothiocyanatobenzyl)-diethylenetriamine-N^1,N^2,N^3,N^3-tetraacetic acid), deferoxamine or DFA (*N'*-[5-[[4-[[5-(acetylhydroxyamino)pentyl]amino]-1,4-dioxobutyl]hydroxyamino]pentyl]-*N*-(5-aminopentyl)-*N*-hydroxybutanediamide) or HYNIC (hydrazino-nicotinamide). Isotopic labelling techniques are known to a person skilled in the art, and are described in more detail in e.g. G.T. Hermanson, *"Bioconjugate Techniques"*, Elsevier, 3rd Ed. **2013**, Chapter 12: *"Isotopic labelling techniques"*, p. 507 - 534, incorporated by reference.

[0123] Polymers suitable for use as a payload D in the compound according to the invention are known to a person skilled in the art, and several examples are described in more detail in e.g. G.T. Hermanson, *"Bioconjugate Techniques"*, Elsevier, 3rd Ed. **2013**, Chapter 18: *"PEGylation and synthetic polymer modification"*, p. 787 - 838, incorporated by reference. When payload D is a polymer, payload D is preferably independently selected from the group consisting of a poly(ethyleneglycol) (PEG), a polyethylene oxide (PEO), a polypropylene glycol (PPG), a polypropylene oxide (PPO), a 1,q-diaminoalkane polymer (wherein q is the number of carbon atoms in the alkane, and preferably q is an integer in the range of 2 to 200, preferably 2 to 10), a (poly)ethylene glycol diamine (e.g. 1,8-diamino-3,6-dioxaoctane and equivalents comprising longer ethylene glycol chains), a polysaccharide (e.g. dextran), a poly(amino acid) (e.g. a poly(L-lysine)) and a poly(vinyl alcohol).

[0124] Solid surfaces suitable for use as a payload D are known to a person skilled in the art. A solid surface is for example a functional surface (e.g. a surface of a nanomaterial, a carbon nanotube, a fullerene or a virus capsid), a metal surface (e.g. a titanium, gold, silver, copper, nickel, tin, rhodium or zinc surface), a metal alloy surface (wherein the alloy is from e.g. aluminum, bismuth, chromium, cobalt, copper, gallium, gold, indium, iron, lead, magnesium, mercury, nickel, potassium, plutonium, rhodium, scandium, silver, sodium, titanium, tin, uranium, zinc and/or zirconium), a polymer surface (wherein the polymer is e.g. polystyrene, polyvinylchloride, polyethylene, polypropylene, poly(dimethylsiloxane) or polymethylmethacrylate, polyacrylamide), a glass surface, a silicone surface, a chromatography support surface (wherein the chromatography support is e.g. a silica support, an agarose support, a cellulose support or an alumina support), etc. When payload D is a solid surface, it is preferred that D is independently selected from the group consisting of a functional surface or a polymer surface.

[0125] Hydrogels are known to the person skilled in the art. Hydrogels are water-swollen networks, formed by cross-links between the polymeric constituents. See for example A. S. Hoffman, *Adv. Drug Delivery Rev.* **2012**, *64*, 18, incorporated by reference. When the payload is a hydrogel, it is preferred that the hydrogel is composed of poly(ethylene)glycol (PEG) as the polymeric basis.

[0126] Micro- and nanoparticles suitable for use as a payload D are known to a person skilled in the art. A variety of suitable micro- and nanoparticles is described in e.g. G.T. Hermanson, *"Bioconjugate Techniques"*, Elsevier, 3rd Ed. **2013**, Chapter 14: *"Microparticles and nanoparticles"*, p. 549 - 587, incorporated by reference. The micro- or nanoparticles may be of any shape, e.g. spheres, rods, tubes, cubes, triangles and cones. Preferably, the micro- or nanoparticles are of a spherical shape. The chemical

composition of the micro- and nanoparticles may vary. When payload D is a micro- or a nanoparticle, the micro- or nanoparticle is for example a polymeric micro- or nanoparticle, a silica micro- or nanoparticle or a gold micro- or nanoparticle. When the particle is a polymeric micro- or nanoparticle, the polymer is preferably polystyrene or a copolymer of styrene (e.g. a copolymer of styrene and divinylbenzene, butadiene, acrylate and/or vinyltoluene), polymethylmethacrylate (PMMA), polyvinyltoluene, poly(hydroxyethyl methacrylate (pHEMA) or poly(ethylene glycol dimethacrylate/2-hydroxyethylmetacrylae) [poly(EDGMA/HEMA)]. Optionally, the surface of the micro- or nanoparticles is modified, e.g. with detergents, by graft polymerization of secondary polymers or by covalent attachment of another polymer or of spacer moieties, etc.

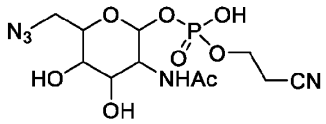
[0127] Payload D may also be a biomolecule. Biomolecules, and preferred embodiments thereof, are described in more detail below. When payload D is a biomolecule, it is preferred that the biomolecule is selected from the group consisting of proteins (including glycoproteins such as antibodies), polypeptides, peptides, glycans, lipids, nucleic acids, oligonucleotides, polysaccharides, oligosaccharides, enzymes, hormones, amino acids and monosaccharides.

[0128] In a preferred embodiment, D is a cytokine selected from IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23,, IL-24, IL-26, IL-28, IL-29, IL-33, IL-36, IL37, IL-38, IFN-α (including IFN-α1/13, IFN-α2, IFN-α4, IFN-α5, IFN-α6, IFN-α7, IFN-α8, IFN-α10, IFN-α14, IFN-α16, IFN-α17, and IFN-α21), IFN- β, IFN-Y, IFN-A, TFN-α, TNF-β, TGF-β1, M-CSF, G-CSF, GM-CSF, and CXL10, more preferably the immune cell engaging polypeptide is IL-2 or IL-15, more preferably IL-15. It is understood that a reference to a cytokine includes mutated variants, cytokines with an attached cofactor. For example, IL-15 may refer to normal IL-15, but also to RLI or a mutated version of IL-15.

[0129] In the context of the present invention, cytotoxic payloads are especially preferred. Thus, D is preferably, a cytotoxin, more preferably selected from the group consisting of colchicine, vinca alkaloids, anthracyclines, camptothecins, doxorubicin, daunorubicin, taxanes, calicheamicins, tubulysins, irinotecans, an inhibitory peptide, amanitins, amatoxins, duocarmycins, epothilones, mytomycins, combretastatins, maytansines, auristatins, enediynes, pyrrolobenzodiazepines (PBDs) or indolinobenzodiazepine dimers (IGN).

Compound (**2**)

[0130] In a second aspect, the invention relates to compound (**2**):

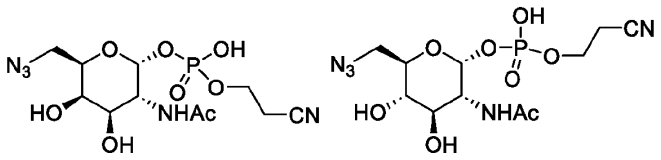


(**2**)

[0131] Compound (**2**) may be in neutral or anionic form. In other words, the phosphate group may be protonated or deprotonated. Compound (**2**) according to the invention may be in crystallized form, as it is

obtained in that form by the process according to the first aspect of the invention. However, compound (**2**) in any other form is also useful and covered by the present invention. For example, the compound may be in solution, e.g. when used as reactant for step (IV), or amorphous after evaporation of a solution of compound (**2**). In an especially preferred embodiment, the compound according to the invention is in crystalline form, preferably as obtainable by the process according to the present invention. Most preferably, compound (**2**) according to the present invention is obtained by the process according to the present invention.

[0132] Preferably, compound (**2**) is an *N*-acetyl-galactosamine derivative according to structure (**2a**) or an *N*-acetyl-glucosamine derivative according to structure (**2b**). Most preferably, compound (**2**) is the *N*-acetyl-galactosamine derivative according to structure (**2a**).



(**2a**) (**2b**).

[0133] Compound (**2**) is useful intermediate in chemical synthesis, in particular in the synthesis of compound (**3**) or in the preparation of bioconjugates wherein a glycoprotein is covalently connected to a payload, preferably in the preparation of an antibody-drug-conjugate (ADC).

Examples

*Example 1: Effect of impurity (**S3**) on the DAR value of an ADC*

[0134] The effect of the impurity in the substrate on the final drug-to-antibody ratio (DAR value) of an ADC has been investigated. The ratio between the reaction rates of the enzymatical transfer of GalNAc-UDP (compound (**S3**)) and 6-azido GalNAc-UDP (compound (**5**)) can be considered as infinite, meaning that when a mixture of GalNAc-UDP and 6-azido GalNAc-UDP is exposed to the reaction conditions for enzymatic transfer, approximately all the UDP-GalNAc is transferred to the antibody before any of the 6-azido GalNAc-UDP is transferred.

[0135] A trimmed antibody has two available glycans for an enzymatical transfer. Thus, the theoretical DAR value can be calculated based on the wt.% of the impurity. The highest DAR value possible is 2, because there are two native glycans on an antibody. Thus, the highest theoretical DAR can be calculated by the formula below. Herein n is the equivalent of substrate per antibody; N3 = 6-azido GalNAc-UDP; OH = GalNAc-UDP; m represents the weight fraction; M represents the mole per weight fraction; and U represents the molecular weight.

$$wt.\%\ of\ OH = \frac{m_{OH}}{m_{tot}} = \frac{m_{OH}}{m_{N3} + m_{OH}} \times 100\% = \frac{m_{OH}}{1} \times 100\%$$

$$mol\%\ of\ OH = \frac{M_{OH}}{M_{tot}} \times 100\% = \frac{M_{OH}}{M_{N3} + M_{OH}} \times 100\% = \frac{\frac{m_{OH}}{U_{OH}}}{\frac{m_{N3}}{U_{N3}} + \frac{m_{OH}}{U_{OH}}} \times 100\%$$

$$mol\%\ of\ OH = \frac{m_{OH}/U_{OH}}{(1 - m_{OH})/U_{N3} + m_{OH}/U_{OH}} \times 100\%$$

$$DAR = 2 - n \times \frac{m_{OH}/U_{OH}}{(1 - m_{OH})/U_{N3} + m_{OH}/U_{OH}}$$

[0136] The impact of the impurity in wt% substrate on the DAR value is illustrated below:

UDP-GalNAc (wt%)	UDP-GalNAc (mol%)	Equivalents per antibody (n)							
		10	12	14	16	18	25	50	75
1	1.041	1.90	1.88	1.85	1.83	1.81	1.74	1.48	1.22
0.1	0.104	1.99	1.99	1.99	1.98	1.98	1.97	1.95	1.92
0.075	0.078	1.99	1.99	1.99	1.99	1.99	1.98	1.96	1.94
0.05	0.052	1.99	1.99	1.99	1.99	1.99	1.99	1.97	1.96
0.025	0.026	2.00	2.00	2.00	2.00	2.00	1.99	1.99	1.98
0.01	0.010	2.00	2.00	2.00	2.00	2.00	2.00	1.99	1.99
0.003	0.003	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00

[0137] Compound 1-phosphate-6-azido GalNAc compound (**3a**) was prepared via three conventional methods (Examples 2a – 2c) and via the method according to the present invention (Examples 11-12), leading to the content of the corresponding 6-OH impurity as given in the table below. Also given are the DAR values of the ADCs when the product of the examples 2a, 2b, 2c and 12 is used in the preparation of an ADC, for the scenario that 25 equivalents of UDP-sugar are used per antibody.

[0138] Impact of synthesis route to impurity content in wt% and DAR value

Example	UDP-GalNAc (wt%)	UDP-GalNAc (mol%)	Theoretical DAR
2a	0.07 – 1.22	0.073 – 1.27	1.98 – 1.68
2b	0.33	0.344	1.91
2c	0.09	0.094	1.98
11-12	< 0.03	< 0.031	> 1.99

*Example 2: Comparative synthesis routes for 1-phosphate-6-azido GalNAc compound (**3a**)*

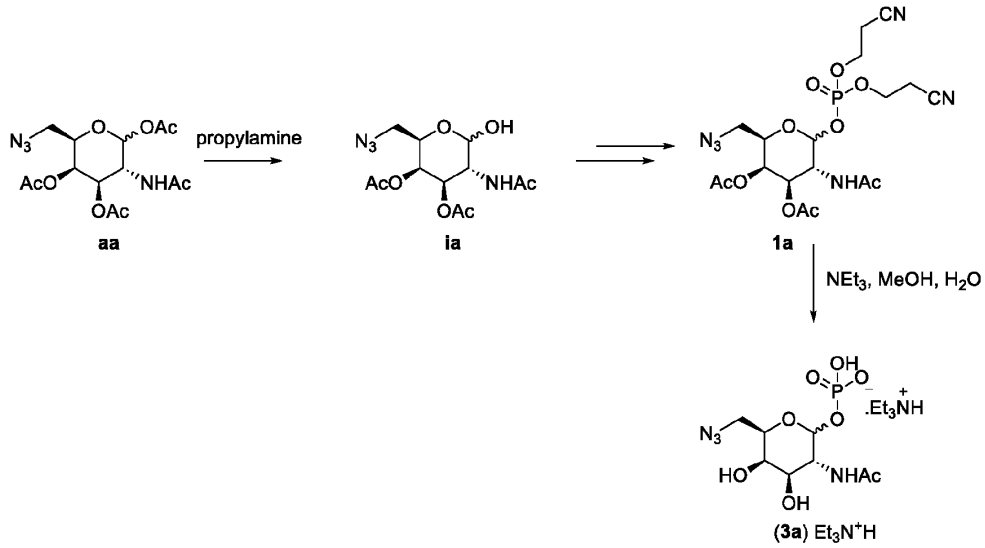
[0139] The effect of the synthesis route for compound (**3a**) has been investigated. Three different procedures have been studied. In Example 2a, the deprotection of **1a** was carried with a mixture of NEt_3/MeOH/H_2O and no crystallization was used. In Example 2b, the deprotection of crude **1a** was carried

out with NaOMe in MeOH in one step. In Example 2c, compound **1a** was first purified, and the deprotection was carried out in NaOMe in MeOH in one step.

[0140] This synthesis route is compared with the deprotection according to the present invention, as given in examples 11 and 12. The deprotection of crude **1a** with NaOMe in MeOH was carried out in two steps with intermittent crystallization. It was found that significantly lower amounts of the the corresponding 6-OH impurity (compound (**S3**)) could be obtained when the method described in Examples 11–12 was employed, as further analysed in Example 1.

Example 2a

[0141] Compound **1a** was prepared and purified. After one-step deprotection with NEt_3 in a mixture of methanol and water, compound **3a** was obtained. The content of the corresponding 6-OH impurity was found to be to be about 0.07 – 1.22 wt%.

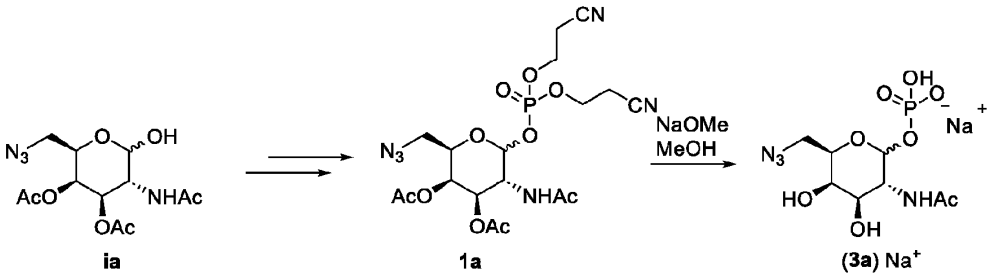


[0142] Compound **aa** (50.0 g, 134 mmol, 1.00 equiv.) was suspended in THF (900 mL) in a 2L round bottom flask under N_2 atmosphere. Propylamine (23.5 mL, 285 mmol, 2.13 equiv.) was added in one portion and the resulting reaction mixture was stirred at r.t. overnight. After 16.5 h reaction time the reaction mixture was concentrated at 40°C under reduced pressure and then co-evaporated from toluene (300 mL). This gave 70.2 g of crude **ia** (which equals 44.4 g pure product) as a yellow oil. The crude material was dissolved in DCM (665 mL) in a 3L three-necked round bottom flask under N_2 atmosphere. 5-(ethylthio)-1H-tetrazole (0.25M in MeCN, 813 mL, 200 mmol, 1.50 equiv.) was added and the resulting reaction mixture was cooled in an ice bath to an internal temperature <10°C. Bis(2-cyanoethyl)-N,N-diisopropyl phosphoramidite (46.2 g, 44.5 mL, 169 mmol, 1.26 equiv.) was then added over 2 min. The reaction mixture was further stirred at <10°C. 3-Chloroperoxybenzoic acid (44.4 g, 185 mmol, 1.4 0equiv.) was dissolved in MeCN (215 mL) and added to the reaction mixture after 20 min over a range of 15 min. The resulting reaction mixture was

allowed to warm to r.t. and stirred for a total of 1 h 20 min. The reaction mixture was diluted with DCM (665 mL) after 1.5 h and the organic layer was washed with aqueous $Na_2S_2O_3$ solution (10%, 665 mL) and aqueous saturated $NaHCO_3$ solution (665 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated at 40°C under reduced pressure. This gave 77.2 g of crude product **1a** as a dark yellow/orange oil to syrup. The crude product was purified by silica gel column chromatography with an initial gradient of PE 40–60°C/EtOAc (3:7 to 0:1) followed by a second gradient of EtOAc/MeCN (1:0 to 3:7). Product containing fractions were combined and concentrated under reduced pressure at 40°C to give 36.3 g of product (52% yield over two steps) as a yellow, sticky oil to foam. From this material, 35.6 g, (68.9 mmol, 1.00 equiv.) was dissolved in MeOH (395 mL) and water (395 mL) in a 3 L round bottom flask. Triethylamine (190 mL, 1.36 mol, 19.8 equiv.) was added rapidly and the resulting reaction mixture heated to 50°C (outer temperature) and stirred overnight. After 28 h, the reaction mixture was stirred at 50°C for a further 17 h. The reaction mixture was concentrated under reduced pressure at 50°C to yield crude material of **3a** as a yellow viscous oil. This residue was dissolved in water (200 mL) and the aqueous layer was extracted with ethyl acetate (10 × 400 mL). The aqueous layer containing the product was then concentrated under reduced pressure at 50°C followed by co-evaporation from MeOH (2 × 200 mL) to obtain compound **3a** in 29.1 g as yellow to light brown oily foam. ^{1}H NMR (400 MHz, D_2O) δ ppm 2.04 (s, 3H), 3.41–3.65 (m, 2H), 3.90–4.09 (m, 2H), 4.15–4.25 (m, 2H), 5.45 (dd, J = 7.24, 3.48, 1H). A content of 0.23% of impurity the corresponding 6-OH impurity measured by LC-MS-SIR analysis.

Example 2b

[0143] Bis-cyanoethyl compound **1a** was prepared and then deprotected in one step with sodium methoxide in methanol, which resulted in the formation of compound **3a**. The content of the corresponding 6-OH impurity was found to be 0.33%.

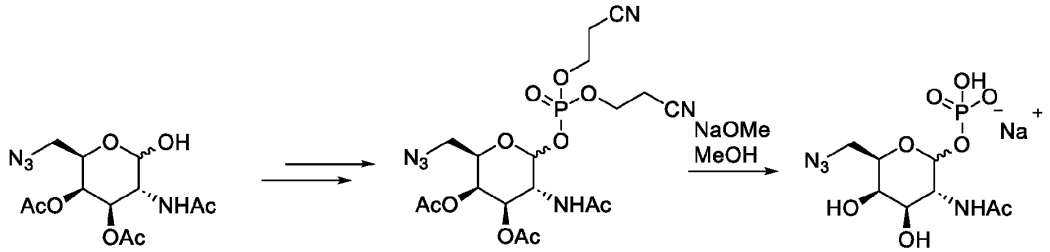


[0144] **Compound ia** was dissolved in a mixture of dichloromethane (288 mL) and acetonitrile (288 mL) in a 2 L flask under N_2 atmosphere. The reaction mixture was cooled to an internal temperature = 5°C in an ice bath. Bis(2-cyanoethyl)-N,N-diisopropyl phosphoramidite (28.02 g, 103 mmol) and after 30 min. 1H-tetrazole (7.2g, 103mmol) were added under stirring. The reaction mixture was further stirred in the ice-bath for 1 hour. The reaction suspension was filtered, the solid (salt) was washed with 10 mL ice-cold dichloromethane and the combined filtrates cooled in an ice bath. 3-Chloroperoxybenzoic acid (75%, 23.68

g, 103 mmol) was added to the cooled reaction suspension. The resulting reaction solution was stirred for 30 min in an ice bath. The reaction mixture was heated to 20°C and extracted with aqueous saturated $NaHCO_3$ solution (590 mL). The aqueous phase was subsequently re-extracted with DCM (100 mL). The organic phases were combined and extracted with a solution of $Na_2S_2O_3$ (5 g) in water (100 mL) and aqueous saturated $NaHCO_3$ solution (100 mL). The aqueous phase was re-extracted with DCM (100 mL). The combined organic phases were dried over $MgSO_4$ (100 g), filtered, diluted with toluene (100 mL) and concentrated at 50°C under reduced pressure in a rotary evaporator. This gave 46.2 g of crude fully protected 6-azido-2,6-dideoxy-alpha-D-GalNAc-1-phosphate as a yellow syrup (crude **1a**). Crude **1a** (5.0 g, 9.7 mmol) was completely deprotected in a mixture of 5.4M methanolic sodium methanolate solution (3.2 g, 17.9 mmol) and MeOH (30 mL) under N_2 atmosphere for 60 min at a bath temperature of 50°C. The reaction solution was mixed with acetic acid (1 g) and ethanol (60 mL) and concentrated at 55°C under reduced pressure to yield a suspension (25 mL). The suspension was stored at 5°C overnight. The resulting thick suspension was filtered. The filter cake was washed with ethanol (3 × 5 mL) and dried at 40°C in vacuum to constant weight. 2.6 g (76%) of **3a** was obtained in form of acetic acid-containing colourless crystals. The identity was confirmed by HPLC. A content of 0.33% of the corresponding 6-OH impurity was measured by LC-MS-SIR analysis.

Example 2c

[0145] Compound **1a** (bis-cyanoethyl compound) was prepared, purified and deprotected in one step with sodium methoxide in methanol, which resulted in the formation of compound **3a**. The content of the corresponding 6-OH impurity was found to be 0.09%.

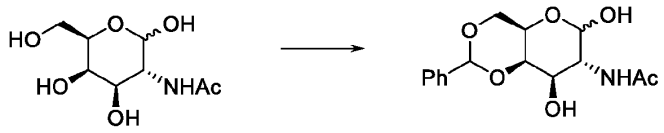


ia **1a** (purified) **(3a)** Na^+

[0146] An 23 g aliquot of the syrup (crude **1a**) was purified by silica gel column with ethyl acetate/acetonitrile (4:1) as the mobile phase. Product containing fractions were combined and concentrated under reduced pressure at 60°C to give 15 g of pure product as voluminous foam (pure **1a**). Pure **1a** (3.55 g, 6.87 mmol) was completely deprotected in a mixture of 5.4M methanolic sodium methanolate solution (2.3 g, 12.8 mmol) and MeOH (9 mL) under N_2 atmosphere for 60 min at a bath temperature of 50°C. The reaction solution was mixed with acetic acid (0.7 g) and ethanol (40 mL) and concentrated at 55°C under reduced pressure to yield a suspension (20 mL). The suspension was stored at 5°C overnight. The resulting thick suspension was filtered. The filter cake was washed with ethanol (3 ×

5 mL) and dried at 40°C in vacuum to constant weight. 1.9 g (80%) of **3a** was obtained in form of acetic acid-containing colourless crystals. The identity was confirmed by HPLC A content of 0.09 % of the the corresponding 6-OH impurity was measured by LC-MS analysis.

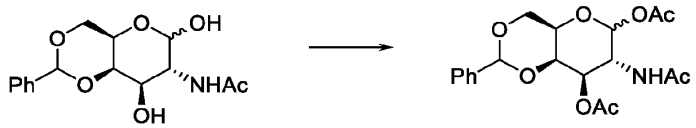
*Example 3: Synthesis of compound (**ba**)*



aa **ba**

[0147] To a three-necked 6 L-flask were added acetonitrile (5 L) and *N*-acetyl galactosamine (**aa**) (0.5 kg, 2.26 mol). To the resulting suspension was added benzaldehyde dimethylacetal (0.825 L, 0.837 kg, 5.50 mol, 2.43 equiv.) followed by camphorsulfonic acid (31.4 g, 0.135 mol, 0.06 equiv.). The mixture was vigorously stirred at r.t. overnight. The precipitated material was filtered off, and the filter cake was washed three times with acetonitrile (0.3 L each) and then four times with 10% EtOAc/iso-hexane (0.5 L each). The product was dried under vacuum at 45 °C to give 0.5 kg (1.617 mol, 71.5%) of compound **ba**. ^{1}H NMR (DMSO-d6) δ ppm 1.83 (s, 3H), 3.80 (s, 1H), 3.84 (dd, 1H), 3.95–4.10 (m, 3H), 4.15 (d, 1H), 5.05 (br. s, 1H), 5.58 (s, 1H), 6.50 (br. d, 1H), 7.33–7.41 (m, 3H), 7.44–7.52 (m, 2H), 7.64 (d, 1H).

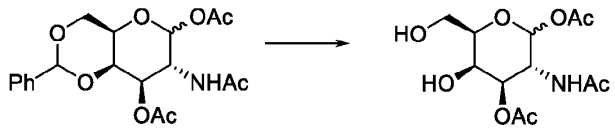
*Example 4: Synthesis of compound (**ca**)*



ba **ca**

[0148] To a three-necked 6 L-flask equipped with a thermometer and a mechanical stirrer were added the benzylidene-protected galactosamine **ba** (1.0 kg, 3.233 mol) and pyridine (3.0 L). The mixture was cooled to initially 10–15 °C, and acetic anhydride (1.0 L) was dropwise added while keeping the internal temperature below 25 °C during addition. The mixture was stirred at rt overnight and then slowly poured into ice/water (10 L) and stirred at rt for at least 3 h. The precipitated product was filtered off, and the filter cake was washed ten times with water (1 L each) and finally twice with methanol (1 L each). The obtained solid was dried under reduced pressure at 45 °C to give 1.14 kg (2.90 mol, 90%) of compound **ca**. ^{1}H NMR (DMSO-d6) δ ppm 1.80 (s, 3H), 2.03 (s, 3H), 2.14 (s, 3H), 3.95 (s, 1H), 4.05 (dt, 2H), 4.43–4.52 (m, 2H), 5.11 (dd, 1H), 5.64 (s, 1H), 6.06 (d, 1H), 7.36–7.46 (m, 5H), 7.99 (d, 1H).

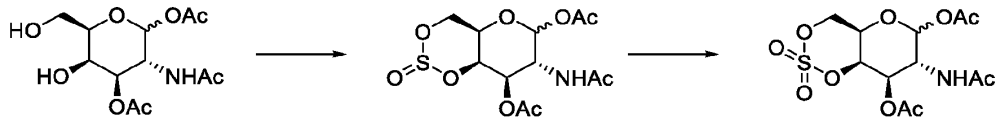
*Example 5: Synthesis of compound (**da**)*



ca **da**

[0149] The protected galactosamine **ca** (0.76 kg, 1.932 mol) was dissolved in a 10 L-hydrogenation reactor in a mixture of methanol (7 L) and dioxane (7 L). Acetic acid (10 mL) and palladium on activated charcoal (5% Pd) (50 g) were added, and the mixture was flushed three times with argon and then three times with hydrogen. The mixture was hydrogenated at 1.5 bar with intensive stirring at 25°C–30 °C overnight. During the reaction, additional palladium on activated charcoal (5% Pd) (4 × 50 g) was added repeatedly to achieve 80%–90% conversion (reaction control by TLC, EtOAc/MeOH 6:1). When the reaction was deemed to be complete, the mixture was filtered over Celite, and the filter cake was washed with methanol. The combined filtrate was concentrated to dryness, and the crude product was purified by chromatography on silica gel. The fractions containing pure product were evaporated, and the resulting oil was repeatedly co-evaporated with methanol and diethyl ether to give the alcohol **da** (0.53 kg, 1.736 mol, 90%) as a white solid. ^{1}H NMR ($CDCl_3$) δ ppm 1.95 (s, 3H), 2.14, 2.16 (2 x s, 6H), 3.84–3.93 (m, 3H), 4.20 (d, 1H), 4.76–4.83 (m, 1H), 5.16 (dd, 1H), 5.58 (d, 1H), 6.62 (d, 1H).

*Example 6: Synthesis of compound (**ea**) and (**fa**)*



da **ea** **fa**

[0150] In the 20 L-reactor connected to a gas scrubber, alcohol **da** (800 g, 2620.5 mmol) was dissolved at r.t. in acetonitrile (5.6 L), the reactor was flushed with nitrogen, and the solution was cooled to an internal temperature of –5°C (jacket temperature –15°C). Thionyl chloride (394 g, 3312 mmol, 1.26 equiv.) was then added dropwise via a dropping funnel within 1 h 15 min, keeping the internal temperature below 0°C. The mixture was stirred for overall 40 min with the jacket temperature remaining at –15°C, which resulted in an internal temperature decreasing from 0°C. After 3 h and 20 min, triethylamine (729 g, 7204 mmol, 2.75 equiv.; minus ca. 50 mL which was retained) was added dropwise over the course of 2.5 h in a way that the internal temperature was kept below 0°C. When the addition had finished, the jacket temperature was kept at –15°C and the pH was checked with wetted pH paper and estimated at 3–4, therefore a small amount of 30% aq. HCl solution was added to achieve a pH of 3 or less. In parallel to the triethylamine addition, a (cloudy) solution of sodium periodate (729 g, 3408 mmol, 1.30 equiv.) in water (9 mL/g salt; 6.7 L) was prepared and cooled in an ice bath. Ruthenium(III) chloride (8.15 g, 39.3 mmol, 0.015 equiv.) was suspended in water (150 mL) and added to the above reaction mixture (vessel of catalyst suspension was

rinsed with some additional water which was also added to the reaction mixture). The (cold) solution of $NaIO_4$ from above was then added in portions to the reaction mixture in 45 min. The reaction mixture was warmed to ca. 17°C within 40 min. After 20 min, the mixture was directly drained from the reactor onto the filter and the filter cake was suction-dried. The reactor was rinsed with MeCN/water 1:1 in several portions, and the filter cake was washed with these and additional portions of solvent mixture until the filtrate was colourless (overall ca. 4 L). The combined filtrate was then carefully filtered twice. The filtrate was concentrated. When half of the original volume was removed by distillation, the mixture was transferred into a 15 L-Büchner flask which was placed in a water/ice-bath, to complete the crystallization of the product. After filtration, the filter cake was washed with water in four portions (4 × 1 L). The product was dried by suction and then in the drying oven at 30 °C for 3 d to give cyclic sulfate **fa** (873.4 g, 91%) as an almost white, sand-like solid. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 1.82 (s, 3H), 2.09 (s, 3H), 2.15 (s, 3H), 4.34–4.45 (m, 2H), 4.72–4.83 (m, 1H), 4.90 (d, J = 11.98, 1H), 5.25 (dd, J = 11.74, 3.06, 1H), 5.44 (d, J = 2.93, 1H), 6.07 (d, J = 3.42, 1H), 8.08 (d, J = 8.56, 1H).

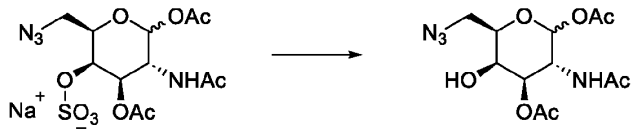
*Example 7: Synthesis of compound (**f1a**)*



fa (**f1a**) Na^+

[0151] A three-necked 3L flask was charged with cyclic sulfate **fa** (344.0 g, 936.6 mmol) and set under nitrogen. The material was dissolved in DMF (5 mL/g) (1.7 L). Sodium azide (62.75 g, 965.2 mmol, 1.03 equiv.) was added in several portions at once at r.t. (without external cooling). The mixture was stirred at r.t. overnight. The solution was concentrated to give a golden oil. The residue was dissolved in 2-propanol (1.58 kg/2.0 L; 4.6 g/g of **fa**) at 45 °C. The pale yellow solution was then quickly filtered while hot into cold (6 °C) and stirred 2-propanol (12 g/g of **fa**) (5.3 L), which resulted in immediate precipitation of a white solid. The flask and filter were rinsed with additional hot (55 °C) 2-propanol (ca. 1.5 L), adding the filtrate to the product suspension. The mixture was stirred in the ice bath for 3 h and then at <–18 °C overnight. The mixture was filtered. The filter cake was washed twice with cold (<–18 °C) 2-propanol (1 L overall), and the filter cake was suction-dried. The filter containing the filter cake was transferred into the drying oven, and the product was dried at 30°C under high vacuum for 6 d. The product was broken up, crushed and mixed in the filter each day. 414 g of **f1a** (102%) was obtained. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 1.79 (s, 3H), 1.93 (s, 3H), 2.11 (s, 3H), 3.34–3.49 (m, 2H), 4.09 (dd, J = 8.80, 3.67, 1H), 4.31–4.39 (m, 1H), 4.56 (d, J = 2.81, 1H), 4.87 (dd, J = 11.86, 3.06, 1H), 5.98 (d, J = 3.55, 1H), 8.04 (d, J = 8.07, 1H).

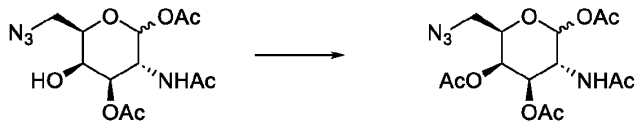
*Example 8: Synthesis of compound (**ga**)*



(**f1a**) Na^+ **ga**

[0152] A 20 L-reactor was equipped with a nitrogen inlet, reflux condenser and dropping funnel. THF (5 mL/g) (5.4 L) was introduced at r.t., followed by water (53.4 mL, 2.97 mol, 1.2 equiv.). The jacket temperature was set to 0°C, and when the internal temperature reached 9°C, sulfuric acid (96%) (289 g, 157 mL, 2.95 mol, 1.2 equiv.) was dropwise added within 60 min. The mixture was again cooled to 7.5 °C, and the azide sulfate **f1a** (1054 g, 2.438 mol) was added in portions. The reaction mixture was stirred at 0°C for ca. 5 min, then the jacket temperature was set to 20°C, and the mixture was stirred for 1 h 10 min while slowly warming up. The mixture was cooled to –3 °C (jacket), and when the internal temperature reached +3°C, triethylamine (435 g, 599 mL, 4.299 mol, 1.763 equiv.) was added dropwise over a period of 40 min. The jacket temperature was set to 10°C and water (1.8 L) was added. Additional triethylamine (145 g, 200 mL, 1.433 mol, 0.588 equiv.) was added within 15 min and the stirrer was turned off when the addition had finished, to allow the phases to separate. The layers were collected while the organic layer was directly drained into a 20 L-Büchner flask containing 554 g of $MgSO_4$. Both phases were stored in the fridge overnight. Next day, a crystalline precipitate was formed in the aqueous layer which was filtered off (filter cake rinsed with 2 × 0.5 L of EtOAc). The aqueous filtrate was extracted with EtOAc (5 × 1 L). The combined organic layers were dried ($MgSO_4$), filtered and the filter cake was washed with EtOAc (0.5 L), and the combined filtrate was concentrated. The obtained residue was dried under vacuum and then placed in the vacuum oven at 30°C overnight. Intermediate **ga** was isolated in a quantity of 796 g (99%). The product was used in the next step without further purification.

*Example 9: Synthesis of compound (**ha**)*

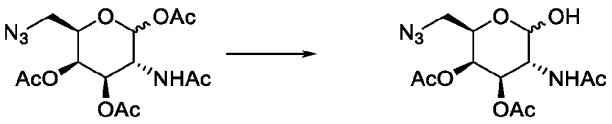


ga **ha**

[0153] In a 20 L-evaporation flask, alcohol **ga** (796 g, 2.410 mol) was suspended in CH_2Cl_2 (4.5 g/g of **ga**) (3.56 kg, 2.7 L) at 40 °C while rotating and the mixture was cooled in an ice bath. Acetic anhydride (295 g, 2.890 mol, 1.20 equiv.) was added at once (temperature rise from 11 to 12°C), and when the internal temperature upon continued cooling and stirring reached 8°C, the addition of 4-dimethylaminopyridine (DMAP) (264 g, 2.161 mol, 0.90 equiv.) started, which was done in small portions over the course of ca. 15 min. The mixture was stirred for 45 min. The reaction mixture was concentrated to dryness to give a thick orange suspension. 2-Propanol (3.85 kg, 4.9 L) was added to the residue, and the mixture was warmed in

the water bath at 45°C until all material was dissolved. The reaction mixture was stirred and after a few minutes, spontaneous crystallization started. When the crystallization had finished, the mixture was stirred in an ice bath for 1 h and was then kept closed in the fridge overnight. The suspension was filtered and the filter cake was washed three times with 2-propanol (pre-cooled in the freezer (overall 3 L). The product was suction-dried and was then further dried in the drying oven at 30°C for 2 d to give **ha** (839.3 g, 1.941 mol, 81%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 1.04 (d, J = 6.11, 6H, corresponding to 14 wt-% IPA-adduct), 1.79 (s, 3H), 1.93 (s, 3H), 2.13 (s, 3H), 2.14 (s, 3H), 3.28–3.36 (m, 2H), 3.41 (dd, J = 12.84, 7.95, 1H), 3.71–3.83 (m, 1H), 4.25–4.39 (m, 3H), 5.09 (dd, J = 11.80, 3.12, 1H), 5.36 (d, J = 2.57, 1H), 6.05 (d, J = 3.55, 1H), 8.11 (d, J = 7.95, 1H).

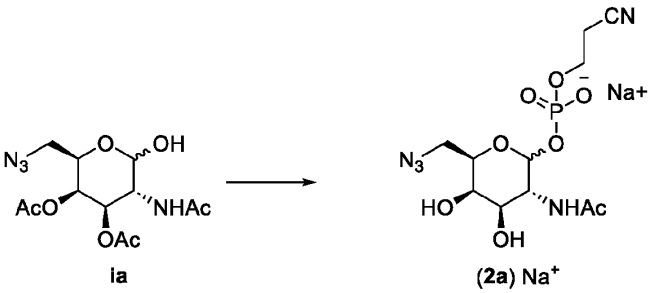
*Example 10: Synthesis of compound (**ia**)*



ha **ia**

[0154] **ha** (680g, 1.57 mol) was suspended in MTBE (4.3Kg) in a 20L round bottom flask under N_2 atmosphere. Morpholine (171 g, 1.95 mol) was added in one portion and the resulting reaction mixture was rotated at 60°C overnight. After 18 h, the reaction mixture was cooled to rt and concentrated at 55°C under reduced pressure. The resulting brown syrup was purified by silica gel column chromatography with ethyl acetate/petroleum ether (60/95) (4.5:1) as mobile phase. Product containing fractions were combined and concentrated under reduced pressure at 60°C to give 407 g of pure product as voluminous foam. MTBE (612 g) was added to the foam (407 g) and the mixture was rotated for 75 min at 40°C on the rotavapor. The suspension was cooled to 20°C and filtered. The residue was washed with MTBE (3 × 500 mL)) and dried at 40°C in vacuum. 325 g (63%) of **ia** was obtained in the form of colourless crystals. ^{1}H NMR (400 MHz, CDCl3) δ ppm 1.99, 2.02 (2 x s, 6H), 2.19 (s, 3H), 3.22 (dd, J = 12.78, 4.58, 1H), 3.43 (dd, J = 12.78, 8.13, 1H), 4.34 (dd, J = 8.13, 4.46, 1H), 4.55 (ddd, J = 11.31, 9.60, 3.55, 1H), 5.26 (dd, J = 11.37, 3.18, 1H), 5.35 (t, J = 2.81, 2H), 5.84 (d, J = 9.54, 1H).

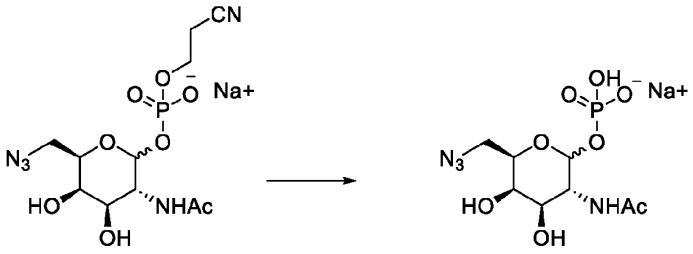
*Example 11: Synthesis of compound (**2a**)*



ia (**2a**) Na^+

[0155] Compound **ia** (345 g, 1.04 mol) was dissolved in a mixture of DCM (2.2 L) and acetonitrile (2.2 L) in a 20L reactor under N_2 atmosphere. The reaction mixture was cooled to an internal temperature = –5°C. 1H-tetrazole (102 g, 1.46 mol) and immediately afterwards bis(2-cyanoethyl)-*N*,*N*-diisopropyl phosphoramidite (326 g, 1.20 mol) were added under stirring. The reaction mixture was further stirred between –5 and 0°C for 1 h. TMantle was set to 10°C and 1H-tetrazole (10.2 g, 0.15 mol) was added and immediately afterwards bis(2-cyanoethyl)-*N*,*N*-diisopropyl phosphoramidite (32.6 g, 0.12 mol) was added under stirring. The reaction mixture was further stirred at an internal temperature between 5–10°C for 1 h. 3-Chloroperoxybenzoic acid (moistened with 25% water, 290 g, 1.26 mol) was dissolved in DCM (2.9L). The organic phase was separated from the water phase, dried over $MgSO_4$ and added to the reaction suspension under cooling (TMantle = –15°C) at an internal temperature of approx. 5°C. The resulting reaction solution was stirred for 30 min at 5°C.The reaction mixture was heated to 20°C and extracted with aqueous saturated $NaHCO_3$ solution (6.9 L) and saturated NaCl solution (1 L). The aqueous phase was subsequently re-extracted with DCM (1.1 L). The organic phases were combined and extracted with a solution of $Na_2S_2O_3$ (182 g, 0.73 mol) in water (3.5 L) and aqueous saturated $NaHCO_3$ solution (3.5 L). The aqueous phase was re-extracted with DCM (1.1 L). The combined organic phases were dried over $MgSO_4$ (430 g), filtered, diluted with toluene (1.5 L) and concentrated at 50°C under reduced pressure in a rotary evaporator. The received syrup was diluted with MTBE (3 L) and vigorously rotated for 30 minutes at a bath temperature of 50°C. The supernatant MTBE phase was sucked off and the syrup was further concentrated at 50°C in vacuo. The raw product was further dried in vacuum at 50°C. This gave 545 g (1.06 mol) of crude fully protected 6-azido-2,6-dideoxy-alpha-D-GalNAc-1-phosphate as a yellow syrup/foam. The crude material (545 g) was dissolved in MeOH (2.4 L) and transferred into the inert 20 L reactor. The flask was rinsed with methanol (0.3 L). The reaction mixture was cooled (TMantle = 0°C) to an internal temperature of 10°C. A 5.4 M methanolic sodium methanolate solution (207 g, 1.15 mol) was poured in one portion into the vigorous stirred reactant solution. The reaction mixture was further stirred under cooling (TMantle = 0°C) for 30 minutes. pH of the reaction mixture was set to 5–6 with acetic acid (about 20 g). The solution was diluted with ethanol (11.7 L) and stirred for 30 min with TMantle = 25°C (crystallisation of the product occurs). Half of the solvent (approx. 8.5 L) was distilled off at TBath = 80°C under reduced pressure (650 mbar). The resulting thick suspension was cooled in an ice bath for 1 h, filtered and the residue was dried at 35 °C in vacuo to constant weight. 324 g (77%) of **2a** was obtained in form of colourless crystals. ^{1}H NMR (400 MHz, MeOH-d4) δ ppm 2.01–2.04 (m, 3H), 2.81 (t, J = 6.30 Hz, 2H), 3.46 (dd, J = 12.59, 5.26 Hz, 1H), 3.61–3.65 (m, 2H), 3.78–3.93 (m, 2H), 4.06–4.18 (m, 3H), 4.30–4.39 (m, 1H), 5.47–5.58 (m, 1H).

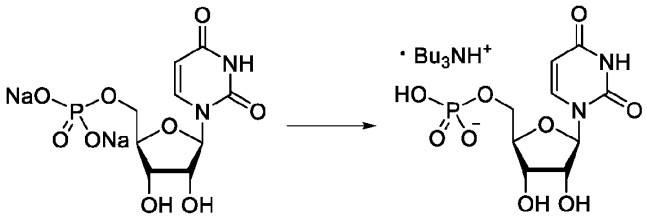
*Example 12: Synthesis of compound (**3a**)*



(**2a**) Na^+ (**3a**) Na^+

[0156] **2a** (688 g, 1.71 mol)) was suspended in a mixture of 5.4 M methanolic sodium methanolate solution (327 g, 1.82 mol) and MeOH (5 L) in a 20L rotary evaporator flask under N_2 atmosphere. The reaction mixture was heated under rotation for 60 min at a bath temperature of 55°C. The reaction solution was mixed with acetic acid (116 g, 1.93 mol) and concentrated at 55°C under reduced pressure to yield crude **3a** as a foam. The crude **3a** was dissolved in acetic acid (1.67 kg) under rotation at 40°C and diluted with ethanol (1.67 kg). Ethanol (5.0 kg) was slowly added to the solution while stirring vigorously. For crystallization, the mixture was seeded with **3a** and stirred for 3 h at rt and afterwards for 1 h in an ice bath. The suspension was stored at 5°C overnight. The resulting thick suspension was filtered. The filter cake was washed with ethanol (3 × 1L) and dried at 40°C in vacuo to constant weight. 521g (91%) of **3a** was obtained in form of acetic acid-containing colourless crystals. To remove the acetic acid, the crystals (521 g) were dissolved in methanol (1.67 kg) at 65°C, the hot solution filtered (wash with 0.2 kg methanol) and diluted with ethanol (12.5 kg) under stirring (the product crystallized). Half of the solvent was distilled off at TBath=60°C under reduced pressure (150 mbar). The resulting suspension was cooled to 5°C for 90 min and filtered. The filter cake was washed with ethanol (3 × 1L). The residue was filtered and dried at 40°C in vacuo overnight. For fine drying, the material was sieved (1mm) and further dried at 40°C under vacuum to constant weight to give **3a** (463 g, 80%) as colourless crystals. ^1H NMR (400 MHz, D_2O) δ ppm 2.02 (s, 3H), 3.41–3.65 (m, 2H), 3.90–4.00 (m, 2H), 4.11–4.23 (m, 2H), 5.42 (dd, *J* = 7.31, 3.55, 1H). The content of the corresponding 6-OH impurity was <0.03% (LC-MS-SIR).

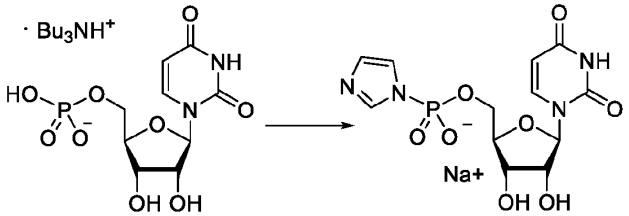
*Example 13: Synthesis of compound (**7**)*



6 (**7**) Bu_3N^+H

[0157] AmberChrom 50WX8-200–400 ion-exchange resin (H^+, 3.7 kg) was washed with water (10.5 L in three equal portions) on a 8L glass frit (Por. 3) applying vacuum until eluate was colourless. The washed resin was mixed with **6** (80%, 999 g, 2.17 mol) and water (5.0 L) in a 20 L round bottom flask. The mixture was agitated on a rotary evaporator with medium rotation speed (75–100rpm) at 25°C for 3 h and then filtered over a 8 L glass frit (Por. 3) into a 15 L round bottom flask under vacuum. The resin was washed twice with 1.5 L of water. Tributylamine (544 mL, 2.29 mol) was added and the reaction mixture was stirred vigorously at r.t. overnight and then lyophilized to give 1117 g (quant.) of **7** as an amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ ppm 0.89 (dd, J=7.5 Hz, 9H), 1.27–1.39 (m, 6H), 1.57–1.68 (m, 6H), 3.04–3.13 (m, 6H), 3.98–4.05 (m, 1H), 4.05–4.12 (m, 1H), 4.21–4.25 (m, 1H), 4.29 (dd, J = 5.1 Hz, J = 4.2 Hz, 1H), 4.33 (dd, J = 5.1 Hz, 1H), 5.91 (d, J = 8.2 Hz, 1H), 5.94 (d, J = 5.0 Hz, 1H), 7.96 (d, J = 8.2 Hz, 1H).

*Example 14: Synthesis of compound (**4**)*

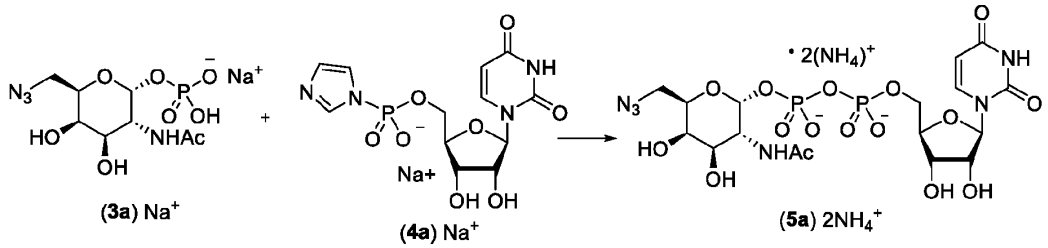


(**7**) Bu_3N^+H (**4**) Na^+

[0158] **7** (98%, 1070 g, 2.06 mol) was dissolved in DMF (8.3 L) in a 100L reactor under nitrogen atmosphere. Imidazole (1217 g, 17.87 mol), 2,2'-dipyridyl disulfide (1157 g, 5.25 mol), triethylamine (1000 g, 9.88 mol) and triphenylphosphine (2605 g, 9.93 mol) were sequentially added and the resulting reaction mixture was stirred at 25°C for 3 h. The reactor content was drained into a stainless-steel vessel. A solution of sodium iodide (2976 g, 19.85 mol) in acetone (60.2 kg) was prepared under stirring in the inert 100 L reactor. The reaction mixture was added to the NaI/acetone solution under stirring at 25°C, which resulted in precipitation of a white solid. The suspension was stirred for a further 30 min and then filtered over an inert 80 L filter unit (with depth filter sheet). The filter cake was washed with acetone (3 × 14.8 L) until most of the yellow color was washed out and then dried in the drying oven at 40°C under vacuum overnight to give 962 g of crude **4a** as an off-white solid. The crude material was dissolved in MeOH (2.5 L) and filtered (rinsed with 0.2 L methanol). Acetone (13.1 L) was placed in an inert 100L reactor and the methanolic product solution was added with stirring at 25°C. The resulting suspension was stirred for a further 30 min and then filtered over an 80 L filter unit (with depth filter sheet). The filter cake was washed with acetone (2 × 6 kg and 24 kg) until all the yellow colour was washed out and then dried in the drying oven at 40°C under vacuum to constant weight to give 855 g of **4** as an electrostatic white solid. A suspension of 855 g of the crude **4a** was stirred in 10.2 L filtered acetone for 1.5 h at 40°C and then filtered. The filter cake was washed with filtered acetone (3 × 1L). The moist solid was again stirred in 10.2 L filtered acetone for 1.5 h at 40°C, filtered, washed (3 × 1 L filtered acetone) and then dried in the drying oven at 40°C under vacuum overnight.

For fine drying, the material was sieved (1 mm) and further dried at 40°C under vacuum to constant weight to give 826 g (96%) of **4** as an electrostatic white solid. ^{1}H NMR (400 MHz, D_2O) δ ppm 3.95–4.05 (m, 1H), 4.07–4.16 (m, 3H), 4.18–4.28 (m, 1H), 5.80 (d, *J*=7.82 Hz, 1H), 5.89 (d, *J* = 4.77 Hz, 1H), 7.08 (s, 1H), 7.26 (s, 1H), 7.58 (d, *J* = 7.95 Hz, 1H), 7.90 (s, 1H).

*Example 15: Synthesis of compound (**5a**)*



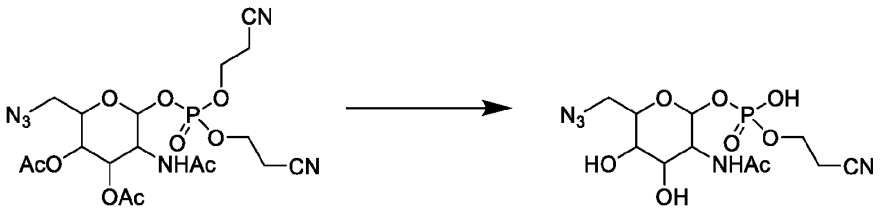
[0159] 130 g AmberLite MAC 3 was slowly stirred for 2 h in 260 g DMSO at room temperature. At the beginning an ice bath was used for cooling. The ion exchanger was filtered through a P2-frit (washed twice with 130 mL DMSO) and then stirred for another 2 h in 260 g DMSO and filtered again (washed twice with 130 g DMSO). DMSO (1000 mL) was placed in a 2L round bottom flask under N_2 atmosphere. Anhydrous $MgCl_2$ (30.6 g, 320 mmol) was added under stirring at rt. Under cooling (water bath), **3a** (100 g, corrected 278 mmol) was added and the reaction mixture was stirred for 5 min. Compound **4a** (136.6 g, corrected 320 mmol)) and the rinsed AmberLite MAC3 ion exchanger were added, and the reaction mixture was stirred at TBath = 33°C. After 23.5 h., the reaction mixture was filtered (filter cake (ion exchanger) washed twice with 130 mL DMSO) and centrifuged. The supernatant clear solution was decanted from the solid. The solid was suspended in 120 mL DMSO and centrifuged again as described above. The supernatant clear solution was decanted from the solid. The two DMSO centrifugates were diluted with 6.9 L of demineralised water and directly purified by ion exchange chromatography (AmberChrom 1x4 200-400 mesh, formate form, gradient of NH_4HCO_3 buffer pH 9.5). Product containing fractions combined, evaporated at 65°C under vacuum to about 3 L and filtered. The filtrate was concentrated at 65°C under vacuum to form a colorless foam, which was further dried overnight at 60°C under vacuum. After sieving (1 mm) and drying the colorless solid at 60°C under vacuum to constant weight, 178 g (93%) purified **5a** was obtained. ^{1}H NMR (400 MHz, D_2O) δ ppm 2.05 (s, 3H), 3.46 (dd, *J* = 12.78, 6.05 Hz, 1H), 3.56 (dd, *J* = 12.78, 7.27 Hz, 1H), 3.87–4.04 (m, 2H), 4.13–4.25 (m, 4H), 4.29–4.36 (m, 2H), 5.50 (dd, *J* = 7.21, 3.42 Hz, 1H), 5.91–5.97 (m,2H), 7.93 (d, *J* = 8.19 Hz, 1H). UDP-GalNAc content < 0.02 wt% (LC-MS-SIR).

CLAIMS

1. A method for preparing crystalized compound (**2**), said method comprising:

(I) providing compound (**1**);

(II) subjecting compound (**1**) to a deprotection step with a base to obtain compound (**2**);

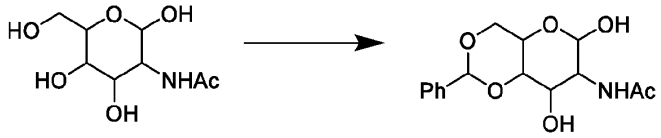


(**1**) (**2**)

(III) crystalizing the product obtained in step (II) to obtain crystalized compound (**2**).

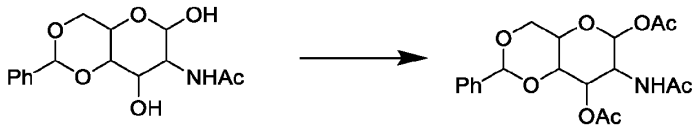
2. The method according to claim 1, wherein compound (**1**) is obtained by subjecting an N-acetyl sugar amine (**a**) to the following reaction steps:

(Ia) protecting the OH groups on the 4 and 6 position of compound (**a**) with a benzylidene acetal group to obtain compound (**b**);



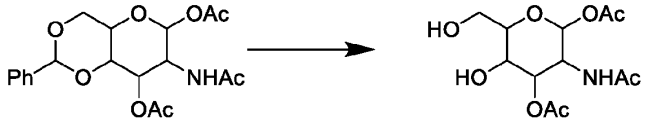
(**a**) (**b**)

(Ib) acetylating the OH groups of compound (**b**) to obtain compound (**c**);



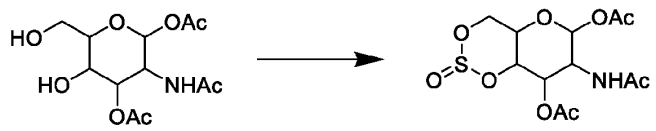
(**b**) (**c**)

(Ic) deprotecting the benzylidene acetal group of compound (**c**) to obtain compound (**d**);



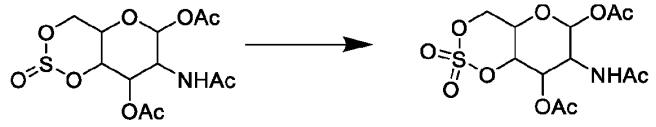
(**c**) (**d**)

(Id) reacting compound (**d**) with $SOCl_2$ to obtain compound (**e**);



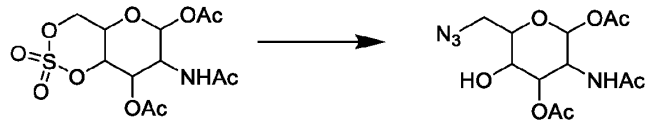
(**d**) (**e**)

(Ie) oxidizing compound (**e**) to obtain compound (**f**);



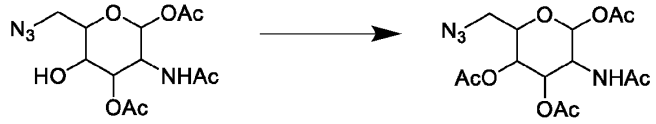
(**e**) (**f**)

(If) reacting compound (**f**) with a salt comprising anionic azide to obtain compound (**g**);



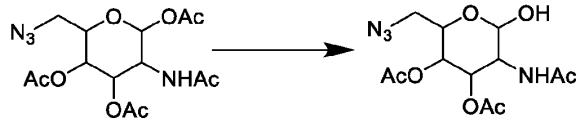
(**f**) (**g**)

(Ig) acetylating compound (**g**) to obtain compound (**h**);



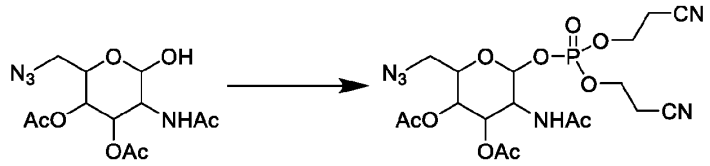
(**g**) (**h**)

(Ih) anomeric deacetylation of compound (**h**) to obtain compound (**i**);



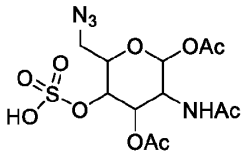
(**h**) (**i**)

(Ii) reacting compound (**i**) with $X\text{-}P(O)(OCH_2CH_2CN)_2$ or with $X\text{-}P(OCH_2CH_2CN)_2$ followed by oxidation, wherein X is a leaving group, to obtain compound (**1**):



(**i**) (**1**).

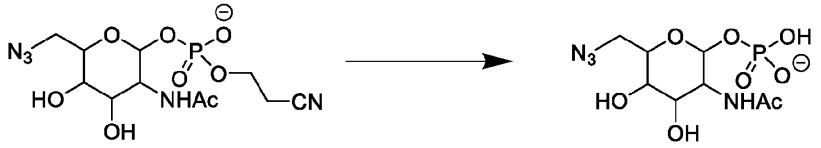
3. The method according to claim 2, wherein step (If) comprises the isolation of the intermediate azide sulfate sugar (**f1**) by crystallization:



(**f1**).

4. A method for preparing compound (**3**), comprising preparing crystallized compound (**2**) according to any one of the preceding claims; and

(IV) subjecting the crystallized compound (**2**) to a second deprotection step with a base to obtain compound (**3**);



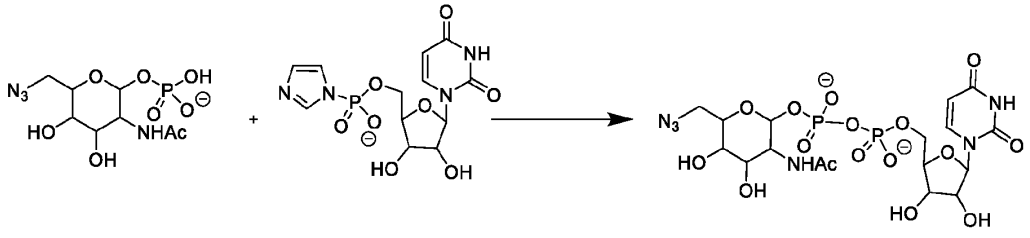
(**2**) (**3**)

(V) optionally crystallizing compound (**3**).

5. The method according to claim 4, wherein the deprotection step (II) is performed under a lower temperature than the deprotection step (IV), preferably step (II) is performed at a temperature below 20 °C and step (IV) at a temperature above 20 °C.

6. A method for preparing compound (**5**), comprising preparing compound (**4**) according to claim 4 or 5; and

(VI) reacting optionally crystalized compound (**3**) with compound (**4**) to obtain an UDP-sugar according to structure (**5**)



(**3**) (**4**) (**5**)

7. A method for modifying a glycoprotein, comprising preparing compound (**5**) according to claim 6; and

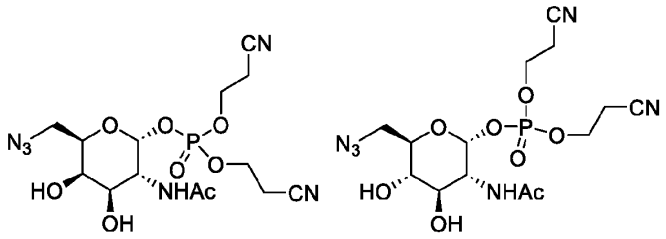
(VII) providing a glycoprotein, which is preferably trimmed to comprise a terminal core-GlcNAc;

(VIII) contacting the glycoprotein with the UDP sugar (**5**) in the presence of a glycosyltransferase to obtain an azido modified glycoprotein.

8. The method according to claim 7, further comprising:

(IX) reacting the azido modified glycoprotein with an azide-reactive click probe, to obtain a functionalized glycoprotein, preferably the functionalized glycoprotein is an antibody-drug conjugate.

9. The method according to any one of the preceding claims, wherein compound (**1**) is an *N*-acetyl-galactosamine derivative or an *N*-acetyl-glucosamine derivative, preferably an *N*-acetyl-galactosamine derivative according to structure (**1a**) or an *N*-acetyl-glucosamine derivative according to structure (**1b**):



(**1a**) (**1b**),

most preferably wherein compound (**1**) is the *N*-acetyl-galactosamine derivative according to structure (**1a**).

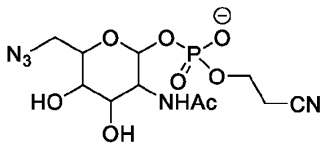
10. The method according to any one of the preceding claims, wherein the base used in step (II) is used as anhydrous solution, preferably the base is an alkoxy compound dissolved in an alcohol, more preferably the base is a methoxide dissolved in methanol.

11. The method according to any one of the preceding claims, wherein step (II) involves the use of 1.0-1.2 equivalent of the base with respect to compound (**1**).

12. The method according to any one of the preceding claims, wherein step (II) and/or (IV), preferably both, comprises a quenching step, preferably wherein the quenching step involves lowering the pH by the addition of an acid, more preferably to obtain a pH in the range of 5.5 – 6.5, most preferably the acid is acetic acid.

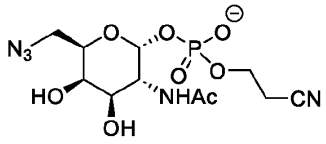
13. The method according to any one of the preceding claims, wherein step (III) is performed in a polar solvent, preferably a protic solvent, more preferably the solvent for step (III) comprises an alcohol.

14. A compound according to structure (**2**)

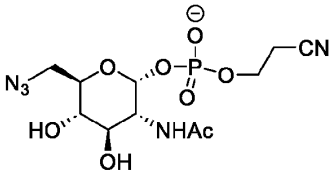


(**2**).

15. The compound according to claim 14, which is an *N*-acetyl-galactosamine derivative according to structure (**2a**) or an *N*-acetyl-glucosamine derivative according to structure (**2b**):



(**2a**)



(**2b**)

most preferably wherein compound (**2**) is the *N*-acetyl-galactosamine derivative according to structure (**2a**).

16. The compound according to claim 14 or 15, which is in crystallized form.