(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 January 2014 (30.01.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/016317 A1

(51) International Patent Classification:
C07H 15/207 (2006.01) *A61K 31/7034* (2006.01)
C08B 37/00 (2006.01) *A61P 33/02* (2006.01)

(21) International Application Number:
PCT/EP2013/065559

(22) International Filing Date:
23 July 2013 (23.07.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
12177588.6 24 July 2012 (24.07.2012) EP

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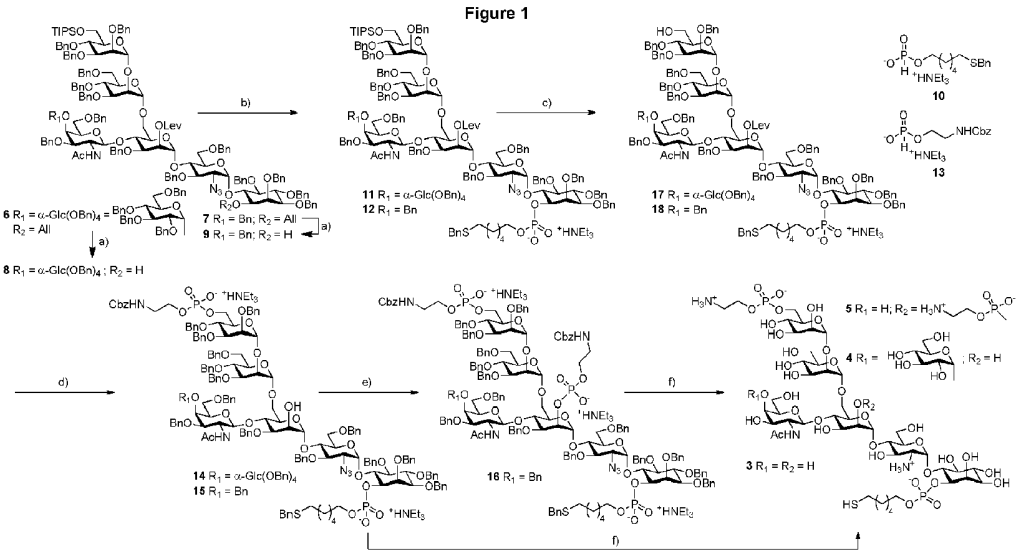
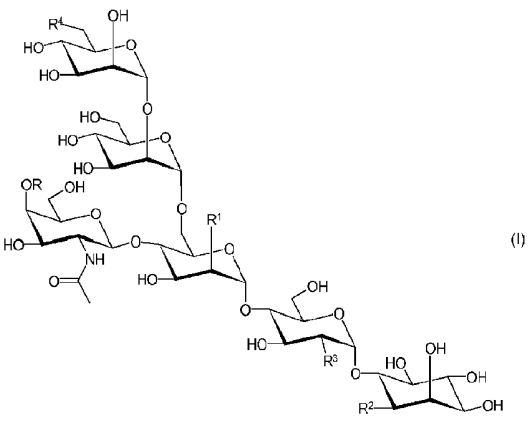
[Continued on next page]

(54) Title: SYNTHESIS OF DIVERSE GLYCOSYLPHOSPHATIDYLINOSITOL GLYCANS FROM TOXOPLASMA GONDII AND THEIR APPLICATION AS VACCINES AND DIAGNOSTICS

Figure 1





(I)

WO 2014/016317 A1

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(57) Abstract: The present invention relates to the synthesis of GPI-related surface antigens of the parasite Toxoplasma gondii (T. gondii) and the resulting products obtained. These synthetic compounds are suitable for diagnosis of toxoplasmosis, as well as vaccine against toxoplasmosis, a diseases caused by infection with T. gondii.

Synthesis of Diverse Glycosylphosphatidylinositol Glycans from *Toxoplasma gondii* and their Application as Vaccines and Diagnostics

The present invention relates to the synthesis of GPI-related surface antigens of the parasite *Toxoplasma gondii* (*T. gondii*) and the resulting products obtained. These synthetic compounds are suitable for diagnosis of toxoplasmosis, as well as vaccine against toxoplasmosis, a diseases caused by infection with *T. gondii.*

Glycosylphosphatidylinositols (GPIs) are complex glycolipids that are found in eukaryotic cells either attached to the C-terminus of proteins or in free form. These complex glycolipids feature a phosphoethanolamine unit connecting the C-terminus of the protein to the glycan, a conserved pseudopentasaccharide core of $H_2N(CH_2)_2OPO_3H6Man\alpha1\rightarrow2Man\alpha1\rightarrow6Man\alpha1\rightarrow4GlcN\alpha1\rightarrow6$*myo*-Ino1-$OPO_3H$ and a lipid attached to the core glycan via a phosphodiester linkage. The conserved GPI structure can be further decorated by various substituents including additional phosphoethanolamine units, an additional fatty acid ester at C2 position of *myo*-inositol and oligosaccharide branch at C3 or C4 of ManI. The constitutive identity of the lipid subunit is variable and may include diacylglycerols, alkylacylglycerols or ceramides, with chains of different length and varying degrees of unsaturation. When GPIs are isolated from natural sources, they are often obtained as heterogeneous mixtures especially in respect to the glycan and lipid subunit. GPIs isolated from different species and, in some cases, from different tissues of the same organism, feature significant structural differences. The primary biological role of GPIs is to localize the attached protein to the outer surface of the plasma membrane bilayer. It is suggested that GPIs are responsible for the association of anchored proteins with lipid rafts and are, thereby, involved in diverse processes such as regulation of innate immunity, protein trafficking, and antigen presentation.

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii (T. gondii)*. *T. gondii* is ubiquitous in all warm blooded animals, but the primary host is the family of Felidae. A third to a half of the human population will have a toxoplasmosis infection at some point in their lives, but very few have symptoms. During the first few weeks after exposure, the infection typically causes no symptoms or a mild, flu-like illness: swollen lymph nodes, high temperature or muscle aches. However in most immunocompetent patients, the infection enters a latent phase, during which only bradyzoites are present, forming cysts in nervous and muscle tissues. Thereafter, the parasite rarely causes any symptoms in otherwise healthy adults. Along with, immunosuppression reactivation of a latent infection may occur and manifests primarily as a toxoplasmic encephalitis. Therefore, anyone with a

compromised immune system is at risk. These individuals include those undergoing chemotherapy, people suffering from HIV/AIDS or other immune disorders and organ-transplant recipients. The parasite can cause encephalitis (inflammation of the brain) and neurologic diseases, and can affect the heart, liver, inner ears, and eyes. Furthermore, primary infection with *T. gondii* during pregnancy can lead to transmission of the parasite from the mother to the unborn child, leading to a congenital toxoplasmosis. Women infected before conception normally do not transmit toxoplasmosis to the fetus. Nevertheless, there are cases known in which women, who already had a latent toxoplasmosis, got reinfected during pregnancy with a highly virulent strain of *T. gondii* that caused congenital toxoplasmosis. Disease in neonates may be severe, particularly if acquired early in pregnancy. Even spontaneous abortion and stillbirth may occur. Other symptoms that may occur are: low birth weight, fever, jaundice, abnormalities of the retina, mental retardation, hydrocephalus, convulsions, and brain calcification.

Universal screening of pregnant women for example is cost saving at an expected cost of $390 per child screened compared to an expected societal cost of congenital toxoplasmosis of $1010 per birth under the "no maternal screening". Countries such as France that have a high prevalence of toxoplasmosis already established a universal maternal screening program. With an estimated 4 million births per year in the U.S. nearly $2.5 billion could be saved annually compared to no maternal screening (*PLoS Negl Trop Dis.* **2011**; *5(9)*, 1333). The diagnosis of toxoplasmosis can be done using a variety of methods. The difficulty lies in the length of time, which is needed and in determining whether the infection is acute or chronic (latent). Acute infection can best be verified by identifying *T. gondii* parasite or *T. gondii* DNA from the patient's blood. Congenital infection of fetuses can be identified by the presence of cysts in the placenta or fetus. Of particular interest is determining acute infection in pregnant women, due to the risk of congenital toxoplasmosis. An acute toxoplamosis is accompanied by a high titer of IgM and low levels of IgG antibodies against *T. gondii* in the blood of a patient. Since high IgM titers can be persistent and detectable for over one year after the primary infection, it is very difficult to distinguish a latent from an acute toxoplasmosis. There are effective diagnostic techniques that monitor changes in the mother's antibody expression over time, but quick diagnosis is greatly preferred because fetuses often rapidly become infected. The Robert-Koch–Institute recommends a serological diagnosis using three subsequently steps:

1. Toxoplasma-antibody screening test: The most commonly used serologic tests detect the presence of anti-*T. gondii* IgG antibodies. IgG antibodies can be detected with the Sabin-Feldman dye test (considered the gold standard), indirect fluorescent

antibody (IFA) or agglutination. If tests applied to specific total antibodies against *T. gondii* as well as to IgG antibodies are negative, then there is neither an infection, nor immunity. If the test for total antibodies is negative an infection can be ruled out. Because screening tests based on IgG used in the early phase of infection can still be negative, they must be supplemented especially in pregnant women with an IgM test.

2. Toxoplasma IgM antibody test: If such test results are negative (but positive IgG-Ab test) it can be assumed that an inactive (latent) toxoplasma infection exists. Further studies are not required. If the test is positive further evaluation must be done, especially during pregnancy or if differentiated clinical symptoms exists.

3. Toxoplasma fact-finding process: This includes in particular the determination of the avidity of IgG antibodies, the IgA antibody detection, immunoblot and quantitative research methods. Such a further determination using PCR and histological tests is very expensive.

Hence, the diagnosis of *T. gondii* infection, especially of acute toxoplasmosis during pregnancy, is still difficult and time consuming. Therefore, there is need for an effective diagnostic test, which allows a fast and reliable diagnosis.

From epidemiological view, the prevention of infection is the most important. It is essential to prevent a primary infection during pregnancy. For this it is necessary to know whether there is immunity or not. So far, the only possibility for prevention is to avoid acquisition of *T. gondii* infection. These persons should avoid contact with materials that may be contaminated with cat feces and the contact with raw meat. The development of a vaccine based on a defined antigen against *T. gondii*, which produces a sterile immunity is therefore of highest interest. In the last 60 years, numerous studies aimed at developing a vaccine against *T. gondii*. However, all these approaches were based on live or inactivated parasites, purified or recombinant proteins, or plasmids encoding protein antigens, and failed to induce protection in mouse models. While some of these antigens increased survival in challenged mice and reduced brain cyst loads, they proved unsuccessful in inhibition of maternal-fetal transmission.

To meet these challenges, the inventors focused on another class of immunogenic molecules, the GPI anchors, contained by the cell membrane of *T. gondii*. The two GPIs contained by the cell membrane of *T. gondii* differ only by the presence of an additional α-glucose (α-Glc) in the side chain. While one GPI is used as a membrane

anchor for proteins and surface antigens of the parasite, the GPI containing the additional α-Glc in the side chain is a free glycolipid on the plasma membrane and is also known as the low molecular weight antigen of *T. gondii* that elicits a specific IgM immune response in humans during an acute toxoplasmosis. Both GPIs of *T. gondii* stimulate the production of the cytokine TNF-α in macrophages. Hence, the GPIs of *T. gondii* seem to be promising candidates for the development of a vaccine and a diagnostic test.

The use of the GPIs as antigens for the development of a vaccine against *T. gondii* eliminates the risk of causing toxoplamosis associated with a vaccine based on attenuated or inactivated parasites. Moreover, *T. gondii* displays a high antigenic variation and passes through various life cycles, for which reason protein-based vaccines could be rendered ineffective through mutations. Since the biosynthesis of carbohydrates is not template-driven, resistance against the vaccines of this invention is not expected. In addition, protein or DNA-based vaccines are very likely limited to induce protective immunity against all strains of the parasite, but the GPI containing the additional α-Glc on the side chain is most probably common in every genotype.

Due to heterogeneity of GPIs isolated from biological samples and their amphiphilic character, which renders purification of GPI structures challenging, homogeneous samples of these glycolipids are only accessible via chemical synthesis. Further literature shows that isolated GPI structures contain also other glycolipids, which could cause false positive results (*Eur. J. Clin. Microbiol. Infect. Dis.*, **2003**, *22*, 418). With this in mind, the inventors initiated a synthetic program to address the need for a diverse set of homogeneous GPIs and their analogues as a basis for vaccines and diagnostic devices.

Therefore, the objective of the present invention is to provide a synthesis of defined compounds derived from GPIs of *T. gondii*, where the resulting products are suitable for use in a diagnostic test of toxoplasmosis and for covalent linkage to a carrier for use as a vaccine for humans and animals against diseases caused by infection with *T. gondii*. The vaccines described herein are directed against a large spectrum of parasite strains. Further preferred embodiments of the present invention are disclosed in the dependent claims, the description and the examples.

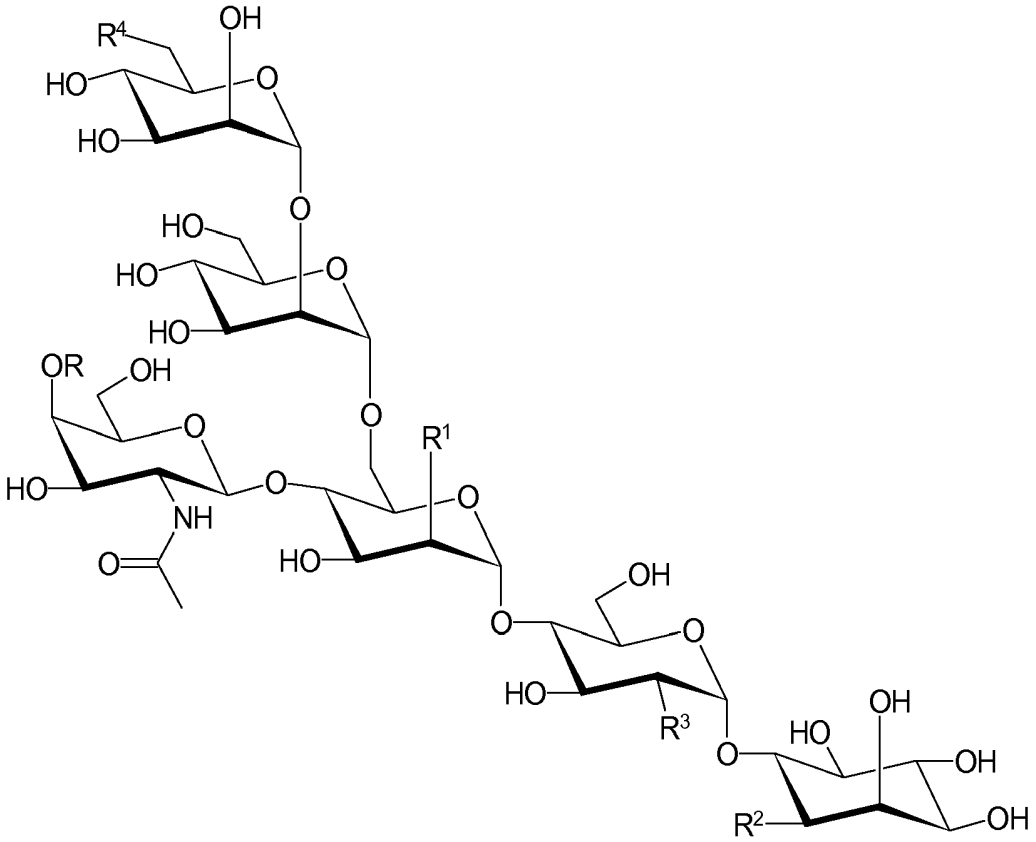
Description of the invention

As used herein, the term "bifunctional linker" refers to a bifunctional molecule containing functional group X and functional group Y, wherein functional group X is capable of reacting with the terminal thiol group of the compounds of general formula (**I**) and the functional group Y is capable of reacting with a carrier.

Saccharides are known by the person skilled in the art as TI-2 (T cell independent-2) antigens and poor immunogens. Therefore, to produce a saccharide-based vaccine, said saccharides are linked or conjugated to a "carrier" to provide a conjugate, which presents an increased immunogenicity in comparison with the saccharide. As used herein, a carrier is a pharmacological or immunological agent that modifies the effect of other agents, such as an active agent or vaccine. The term "carrier" as used herein refers to a compound used as a carrier protein, to which a compound of general formula (**I**) is linked and which enhances the recipient's immune response to the compound of general formula (**I**). In a preferred embodiment, the term "carrier" as used herein refers to a glycosphingolipid with immunomodulatory properties, to which a compound of general formula (**I**) is linked and which enhances the recipient´s immune response to the compound of general formula (**I**).

The term "conventional pharmaceutically acceptable adjuvant" as used herein refers to an immunological adjuvant i.e. a material used in a vaccine composition that modifies or augments the effects of said vaccine by enhancing the immune response to a given antigen contained in the vaccine without being antigenically related to it. For the persons skilled in the art, classically recognized examples immunological adjuvants include, but are not restricted to oil emulsions (e.g., Freund's adjuvant), saponins, aluminium or calcium salts (e.g., alum), non-ionic block polymer surfactants, and many others.

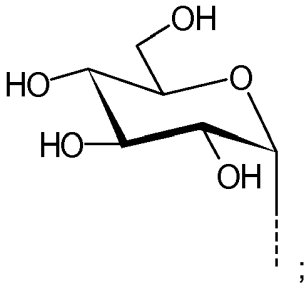
Thus, the present invention relates to compounds of general formula (I)



(I)

wherein

R represents –H, $-CH_3$, $-C_2H_5$, $-C_3H_7$, $-CH(CH_3)_2$, $-C_4H_9$, $-CH_2-CH(CH_3)_2$, $-CH(CH_3)-C_2H_5$, $-C(CH_3)_3$, $-C_5H_{11}$, $-CH(CH_3)-C_3H_7$, $-CH_2-CH(CH_3)-C_2H_5$, $-CH(CH_3)-CH(CH_3)_2$, $-C(CH_3)_2-C_2H_5$, $-CH_2-C(CH_3)_3$, $-CH(C_2H_5)_2$, $-C_2H_4-CH(CH_3)_2$, $-C_6H_{13}$, $-C_3H_6-CH(CH_3)_2$, $-C_2H_4-CH(CH_3)-C_2H_5$, $-CH(CH_3)-C_4H_9$, –Ph, $-CH_2$–Ph, or



;

R^1 and R^4 represent independently of each other –OH, or $-OP(O)(OH)-O-X-NH_2$;

X represents $-CH_2-$, $-C_2H_4-$, $-C_3H_6-$, $-C_4H_8-$, $-C_5H_{10}-$, $-C_6H_{12}-$,

R^2 represents $-SO_2(R^5)$, $-OSO_2(R^5)$, $-OSO_2(OR^5)$, or $-OP(O)(OR^5)(OR^6)$;

R^3 represents –H, –OH, $–NH_2$, $–NHCOCH_3$, $–NHCOCH_2CH_3$, $–NHCOCH_2CH_2CH_3$ or $–N_3$;

R^5 and R^6 represent independently of each other –H, –L–SH, $–(C_2H_4O)_r–CH_2–SH$ or $–(C_2H_4O)_r–C_2H_4–SH$ and R^5 and R^6 cannot be simultaneously –H;

L represents a linker;

and r is an integer from 1 to 40.

Preferably one of R^5 and R^6 is hydrogen, and more preferably R^6 is hydrogen. Thus, R^2 represents preferably $–SO_2(R^5)$, $–OSO_2(R^5)$, $–OSO_2(OR^5)$, or $–OP(O)(OR^5)(OH)$.

Instead of using the disclaimer in the definition of R^5 and R^6, R^5 and R^6 can be defined as follows: R^5 represents –L–SH, $–(C_2H_4O)_r–CH_2–SH$ or $–(C_2H_4O)_r–C_2H_4–SH$ and R^6 represents –H, –L–SH, $–(C_2H_4O)_r–CH_2–SH$ or $–(C_2H_4O)_r–C_2H_4–SH$.

Preferred are compounds of general formula (**I**), wherein R and R^1 to R^4, L, X and r have the meaning as disclosed herein and especially the meanings as disclosed above and wherein R^6 represents hydrogen and R^5 represents –L–SH, $–(C_2H_4O)_r–CH_2–SH$ or $–(C_2H_4O)_r–C_2H_4–SH$ and more preferred R^5 represents –L–SH.

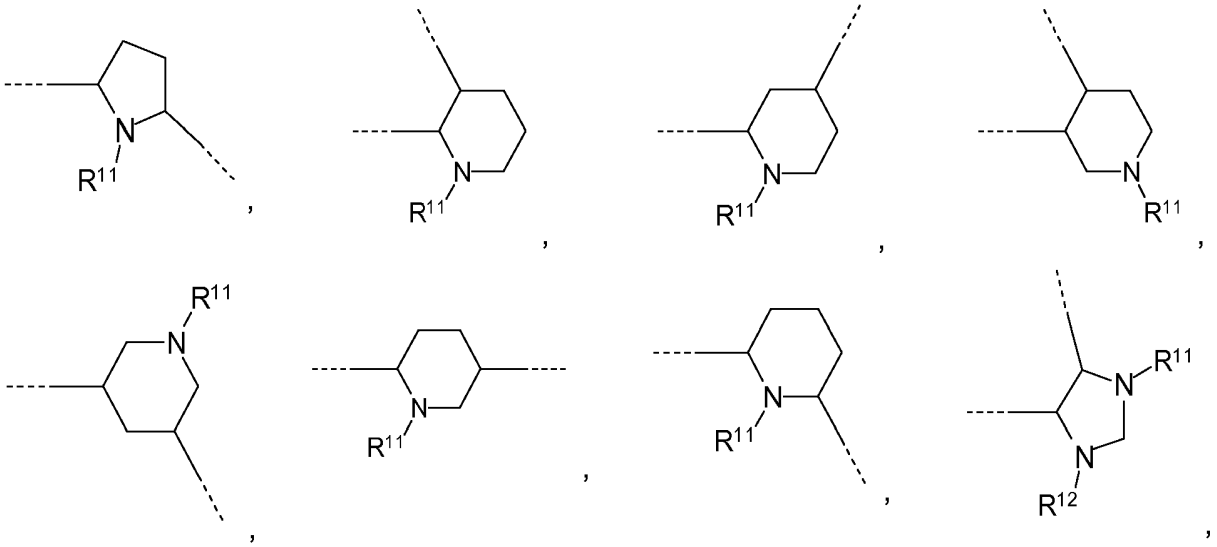
Even more preferred are compounds of general formula (**I**), wherein R^2 represents $–SO_2(L–SH)$, $–OSO_2(L–SH)$, $–OSO_2(O–L–SH)$, or $–OP(O)(O–L–SH)(OH)$ and still more preferred wherein R^2 represents $–OSO_2(O–L–SH)$, or $–OP(O)(O–L–SH)(OH)$ and most preferred wherein R^2 represents $–OP(O)(O–L–SH)(OH)$.

In above formula (**I**), L represents any suitable linker. Preferably L represents a linker containing up to 50 carbon atoms. Further preferred is that this linker L is linked through a carbon atom of the linker to the SH group and through the same or preferably another carbon atom of the linker to the $–SO_2–$, $–OSO_2–$, $–OSO_2–O–$ or the phosphate group $–OP(O)(O–)(O–)$ in the residues $–SO_2–L–SH$, $–OSO_2–L–SH$, $–OSO_2–O–L–SH$, $–OP(O)(OH)(O–L–SH)$ or $–OP(O)(O–L–SH)(O–L–SH)$. This carbon atom linked linker contains up to 50 carbon atoms and preferably up to 40 carbon atoms and more preferably between 3 and 35 carbon atoms and most preferably between 5 and 30 carbon atoms.

More preferably L represents $-L^1-L^2-L^3-$, $-L^1-L^3-$, $-L^1-$, or $-L^1-L^2-L^4-L^5-L^3-$, wherein

L^1 and L^4 represent independently of each other $-(CH_2)_n-$, $-(CH_2)_m-$, $-CHR^7-(CH_2)_m-$, $-(CH_2)_n-CR^7R^8-(CH_2)_m-$, $-o-C_6H_4-$, $-m-C_6H_4-$, $-p-C_6H_4-$;

L^2 and L^5 represent independently of each other $-(CH_2)_p-$, $-(CH_2)_q-$, $-CHR^9-$, $-CR^9R^{10}-$, $-O-$, $-S-$, $-CO-$, $-COO-$, $-O-CO-$, $-NH-CO-$, $-CO-NH-$, $-NH-CO-NH-$, $-o-C_6H_4-$, $-m-C_6H_4-$, $-p-C_6H_4-$, $-NR^{11}-$, $-CH=CH-$;



L^3 represents $-(CH_2)_r-$, $-(CH_2)_r-CR^{13}R^{14}-(CH_2)_s-$, $-o-C_6H_4-$, $-m-C_6H_4-$, $-p-C_6H_4-$;

Compounds of general formula (**I**) are more preferred, wherein R^2 represents $-SO_2(-L^1-L^2-L^3-SH)$, $-OSO_2(-L^1-L^2-L^3-SH)$, $-OSO_2(O-L^1-L^2-L^3-SH)$, or $-OP(O)(O-L^1-L^2-L^3-SH)(OH)$ and still more preferred wherein R^2 represents $-OSO_2(O-L^1-L^2-L^3-SH)$, or $-OP(O)(O-L^1-L^2-L^3-SH)(OH)$ and most preferred wherein R^2 represents $-OP(O)(O-L^1-L^2-L^3-SH)(OH)$ and again even more preferred are compounds of general formula (**I**), wherein R^2 represents $-SO_2(-L^1-L^3-SH)$, $-OSO_2(-L^1-L^3-SH)$, $-OSO_2(O-L^1-L^3-SH)$, or $-OP(O)(O-L^1-L^3-SH)(OH)$ and still more preferred wherein R^2 represents $-OSO_2(O-L^1-L^3-SH)$, or $-OP(O)(O-L^1-L^3-SH)(OH)$ and most preferred wherein R^2 represents $-OP(O)(O-L^1-L^3-SH)(OH)$ and again even more preferred are compounds of general formula (**I**), wherein R^2 represents $-SO_2(-L^1-SH)$, $-OSO_2(-L^1-SH)$, $-OSO_2(O-L^1-SH)$, or $-OP(O)(O-L^1-SH)(OH)$ and still more preferred wherein R^2 represents $-OSO_2(O-L^1-SH)$, or $-OP(O)(O-L^1-SH)(OH)$ and most preferred wherein R^2 represents $-OP(O)(O-L^1-SH)(OH)$.

In these compounds wherein R^2 has the meaning as defined in the afore-mentioned paragraph it is moreover preferred that R^1 represents –OH and/or that R^4 represents –OP(O)(OH)–O–X–NH_2 and especially –OP(O)(OH)–O–CH_2–CH_2–NH_2.

R^7 to R^{10}, R^{13} and R^{14} represent independently of each other –H, –NH_2, –OH, –OCH_3, –OC_2H_5, –OC_3H_7, cyclo-C_3H_5, cyclo-C_4H_7, cyclo-C_5H_9, cyclo-C_6H_{11}, cyclo-C_7H_{13}, cyclo-C_8H_{15}, –Ph, –CH_2–Ph, –CPh_3, –CH_3, –C_2H_5, –C_3H_7, –$CH(CH_3)_2$, –C_4H_9, –CH_2–$CH(CH_3)_2$, –$CH(CH_3)$–C_2H_5, –$C(CH_3)_3$, –C_5H_{11}, –$CH(CH_3)$–C_3H_7, –CH_2–$CH(CH_3)$–C_2H_5, –$CH(CH_3)$–$CH(CH_3)_2$, –$C(CH_3)_2$–C_2H_5, –CH_2–$C(CH_3)_3$, –$CH(C_2H_5)_2$, –C_2H_4–$CH(CH_3)_2$, –C_6H_{13}, –C_3H_6–$CH(CH_3)_2$, –C_2H_4–$CH(CH_3)$–C_2H_5, –$CH(CH_3)$–C_4H_9, –CH_2–$CH(CH_3)$–C_3H_7, –$CH(CH_3)$–CH_2–$CH(CH_3)_2$, –$CH(CH_3)$–$CH(CH_3)$–C_2H_5, –CH_2–$CH(CH_3)$–$CH(CH_3)_2$, –CH_2–$C(CH_3)_2$–C_2H_5, –$C(CH_3)_2$–C_3H_7, –$C(CH_3)_2$–$CH(CH_3)_2$, –C_2H_4–$C(CH_3)_3$, –$CH(CH_3)$–$C(CH_3)_3$, –$CH=CH_2$, –CH_2–$CH=CH_2$, –$C(CH_3)=CH_2$, –CH=CH–CH_3, –C_2H_4–$CH=CH_2$, –C_7H_{15}, –C_8H_{17}, –CH_2–CH=CH–CH_3, –CH=CH–C_2H_5, –CH_2–$C(CH_3)=CH_2$, –$CH(CH_3)$–CH=CH, –$CH=C(CH_3)_2$, –$C(CH_3)$=CH–CH_3, –CH=CH–CH=CH_2, –C_3H_6–$CH=CH_2$, –C_2H_4–CH=CH–CH_3, –CH_2–CH=CH–C_2H_5, –CH=CH–C_3H_7, –CH_2–CH=CH–CH=CH_2, –CH=CH–CH=CH–CH_3, –CH_2NH_2, –CH_2OH, –CH_2–CH_2NH_2, –C_6H_4–OCH_3, –C_6H_4–OH, –CH_2–CH_2–OCH_3, –CH_2–CH_2OH, –CH_2–OCH_3, –CH_2–C_6H_4–OCH_3, –CH_2–C_6H_4–OH,

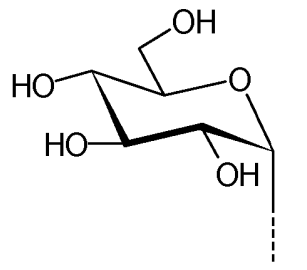
R^{11} and R^{12} represent independently of each other cyclo-C_3H_5, cyclo-C_4H_7, cyclo-C_5H_9, cyclo-C_6H_{11}, cyclo-C_7H_{13}, cyclo-C_8H_{15}, –Ph, –CH_2–Ph, –CPh_3, –CH_3, –C_2H_5, –C_3H_7, –$CH(CH_3)_2$, –C_4H_9, –CH_2–$CH(CH_3)_2$, –$CH(CH_3)$–C_2H_5, –$C(CH_3)_3$, –C_5H_{11}, –$CH(CH_3)$–C_3H_7, –CH_2–$CH(CH_3)$–C_2H_5, –$CH(CH_3)$–$CH(CH_3)_2$, –$C(CH_3)_2$–C_2H_5, –CH_2–$C(CH_3)_3$, –$CH(C_2H_5)_2$, –C_2H_4–$CH(CH_3)_2$, –C_6H_{13}, –C_3H_6–$CH(CH_3)_2$, –C_2H_4–$CH(CH_3)$–C_2H_5, –$CH(CH_3)$–C_4H_9, –CH_2–$CH(CH_3)$–C_3H_7, –$CH(CH_3)$–CH_2–$CH(CH_3)_2$, –$CH(CH_3)$–$CH(CH_3)$–C_2H_5, –CH_2–$CH(CH_3)$–$CH(CH_3)_2$, –CH_2–$C(CH_3)_2$–C_2H_5, –$C(CH_3)_2$–C_3H_7, –$C(CH_3)_2$–$CH(CH_3)_2$, –C_2H_4–$C(CH_3)_3$, –$CH(CH_3)$–$C(CH_3)_3$, –$CH=CH_2$, –CH_2–$CH=CH_2$, –$C(CH_3)=CH_2$, –CH=CH–CH_3, –C_2H_4–$CH=CH_2$, –C_7H_{15}, –C_8H_{17}, –CH_2–CH=CH–CH_3, –CH=CH–C_2H_5, –CH_2–$C(CH_3)=CH_2$, –$CH(CH_3)$–CH=CH, –$CH=C(CH_3)_2$, –$C(CH_3)$=CH–CH_3, –CH=CH–CH=CH_2, –C_3H_6–$CH=CH_2$, –C_2H_4–CH=CH–CH_3, –CH_2–CH=CH–C_2H_5, –CH=CH–C_3H_7, –CH_2–CH=CH–CH=CH_2, –CH=CH–CH=CH–CH_3, –CH_2NH_2, –CH_2OH, –CH_2–CH_2NH_2,

$-C_6H_4-OCH_3$, $-C_6H_4-OH$, $-CH_2-CH_2-OCH_3$, $-CH_2-CH_2OH$, $-CH_2-OCH_3$, $-CH_2-C_6H_4-OCH_3$, $-CH_2-C_6H_4-OH$,

n, m, r and s represent independently of each other an integer from 1 to 20;
p and q represent independently of each other an integer from 0 to 5.

The compounds of the general formula (**I**) are capable of evoking a very specific immune response in such a way that antibodies are produced in a host, which do not show any significant cross activities to other related compounds.

Further, the compounds of the general formula (**I**) are particular useful since these compounds are designed of being capable of binding to a carrier useful in vaccination, ensuring the correct orientation of the glycan in presence of free amine groups, which are characteristic to GPIs. The correct orientation of the glycan in presence of free amine is ensured by the substituent(s) R^5 and/or R^6 that present a terminal thiol group. The free terminal thiol group is more nucleophilic than the free amines under neutral or acid pH conditions, which are specific to the conjugation reaction, and therefore ensures natural orientation of the glycan on the carrier. This is a major advantage of using the terminal thiol group over, for instance, an amino or hydroxyl group.

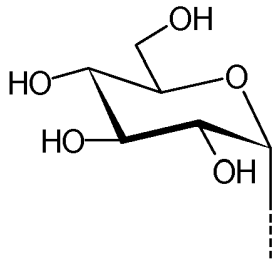
Preferred substituents for R are: –H and 

Preferred substituents R^3 are: $-NH_2$, –OH, and $-NHCOCH_3$.

Preferred substituents for R^5 and R^6 are: $-CH_2-SH$, $-C_2H_4-SH$, $-C_3H_6-SH$, $-C_4H_8-SH$, $-C_5H_{10}-SH$, $-C_6H_{12}-SH$, $-C_7H_{14}-SH$, $-C_8H_{16}-SH$, $-C_9H_{18}-SH$, $-C_{10}H_{20}-SH$, –CH=CH–SH, $-C(=O)-(CH_2)_n-SH$, more preferred $-C_5H_{10}-SH$, $-C_6H_{12}-SH$, and $-C_7H_{14}-SH$, and most preferred $-C_6H_{12}-SH$.

Further, in a preferred embodiment of the compounds of formula (**I**) according to the present invention R represents –H.

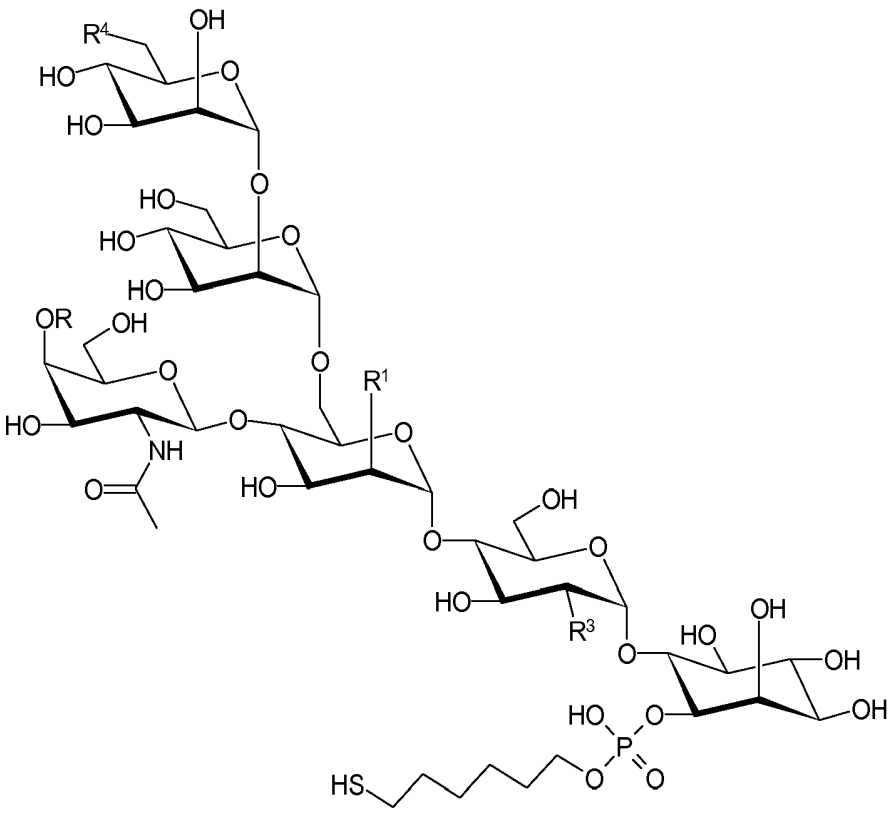
A further preferred embodiment of the present invention refers to compounds of formula (**I**) wherein R represents



In another preferred embodiment of the compounds of formula (**I**) according to the present invention R^1 represents –OH. More preferred are compounds of general formula (**I**), wherein R^1 represents –OH and R represents –H.

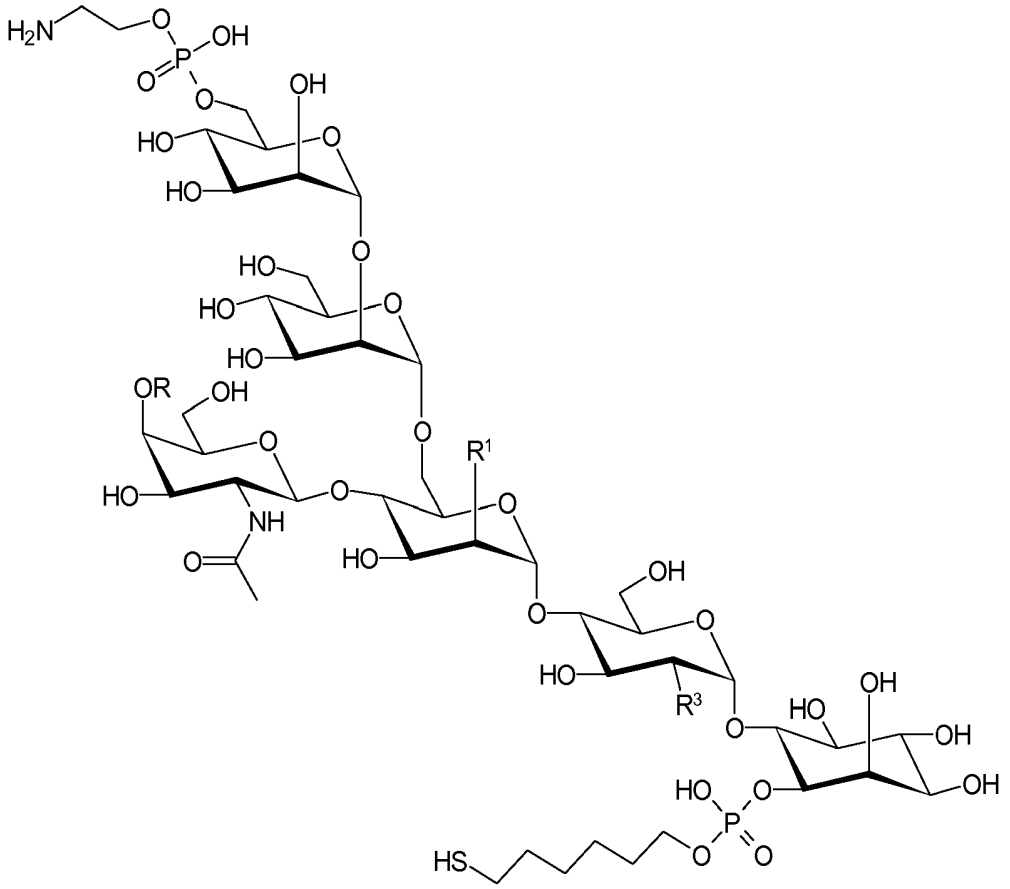
In a particularly preferred embodiment of the compounds of formula (**I**) according to the present invention R^2 represents $-OP(O)(OR^5)(OR^6)$, wherein R^5 represents –H, and R^6 represents $-C_6H_{12}-SH$.

Thus, especially preferred are compounds of the formula (**II**) and (**III**):



(II)

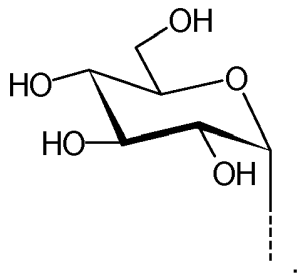
and



(III)

wherein the substituents R^1, R^3 and R^4 in (**II**) and R^1 and R^3 in (**III**) have the meanings as defined herein.

In the formulae (**II**) and (**III**)
R represents preferably –H or



.

The compounds falling under general formula (**I**) – (**III**) are novel so that the present invention relates also to compounds of general formula (**I**) – (**III**), as well as stereoisomers, mixtures of enantiomers, mixtures of diastereomers, tautomers, hydrates, solvates and racemates and pharmaceutically acceptable salts of these compounds.

In yet another preferred embodiment of the present invention, the compound according to the general formula (**I**) is selected from the group comprising or consisting of:

6-*O*-(aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-4-*O*-(α-D-glucopyranosyl-(1→4)-2-deoxy-2-acetamido-β-D-galactopyranosyl)-α-D-manno-pyranosyl-(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(thiohexyl phosphono)-D-*myo*-inositol

6-*O*-(aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-(2-*O*-(aminoethyl phosphono)-4-*O*-(α-D-glucopyranosyl-(1→4)-2-deoxy-2-acetamido-β-D-galactopyranosyl))-α-D-manno-pyranosyl -(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(thiohexyl phosphono)-D-*myo*-inositol

α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-(2-*O*-(aminoethyl phosphono)-4-*O*-(α-D-glucopyranosyl-(1→4)-2-deoxy-2-acetamido-β-D-galactopyranosyl))-α-D-manno-pyranosyl -(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(thiohexyl phosphono)-D-*myo*-inositol

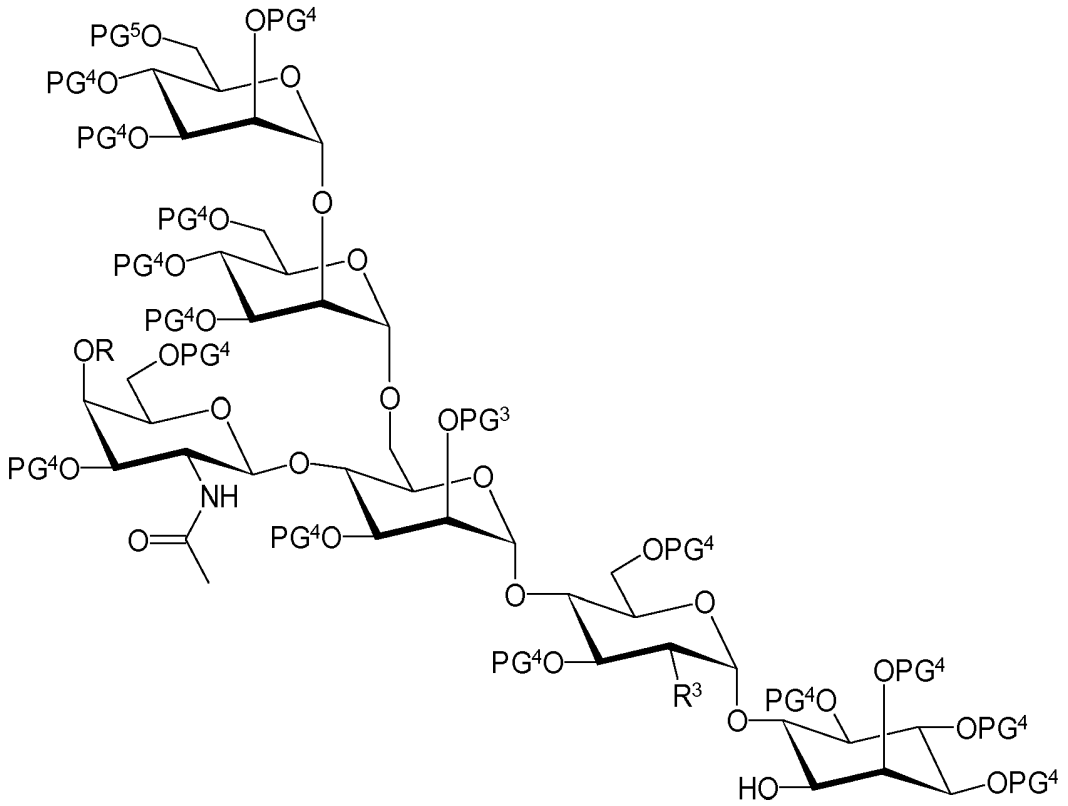
6-*O*-(aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-4-*O*-(2-deoxy-2-acetamido-β-D-galactopyranosyl) -α-D-manno-pyranosyl -(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(thiohexyl phosphono)-D-*myo*-inositol

6-*O*-(aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-(2-*O*-(aminoethyl phosphono)-4-*O*-(2-deoxy-2-acetamido-β-D-galactopyranosyl))-α-D-manno-pyranosyl -(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(thiohexyl phosphono)-D-*myo*-inositol

α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-(2-*O*-(aminoethyl phosphono)-4-*O*-(2-deoxy-2-acetamido-β-D-galactopyranosyl))-α-D-manno-pyranosyl -(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(thiohexyl phosphono)-D-*myo*-inositol

Another aspect of the present invention relates to a method for synthesis of a compound of formula (**I**) according to the following procedures:

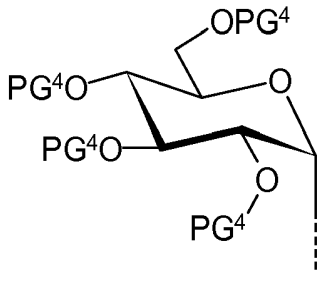
a) providing a compound of the general formula (**IV**)



(**IV**)

wherein

R represents –H, $-CH_3$, $-C_2H_5$, $-C_3H_7$, $-CH(CH_3)_2$, $-C_4H_9$, $-CH_2-CH(CH_3)_2$, $-CH(CH_3)-C_2H_5$, $-C(CH_3)_3$, $-C_5H_{11}$, $-CH(CH_3)-C_3H_7$, $-CH_2-CH(CH_3)-C_2H_5$, $-CH(CH_3)-CH(CH_3)_2$, $-C(CH_3)_2-C_2H_5$, $-CH_2-C(CH_3)_3$, $-CH(C_2H_5)_2$, $-C_2H_4-CH(CH_3)_2$, $-C_6H_{13}$, $-C_3H_6-CH(CH_3)_2$, $-C_2H_4-CH(CH_3)-C_2H_5$, $-CH(CH_3)-C_4H_9$, –Ph, $-CH_2$–Ph, or



,

R^3 represents $-N_3$, and

PG^3 to PG^5 represent suitable protecting groups for hydroxyl functional groups;

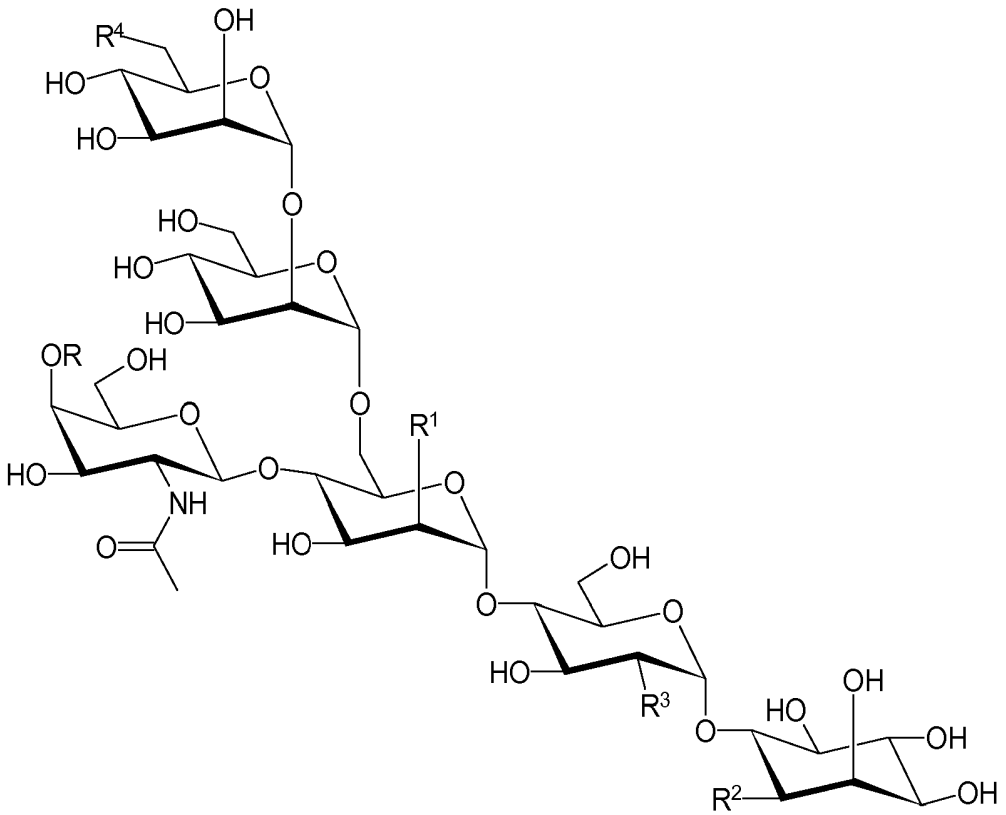
b) introducing the substituent R^2 by reacting a compound of step a) with an acid derivative selected from $H-SO_2(OR^{5'})$ or $H-P(O)(OR^{5'})(OR^{6'})$ or a salt thereof,

wherein $R^{5'}$ and $R^{6'}$ represent independently of each other –H, $–CH_2–S–PG^6$, $–C_2H_4–S–PG^6$, $–C_3H_6–S–PG^6$, $–C_4H_8–S–PG^6$, $–C_5H_{10}–S–PG^6$, $–C_6H_{12}–S–PG^6$, $–C_7H_{14}–S–PG^6$, $–C_8H_{16}–S–PG^6$, $–C_9H_{18}–S–PG^6$, $–C_{10}H_{20}–S–PG^6$, $–CH=CH–S–PG^6$, $–C(=O)–(CH_2)_n–S–PG^6$ and $R^{5'}$ and $R^{6'}$ are not simultaneously –H, wherein PG^6 is a suitable protecting group for a thiol,

c) introducing the substituent R^4 by removing PG^5 or by removing PG^5 and subsequent conversion with an acid of the formula $H–P(O)(OH)–O–C_2H_4–NH(PG^7)$ or a salt thereof, wherein PG^7 is a suitable protecting group for an amine;

d) introducing the substituent R^1 by removing PG^3 or by removing PG^3 and subsequent conversion with an acid of the formula $H–P(O)(OH)–O–C_2H_4–NH(PG^7)$ or a salt thereof, wherein PG^7 is a suitable protecting group for an amine;

e) deprotecting the compound of step d) by removing the protection groups PG^4, PG^6 and PG^7 resulting in a compound of formula (**I**)



(**I**).

wherein R, R^1 – R^4 have the meanings as defined herein,

and wherein the steps c) and d) can be performed consecutively in the row c) and then d) or d) first and then c) thereafter or simultaneously.

The protecting groups PG^3, PG^4, PG^5, PG^6 and PG^7 are commonly used protecting groups in organic synthesis, preferably for amines, hydroxyl groups, thiols, imines, carbonyls or other common functional groups.

More specifically, PG^3, PG^4 and PG^5 preferably are suitable protecting groups for hydroxyl groups, more preferably different suitable protecting groups for hydroxyl groups capable of being removed subsequently one after another by a suitable sequence of deprotection reactions. Preferred protection groups for hydroxyl groups are benzyl, benzoyl, 4-*O*-*p*-methoxybenzyl, allyl, acetyl, methylsulfonylethoxycarbonyl, levulinyl, dimethoxytrityl, 2-naphthylmethyl, triisopropylsilyl, *tert*-butyldimethylsilyl, *tert*-butyldiphenylsilyl, 2-trimethyl-silylethoxymethyl. More specifically, in a preferred embodiment of the present invention protecting group PG^3 may be levulinyl, protecting group PG^4 may be benzyl and protecting group PG^5 may be triisopropylsilyl.
Preferred protecting groups for amines form carbamates such as *tert*-*b*utyloxy carbonyl, 9-fluorenylmethyl carbonyl, allyl carbonyl, trichloroethyl carbonyl, benzylcarboxy carbonyl; or form amides such as acetyl or trichloro acetyl. In a preferred embodiment of the present invention protecting group PG^7 is a benzylcarboxy carbonyl group.

Also, protecting groups for hydroxyl groups may serve as well as protecting groups for thiols. Therefore, preferred protecting groups for thiols groups are benzyl, benzoyl, 4-*O*-*p*-methoxybenzyl, allyl, acetyl, methylsulfonylethoxycarbonyl, levulinyl, dimethoxytrityl, 2-naphthylmethyl, triisopropylsilyl, *tert*-butyldimethylsilyl, *tert*-butyldiphenylsilyl, 2-trimethylsilylethoxymethyl. Specifically, in a preferred embodiment of the present invention protecting group PG^6 is a benzyl group.

According to the present invention steps c) and d) are not supposed of being strictly performed in the order that step c) is accomplished before step d) is performed. Therefore, in one embodiment of the present invention step c) is performed before step d). In another embodiment of the present invention step d) is first performed and afterwards step c) is conducted. Thus, step c) is performed after step d). Therefore, these are two options wherein steps c) and step d) are performed consecutively. However, in another preferred embodiment of the present invention step c) and step d) are performed simultaneously. In such embodiment protection groups PG^3 and PG^5 are removed together and then optionally, substituents R^1 and R^4 being

$H–P(O)(OH)–O–C_2H_4–NH(PG^7)$ or a salt thereof with PG^7 having the meaning as defined herein, are introduced at the same time.

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

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

Further, it is also possible that the compounds of the present invention bear simultaneously basic and acid groups. Further, it may also occur that these basic and acid groups appear to be in close vicinity to one another enabling an intramolecular proton transfer from the acidic group the basic group. Therefore, in a preferred embodiment of the present invention the compound of the formula (**I**) may be zwitter-ionic, bearing at least e.g. one $–O^-$ and one $–NH_3^+$ group.

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

The possibility of a synthesis of the compounds according to formula (**I**) enables production of sufficient and pure amounts of the desired GPI structure, which may support research towards understanding of the pathomechanism of *T. gondii* infection and enables designing a vaccine against *T. gondii*.

Surprisingly it was found that the compounds of general formula (**I**) are suitable to raise an immune response in an animal, and are suitable for vaccination against infectious diseases. Therefore, another aspect of the present invention relates to the use of a compound of general formula (**I**) for vaccination against toxoplasmosis. One embodiment of the invention is further a compound of the general formula (**I**) for vaccination against an infection with *T. gondii*. The invention relates also to the use of a compound of general formula (**I**) for the manufacture of a vaccine against toxoplasmosis.

Further was found that extraordinary potent and stable vaccine can be derived when a compound of general formula (**I**) is covalently linked to a carrier through the terminal thiol group, preferably as thio ether or thio ester. Saccharides are known by the person skilled in the art as TI-2 (T cell independent-2) antigens and poor immunogens. Therefore, to produce a saccharide-based vaccine, said saccharides are linked or conjugated to a "carrier" to provide a conjugate, which presents an increased immunogenicity in comparison with the saccharide. Thus, a compound of general formula (**I**) is linked to a carrier through the terminal thiol group to provide a conjugate consisting on the compound of general formula (**I**) covalently linked to a carrier.

The immune response raised against the conjugate consisting of compound **3** covalently linked to CRM_{197} clearly recognized the parasite (see **Figure 4**, immunofluorescence picture). Analysis of the polyclonal antibodies revealed that the immune response against the conjugate was very specific. The serum antibodies bound compound **3**, but did not show cross reactivity to the structurally close related compound **5,** which is a mammalian structure (see **Figure 3**). It is important to note that compound **3** does not induce cross-reactivity to human structures, which excludes the possibility of raising an autoimmune response.

The immune response raised against the conjugate consisting of compound **4** covalently linked to CRM_{197} clearly recognized the parasite and located the GPI at the apical end of the parasitic cell (see **Figure 13**, immunofluorescence picture). These findings are very important because the apical complex of *T. gondii* is used for invading host cells. Induction of an immune response against this site of the parasite

is capable of blocking the invasion mechanism of *T. gondii*. Together with the opsonizing properties of the raised antibodies, this vaccine can be capable of inducing immunity against this parasite. Analysis of the polyclonal antibodies revealed that the immune response against the conjugate was very specific. The serum antibodies bound compound **4**, but did not show cross reactivity to substructures of this GPI carbohydrate (see **Figure 12**).

The specific immune response both conjugates elicited show that only compounds **3** and **4**, presenting the full glycan structure, are able to induce the production of antibodies *in vivo* that are able to recognize the parasite *T. gondii*. Although WO 1997010249 A1 shows that substructures of GPI carbohydrate covalently attached to a carrier are sufficient to raise an immune response that recognizes the parasite *T. gondii*, our results indicate that these substructures of compound **4** (see **Figure 12**; one trisaccharide and two pentasaccharides with a varying degree of phosphorylation) seem not be sufficient, because they are not recognized by the polyclonal antibody response.

The present invention relates therefore to a compound of general formula (**I**) covalently linked to a carrier. Particularly preferred is that said carrier is a peptidic compound and even more preferred a bacterial peptide or a compound derived from a bacterial peptide. In another preferred embodiment, said carrier is a glycosphingolipid with immunomodulatory properties.

As used herein, a carrier is a pharmacological or immunological agent that modifies the effect of other agents, such as an active agent or vaccine. The term “carrier” as used herein refers to a compound used as a carrier protein, to which a compound of general formula (**I**) is linked and which enhances the recipient's immune response to the compound of general formula (**I**). In a preferred embodiment, the term “carrier” as used herein refers to a glycosphingolipid with immunomodulatory properties, to which a compound a compound of general formula (**I**) is linked and which enhances the recipient´s immune response to the compound of general formula (**I**).

It is preferred that the carrier to which the compound of general formula (**I**) is covalently linked is a carrier protein. For the person skilled in the art, a carrier protein is a protein selected from the group comprising or consisting of: a diphtheria toxoid, a mutated diphtheria toxoid, a modified diphtheria toxoid, a mutated and modified diphtheria toxoid, a tetanus toxoid, a modified tetanus toxoid, a mutated tetanus toxoid, outer membrane protein (OMP), bovine serum albumin (BSA), keyhole limpet hemocyanine (KLH) or cholera toxoid (CT).

It is particularly preferred that the carrier to which the compound of general formula (**I**) is covalently linked, is selected from the group comprising or consisting of: a diphtheria toxoid, a mutated diphtheria toxoid, a modified diphtheria toxoid, a mutated and modified diphtheria toxoid, a tetanus toxoid, a modified tetanus toxoid or a mutated tetanus toxoid. The term "toxoid" as used herein refers to a bacterial toxin (usually an exotoxin) whose toxicity has been inactivated or suppressed either by chemical (formalin) or heat treatment, while other properties, typically immunogenicity, are maintained. A mutated toxoid as used herein is a recombinant bacterial toxin, which has been amended to be less toxic or even non-toxic by amending the wild-type amino acid sequence. Such a mutation could be a substitution of one or more amino acids. A modified toxoid, as used herein, is a bacterial toxoid, on which a functional group X has been introduced by reacting said bacterial toxoid with a bifunctional linker. Thus, the modified toxoid presents a or is modified with functional group X, said functional group X being capable of reacting with the terminal thiol group of the compounds of general formula (**I**). The term "bifunctional linker" refers to a bifunctional molecule containing functional group X and functional group Y, wherein functional group X is capable of reacting with the terminal thiol group on the compounds of general formula (**I**) and the functional group Y is capable of reacting with a carrier. It is especially preferred that the compound of general formula (**I**) is covalently linked to the non-toxic mutated diphtheria toxin CRM_{197}, which is modified with the functional group X. Preferably, the compound of general formula (**I**) is covalently linked to the non-toxic mutated diphtheria toxin CRM_{197}, which is modified with maleimide. In the most preferred embodiment, the compound of general formula (**I**) is covalently linked to the non-toxic mutated diphtheria toxin CRM_{197}, which is modified with α-iodoacetamide.

CRM_{197} like wild-type diphtheria toxin is a single polypeptide chain of 535 amino acids (58 kD) consisting of two subunits linked by disulfide bridges having a single amino acid substitution of glutamic acid for glycine. It is used as a carrier protein in a number of approved conjugate vaccines, such as the pneumococcal vaccine Prevnar 13® (Pfizer Inc.).

In one aspect of the present invention the compounds of the formula (**I**) are covalently linked to a carrier for the use in a vaccine. The binding or covalent linkage to the carrier can be accomplished by first providing a suitable carrier capable of stimulating the immune system's response to a target antigen, but does not in itself confer immunity as defined above. Examples of suitable carriers include, but they are not restricted to peptidic compounds, bacterial peptides, compounds derived from

a bacterial peptides, mutated toxoids and glycosphingolipids with immunomodulatory properties.

In a preferred embodiment of the present invention such a suitable carrier may be CRM_{197}. Said suitable carriers are able to react with the functional group Y of the bifunctional linker to provide a carrier modified with a functional group X. In a preferred embodiment of the present invention the carrier is modified by at least one functional group X of the group comprising or consisting of maleimide; α-iodoacetyl; α-bromoacetyl; *N*-hydroxysuccinimide ester (NHS), 2-pyridyldithiols, thiol and vinyl (see also **Figure 5A**). The introduction of such functional group X on the carrier is preferably accomplished by reaction of a suitable carrier with a bifunctional linker that bears on one side the functional group Y prone of reacting with the suitable carrier and on the other side a functional group X prone to react with the terminal thiol group of the compounds of general formula (**I**).

In a preferred embodiment of the present invention such a bifunctional linker bears on one side a vinyl functional group X prone to react with the terminal thiol group of the compound of general formula (**I**), and on the other side a *N*-hydroxysuccinimide ester functional group Y that is prone of reacting with lysine side amino group of a peptidic compound.

In another preferred embodiment of the present invention such a bifunctional linker bears on one side a maleimide functional group X prone to react with the terminal thiol group of the compound of general formula (**I**), and on the other side a *N*-hydroxysuccinimide ester functional group Y that is prone of reacting with lysine side amino group of a peptidic compound.

Preferably, such a bifunctional linker bears on one side an α-iodoacetyl functional group X prone to react with the terminal thiol group of the compound of general formula (**I**), and on the other side a *N*-hydroxysuccinimide ester functional group Y that is prone of reacting with lysine side amino group of a peptidic compound.

In another embodiment, said carrier is preferably a glycosphingolipid with immunomodulatory properties, and more preferably (2*S*,3*S*,4*R*)-1-(α-D-galactopyranosyl)-2-hexacosanoylaminooctadecane-3,4-diol.

The term glycosphingolipid with immunomodulatory properties, as used herein, refers to a suitable glycosphingolipid capable of stimulating the immune system's response to a target antigen, but which does not in itself confer immunity as defined above.

Glycoshingolipids as used herein are compounds containing a carbohydrate moiety α–linked to a sphingolipid. Preferably, the carbohydrate moiety is a hexopyranose and most preferably is α-D-galactopyranose. For the person skilled in the art, sphingolipids are a class of lipids containing a C18 amino alcohol connected via an amide bond to a fatty acid. The C18 amino alcohol is preferably mono-, di- or polysubstituted with hydroxyl groups. Especially preferred, the C18 amino alcohol is phytosphingosine. The fatty acid is preferably a monocarboxylic acid having a saturated alkyl chain of a number of carbons ranging from 16 to 28 and more preferably from 18 to 26. Glycosphingolipids with immunomodulatory properties include, but they are not restricted to (2*S*,3*S*,4*R*)-1-(α-D-galactopyranosyl)-2-hexacosanoylaminooctadecane-3,4-diol, which can stimulate natural killer (NK) activity and cytokine production by natural killer T (NKT) cells and exhibits potent antitumor activity *in vivo* (*Proc. Natl Acad. Sci.* USA, **1998**, *95*, 5690).

The conjugates of the compounds of general formula (**I**) to the glycosphingolipid with immunomodulatory properties have the advantage of being heat stable. To be suitable for covalent linkage to the compounds of general formula (**I**), on the glycosphingolipid with immunomodulatory properties a functional group X is introduced by reacting the glycosphingolipid with immunomodulatory properties with a bifunctional linker. Thus, a modified glycosphingolipid with immunomodulatory properties presents or is modified with a functional group X prone to react with the terminal thiol group of the compounds of general formula (**I**) preferably by formation of a covalent bond. Preferably, the glycosphingolipid with immunomodulatory properties is modified at the C6 of the carbohydrate moiety. In a preferred embodiment of the present invention the carrier is modified by at least one functional group X of the group comprising or consisting of maleimide, α-iodoacetyl, α-bromoacetyl, *N*-hydroxysuccinimide ester (NHS), 2-pyridyldithiols, thiol and vinyl (see also **Figure 5A**). The introduction of such functional group X on the glycosphingolipid with immunomodulatory properties is preferably accomplished by reaction of a suitable glycosphingolipid with immunomodulatory properties with a bifunctional linker that bears on one side a functional group X prone of reacting with the terminal thiol group of the compound of general formula (**I**) and on the other side a functional group Y that is prone to react with the glycosphingolipid with immunomodulatory properties.

In a preferred embodiment of the present invention such a bifunctional linker bears on one side α-iodoacetyl functional group X prone to react with the terminal thiol group of a compound of general formula (**I**), and on the other side a *N*-

hydroxysuccinimide ester functional group Y that is prone of reacting with an amino group of a glycosphingolipid with immunomodulatory properties.

In another preferred embodiment of the present invention such a bifunctional linker bears on one side maleimide functional group X prone to react with the terminal thiol group of a compound of general formula (**I**), and on the other side a *N*-hydroxysuccinimide ester functional group Y that is prone of reacting with an amino group of a glycosphingolipid with immunomodulatory properties.

Preferably, such a bifunctional linker bears on one side vinyl functional group X prone to react with the terminal thiol group of a compound of general formula (**I**), and on the other side *N*-hydroxysuccinimide ester functional group Y that is prone of reacting with an amino group of a glycosphingolipid with immunomodulatory properties.

For the preferred embodiment wherein the carrier is modified with a vinyl functional group X, a compound of the formula (**I**) can be covalently linked to the vinyl functional group X by a thiol-ene reaction by means of irradiation with UV light and/or in the presence of an initiator to form a stable thioether bond (see also **Figure 5b**).

For the preferred embodiment wherein the carrier is modified with a maleimide functional group X, the terminal thiol group of a compound of general formula (**I**) adds via a Michael addition to the maleimide moiety.

For the preferred embodiment wherein the carrier is modified with a α-iodo-acetamide functional group X, the terminal thiol group of a compound of the formula (**I**) substitutes the iodide of the α-iodo-acetyl moiety.

Further, in the preferred embodiments wherein the carrier is a protein carrier, these functionalization reactions are performed in such a way that more than one functional group X is introduced on the carrier. Thus, the said protein carrier presents or is modified with more than one functional group X. However, it is also possible that just one functional group X is introduced on the carrier. In a preferred embodiment of the present invention the number of functional groups X introduced on the carrier after the functionalization reaction ranges preferably from 5 to 100, more preferably from 10 to 50, and most preferably from 10 to 40. After the carrier was modified with functional group X by one said functionalization reactions, the compounds of the present invention are added in order to bind the terminal thiol group, e.g. to the maleimide double bond by an addition-like reaction. Therein, it is possible to adjust the number of molecule of the formula (**I**) being linked to the carrier. The number of

molecules of the formulas (**I**) being linked to the carrier can range preferably from 1 to 100, more preferably from 3 to 50, and most preferably from 5 to 15. In a preferred embodiment the number of molecules of the formulas (**I**) being linked to a carrier ranges from 1 to 15, more preferably from 2 to 10 and especially preferred from 3 to 7.

Another aspect of the present invention relates to the use of the compound of general formula (**I**) covalently linked to a carrier for vaccination against toxoplasmosis. One embodiment of the invention is further a compound of the general formula (**I**) covalently linked to a carrier for vaccination against an infection with *T. gondii*. The invention relates also to the use of a compound of general formula (**I**) covalently linked to a carrier for the manufacture of a vaccine against toxoplasmosis.

The examples of the present invention show that the compounds of general formula (**I**) linked to a carrier elicited a highly specific antibody response to the compounds of general formula (**I**) in mice, including isotype switching and affinity maturation (see **example 14** and **example 15**). Moreover, the generated antibodies recognized the natural GPI on the parasite (see **example 16**).

Another aspect of the present invention relates to pharmaceutical formulations and pharmaceutical compositions for vaccination containing a compound of general formula (**I**) optionally covalently linked to a carrier as an active ingredient, together with at least one pharmaceutically acceptable carrier, excipient, solvent and/or diluents.

Further preferred, the pharmaceutical composition is formulated in the form of a lyophilisate or liquid buffer solution.

The compound of general formula (**I**) optionally covalently linked to a carrier can also be administered in form of its pharmaceutically active salt optionally using substantially nontoxic pharmaceutically acceptable carrier, excipients, adjuvants or diluents. The pharmaceutical composition, which is used as a vaccine is prepared in a conventional solid or liquid carrier or diluents and may comprise a conventional pharmaceutically acceptable adjuvant at suitable dosage level in a known way. Classically recognized examples of conventional pharmaceutically acceptable adjuvants include oil emulsions (e.g., Freund's adjuvant), saponins, aluminium or calcium salts (e.g., alum), non-ionic block polymer surfactants, and many others.

The inventive pharmaceutical composition may be administered by any appropriate means, including but not limited to inhalation, injection (intravenous, intraperitoneal, intramuscular, subcutaneous) by absorption through epithelial or mucocutaneous linings (oral mucosa, rectal and vaginal epithelial linings, nasopharyngial mucosa, intestinal mucosa); orally, rectally, transdermally, topically, intradermally, intragastrically, intracutaneously, intravaginally, intravasally, intranasally, intrabuccally, percutaneously, sublingually, or any other means available within the pharmaceutical arts. The compounds of the invention of the general formula (**I**) are present in said vaccine formulation in the range of 10 to 1000 µg/g.

It was shown that the GPI of *T. gondii* containing the additional α-Glc in the side chain is identical with the known "low molecular weight antigen" of this parasite and IgM antibodies against this structure could be detected in sera from patients with toxoplasmosis (*J. Biol. Chem.* **1992,** *267*, 11721.). The current ELISA-based diagnostic techniques in order to identify acute toxoplasmosis have a high rate of false-positive results because high IgM responses can be detected even more than a year after a primary infection. Moreover, so far isolates of the parasite are used as antigens for the test, and therefore their quality of production can vary extremely. In literature it has been shown that other glycolipids are included in these isolates, which is one of the reasons for false-positive results (*J. Clin. Microbiol. Infect. Dis.* **2003**, *22*, 418). Disadvantages of overtreatment of healthy and infected patients, especially pregnant women, who would require a stressful diagnosis and need medical treatment including pyrimethamine, sulfadiazine, and folinic acid (PSF) after 18 weeks of gestation, which are partially inhibitors of the enzyme dihydrofolate reductase and cause severe side effects such as cardiac arrhythmias and leukopenia, are obvious. Furthermore inhibition of the dihydrofolate reductase can lead to congenital malformations, including neural tube defects, which are of course harmful for the unborn child (*PLoS Negl Trop Dis.* **2011**; *5(9)*, 1333). A test based on a single synthetic antigen is therefore a clear advantage over the commercially available diagnostic tools.

Moreover the inventors could show that the compounds according to formula (**I**) can be used in immunological assays for diagnosis of diseases caused by *T. gondii*. Such assays comprise, for instance, microarray and ELISA useful for diagnosis of diseases caused by *T. gondii*. Therefore another aspect of the present invention refers to the use of a compound of formula (**I**) for diagnosis of toxoplasmosis. Thus, especially preferred embodiments of the present invention relate to pure synthetic compounds of formula (**I**) for diagnosis of toxoplasmosis.

It is preferred that the compound of formula (**I**) is used for diagnosis of acute toxoplasmosis. Acute toxoplasmosis is characterized by a high-titer of IgM in the serum of the patients and the fact that IgG is not present or only with a low-titer. It is preferred that the compound of formula (**I**) is used for the differential diagnosis of acute toxoplasmosis, that means that the compound of formula (**I**) is used in a diagnostic test which allows not only to determine if a patient is infected with *T. gondii*, but also to differentiate between an acute infection and a latent or chronic infection.

Thus, one especially preferred embodiment of the present invention relates to the use of only one specific defined compound of formula (**I**) for diagnosis of toxoplasmosis. It is further preferred that the compound of formula (**I**) used for diagnosis of toxoplasmosis is substantially pure, having a purity of ≥ 95%, preferably ≥ 96%, more preferably ≥ 97%, still more preferably ≥ 98%, and most preferably ≥ 99%. In addition, the chemically synthesized compound of formula (**I**) does not have any microheterogenicity as the oligosaccharides from biological sources do. Nevertheless, the use of a mixture of different compounds of formula (**I**) for diagnosis of toxoplasmosis is possible, but is less preferred.

There are different possibilities for the choice of an assay system in which a compound of formula (**I**) is used for diagnosis of toxoplasmosis. An assay conducted for diagnostic purposes according to the invention may be an immune assay like a solid-phase enzyme immunoassay (EIA), an enzyme linked immunosorbent assay (ELISA), especially an “indirect" ELISA, a radioimmune assay (RIA) or a fluorescence polarization immunoassay. For the use of a compound of formula (**I**) in such assays it could be necessary to immobilize the compound of formula (**I**) on a carrier material, preferably a solid carrier material.

Therefore a compound of formula (**I**) may be immobilized on a carrier material, particularly for diagnostic applications. One preferred embodiment of the present invention is a compound of general formula (**I**) immobilized on a carrier material by covalent bonding. One particularly preferred embodiment of the present invention is a compound of general formula (**I**) immobilized on a carrier material by direct or indirect covalent bonding. Thereby direct covalent bonding is especially preferred.

There are also carrier materials commercially available made from polymers with reactive functional introduced for covalent bonding. One example are microplates named CovaLink™ NH by Thermo scientific, which allow covalent binding through a secondary amine group.

In a preferred embodiment the solid carrier material is selected from the group comprising or consisting of: glass slides, microtitre plates, test tubes, microspheres, nanoparticles or beads.

It is particularly preferred that the carrier material is a glass slide or a microtitre plate. A microtitre plate or microplate or microwell plate, is a flat plate with multiple "wells" used as small test tubes. Typically a microtitre plate having 6, 24, 96, 384 or even 1536 sample wells can be used. Microplates are produced from many different materials, like polycarbonate for microtitre plate used for PCR. The most common is polystyrene as used for most optical detection microplates. It can be colored white by the addition of titanium dioxide for optical absorbance or luminescence detection or black by the addition of carbon for fluorescent biological assays.

"Direct covalent bonding" as used herein refers to immobilization of a compound of general formula (**I**) by reacting a functional group of the compound of general formula (**I**) with a functional group of the material the carrier material is made from. It is preferred that the functional group of the compound of general formula (**I**) is R^2 as defined above. Possible reactive, functional groups of the carrier material may be: thiols, carbonyls, carboxyls, vinyls, halides such as fluorides, chlorides, bromides and iodides, maleimides, succinimide esters.

"Indirect covalent bonding" as used herein refers to immobilization of a compound of general formula (**I**) on a carrier material, wherein the compound of general formula (**I**) is covalently linked to a second compound, which mediates the immobilization to the carrier material. It is preferred that this second compound is a protein, which does not cause an immune reaction. It is important that the second compound itself is most probably not bound by any antibody present in the blood or serum of a patient to avoid false positive results. Further the second compound should be able to be immobilized on the carrier material, by covalent or non-covalent bonding. It is preferred that this second compound is selected from the group comprising or consisting of bovine serum albumin (BSA), human serum albumin (HAS), gelatin or casein. The immobilization using indirect covalent bonding therefore refers preferably to covalent bonding of a compound of general formula (**I**) to a protein as a second compound (e.g. using the free amino groups of a protein) and subsequently binding of the protein to the carrier material by covalent bonding or non covalent interaction between the carrier material and the protein. Possible non-covalent interactions are: hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions. Many polymers, such as polystyrene and polypropylene are

hydrophobic in nature. Nevertheless there are also manufacturers, which supply carrier materials having specialized surfaces optimized for different adhesion conditions.

However, immobilization, especially using indirect covalent bonding, may also occur by strong adhesion. Thus, an effective immobilization according to the present invention may be realized not only by chemical bonding, but also unbound by immobilization related to physisorption. As key feature for physisorption acts the phenomenon that the force for adhesion is caused by van der Waals force. The term "unbonded" refers to a bonding other than covalent bonding.

Chemisorption as immobilization form according to the present invention uses chemical bonds between the carrier material and a compound of formula (**I**). Such bond may be covalent, but may also be ionic. Compounds of the general formula (**I**) can therefore be covalently attached via chemisorption to for example gold or silver nanoparticles as well as surfaces consisting of those noble metals and CdSe quantum dots.

In a preferred embodiment of the present invention immobilization of a compound of the formula (**I**) on a carrier material is realized by direct covalent bonding namely a chemical reaction between these two reactants, preferably by a substitution reaction. In a more preferred embodiment of the present invention the carrier material is modified with a functional group, which is capable of leaving the carrier material upon reaction with the compound of the present invention. Such functional group may be bound directly to a composing molecule of the carrier material or may be bound to a linker, which is directly bound to the composing molecule of the carrier material. Thus, in a more preferred embodiment of the present invention the carrier material is modified to bear a suitable leaving group. Suitable leaving groups may be halides such as chlorides, bromides and iodides, succinimide esters, and esters. Such leaving groups may be or may be incorporated in maleimide, α-iodoacetyl, α-bromoacetyl, *N*-hydroxysuccinimide ester (NHS) and 2-pyridyldithiols. In yet more preferred embodiment, the leaving group on the carrier material is capable to preferably react with thiols, preferably upon proton exchange. In a preferred embodiment of the present invention the carrier material is functionalized with a succinimidyl hydroxide functional group, more preferably *N*-succinimidyl hydroxide, which will leave the carrier material upon reaction with a compound of the present invention as *N*-hydroxysuccinimide.

Modification of the carrier material by introduction of a suitable leaving group is preferably carried out by reaction of an unmodified carrier material with a reactive bifunctional molecule A, preferably a bifunctional molecule with a molecular bridge or spacer arm between the two functional groups: a functional group A1 prone to react with the carrier material and a functional group A2, which is a suitable leaving group. In a preferred embodiment of the present invention functional groups A1 willingly reacting with the carrier material comprises sulfosuccinimide esters and succinimides. One further preferred aspect of the bifunctional molecules A is the ability of providing the functional group meant to bind with a compound of the formula (**I**) is an appropriate distance to the carrier material. Such an appropriate distance is provided by a molecular bridge or spacer arm of suitable length. Such a molecular bridge or spacer arm may have a length preferably from 3 Å (10^{-10} m) to 10 nm, more preferably from 5 Å to 50 Å, and most preferably from 6 Å to 30 Å. Suitable reactive bifunctional molecules for modification of the carrier material comprise succinimidyl (4-iodoacetyl) aminobenzoate (sulfo-SIAB), succinimidyl-3-(bromoacetamido)propionate (SBAP), disuccinimidyl glutarat (DSG), 2-pyridyldithiol-tetraoxatetradecane-N-hydroxysuccinimide (PEG-4-SPDP) (see **Figure 7**).

In a preferred embodiment the assay conducted for diagnostic purposes according to the invention is a fluorescence polarization assay. For such assay, the compound of general formula (**I**) is immobilized on a fluorescent carrier material. The compound of general formula (**I**) covalently bound to a fluorescent carrier material could be used for detection of antibodies in human sera specific to the compounds or general formula (**I**) by fluorescence polarization. Fluorescence polarization is a known powerful method for the rapid and homogeneous analysis of molecular interactions in biological and chemical systems. The principles of fluorescence polarization are based on the excitation of a fluorescent molecule with polarized light. This results in the emission of photons in the plane, which is parallel and perpendicular to the excitation plane and yields information about the local environment of the fluorescent molecule.The rotation of fluorescent molecule in solution can be observed by measuring the rotation of the plane of polarization of the light that was originally beamed in. The observed rotation depends on the rotation relaxation time and is only influenced by the temperature, the viscosity and the molecular weight of the fluorescent molecule. Thus, fluorescence polarization is a suitable method for measuring these parameters and in particular changes in these parameters. The term “fluorescent carrier material” as used herein refers to a compound that absorbs light energy of a specific wavelength and re-emits light at a longer wavelength. In a preferred embodiment, the fluorescent carrier material is fluorescein isothiocyanate

(FITC), which has an excitation/emission peak at 495/517 nm and can be coupled to the compounds of general formula (**I**) through the isothiocyanate group.

Another aspect of the present invention is the use of a compound of general formula (**I**) immobilized on a carrier material by covalent bonding for diagnosis of toxoplasmosis. The diagnosis of acute toxoplasmosis is preferred.

One embodiment of the present invention relates to a kit comprising at least one compound of general formula (**I**) immobilized on a carrier material by covalent bonding or the compound of general formula (**I**) for immobilization on a carrier.

A kit in molecular biology or in medical diagnostics is a package, which includes all necessary ingredients for performing a certain method or singular step. Standard chemicals as present in any standard molecular biology or medical laboratory are normally not included. Nevertheless, some of these standard chemicals may be indispensable to carry out the diagnosis or the immobilization properly. It is understood that all ingredients are provided in quantities that allow for a proper execution of the desired reactions for the majority of scientific, diagnostic and industrial applications.

Often, but not always, these ingredients are provided in already prepared solutions ready- or close to ready-for-use. There may be also combinations of different ingredients already added together. A further advantage is that such kits use to be verified. Therefore the operator doesn't have to prove again the viability of the diagnostic method and can save on at least some control experiments.

Such a kit according to the invention shall include at least the following components:

A) compound of general formula (**I**) immobilized on a carrier material by covalent bonding
B) at least one antibody, like detection antibody
C) a standard solution

The following components may also be included in such kits:

D) blocking solution
E) wash solution
F) sample buffer

An antibody in the kit may be a specific antibody, which can be used as a capture antibody. But preferably it is at least an enzyme-linked secondary antibody used as detection antibody that binds specifically to antibody's Fc region. For quantitative determinations, the optical density (OD) or fluorescence of the sample is compared to a standard curve, which is typically a serial dilution of a known-concentration solution of the target molecule (a standard solution). A blocking solution may be a solution of a non-reacting protein, such as bovine serum albumin or casein, which is added to block any plastic surface in the well that remains uncoated by the antigen. Washing solutions are used to remove unbound components. A sample buffer may be used to dilute the sample of the patient (blood, serum, urine) so that the concentration of the target molecule is in the range which can normally be detected by the test system used.

If the kit shall be allow for the immobilization of a compound of general formula (**I**) on a solid carrier material the kit should include at least:

A) A compound of general formula (**I**)
B) A carrier material, like a microtiter plate

Thereby the carrier material may be modified, for example the carrier material may be modified with a functional group as described above.

The following components may also be included in such kits:

C) blocking solution
D) wash solution
E) reaction buffer

Description of the Figures

Figure 1: Reaction scheme for the preparation of glycans **3**, **4** and **5** as examples for a phosphate linked GPI.

Figure 2: Reaction scheme for the preparation of glycan **22** as example for a sulfone linked GPI.

Figure 3: left side: Analysis of pooled serum that was obtained 4 weeks after 1st immunization of Balb/c mice with a conjugate consisting of CRM_{197} covalently linked to compound **3;**
right side: Printing pattern: all compounds were printed in a 3x3 pattern and in a concentration of 1 mM; left, upper corner: compound **5**; left lower corner: compound 3; right, upper corner: compound **4**; right lower corner: (α-D-Mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→6)-(α-D-mannopyranosyl)-(1→4)-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→6)-1-O-(6-thiohexyl phosphono)-D-myo-inositol (*Chem. Eur. J.* **2005**, *11*, 2493). From the fluorescence pattern it can be seen that such specific antibodies were produced by the mouse that these antibodies specifically evoke a binding to compound **3**, and not to the structurally very related compounds **4**, **5** and (α-D-Mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→6)-(α-D-mannopyranosyl)-(1→4)-(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→6)-1-O-(6-thiohexyl phosphono)-D-myo-inositol.

Figure 4: Fluorescence microscopic image of tachyzoiten stained with serum that was obtained after immunization of Balb/c mice with a conjugate consisting of compound **3** covalently linked to CRM_{197}; blue: cell nucleus, DAPI; green: fluorescent secondary antibody. The fluorescence image shows that the mouse serum which was derived from a mouse immunized with a conjugate consisting of compound **3** covalently linked to CRM_{197} effectively binds to tachyzoites of *T. gondii.*

Figure 5: **A)** Possible functional groups X being attached to a suitable carrier; **B)** Possible reaction pathway of attaching a compound of the general formula (I) to a carrier modified with a vinyl functional group X by an thiol-ene reaction upon activation by irradiation of light and/or by a radical starter.

Figure 6: Preparation of conjugates of compounds **3** and **4** with CRM_{197} for immunization: a) **maleimide-modification of CRM_{197}** PBS, pH = 7.4, room temperature, 2 h b) **coupling of compounds 3 and 4:** PBS, pH = 7.4, room temperature, 3 h.

Figure 7: Selection of reactive bifunctional molecules A suitable for modifying a carrier material for subsequent introduction of a compound of the formula (**I**) on the carrier material by direct bonding.

Figure 8: Preparation of the conjugate of compound **4** with CRM_{197} for immunization: a) **α–iodoacetamide modification of CRM_{197}**: PBS, pH = 7.4, 1 h, room temperature; b) **coupling of compound 4**: compound **4**, PBS, pH = 8.5, 3 h.

Figure 9: MALDI-TOF analysis of **A)** CRM_{197} (blue, 58.5 kDa), **B)** CRM_{197}-iodoacetamide (red, 68 kDa) and **C)** CRM_{197}-GPI conjugate of compound **4** with CRM_{197} (black, 72 kDa); **D)** Comparison of the MALDI-TOF analyses of CRM_{197} (blue, 58.5 kDa), CRM_{197}-iodoacetamide (red, 68 kDa) and CRM_{197}-GPI conjugate of compound **4** with CRM_{197} (black, 72kDa). As a Matrix 2',4',6' – Trihydroxyacetophenone (THAP) was used.

Figure 10: In flow preparation of the conjugate of compound **4** with a vinyl-modified glycosphingolipid with immunomodulatory properties.

Figure 11: Serum antibody levels against compound **4** in mice immunized with the conjugate obtained as described in example 11e: **A)** Total serum IgG levels; **B)** IgG subclass levels; Bars represent mean values averaged over all mice including standard error of the mean; FI = fluorescence intensity.

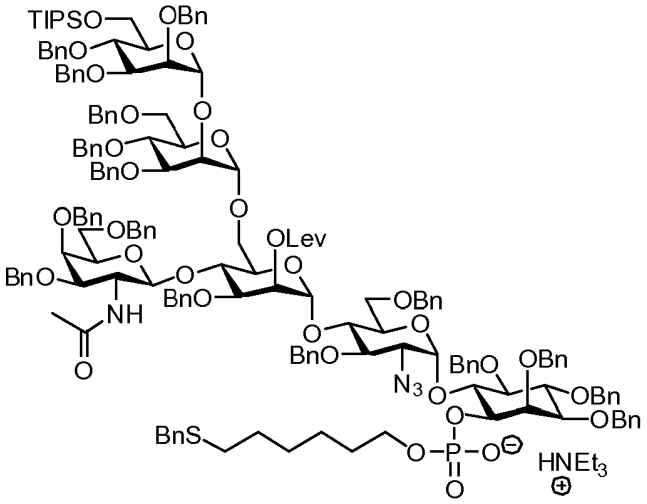
Figure 12: Specificity and epitope recognition of the antibody response: Pictures of microarrays incubated with serum (dilution 1 : 1000) of the three mice six weeks after 1^{st} immunization and a secondary fluorescent antibody directed against mouse IgG. Compound **4** as well as the shown substructures were printed at 100µM.

Figure 13: Recognition of the natural GPI antigen displayed on the *T. gondii* parasite by the antibodies raised against compound **4**: IF pictures of paraformaldehyde-fixed purified *T. gondii* tachyzoites grown in human foreskin fibroblasts stained with (A) DAPI (B) pooled serum from immunized mice and a secondary FITC-conjugated anti-mouse-IgG (C) differential interference contrast picture and (D) merge of (A), (B) and (C). Full circle in (C) indicates the apical and half circle the basolateral end of the parasite (white bar = 5 µm).

Experimental Part

Part A1 : Preparation of phosphate linked thiol functionalized GPI

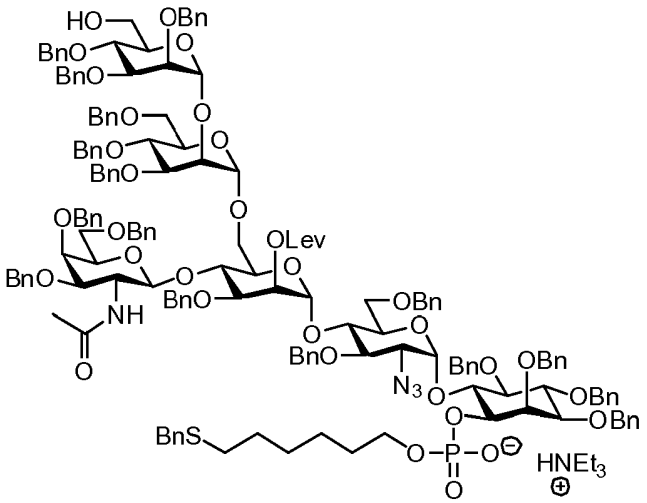
Example 1: Triethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-tri*iso*propylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-3-*O*-benzyl-2-*O*-levulinyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (compound 12)



2,3,4-Tri-*O*-benzyl-6-*O*-tri*iso*propylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-3-*O*-benzyl-2-*O*-levulinyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-allyl-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**compound 9**) (20mg, 7.26µmol, 1equiv) and triethylammonium 6-(benzylthio)hexyl phosphonate (**compound 10**) (12.7mg, 33µmol, 4.5equiv) are co-evaporated 3 times with 2mL dry pyridine. The residue is dissolved in 2mL dry pyridine and PivCl (6.70µL, 54µmol, 7.5equiv) is added. The solution is stirred for 2h at r.t. before water (10µL, 0.56mmol, 76equiv) and iodine (10.1mg, 40µmol, 5.5 equiv) are added. The red solution is stirred for 1h and is quenched with sat. $Na_2S_3O_3$. The reaction mixture is diluted with 10mL CHCl3 and dried over Na_2SO_4. The solvents are removed *in vacuo* and the residue is purified through flash column chromatography (starting from CHCl3/MeOH 0%->5% MeOH) to yield yellow oil (18mg, 5.9µmol, 82%).

$[\alpha]_D^{20}$ = + 32.6 (c =1.00 in $CHCl_3$); ν_{max} (neat) 2926, 2864, 2107, 1742, 1720, 1677, 1454, 1098, 1059, 1028cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.35 (d, *J* = 7.4 Hz, 2H), 7.31 – 7.04 (m, 81H), 6.99 (dd, *J* = 6.6, 2.8 Hz, 2H), 5.94 (d, *J* = 8.8 Hz, 1H, N**H**), 5.86 (d, *J* = 3.7 Hz, 1H, GlcNH$_2$-1), 5.27 – 5.24 (m, 2H, ManI-2), 5.09 (d, *J* = 1.2 Hz, 1H), 4.96 (d, *J* = 12.0 Hz, 1H, CH_2 of Bn), 4.92 – 4.81 (m, 3H, CH_2 of Bn), 4.81 – 4.57 (m, 11H), 4.57 – 4.48 (m, 4H), 4.48 – 4.38 (m, 4H), 4.38 – 4.19 (m, 12H), 4.11 – 3.72 (m, 17H), 3.69 (dd, *J* = 9.7, 7.0 Hz, 1H), 3.63 – 3.54 (m, 8H), 3.52 (t, *J* = 6.4 Hz, 1H), 3.50 – 3.30 (m, 8H), 3.25 (dd, *J* = 7.8, 3.9 Hz, 2H), 3.05 (dd, *J* = 10.2, 3.7 Hz, 1H, GlcNH$_2$-2), 2.86 (q, *J* = 7.3 Hz, 6H, NC**H**$_2$CH$_3$), 2.28 (t, 2H, -S-C**H**$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-O), 2.23 – 2.08 (m, 4H, CH_2 of Lev), 1.83 (s, 3H, NHAc), 1.56 – 1.48 (m, 5H, CH_3 of Lev, -S-CH$_2$-CH$_2$-CH$_2$-CH$_2$-C**H**$_2$-CH$_2$-O), 1.42 ($m_{centered}$, 2H, -S-CH$_2$-C**H**$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-O), 1.26 – 1.14 (m, 13H, NCH$_2$C**H**$_3$, -S-CH$_2$-CH$_2$-C**H**$_2$-C**H**$_2$-CH$_2$-CH$_2$-O), 1.00 – 0.93 (m, 21H, TIPS); ^{13}C NMR (151 MHz, $CDCl_3$) δ 205.92 (ketone of Lev), 171.77 (**C**O of Lev), 170.35 (**C**ONH), 140.05, 139.05, 138.95, 138.88, 138.83, 138.75, 138.68, 138.58, 138.57, 138.40, 138.33, 138.24, 138.05, 137.99, 137.71, 128.92, 128.64, 128.62, 128.59, 128.57, 128.54, 128.46, 128.42, 128.40, 128.36, 128.32, 128.28, 128.26, 128.21, 128.14, 128.11, 128.09, 128.08, 128.00, 127.96, 127.84, 127.77, 127.72, 127.68, 127.67, 127.61, 127.56, 127.51, 127.49, 127.44, 127.28, 127.14, 126.97, 126.94, 100.58, 99.48, 98.79, 98.66, 96.51 (GlcNH$_2$-1), 81.99, 81.75, 81.23, 80.89, 80.52, 79.77, 79.24, 76.01, 75.76, 75.73, 75.57, 75.44, 75.34, 75.13, 74.76, 74.57, 74.40, 74.37, 74.26, 74.24, 74.20, 73.93, 73.85, 73.62, 73.27, 73.15, 72.96, 72.68, 72.40, 72.37, 72.32, 72.28, 72.09, 71.57, 71.49, 71.07, 70.77, 69.95 (ManI-2), 69.76, 68.98, 68.74, 66.74, 65.73, 65.69, 63.71 (GlcNH$_2$-2), 62.94, 53.25, 45.58, 37.90, 36.38, 31.46, 31.07, 31.02, 29.69, 29.30, 28.82, 28.06, 25.55, 23.22, 18.24, 18.18, 12.17, 8.74; ^{31}P NMR (162 MHz, $CDCl_3$) δ -0.30; *m/z* (ESI) Found: $[M+Na]^+$, 3062.3573 $C_{177}H_{205}N4O_{35}PSSi$ requires $[M+Na]^+$, 3062.3577.

Example 2: Triethylammonium 2,3,4-Tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-3-*O*-benzyl-2-*O*-levulinyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (compound 18)

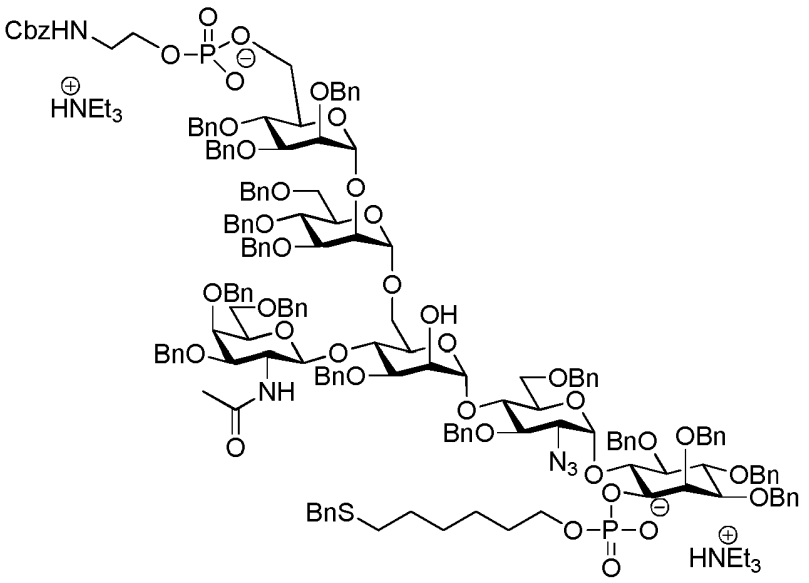


Triethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-tri*iso*propylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-3-*O*-benzyl-2-*O*-levulinyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**compound 12**) (59mg, 19µmol, 1 equiv) is dissolved in 2mL MeCN. Water (13.5µL, 0.75mmol, 40equiv) and $Sc(TfO)_3$ (18.5mg, 38µmol, 2equ.) are added and the solution is heated up to 50°C for 5h. The reaction is quenched with pyridine (7.6µL, 94µmol, 5 equiv) and the solvents are removed *in vacuo*. The residue is purified through flash column chromatography (starting from CHCl3/MeOH 0%->5% MeOH) to yield colorless oil (52mg, 18µmol, 93%).

$[\alpha]_D^{20}$ = + 31.3 (c =1.10 in $CHCl_3$); ν_{max} (neat) 3346, 2925, 2107, 1742, 1719, 1669, 1497, 1454, 1362, 1048, 912cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 – 6.88 (m, 85H), 6.01 (d, *J* = 8.2 Hz, 1H, N**H**), 5.88 (d, *J* = 3.7 Hz, 1H, $GlcNH_2$-1), 5.27 – 5.14 (m, 2H, ManI-2), 4.94 (m, 1H), 4.90 – 4.63 (m, 13H), 4.59 (d, *J* = 10.7 Hz, 1H), 4.55 – 4.28 (m, 18H), 4.25 – 4.20 (m, 1H), 4.17 (dd, *J* = 11.8, 5.3 Hz, 2H), 4.13 – 3.97 (m, 3H), 3.95 (t, *J* = 2.2Hz, 1H), 3.90 (t, *J* = 9.6Hz, 1H), 3.87 – 3.50 (m, 23H), 3.49 – 3.38 (m, 7H), 3.16 (dd, *J* = 6.9, 3.1 Hz, 1H), 3.06 (dd, *J* = 10.2, 3.7 Hz, 1H, $GlcNH_2$-2), 2.76 (q, *J* = 7.2 Hz, 6H, $NC\mathbf{H}_2CH_3$), 2.34 – 2.08 (m, 6H, CH_2 of Lev, -S-C**H**$_2$-CH_2-CH_2-CH_2-CH_2-CH_2-O), 1.85 (s, 3H, CH_3 of Lev), 1.64 (s, 3H, NHAc), 1.54 – 1.47 (m, 2H, -S-CH_2-CH_2-CH_2-CH_2-C**H**$_2$-CH_2-O), 1.45 – 1.37 (m, 2H, -S-CH_2-C**H**$_2$-CH_2-CH_2-CH_2-CH_2-O), 1.25 – 1.16 (m, 4H, -S-CH_2-CH_2-C**H**$_2$-C**H**$_2$-CH_2-CH_2-O), 1.13 (t, *J* = 7.3 Hz, 9H, $NCH_2C\mathbf{H}_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.17 (ketone of Lev), 171.72, 170.70, 140.03, 139.03, 138.87, 138.74, 138.69, 138.65, 138.62, 138.52, 138.48, 138.44, 138.41, 138.35, 138.19, 138.10, 138.00, 128.91, 128.63, 128.54, 128.53, 128.47, 128.45, 128.39, 128.36, 128.34, 128.33, 128.30, 128.28, 128.25, 128.11, 128.09, 128.08, 128.02, 128.00, 127.96, 127.81, 127.81, 127.77, 127.73, 127.67, 127.60, 127.56, 127.52, 127.47, 127.45, 127.43, 127.34, 127.24, 127.12, 126.93, 100.75,

99.86, 99.32, 98.90, 96.39 ($GlcNH_2$-1), 81.92, 81.79, 81.18, 80.09, 80.05, 79.55, 79.07, 76.08, 75.92, 75.72, 75.41, 75.26, 75.14, 75.10, 75.05, 74.94, 74.73, 74.55, 74.10, 73.95, 73.52, 73.48, 73.43, 73.09, 73.03, 72.42, 72.36, 72.29, 72.23, 72.18, 71.72, 71.28, 69.97, 69.67, 69.46 (ManI-2), 68.90, 68.67, 67.15, 65.71, 65.67, 63.39 (Glc-NH_2-2), 62.37, 54.27, 45.89 (NCH_2CH_3), 37.88, 36.36, 31.45 (-S-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-O), 31.06, 31.01, 29.70, 29.29, 28.81, 28.01, 25.54, 23.44, 9.62 (NCH_2CH_3); ^{31}P NMR (243 MHz, $CDCl_3$) δ -1.08; *m/z* (ESI) Found: $[M+Na]^+$, 2922.2032 $C_{168}H_{185}N_4O_{35}PSSi$ requires $[M+Na]^+$, 2922.1969.

Example 3: Bistriethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-(2-(*N*-benzyloxy carbonyl) aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-3-*O*-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (compound 15)

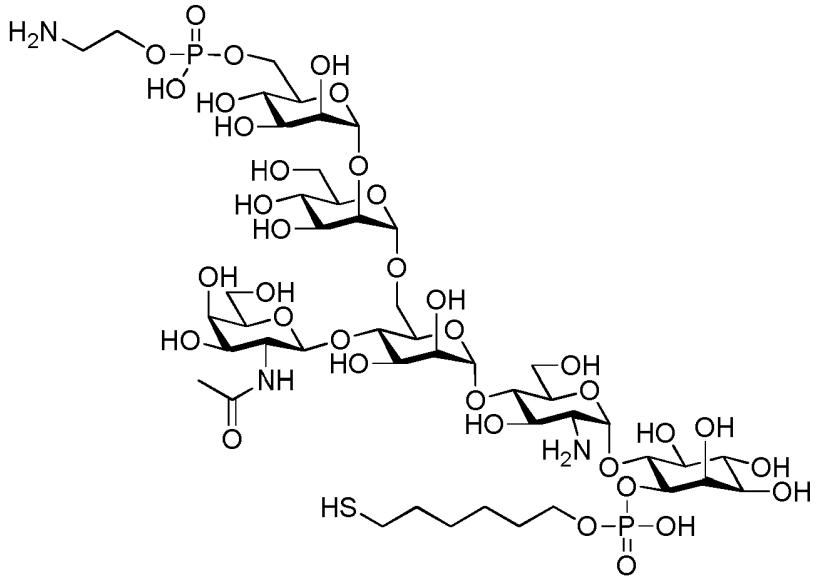


Triethylammonium 2,3,4-Tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-3-*O*-benzyl-2-*O*-levulinyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**compound 18**) (28.3mg, 78μmol, 4.5equiv) and triethylammonium 2-(((benzyloxy)carbonyl)amino)ethyl phosphonate (**compound 13**) (28.3mg, 78μmol, 4.5equiv) are co evaporated 3 times with 2mL dry pyridine. The residue is dissolved in 2mL dry pyridine and PivCl (16.1μL, 131μmol, 7.5equiv) is added. The solution is stirred for 2h at r.t. before water (15.6μL, 0.87mmol, 50equiv) and iodine (24.3 mg, 96μmol, 5.5 equiv) are added. The red

solution is stirred for 1h and is quenched with hydrazine (1M in THF; 300µL, 0.3mmol, 17equiv).The reaction mixture is stirred for 18h. The solvents are removed *in vacuo* and the residue is purified through flash column chromatography (starting from CHCl3/MeOH: 97/3→90/10) to yield yellow oil (49.5mg, 15µmol, 88%).

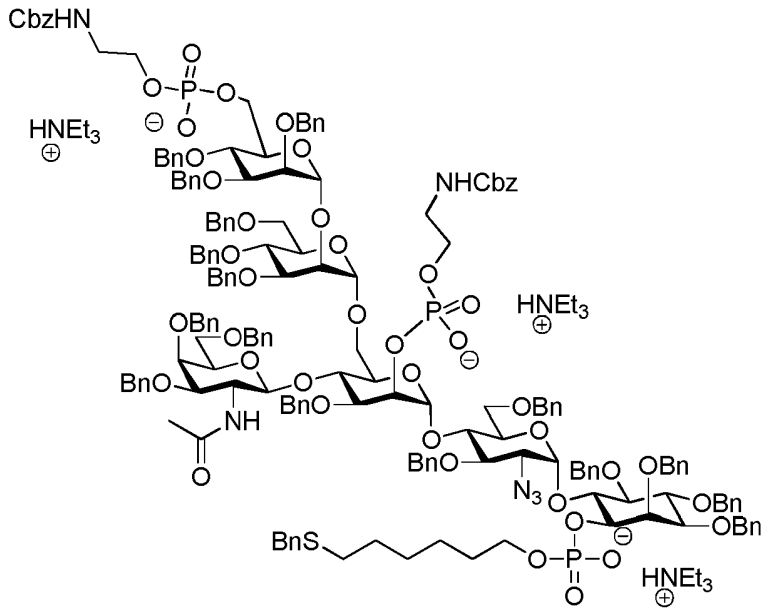
$[\alpha]_D^{20}$ = + 32.5 (c =1.00 in $CHCl_3$); ν_{max} (neat) 3387, 3063, 2929, 2108, 1672, 1497, 1057, 1029, 839cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 – 6.90 (m, 90H), 6.28 (s, 1H, N**H**Ac), 5.89 (d, *J* = 3.5 Hz, 1H, $GlcNH_2$-1), 5.19 (d, *J* = 1.6 Hz, 1H), 5.05 – 4.15 (m, 40H), 4.14 – 4.02 (m, 3H), 3.98 – 3.35 (m, 36H), 3.28 – 3.20 (m, 1H), 3.14 (dd, *J* = 9.2, 4.5 Hz, 1H), 3.05 (dd, *J* = 10.2, 3.5 Hz, 1H, $GlcNH_2$-2), 2.61 (q, *J* = 7.3 Hz, 12H, $NC\mathbf{H}_2CH_3$), 2.28 (t, *J* = 7.4 Hz , 2H, -S-C$\mathbf{H}_2$-CH_2-CH_2-CH_2-CH_2-CH_2-O), 1.92 – 1.76 (m, 3H, $COC\mathbf{H}_3$), 1.58 – 1.37 (m, 4H, -S-CH_2-C$\mathbf{H}_2$-CH_2-CH_2-C$\mathbf{H}_2$-CH_2-O), 1.26 – 1.13 (m, 4H, -S-CH_2-CH_2-C$\mathbf{H}_2$-C$\mathbf{H}_2$-CH_2-CH_2-O), 0.97 (t, *J* = 7.3 Hz, 18H, $NCH_2C\mathbf{H}_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.84 ($\mathbf{C}OCH_3$), 156.58 (O(CO)NH), 138.71, 128.87, 128.50, 128.44, 128.36, 128.30, 128.21, 128.03, 127.94, 127.86, 127.76, 127.50, 126.91, 102.05, 101.15, 100.26, 98.71, 96.19, 82.02, 81.79, 81.06, 80.17, 80.03, 79.66, 77.37, 77.16, 76.95, 76.36, 75.93, 75.58, 75.19, 74.84, 74.80, 74.65, 74.03, 73.49, 72.79, 72.29, 71.62, 71.56, 71.49, 71.41, 70.08, 69.75, 69.64, 68.97, 66.34, 65.70, 65.04, 64.09, 63.49, 58.17, 45.70, 38.76, 36.34, 32.00, 31.44, 29.78, 29.59, 29.44, 29.27, 28.80, 27.54, 25.50, 22.77, 14.21, 8.71; ^{31}P NMR (243 MHz, $CDCl_3$) δ -0.03, -1.67; *m/z* (ESI) Found: $[M-H]^-$, 3041.2393 $C_{173}H_{191}N_5O_{38}P_2S$ requires $[M-H]^-$, 3041.2353.

Example 4: 6-*O*-(aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-4-*O*-(2-deoxy-2-acetamido-β-D-galactopyranosyl)-α-D-mannopyranosyl-(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(thiohexyl phosphono)-D-*myo*-inositol (compound 3)



Approximately 10 mL ammonia were condensed in a flask and *tert*-BuOH (2 drops) was added. Afterwards small pieces of sodium were added till a dark blue colour was established. Bistriethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-(2-(*N*-benzyloxycarbonyl) aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-3-*O*-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**compound 15**) (28 mg, 8.6 µmol) was dissolved in dry THF (1.5 mL) and added to the ammonium solution at -78 °C. The reaction was stirred for 30 min at this temperature. The reaction was quenched with dry MeOH (2 mL) and the ammonia was blown off using a stream of nitrogen. The pH of the resulting solution was adjusted with concentrated acetic acid to 8-9. Solvents were removed in vacuo and the residue was purified using a small G10 column (GE Healthcare) to yield **X** as white solid (6.7 mg, 4.9 µmol, 58%): ^{1}H NMR (600 MHz, D_2O) δ 5.54 (d, J = 3.9 Hz, 1H, $GlcNH_2$), 5.23 (s, 1H), 5.19 (s, 1H), 5.03 (s, 1H), 4.51 (d, J = 8.3 Hz, 1H, GalNAc-1), 4.29 – 3.66 (m, 36H), 3.63 – 3.53 (m, 2H), 3.45 (td, J = 9.3, 4.3 Hz, 1H), 3.38 (dd, J = 10.9, 4.3 Hz, 1H, $GlcNH_2$-2), 3.34 – 3.29 (m, 2H), 2.80 (t, J = 7.1 Hz, 1H), 2.58 (t, J = 7.1 Hz, 1H), 2.12 (s, 3H, Me of NHAc), 1.80 – 1.59 (m, 4H, linker), 1.51 – 1.36 (m, 4H, linker); ^{13}C NMR (151 MHz, D_2O) δ 177.30 (amide), 105.02, 104.34 (GalNAc-1), 104.09, 101.16, 98.15 (Glc-NH_2-1), 81.69, 79.37, 78.78, 78.01, 75.69, 75.40, 74.84, 74.60, 74.05, 73.92, 73.62, 73.15, 73.08, 72.82, 72.64, 72.49, 72.03, 71.56, 70.32, 69.61, 69.21, 68.85, 67.28, 64.53, 64.50, 63.76, 63.70, 62.81, 56.64 ($GlcNH_2$-2), 55.19, 42.66, 40.73, 35.53, 32.31, 30.88, 29.67, 27.20, 27.00, 26.29, 24.96 (Me of NHAc); ^{31}P NMR (243 MHz, D_2O) δ -2.62, -2.83; *m/z* (ESI) Found: $[M-2H]^{2-}$, 673.7104 $C_{46}H_{85}N_3O_{36}P_2S$ requires $[M-2H]^{2-}$, 673.6981.

Example 5: Tristriethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-(2-(*N*-benzyloxycarbonyl) aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-2-(2-(*N*-benzyloxycarbonyl) aminoethyl phosphono)-3-*O*-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (compound 16)

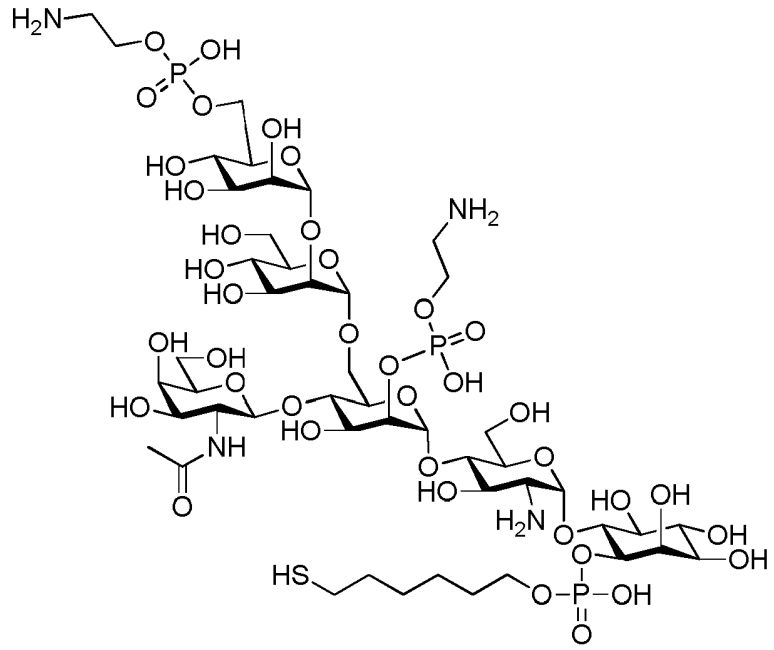


Bistriethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-(2-(*N*-benzyloxycarbonyl) aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-3-*O*-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**compound 15**) (15mg, 4.6µmol, 1equiv) and triethylammonium 2-(((benzyloxy)carbonyl)amino)ethyl phosphonate (**compound 13**) (8.1mg, 22.5µmol, 4.5equiv) are co evaporated 3 times with 2mL dry pyridine. The residue is dissolved in 2mL dry pyridine and PivCl (4.6µL, 36.8µmol, 7.5equiv) is added. The solution is stirred for 2h at r.t. before water (10µL, 0.56mmol, 76equiv) and iodine (6.8, 27µmol, 5.5 equiv) are added. The red solution is stirred for 1h and is quenched with sat. $Na_2S_3O_3$. The reaction mixture is diluted with 10mL CHCl3 and dried over Na_2SO_4. The solvents are removed *in vacuo* and the residue is purified through flash column chromatography (CHCl3/MeOH 100/0→80/20) to yield yellow oil (13.5mg, 3.8µmol, 76%).

$[\alpha]_D^{20}$ = + 22.0 (c =1.00 in $CHCl_3$); ν_{max} (neat) 3358, 2927, 2108, 1641, 1454, 1398, 1054, 7028, 838, 804cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.56 – 6.77 (m, 90H), 6.44 (s, 2H, CbzN**H**), 6.22 (s, 1H, N**H**COCH$_3$), 5.87 (s, 1H, GlcNH$_2$-1), 5.49 (s, 1H), 5.09 – 3.35 (m, 83H), 3.30 (dd, *J* = 14.2, 7.1 Hz, 1H), 3.26 – 3.17 (m, 2H), 3.17 – 3.08 (m, 2H), 3.07 – 2.79 (m, 1H), 2.58 (q, *J* = 7.2 Hz, 18H, NC**H**$_2$CH$_3$), 2.27 (t, *J* = 7.4Hz, 2H, -S-C**H**$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-CH$_2$-O), 2.05 – 1.98 (m, 2H), 1.90 – 1.83 (m, 3H, NHCOC**H**$_3$), 1.59 – 1.36 (m, 4H, -S-CH$_2$-C**H**$_2$-CH$_2$-CH$_2$-C**H**$_2$-CH$_2$-O), 1.27 – 1.12 (m, 4H, -S-CH$_2$-CH$_2$-C**H**$_2$-C**H**$_2$-CH$_2$-CH$_2$-O), 0.99 (t, *J* = 7.2 Hz, 27H, NCH$_2$C**H**$_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.57 (O**C**ONH), 140.07, 139.03, 138.78, 138.73, 138.67, 138.32, 137.23, 128.93, 128.54, 128.49, 128.44, 128.39, 128.36, 128.31, 128.28, 128.25, 128.23, 128.18, 128.03, 128.01, 127.97, 127.93, 127.82, 127.71, 127.60,

127.57, 127.54, 127.51, 127.41, 127.38, 127.30, 127.15, 127.06, 126.94, 126.84, 100.59, 98.53, 96.54 ($GlcNH_2$-1), 81.89, 81.20, 75.58, 75.04, 74.83, 74.67, 73.28, 72.84, 72.30, 66.39, 66.23, 65.70, 63.97, 45.85 ($N\mathbf{C}H_2CH_3$), 42.97, 42.52, 40.10, 36.39, 34.58, 33.94, 32.05, 31.56, 31.50 (S-$\mathbf{C}H_2$-CH_2-CH_2-CH_2-CH_2-CH_2-O), 31.08, 31.03, 30.33, 29.82, 29.79, 29.74, 29.63, 29.48, 29.32, 29.28, 29.08, 28.84, 25.55, 22.81, 21.56, 14.99, 14.30, 14.24, 13.23, 9.91 ($NCH_2\mathbf{C}H_3$); ^{31}P NMR (243 MHz, $CDCl_3$) δ 0.17, -0.02, -1.15; *m/z* (ESI) Found: $[M+5Na-3H]^{2+}$, 1705.6285 $C_{183}H_{203}N_6O_{43}P_3S$ requires $[M+5Na-3H]^{2+}$, 1705.6062.

Example 6: 6-*O*-(aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-2-O--(aminoethyl phosphono)-4-*O*-(2-deoxy-2-acetamido-β-D-galactopyranosyl)-α-D-mannopyranosyl-(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(thiohexyl phosphono)-D-*myo*-inositol (compound 5)

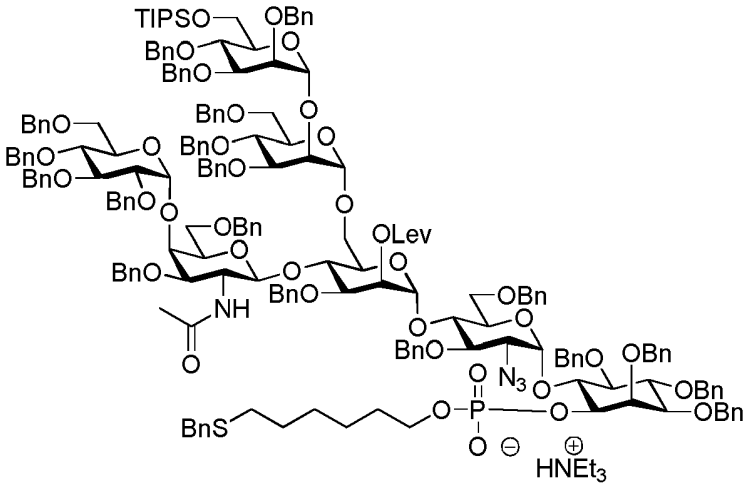


Tristriethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-(2-(*N*-benzyloxycarbonyl) aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-2-(2-(*N*-benzyloxycarbonyl) aminoethyl phosphono)-3-*O*-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**compound 16**) (22mg, 6.1μmol, 1equiv) was dissolved in dry THF (15mL) and dry *tert*-BuOH (0.1mL). The solution is cooled down to -78°C and approximately 20mL ammonia is condensed in the flask. Afterwards small pieces of sodium are added. The solution is

warmed to about -40°C till a dark blue colour is established. Then the solution is cooled down to -78°C and the reaction is stirred for 1h at this temperature. The reaction is quenched with 2mL dry MeOH and the ammonia is blown off of using a stream of nitrogen. Solvents are afterwards evaporated and the residue is dissolved in 5mL water. The pH of the solution is adjusted with concentrated acetic acid to 4-7. Water is removed by freeze drying and the residue is purified using a small G25 column (1cmx20cm) to yield a white solid (2.6 mg, 1.8µmol, 29%).

^{1}H NMR (400 MHz, D_2O) δ 5.57 – 5.52 (m, 1H, GlcNH$_2$-1), 5.45 (s, 1H, ManI-1), 5.19 (s, 1H), 5.04 (s, 1H), 4.53 (d, J = 8.4 Hz, 2H, GalNAc-1, ManI-2), 4.29 – 3.64 (m, 37H), 3.62 – 3.53 (m, 2H), 3.44 (t, J = 9.3 Hz, 1H), 3.41 – 3.34 (m, 1H), 3.34 – 3.26 (m, 4H), 2.79 (t, J = 7.3 Hz, 2H, HS-CH$_2$), 2.11 (s, 3H, Me of NHAc), 1.81 – 1.57 (m, 4H), 1.53 – 1.36 (m, 4H).; ^{31}P NMR (162 MHz, D_2O) δ 0.36, 0.14, -0.81; *m/z* (ESI) Found: $[M-3H]^{-3}$, 979.96 $C_{96}H_{180}N_8O_{78}P_6S_2$ requires $[M-3H]^{-3}$, 979.93.

Example 7: Triethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-tri*iso*propylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D- mannopyranosyl-(1→6)-3-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-2-*O*-levulinyl-α-D-manno-pyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (compound 11)

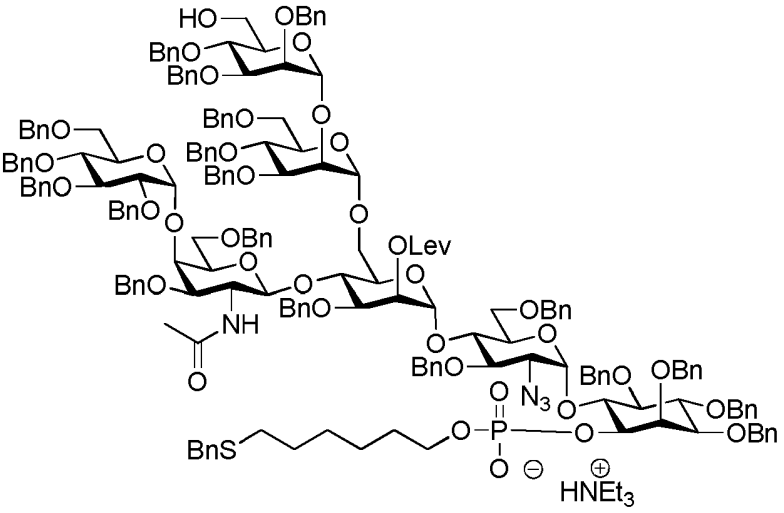


2,3,4-Tri-*O*-benzyl-6-*O*-tri*iso*propylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D- mannopyranosyl-(1→6)-3-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-

2-*O*-levulinyl-α-D-manno-pyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**compound 8**) (50mg, 16μmol, 1equiv) and triethylammonium 6-(benzylthio)hexyl phosphonate (**compound 10**) (39mg, 100μmol, 6.4equ.) are co evaporated 3 times with 2mL dry pyridine. The residue is dissolved in 2mL dry pyridine and PivCl (14.5μL, 118μmol, 7.5equiv) is added. The solution is stirred for 2h at r.t. before water (14μL, 0.79mmol, 50equiv) and iodine (29.9mg, 118μmol, 7.5 equiv) are added. The red solution is stirred for 1h and is quenched with sat. $Na_2S_3O_3$. The reaction mixture is diluted with 10mL CHCl3 and dried over Na_2SO_4. The solvents are removed *in vacuo* and the residue is purified through flash column chromatography ($CHCl_3$/MeOH 100/0→95/5) to yield yellow oil (49mg, 14μmol, 87%).

$[\alpha]_D^{20}$ = + 42.3 (c =1.00 in $CHCl_3$); ν_{max} (neat) 3064, 3032, 2926, 2865, 2107, 1742, 1720, 1678, 1497, 1454, 1362, 1054, 1028, 913cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49 – 6.87 (m, 100H), 6.07 (d, *J* = 9.0 Hz, 1H, N**H**), 5.78 (s, 1H), 5.25 (s, 2H, ManI-2), 5.07 (s, 1H), 4.94 (d, *J* = 11.9 Hz, 1H, CH_2 of Bn), 4.89 – 3.89 (m, 49H), 3.86 – 3.74 (m, 11H), 3.73 – 3.66 (m, 2H), 3.63 (dd, *J* = 8.9, 2.9 Hz, 1H), 3.60 – 3.45 (m, 11H), 3.44 – 3.36 (m, 4H), 3.34 – 3.22 (m, 5H), 3.03 (dd, *J* = 10.1, 3.6 Hz, 1H), 2.85 (q, *J* = 7.3 Hz, 6H, NC**H**$_2$CH$_3$), 2.78 (d, *J* = 9.4 Hz, 1H), 2.27 (t, *J* = 7.4 Hz, 2H, BnS-C**H**$_2$), 2.24 – 2.04 (m, 4H, CH_2 of Lev), 1.80 (s, 3H, CH_3 of Lev), 1.70 (s, 3H, NHCOC**H**$_3$), 1.55 – 1.34 (m, 4H, -S-CH$_2$-C**H**$_2$-CH$_2$-CH$_2$-C**H**$_2$-CH$_2$-O), 1.23 – 1.16 (m, 4H, -S-CH$_2$-CH$_2$-C**H**$_2$-C**H**$_2$-CH$_2$-CH$_2$-O), 1.12 (t, *J* = 7.6 Hz, 9H, NCH$_2$C**H**$_3$), 1.00 – 0.94 (m, 21H, TIPS); ^{13}C NMR (151 MHz, $CDCl_3$) δ 205.92 (ketone of Lev), 171.76 (CO of Lev), 170.21 (NH**C**OCH$_3$), 140.02, 139.09, 139.03, 138.94, 138.91, 138.84, 138.76, 138.74, 138.69, 138.60, 138.57, 138.30, 138.15, 138.10, 138.01, 137.54, 128.92, 128.77, 128.72, 128.65, 128.62, 128.57, 128.54, 128.46, 128.44, 128.39, 128.36, 128.33, 128.31, 128.28, 128.27, 128.25, 128.24, 128.22, 128.16, 128.12, 128.10, 128.07, 127.98, 127.87, 127.84, 127.82, 127.77, 127.71, 127.67, 127.65, 127.63, 127.57, 127.55, 127.52, 127.48, 127.44, 127.29, 127.27, 127.18, 127.14, 126.94, 126.45, 101.71, 100.84, 99.65, 98.84, 98.65, 96.71, 82.20, 81.99, 81.51, 81.19, 81.03, 80.51, 80.12, 79.80, 79.13, 77.95, 77.53, 76.81, 75.93, 75.74, 75.43, 75.35, 75.26, 75.15, 74.75, 74.69, 74.54, 74.43, 74.03, 73.86, 73.63, 73.48, 73.23, 73.20, 73.00, 72.85, 72.39, 72.36, 72.07, 71.53, 71.37, 70.88, 70.79, 70.06 (ManI-2), 69.67, 68.77, 68.59, 67.74, 66.87, 65.80, 63.72, 62.90, 52.87, 45.42 (N**C**H$_2$CH$_3$), 38.57, 37.83, 36.38, 31.46, 31.00, 30.95, 29.82, 29.66, 29.30 (CH_3 of Lev), 28.81, 28.04, 27.69, 27.41, 25.52, 23.31 (NHCO**C**H$_3$), 18.24, 18.18, 14.25, 12.18, 8.55 (NCH$_2$**C**H$_3$); ^{31}P NMR (243 MHz, $CDCl_3$) δ -1.45; *m/z* (ESI) Found: $[M-2H]^{2-}$, 1734.7564 $C_{204}H_{233}N_4O_{40}PSSi$ requires $[M-2H]^{2-}$, 1734.7730.

Example 8: Triethylammonium 2,3,4-Tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D- mannopyranosyl-(1→6)-3-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-2-*O*-levulinyl-α-D-manno-pyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (compound 17)

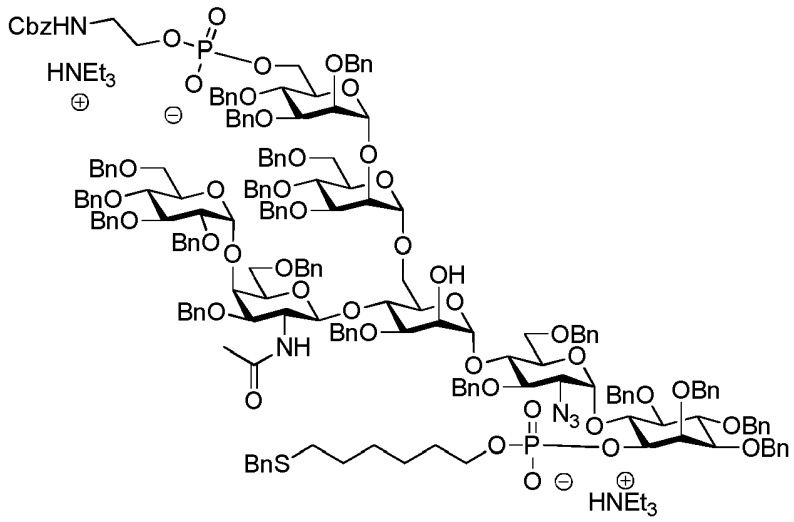


Triethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-tri*iso*propylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D- mannopyranosyl-(1→6)-3-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-2-*O*-levulinyl-α-D-manno-pyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**compound 11**) (43mg, 12µmol, 1 equiv) is dissolved in 2mL MeCN. Water (8.7µL, 0.48mmol, 40equiv) and $Sc(TfO)_3$ (11.8mg, 24µmol, 2equiv) are added and the solution is heated up to 50°C for 5h. The reaction is quenched with pyridine (4.8µL, 60µmol, 5 equiv) and the solvents are removed *in vacuo*. The residue is purified through flash column chromatography ($CHCl_3$/MeOH 100/0→95/5) to yield colorless oil (32mg, 9.4µmol, 78%).

$[a]_D^{20}$ = + 47.4 (c =1.00 in $CHCl_3$); ν_{max} (neat) 3363, 3031, 2926, 2862, 2107, 1742, 1719, 1671, 1497, 1454, 1362, 1068, 1049, 1028, 697cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.55 – 6.71 (m, 100H), 6.19 (s, 1H, N**H**), 5.87 (s, 1H), 5.20 (s, 1H), 5.17 (s, 1H), 5.03 – 4.16 (m, 40H), 4.15 – 3.33 (m, 43H), 3.28 (d, *J* = 10.6 Hz, 1H), 3.21 – 3.11 (m, 1H), 3.02 (d, *J* = 7.7 Hz, 1H), 2.87 (d, *J* = 10.1 Hz, 1H), 2.80 (q, *J* = 7.0 Hz, 6H, NCH_2CH_3), 2.35 – 2.13 (m, 6H, BnS-CH_2, CH_2 of Lev), 1.85 (s, 3H, CH_3 of Lev), 1.75 (s, 3H, $NHCOCH_3$), 1.59 – 1.35 (m, 4H, -S-CH_2-CH_2-CH_2-CH_2-CH_2-O), 1.28 – 1.13 (m, 4H, -S-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-O), 1.10 (t, *J* = 7.3 Hz, 1H, NCH_2CH_3);

^{13}C NMR (151 MHz, $CDCl_3$) δ 206.20 (ketone of Lev), 171.68 (CO of Lev), 170.78 (NH**C**O), 149.97, 140.04, 139.04, 138.99, 138.87, 138.78, 138.68, 138.64, 138.54, 138.49, 138.30, 138.20, 138.10, 128.93, 128.82, 128.55, 128.49, 128.48, 128.43, 128.39, 128.37, 128.33, 128.30, 128.17, 128.08, 128.03, 127.96, 127.90, 127.80, 127.75, 127.66, 127.62, 127.59, 127.55, 127.53, 127.45, 127.35, 127.12, 126.95, 126.82, 101.45, 100.42, 100.04, 99.52, 99.19, 96.41, 82.15, 81.91, 81.21, 80.37, 79.99, 79.65, 77.96, 76.20, 75.86, 75.70, 75.36, 75.29, 75.13, 75.04, 74.93, 74.74, 74.10, 73.60, 73.53, 73.36, 73.29, 73.00, 72.56, 72.37, 72.31, 72.26, 72.17, 71.69, 71.20, 70.79, 69.84, 69.48, 69.26 (ManI-2), 69.00 67.98, 67.79, 65.75, 65.71, 63.32, 62.50, 54.82, 45.44 (N**C**H_2CH_3), 37.88, 36.39, 32.06, 31.47, 31.06, 29.91, 29.83, 29.72, 29.45, 29.39, 29.31, 28.84, 28.03, 27.71, 27.36, 25.55, 23.59, 22.83, 17.85, 14.26, 12.43, 8.60 (NCH$_2$**C**H$_3$); ^{31}P NMR (243 MHz, $CDCl_3$) δ -1.12; *m/z* (ESI) Found: $[M+Cl-H]^{2-}$, 1673.6842 $C_{195}H_{213}N_4O_{40}PS$ requires $[M+Cl-H]^{2-}$, 1673.6918.

Example 9: Bistriethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-(2-(*N*-benzyloxycarbonyl) aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-3-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-α-D-manno-pyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (compound 14)

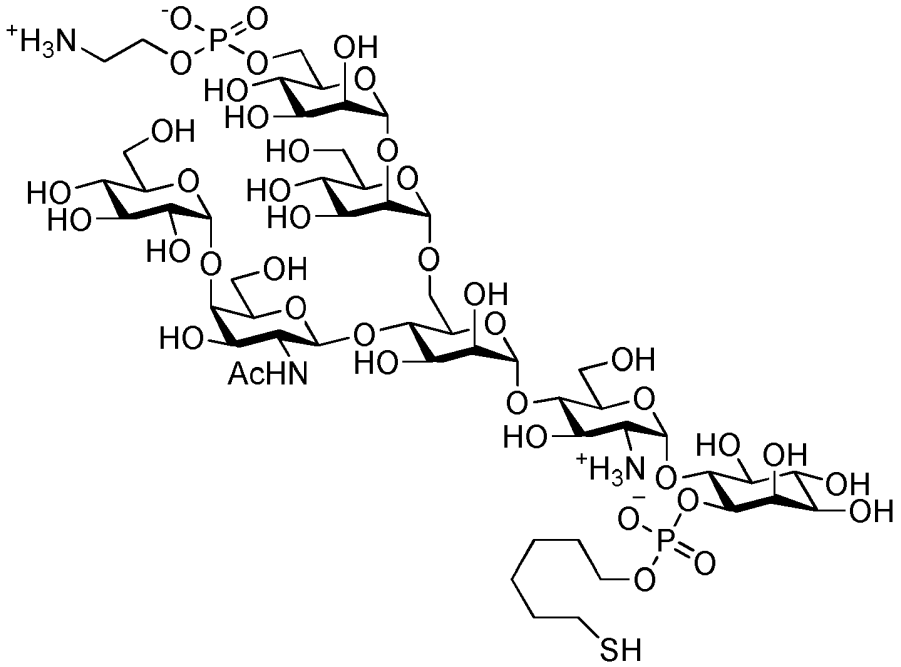


Triethylammonium 2,3,4-Tri-*O*-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-3-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-2-*O*-levulinyl-α-D-manno-pyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-

glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**compound 17**) (31mg, 9.1µmol, 1equiv) and triethylammonium 2-(((benzyloxy)carbonyl)amino)ethyl phosphonate (**compound 13**) (14.7mg, 41µmol, 4.5equiv) are co evaporated 3 times with 2mL dry pyridine. The residue is dissolved in 2mL dry pyridine and PivCl (8.4µL, 68µmol, 7.5equiv) is added. The solution is stirred for 2h at r.t. before water (8.2µL, 0.45mmol, 50equiv) and iodine (12.7mg, 50µmol, 5.5 equiv) are added. The red solution is stirred for 1h and is quenched with hydrazine (1M in THF, 227µL, 0.28mmol, 25equiv).The reaction mixture is stirred for 18h. The solvents are removed *in vacuo* and the residue is purified through flash column chromatography ($CHCl_3$/MeOH 100/0→90/10) to yield yellow oil (25.3mg, 6.9µmol, 76%).

$[a]_D^{20}$ = + 46.0 (c =1.00 in $CHCl_3$); ν_{max} (neat) 3344, 2926, 2864, 2108, 1683, 1497, 1454, 1363, 1093, 1071, 1028, 863cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 – 6.79 (m, 105H), 6.23 (s, 1H, N**H**COCH$_3$), 5.87 (d, *J* = 3.4 Hz, 1H), 5.15 (s, 1H), 5.00 – 3.30 (m, 90H), 3.24 (d, *J* = 10.3 Hz, 1H), 3.11 (d, *J* = 4.6 Hz, 1H), 3.03 (d, *J* = 8.0 Hz, 1H), 2.80 (d, *J* = 10.2 Hz, 1H), 2.69 (q, *J* = 7.2 Hz, 12H, NC**H**$_2$CH$_3$), 2.28 (t, *J* = 7.4 Hz, 2H, BnS-C**H**$_2$), 1.86 (s, 1H, NHCOC**H**$_3$), 1.59 – 1.36 (m, 4H, -S-CH$_2$-C**H**$_2$-CH$_2$-CH$_2$-C**H**$_2$-CH$_2$-O), 1.26 – 1.11 (m, 4H, -S-CH$_2$-CH$_2$-C**H**$_2$-C**H**$_2$-CH$_2$-CH$_2$-O), 1.02 (t, *J* = 7.3 Hz, 18H, NCH$_2$C**H**$_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.72 (NH**C**OCH$_3$), 155.80 (O-**C**O-NH), 139.25, 138.46, 138.30, 138.23, 137.98, 137.70, 137.48, 137.27, 137.00, 136.33, 128.69, 128.25, 128.19, 128.13, 127.85, 127.79, 127.62, 127.27, 127.13, 127.07, 126.80, 126.74, 126.54, 126.26, 125.68, 101.37, 100.31, 99.20, 98.96, 98.52, 94.76, 81.97, 81.82, 81.78, 81.51, 80.99, 80.93, 80.86, 80.84, 80.52, 80.17, 79.87, 79.48, 79.22, 78.90, 78.51, 77.79, 77.30, 75.92, 75.62, 74.89, 74.68, 74.57, 74.19, 73.94, 73.35, 72.99, 72.41, 72.11, 71.48, 71.20, 71.01, 70.46, 69.50, 69.23, 68.93, 67.83, 67.04, 66.60, 66.06, 65.93, 65.65, 64.95, 64.68, 64.19, 64.01, 63.31, 63.23, 62.40, 62.26, 52.47, 51.52, 45.62, 44.66, 43.74, 36.52, 35.62, 34.73, 31.66, 31.10, 30.71, 30.29 (BnS-**C**H$_2$), 29.89, 29.82, 29.40, 29.06, 28.55, 28.23, 28.08, 27.72, 27.25, 26.36, 25.59, 24.80, 23.96, 23.12, 22.28 (NHCO**C**H$_3$), 9.11, 8.26, 7.41, 6.56; ^{31}P NMR (243 MHz, $CDCl_3$) δ 0.00, -1.32; *m/z* (ESI) Found: $[M-2H]^{2-}$, 1735.2054 $C_{200}H_{219}N_5O_{43}P_2S$ requires $[M-2H]^{2-}$, 1735.2077.

Example 10: 6-*O*-(aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)- α-D-mannopyranosyl-(1→6)-4-*O*-(α-D-glucopyranosyl-(1→4)-2-deoxy-2-acetamido-β-D-galactopyranosyl)-α-D-manno-pyranosyl-(1→4)-2-amino-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(thiohexyl phosphono)-D-*myo*-inositol (compound 4)



Bistriethylammonium 2,3,4-Tri-*O*-benzyl-6-*O*-(2-(*N*-benzyloxycarbonyl) aminoethyl phosphono)-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D- mannopyranosyl-(1→6)-3-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-α-D-manno-pyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→6)-1-*O*-(6-(*S*-benzyl)thiohexyl phosphono)-2,3,4,5-tetra-*O*-benzyl-D-*myo*-inositol (**compound 14**) (20mg, 5.4µmol, 1equiv) was dissolved in dry THF (3mL) and dry *tert*-BuOH (2 drops). The solution is cooled down to -78°C and approximately 10mL ammonia is condensed in the flask. Afterwards small pieces of sodium are added till a dark blue colour is established. Then the solution is stirred for 35 min at this temperature. The reaction is quenched with 2mL dry MeOH and the ammonia is blown off of using a stream of nitrogen. Solvents are afterwards evaporated and the residue is dissolved in 5mL water. The pH of the solution is adjusted with concentrated acetic acid to 7. Water is removed by freeze drying and the residue is purified using a small G25 column (1cmx20cm) to yield a white solid (4.5mg, 3.0µmol, 55%).

ν_{max} (neat) 3350, 2918, 1646, 1025 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 5.59 (d, *J* = 3.6 Hz, 1H, $GlcNH_2$-1), 5.27 (s, 1H), 5.23 (s, 1H), 5.07 (s, 1H), 4.99 (d, *J* = 3.8 Hz, 1H, Glc-1), 4.60 (d, *J* = 8.2 Hz, 1H, GalNAc-1), 4.31 – 3.67 (m, 39H), 3.60 (dd, *J* = 10.1, 3.6 Hz, 2H), 3.55 – 3.39 (m, 3H), 3.35 (t, 2H), 2.83 (t, *J* = 7.2 Hz, 1H), 2.61 (t, *J* = 7.1 Hz, 1H), 2.14 (s, 3H), 1.82 – 1.58 (m, 4H), 1.54 – 1.38 (m, 4H); ^{13}C NMR (151 MHz, D_2O) δ 177.28 (amide), 105.01, 104.76 (GalNAc-1), 103.91, 102.82 (Glc-1), 101.11, 99.97 ($GlcNH_2$-1), 81.64, 79.91, 79.30, 79.25, 79.15, 78.84, 78.04, 75.70, 75.69,

75.39, 75.36, 75.29, 74.84, 74.61, 74.55, 74.40, 74.32, 74.08, 73.94, 73.62, 73.16, 72.86, 72.83, 72.66, 72.58, 72.55, 72.50, 72.27, 72.18, 72.12, 72.06, 71.82, 71.62, 69.60, 69.22, 68.87, 67.29, 64.51, 64.48, 63.77, 62.98, 62.82, 62.52, 56.58, 55.08, 42.72, 42.67, 40.75, 35.52, 32.28, 30.88, 29.80, 29.67, 27.30, 27.18, 26.99, 26.52, 26.29, 25.84, 24.96; ^{31}P NMR (162 MHz, D_2O) δ 0.40, 0.22; *m/z* (ESI) Found: $[M-H]^-$, 1510.4612 $C_{52}H_{93}N_3O_{41}P_2S$ requires $[M-H]^-$, 1510.4564.

Part A2 : Preparation of sulfone linked thiol functionalized GPI

Formation of the mesilate ester with mesityl chloride and triethylamine followed by a S_{N2} reaction leads to bromide **19a** that is substituted by the thiol linker under inversion of stereochemistry to generate **19**. Oxidation with hydrogen peroxide yields the sulfone **20**, which is deprotected under acidic conditions. Introduction of a protected phosphoethanolamine and cleavage of the levulinic ester using hydrazine produces oligosaccharide **21**. Final hydrogenolysis yields glycan **22** ready for conjugation. The reaction scheme is shown under **Figure 2**.

B Experimental data for Vaccination

Example 11: Conjugation to a carrier

Vaccines based on polysaccharides are characterized by a T-cell independent immune response without inducing an immunological memory. Immunogenicity of polysaccharide vaccines in infants, elderly and immunocompromised patients are weak. Conjugation of carbohydrates to a carrier, such as a carrier protein or a glycosphingolipid with immnunomodulatory properties, creates a T-cell dependent immune response against the carbohydrate. As carrier protein, the nontoxic diphtheria toxoid variant CRM_{197} was used, since it has been approved as a constituent of licensed vaccines.

Example 11a: Conjugation to a maleimide-modified protein (Figure 6)

1mg (17nmol) CRM_{197} was dissolved in 500µL PBS to yield a 40µM CRM_{197} solution. 3mg Sulfo-GMBS (Pierce) was dissolved in 500µL PBS (1.6mM, 40equiv.) and added to the protein solution. The solution was incubated for 2 h at room temperature, before it was concentrated and washed with water (4x500µL) in an Amicon Ultra-0.5 mL centrifugal filter (Millipore™). Afterwards 250µg (170nmol) of GPI **3** was incubated with an equimolar amount of TCEP for 1h in 500µL PBS. The GPI solution was added to the concentrated maleimide-modified CRM_{197} and the solution was incubated for 3 h at room temperature. The conjugate was purified using a G25 column (10mmx140mm, eluent 5%EtOH in water) and the fractions containing the protein were identified using Bradford solution. The fractions containing the

conjugate were pooled and the protein concentration was determined by BCA Protein Assay (Pierce). Finally the solution was lyophilized to yield the conjugate as a white solid. Purity and loading were determined via MALDI mass analysis.

Example 11b: In batch conjugation to an olefin-modified protein at 254 nm

Compound of general formula (**I**) (10 equiv.) and olefin-modified CRM_{197} (1 equiv., p. *Angew. Chem.* **2007**, *119,* 5319) were dissolved in a quartz glass reaction vessel under argon atmosphere in degassed PBS at pH = 7.4. The solution was stirred for 6 h under irradiation with light emitted by a low pressure mercury lamp (λ = 254, 77 W). Afterwards the solution was frozen dried and the crude material was purified using size exclusion chromatography (Sephadex-G25, 5% EtOH in water, 10 mmx150 mm) to yield the conjugates of the compound of general formula (**I**) covalently linked to the olefin modified CRM_{197}, as white solids.

Example 11c: **In flow conjugation to an olefin-modified protein at 254 nm**

By using a photochemical flow reactor (*Chem. Eur. J.* **2013**, *19*, 3090) that was fitted with a loop of Teflon AF2400 tubing (566 µL), a solution of compound of general formula (**I**) (10 equiv.) in water (300 µL) was reacted with olefin-modified CRM_{197} (1 equiv., *Angew. Chem.* **2007**, *119,* 5319) in water (300 µL) and AcOH (8 µL; residence time: 10 min, flow rate: 28.3 $\mu L/min^{-1}$ per syringe). The reactor output was lyophilized and the crude material was purified using size exclusion chromatography (Sephadex-G25, 5% EtOH in water, 10 mmx150 mm) to yield the conjugates of the compounds of general formula (**I**) covalently linked to the olefin modified CRM_{197} as white solid.

Example 11d: **In flow conjugation to an olefin-modified protein at 366 nm**

By using a photochemical flow reactor (*Chem. Eur. J.* **2013**, *19*, 3090) that was fitted with a loop of Teflon AF2400 tubing (566 µL), a solution of compound of general formula (**I**) (10 equiv.) in water (300 µL) was reacted with olefin modified CRM_{197} (1 equiv., *Angew. Chem.* **2007**, *119,* 5319) in water (300 µL) and AcOH (8 µL; residence time: 30 min, flow rate: 9.4 $\mu L/min^{-1}$ per syringe). The reactor output was lyophilized and the crude material was purified using size exclusion chromatography (Sephadex-G25, 5% EtOH in water, 10 mmx150 mm) to yield the conjugate of the compound of general formula (**I**) covalently linked to the olefin modified CRM_{197} as white solid.

Example 11e: **Conjugation to a α-iodoacetamide-modified protein**

Conjugation of the compound **4** to CRM_{197} protein is performed as described in **Figure 8**. CRM_{197} (1 mg, 0.017 µmol) was dissolved in sterile filtered double-distilled water (1 mL) and transferred to an Amicon® Ultra-4 centrifugal filter unit (10 kDa cut-off). To wash away the additive sucrose the solution was concentrated to 200 µL,

sterile filtered double-distilled water (800 µL) was added and the solution was concentrated again to 200 µL volume. Phosphate buffer (50 mM NaH_2PO_4, pH 8.5, 800 µL) was added to the solution, which was transferred to an eppendorf tube. Sulfo-SIAB (0.9 mg, 1.7 µmol, Thermo Scientific) was added to the solution, which was agitated for 1 h under the exclusion of light. To wash away unreacted linker the solution was concentrated to 200 µL. Sterile filtered double-distilled water (800 µL) was added and the solution was concentrated again to 200 µL volume. This step was repeated one time. Afterwards, PBS sodium phosphate (pH 8.5, 500 µL) was added to the solution, which was transferred to an eppendorf tube. Compound **4** (250 µg, 0.165 µmol; in 250 µL double-distilled water) that was already incubated for 1 h with an equimolar amount of TCEP·HCl (tris(2-carboxyethyl)phosphine hydrochlorid, Thermo Scientific) was added to the solution. The reaction mixture was agitated for 3 h under the exclusion of light, before a cysteine solution (30 µL, 310 mM) was added to quench unreacted iodoacetamine groups. The conjugate was concentrated again to 200 µL volume. Sterile filtered double-distilled water (800 µL) was added and the solution was concentrated again to 200 µL volume. This step was repeated one time. Sterile filtered double-distilled water (800 µL) was added to the conjugate solution, which was divided in four aliquots of 250 µL each and lyophilized. The white powder was stored at -25°C before use. Maldi-TOF analysis shows the formation of the target conjugate and that on average three compounds **4** were covalently linked to one carrier protein (see **Figure 9**).

Example 11f: In flow conjugation of the compound 4 to a vinyl-modified glycosphingolipid with immunomodulatory properties

By using a photochemical flow reactor (*Chem. Eur. J.* **2013**, *19*, 3090) that was fitted with a loop of Teflon AF2400 tubing (566 µL), a solution of compound **4** (1.5 equiv.) in water (300 µL) was reacted with pentenyl modified (2*S*,3*S*,4*R*)-1-(α-D-galactopyranosyl)-2-hexacosanoylaminooctadecane-3,4-diol (1 equiv.) in water (300 µL) and AcOH (8 µL; residence time: 10 min, flow rate: 28.3 µL/min^{-1} per syringe) (see **Figure 10**). The reactor output was lyophilized and the crude material was purified using size exclusion chromatography (Sephadex-G25, 5% EtOH in water, 10 mmx150 mm) to yield the conjugate of compound **4** covalently linked to the pentenyl modified (2*S*,3*S*,4*R*)-1-(α-D-galactopyranosyl)-2-hexacosanoylaminooctadecane-3,4-diol as white solid.

Example 12: Immunizations with the conjugate consisting of compound 3 covalently linked to CRM_{197} protein

Three female BALB/c mice were immunized *s.c.* with 35 µg conjugate, prepared as described in example **11a**, in Freund's complete adjuvant. All mice were boosted two times with 35µg conjugate in Freund's incomplete adjuvant in two-week intervals. After the second immunization, serum was collected and the antibody titer (total IgG) was determined by microarray six weeks after the first immunization. The results are shown in **Figure 3**.

Example 13: Detection of *T. gondii* GPIs by indirect immunofluorescence

Extracellular tachyzoites collected from cell culture supernatants were fixed with 4% (w/v) paraformaldehyde in PBS for 30 min. Cells were washed three times with PBS, and incubated for 1 h with mice sera raised against compounds 3 or 4 diluted to 100 in PBS, 10% BSA. Cells were washed three times with PBS before incubated for 1 h with secondary FITC-conjugated anti-mouse immunoglobulin antibody (DakoCytomation, Glostrup) containing 10% BSA and washed finally three times with PBS. After three final washes with PBS, aliquot were spotted on microscope slides followed by a glass cover slides, mounted in Fluoroprep (Dako) and recorded by using a 100× Plan-NeoFluar oil objective lens with NA 1.30 using an Axiophot microscop (Zeiss). The results are shown in **Figure 4**.

Example 14: Immunizations with the conjugate consisting of compound 4 covalently linked to CRM_{197} protein.

To evaluate the immunogenic properties of the conjugate consisting of compound **4** covalently linked to CRM_{197} protein, obtained as described at example 11e, BALB/c mice were immunized and boosted two times with 35 µg conjugate (in each case) in Freund's incomplete adjuvant in two-week intervals. The conjugate proved immunogenic in all mice and immunoglobulin (Ig) class-switching and affinity maturation were detected by carbohydrate microarray analysis (see **Figure 11A**). IgG antibodies against compound **4** were detected up to a dilution of 1:1000 in sera of all mice six weeks after the first immunization. The nature of the IgG response was further evaluated, demonstrating that antibodies raised against compound **4** mainly consisted in IgG_1 and IgG_{2a} subclasses, while IgG_3 was almost indetectable (see **Figure 11B**), which is in agreement with previous results (*Infect. Immun.* **1999**, *67*, 4862). The high abundance of IgG_{2a}, which exhibits strong antibody-dependent cellular and complement-dependent cytotoxicity, suggests that the immune response to the conjugate of compound **4** to CRM_{197} can induce phagocytosis or lysis of the parasite *in vivo*, assuming that the antibodies recognize the natural antigen on *T. gondii* cells.

Example 15: Specificity and epitope recognition of the antibody response against compound 4

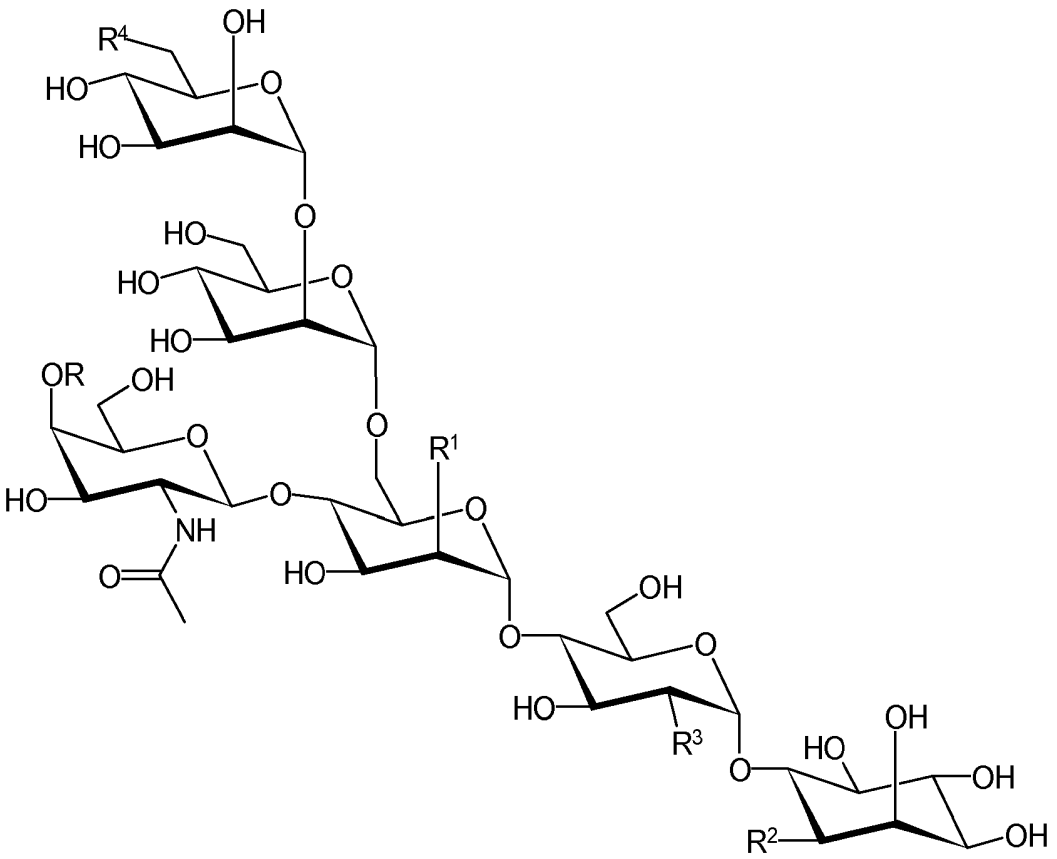
To address the specificity and epitope recongnition of the antibody response, carbohydrate microarray analysis with substructures of compound **4** was employed (see **Figure 12**). The immune response to all animals was highly specific towards compound **4**, as antibodies did not recognize any of the substructures of compound **4** (see **Figure 12**) at a dilution of 1: 1000. This indicates a possible conformational change induced by the α-$GcNH_2$-(1→6)-*myo*-Ino moiety that affects the whole glycan **4**, since none of the substructures contains this element. Therefore, the structural conformation of compound **4** likely differs from the analyzed substructures, which could explain the preference of the polyclonal antibodies. Another explanation for this specificity might be that the raised antibody recognizes multiple epitopes on compound **4**. Hence the avidity of IgGs is significantly lower when one or more structural features are not present.

Example 16: Recognition of the natural GPI antigen displayed on the *T. gondii* parasite by the antibodies raised against compound 4

To confirm that the antibodies raised against compound **4** recognize the natural GPI antigen displayed on the parasite, *T. gondii* tachyzoites were incubated with serum of immunized mice and analyzed with immunofluoresecence (IF) confocal microscopy (see **Figure 13**). The antibodies bound to the surface of the parasite and showed preferential localization of the GPI containing the additional α-glucose in the side chain at the apical end of the cell. In contrast, antibodies binding to the parasite in sera of mice before immunization could not be detected. These results indicate that the GPI containing the additional α-glucose in the side chain potentially plays a role in the formation or function of the apical complex, which is essential for invasion of host cells and plays a critical role during replication of *T. gondii*. Tachyzoites secrete factors for attachment, invasion and formation of the parasitophorous vacuole, which is surrounding and protecting the parasite inside the host cell from endocytosis, in a regulated fashion from the apical region. Blocking the site of attachment with opsonizing antibodies directed against the GPI structure containing the α-Glc in the side chain and clustering of this antigen could disturb the organization of the apical membrane leading to inhibition of the cell invasion. This dual mechanism of action has great potential to induce sterile immunity against *T. gondii*

Claims

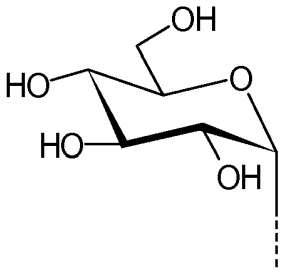
1. Compound of general formula (I)



(I)

wherein

R represents –H, $-CH_3$, $-C_2H_5$, $-C_3H_7$, $-CH(CH_3)_2$, $-C_4H_9$, $-CH_2-CH(CH_3)_2$, $-CH(CH_3)-C_2H_5$, $-C(CH_3)_3$, $-C_5H_{11}$, $-CH(CH_3)-C_3H_7$, $-CH_2-CH(CH_3)-C_2H_5$, $-CH(CH_3)-CH(CH_3)_2$, $-C(CH_3)_2-C_2H_5$, $-CH_2-C(CH_3)_3$, $-CH(C_2H_5)_2$, $-C_2H_4-CH(CH_3)_2$, $-C_6H_{13}$, $-C_3H_6-CH(CH_3)_2$, $-C_2H_4-CH(CH_3)-C_2H_5$, $-CH(CH_3)-C_4H_9$, –Ph, $-CH_2$–Ph, or



;

R^1 and R^4 represent independently of each other –OH or –OP(O)(OH)–O–X–NH_2;

X represents $–CH_2–$, $–C_2H_4–$, $–C_3H_6–$, $–C_4H_8–$, $–C_5H_{10}–$, or $–C_6H_{12}–$,

R^2 represents $–SO_2(R^5)$, $–OSO_2(R^5)$, $–OSO_2(OR^5)$, or $–OP(O)(OR^5)(OR^6)$;

R^3 represents –H, –OH, $–NH_2$, $–NHCOCH_3$, $–NHCOCH_2CH_3$, $–NHCOCH_2CH_2CH_3$, or $–N_3$;

R^5 and R^6 represent independently of each other –H, –L–SH, $–(C_2H_4O)_r–CH_2–SH$ or $–(C_2H_4O)_r–C_2H_4–SH$ and R^5 and R^6 cannot be simultaneously –H;

L represents a linker;

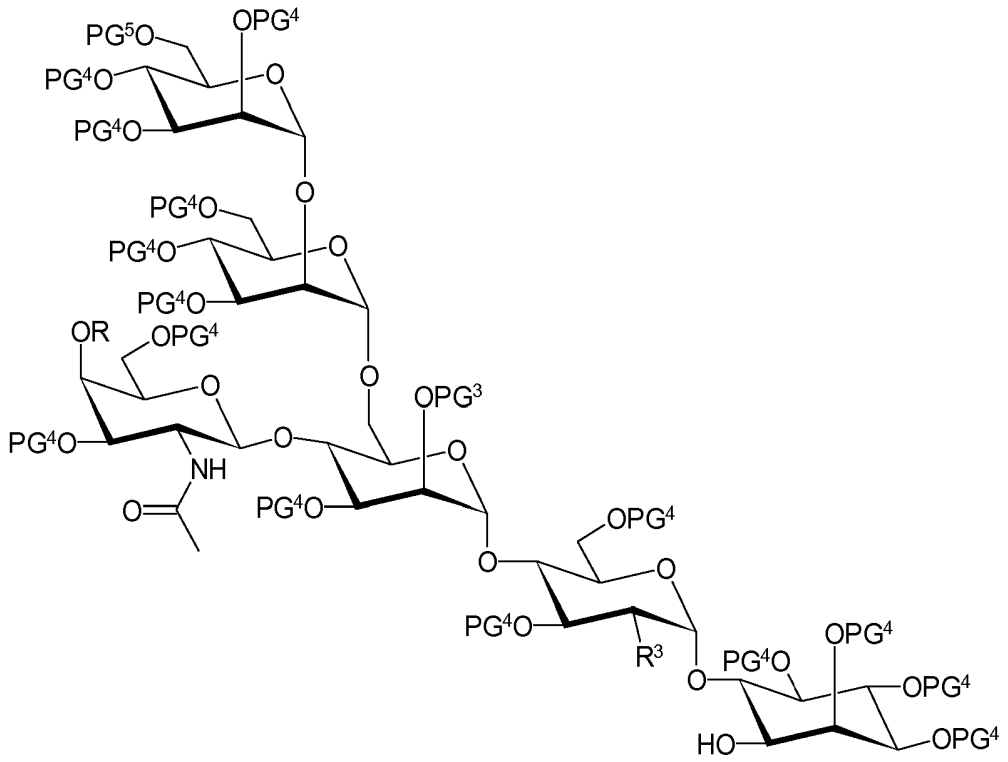
and r is an integer from 1 to 40.

2. Compound according to claim 1, wherein R represents –H.

3. Compound according to claim 1 or 2, wherein R^1 represents –OH.

4. Compound according to any of the preceding claims, wherein R^2 represents $–OP(O)(OR^5)(OR^6)$,
R^5 represents –H, and
R^6 represents $–C_6H_{12}–SH$.

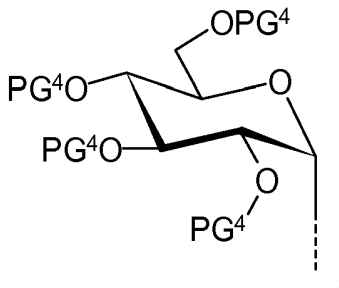
5. Method for synthesis of a compound of formula (**I**) according to the following procedures:
a) providing a compound of the general formula (**IV**)



(IV)

wherein

R represents –H, $-CH_3$, $-C_2H_5$, $-C_3H_7$, $-CH(CH_3)_2$, $-C_4H_9$, $-CH_2-CH(CH_3)_2$, $-CH(CH_3)-C_2H_5$, $-C(CH_3)_3$, $-C_5H_{11}$, $-CH(CH_3)-C_3H_7$, $-CH_2-CH(CH_3)-C_2H_5$, $-CH(CH_3)-CH(CH_3)_2$, $-C(CH_3)_2-C_2H_5$, $-CH_2-C(CH_3)_3$, $-CH(C_2H_5)_2$, $-C_2H_4-CH(CH_3)_2$, $-C_6H_{13}$, $-C_3H_6-CH(CH_3)_2$, $-C_2H_4-CH(CH_3)-C_2H_5$, $-CH(CH_3)-C_4H_9$, –Ph, $-CH_2-Ph$, or



,

R^3 represents $-N_3$, and

PG^3 to PG^5 represent suitable protecting groups for hydroxyl functional groups;

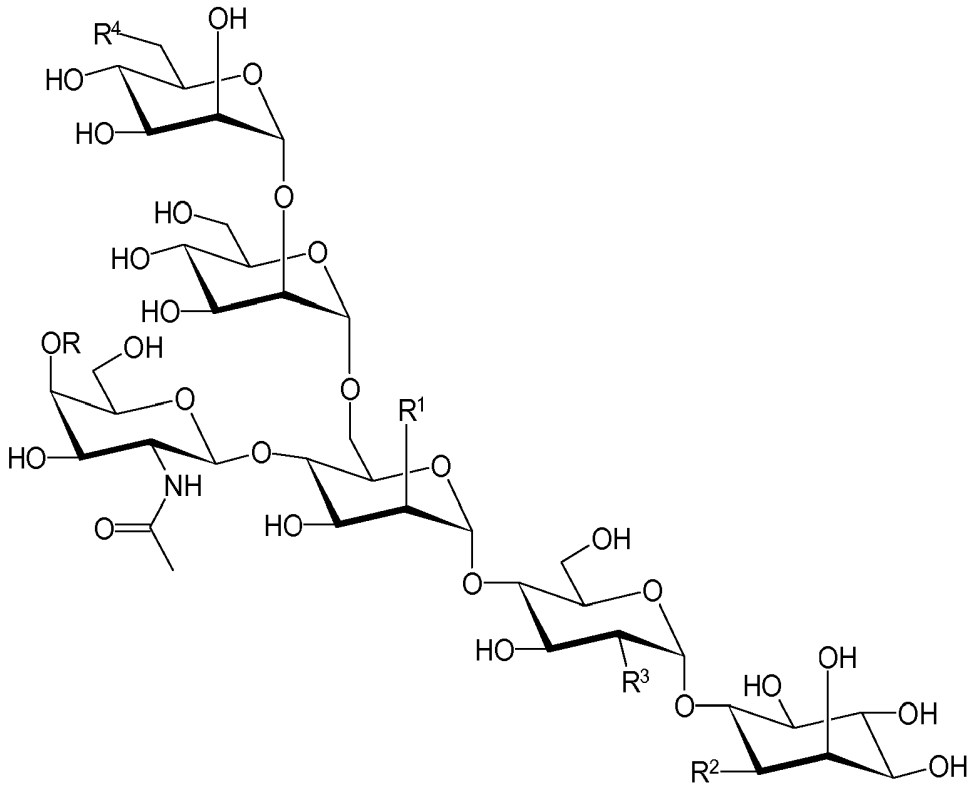
b) introducing the substituent R^2 by reacting a compound of step a) with an acid derivative selected from $H-SO_2(OR^{5'})$ or $H-P(O)(OR^{5'})(OR^{6'})$ or a salt thereof,

wherein $R^{5'}$ and $R^{6'}$ represent independently of each other –H, $–CH_2–S–PG^6$, $–C_2H_4–S–PG^6$, $–C_3H_6–S–PG^6$, $–C_4H_8–S–PG^6$, $–C_5H_{10}–S–PG^6$, $–C_6H_{12}–S–PG^6$, $–C_7H_{14}–S–PG^6$, $–C_8H_{16}–S–PG^6$, $–C_9H_{18}–S–PG^6$, $–C_{10}H_{20}–S–PG^6$, $–CH=CH–S–PG^6$, $–C(=O)–(CH_2)_n–S–PG^6$ and $R^{5'}$ and $R^{6'}$ cannot be simultaneously –H and wherein PG^6 is a suitable protecting group for a thiol,

c) introducing the substituent R^4 by removing PG^5 or by removing PG^5 and subsequent conversion with an acid of the formula $H–P(O)(OH)–O–C_2H_4–NH(PG^7)$ or a salt thereof, wherein PG^7 is a suitable protecting group for an amine;

d) introducing the substituent R^1 by removing PG^3 or by removing PG^3 and subsequent conversion with an acid of the formula $H–P(O)(OH)–O–C_2H_4–NH(PG^7)$ or a salt thereof, wherein PG^7 is a suitable protecting group for an amine;

e) deprotecting the compound of step d) by removing the protection groups PG^4, PG^6 and PG^7 resulting in a compound of formula (I)



wherein R, R^1 – R^4 have the meanings as defined in claim 1,

characterized in that steps c) and d) can be performed consecutively or simultaneously.

6. Method according to claim 5, wherein step c) is performed after step d).

7. Method according to claim 5, wherein in steps b), c) and d) ammonium salts of the corresponding acids are used.

8. Compound of any one of claims 1 – 4 covalently linked to a carrier.

9. Compound according to claim 8, wherein the carrier is selected from the group comprising a diphtheria toxoid, a mutated diphtheria toxoid, a modified diphtheria toxoid or tetanus toxoid.

10. Compound of any one of claims 1 – 4 immobilized on a carrier material by covalent bonding.

11. Compound according to claim 10 wherein the carrier material is selected from the group comprising a glass slide, a microtiter plate, test tubes, microspheres, nanoparticles or beads.

12. Compound according to any one of claims 1 – 4, 8 or 9 for vaccination against toxoplasmosis.

13. Use of the compound according to any one of the claims 1 – 4, 10 or 11 for diagnosis of toxoplasmosis.

14. Use according to claim 13 for diagnosis of acute toxoplasmosis.

15. A kit including at least the following components:

A) A compound of general formula (**I**) immobilized on a carrier material by covalent bonding;
B) at least one antibody:
and
C) a standard solution.

Figure 1

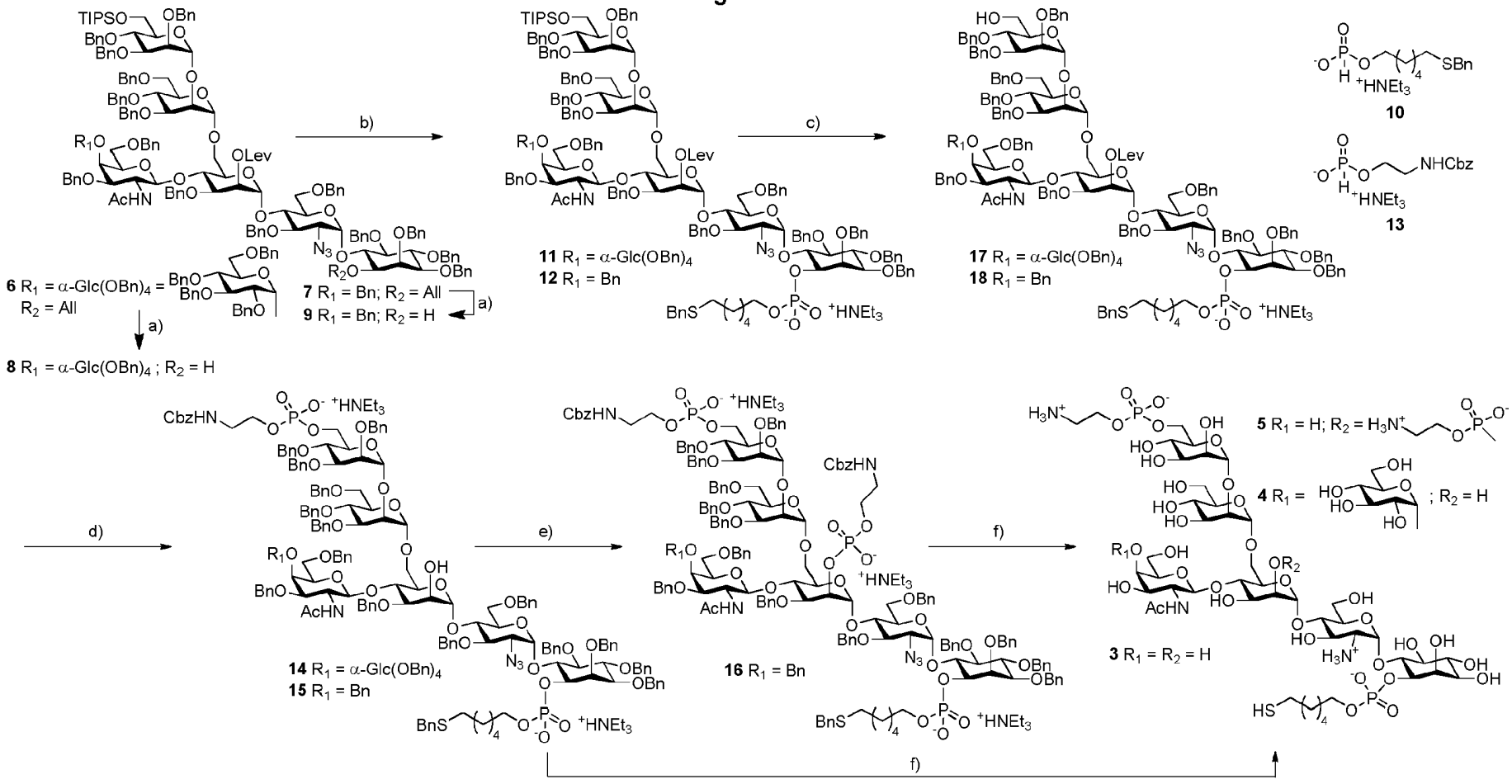
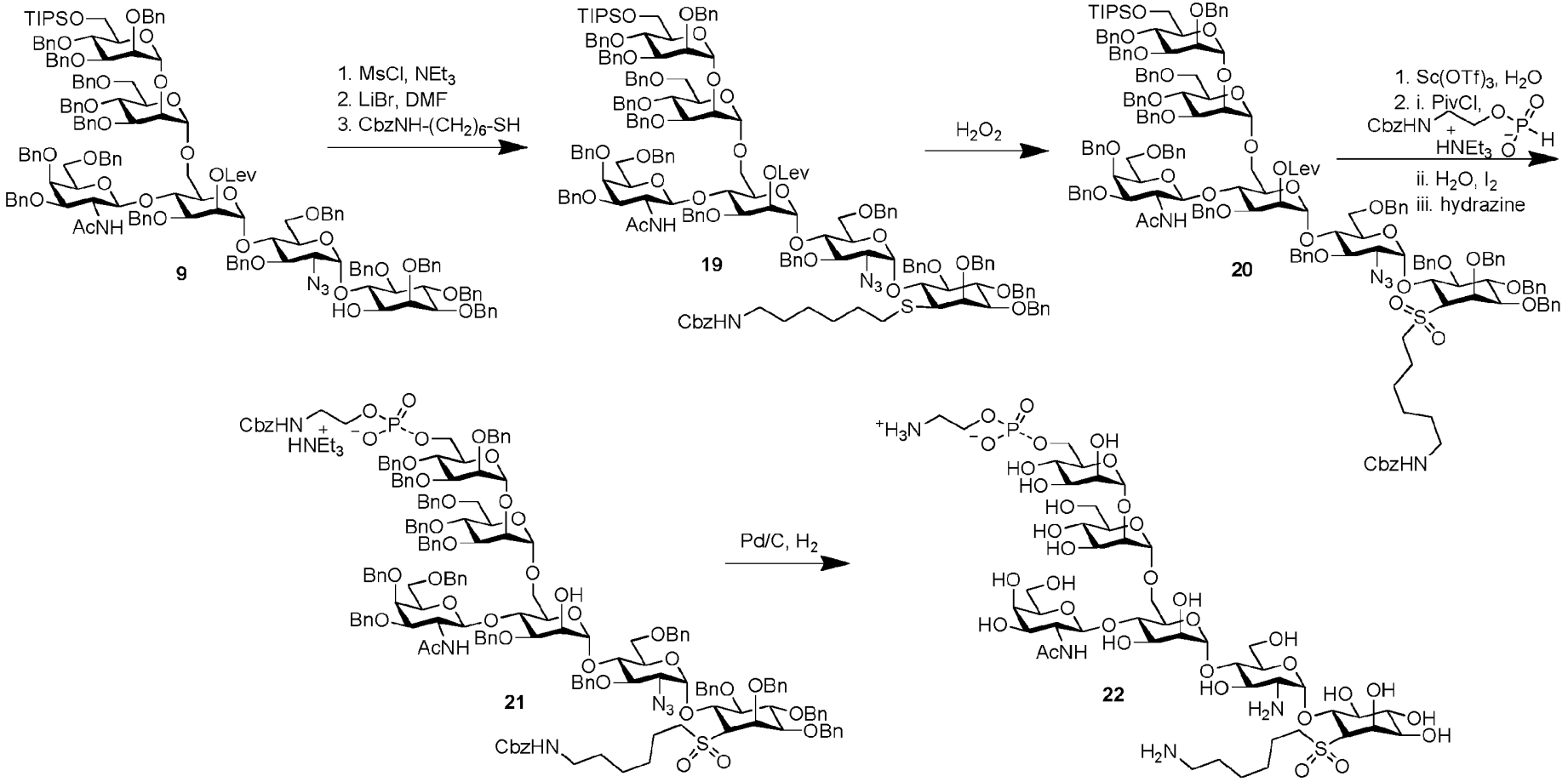

6 R1 = α-Glc(OBn)4 = R2 = All
7 R1 = Bn; R2 = All
9 R1 = Bn; R2 = H
8 R1 = α-Glc(OBn)4 ; R2 = H
11 R1 = α-Glc(OBn)4
12 R1 = Bn
17 R1 = α-Glc(OBn)4
18 R1 = Bn
10
13
14 R1 = α-Glc(OBn)4
15 R1 = Bn
16 R1 = Bn
5 R1 = H; R2 = H3N+
4 R1 = ; R2 = H
3 R1 = R2 = H
a)
b)
c)
d)
e)
f)

Figure 2


1. MsCl, NEt3
2. LiBr, DMF
3. CbzNH-(CH2)6-SH
H2O2
1. Sc(OTf)3, H2O
2. i. PivCl,
ii. H2O, I2
iii. hydrazine
Pd/C, H2
9
19
20
21
22

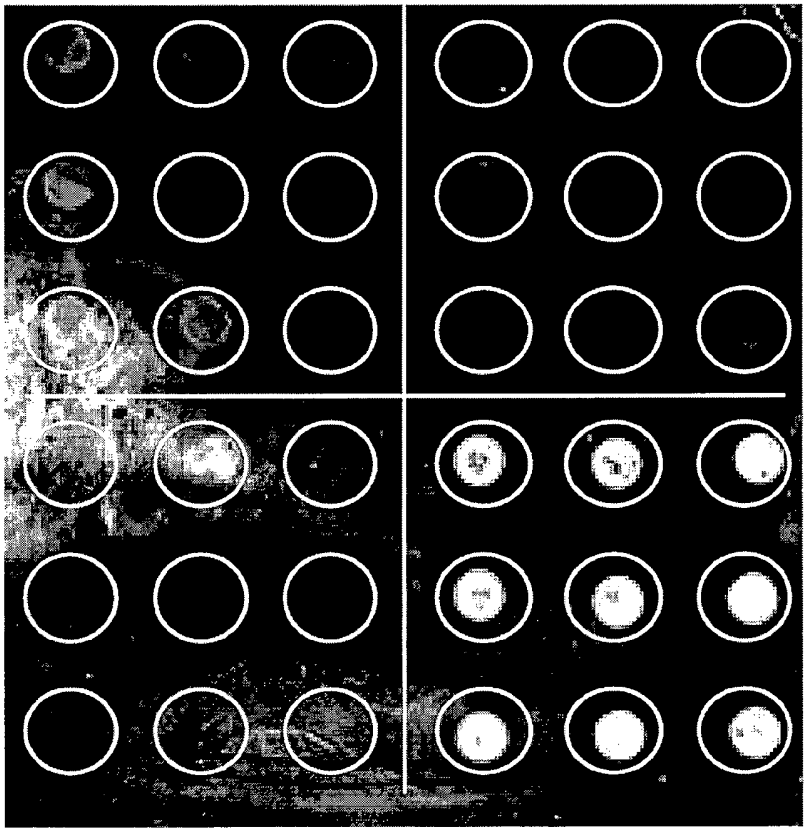


Figure 3

Figure 4

Figure 5

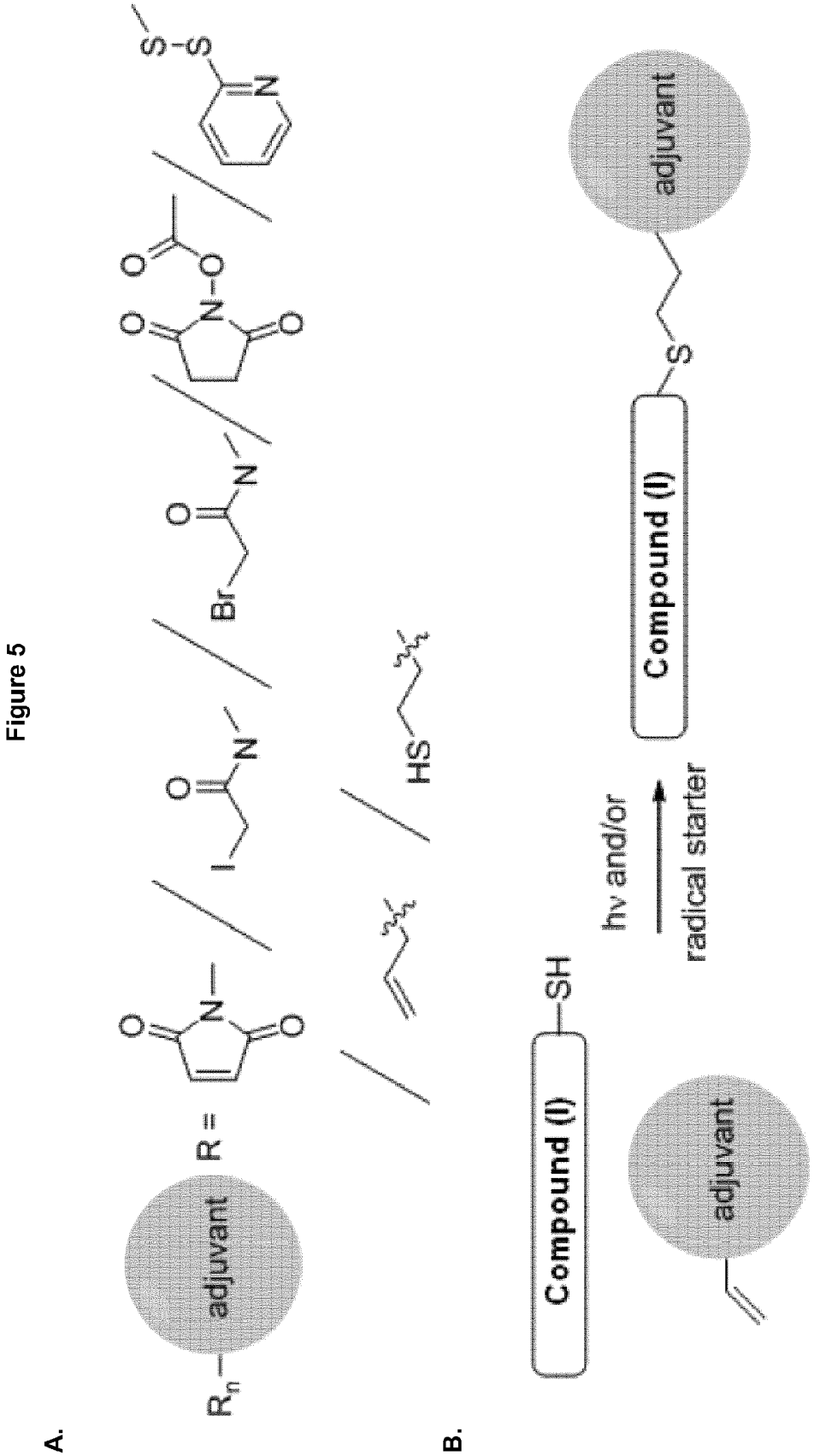

A.
adjuvant
R_n
R =
B.
Compound (I)
SH
hν and/or
radical starter
adjuvant
Compound (I)
S
adjuvant

Figure 6

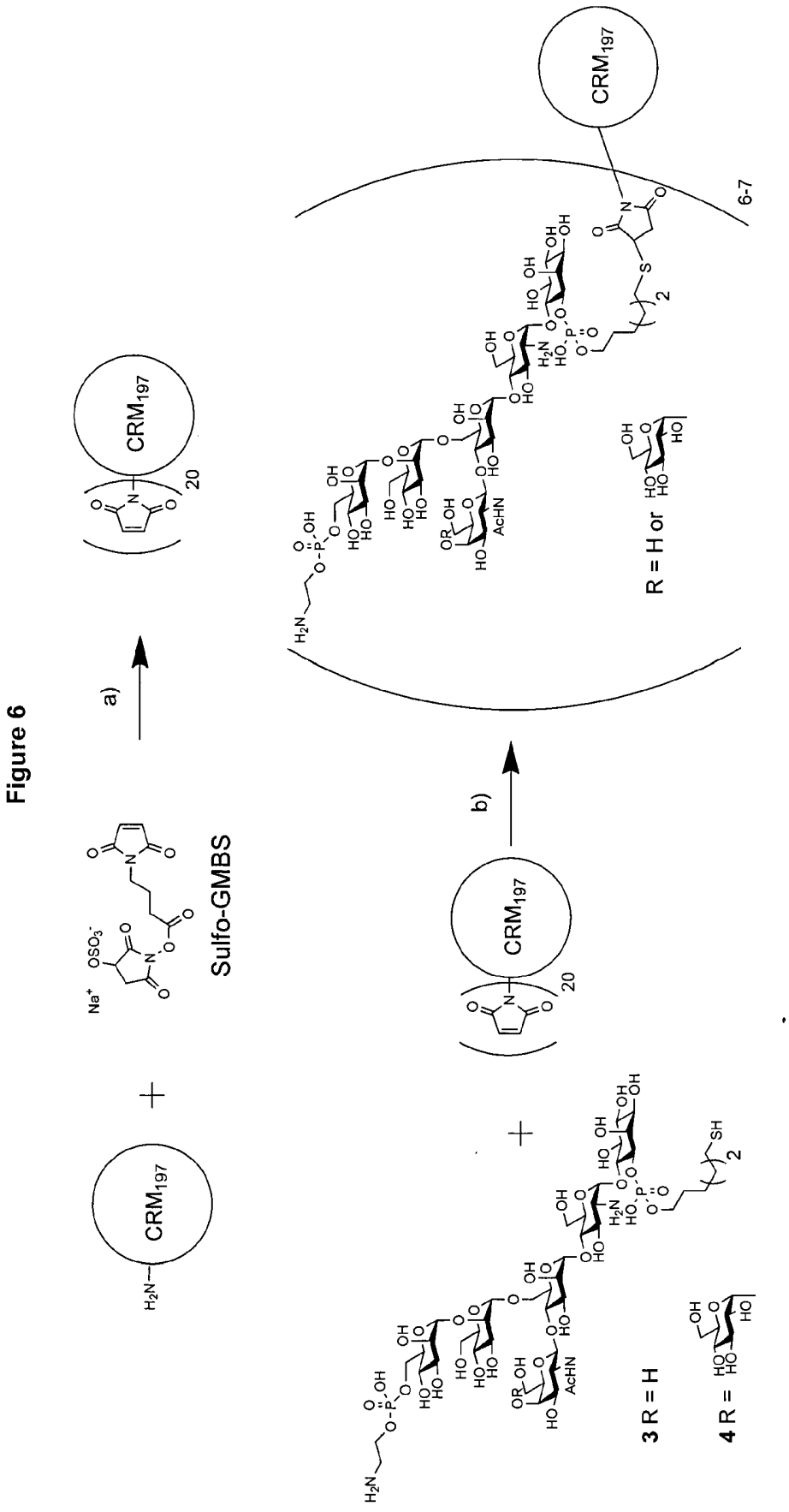
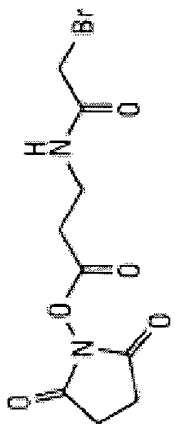
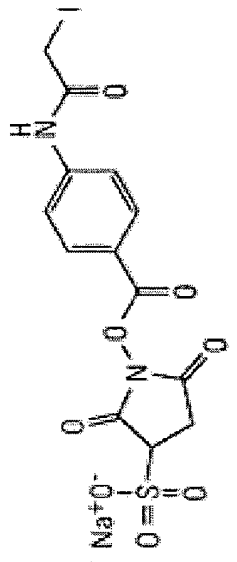


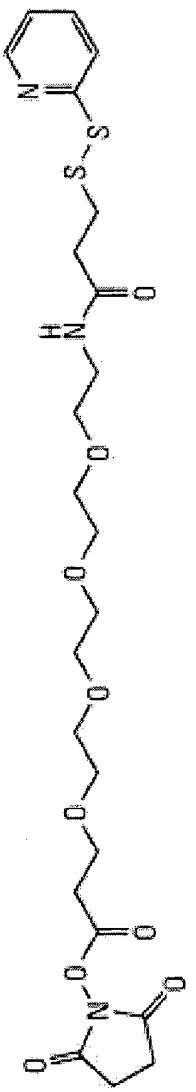
Figure 7



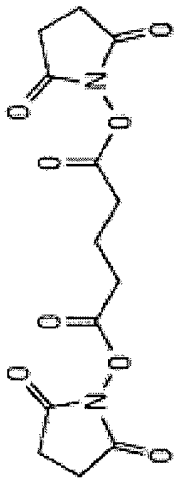
SBAP
Succinimidyl-3-(bromoacetamido)propionate
MW 307.10
Spacer Arm 6.2 Å



Sulfo-SIAB
Sulfosuccinimidyl (4-iodoacetyl) aminobenzoate
MW 504.19
Spacer Arm 10.6 Å



PEG4-SPDP
2-Pyridyldithiol-tetraoxatetradecane-*N*-hydroxysuccinimide
MW 559.17
Spacer Arm 25.7 Å



DSG
Disuccinimidyl glutarate
MW 326.26
Spacer Arm 7.7 Å

Figure 8

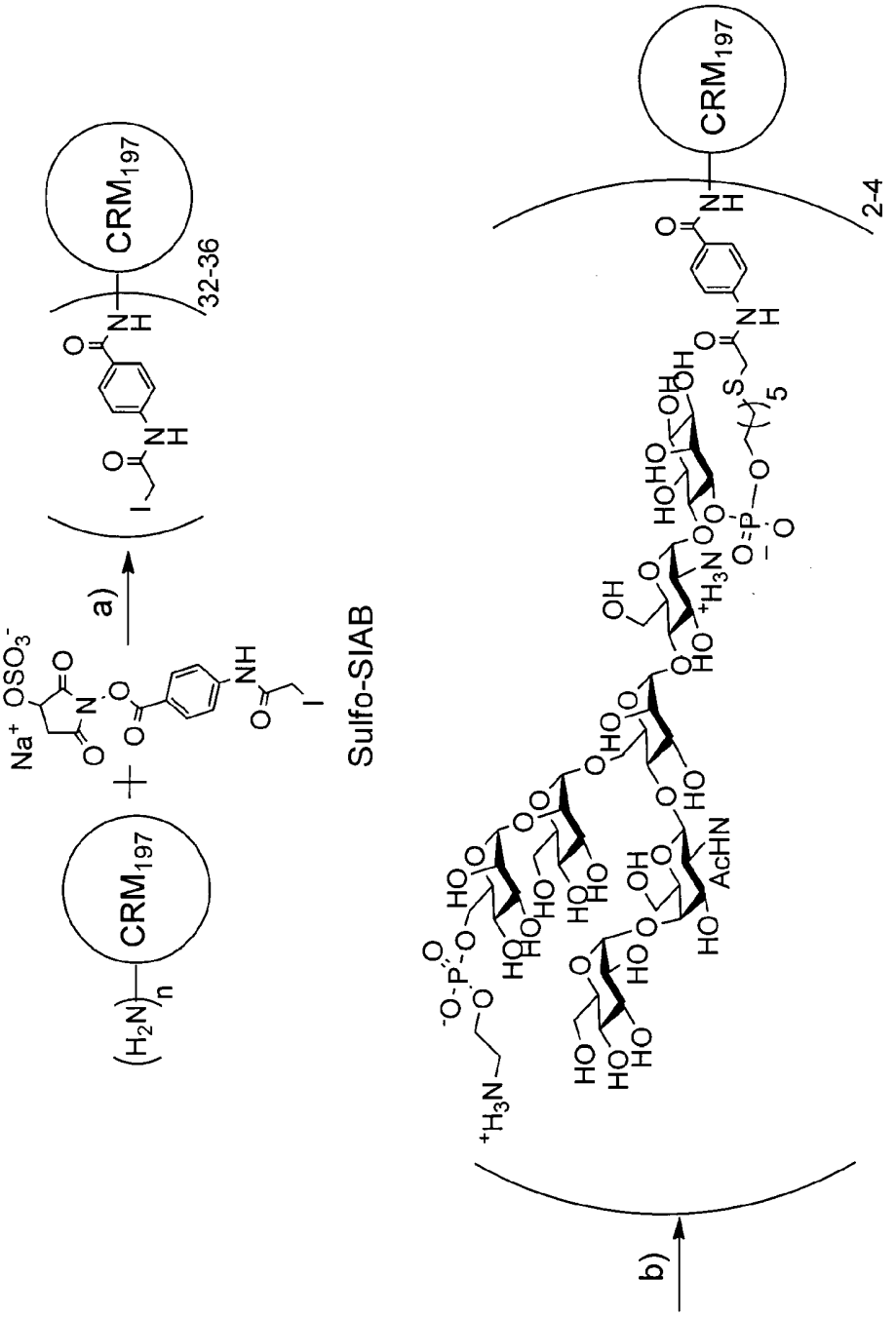
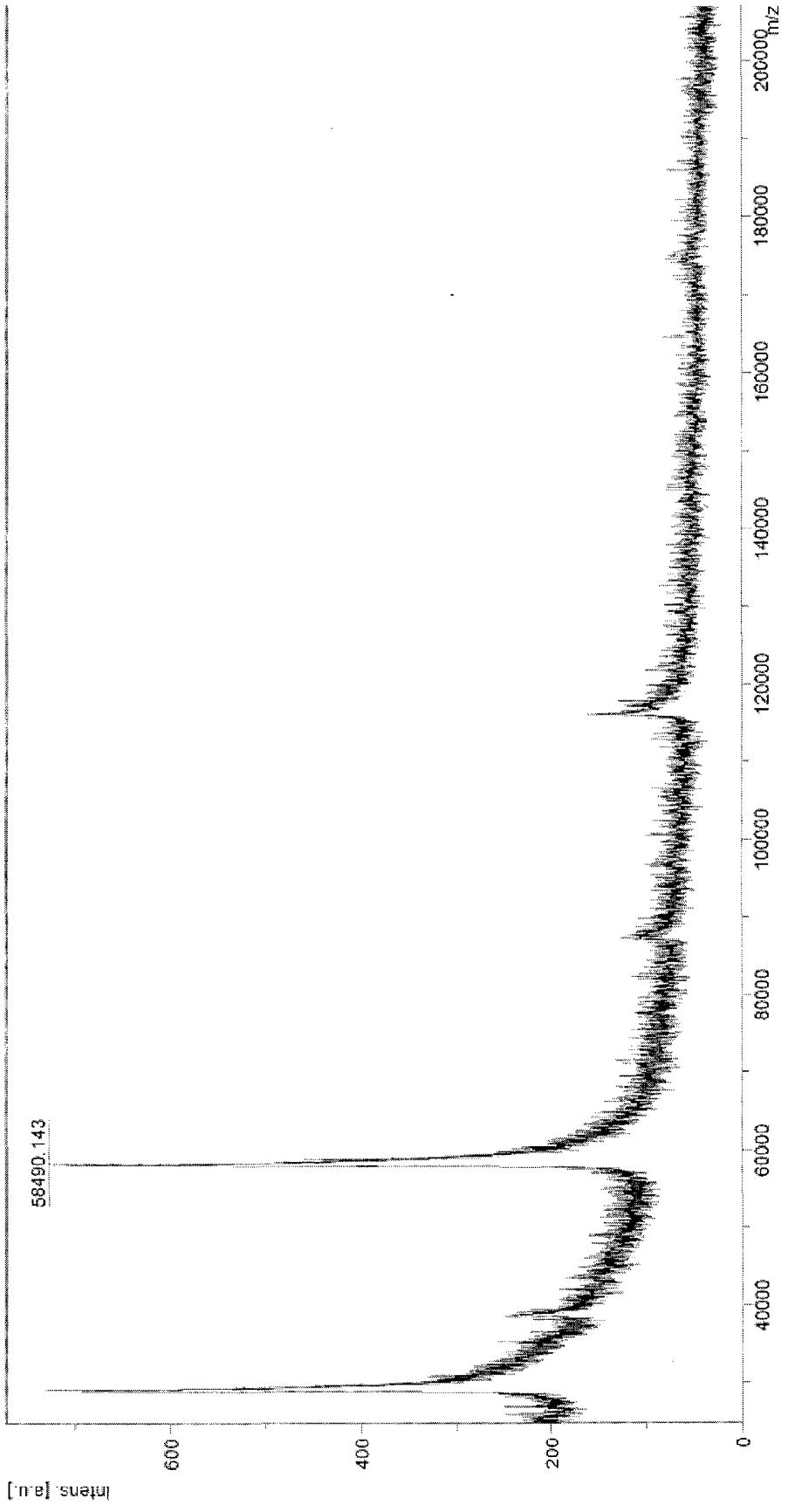
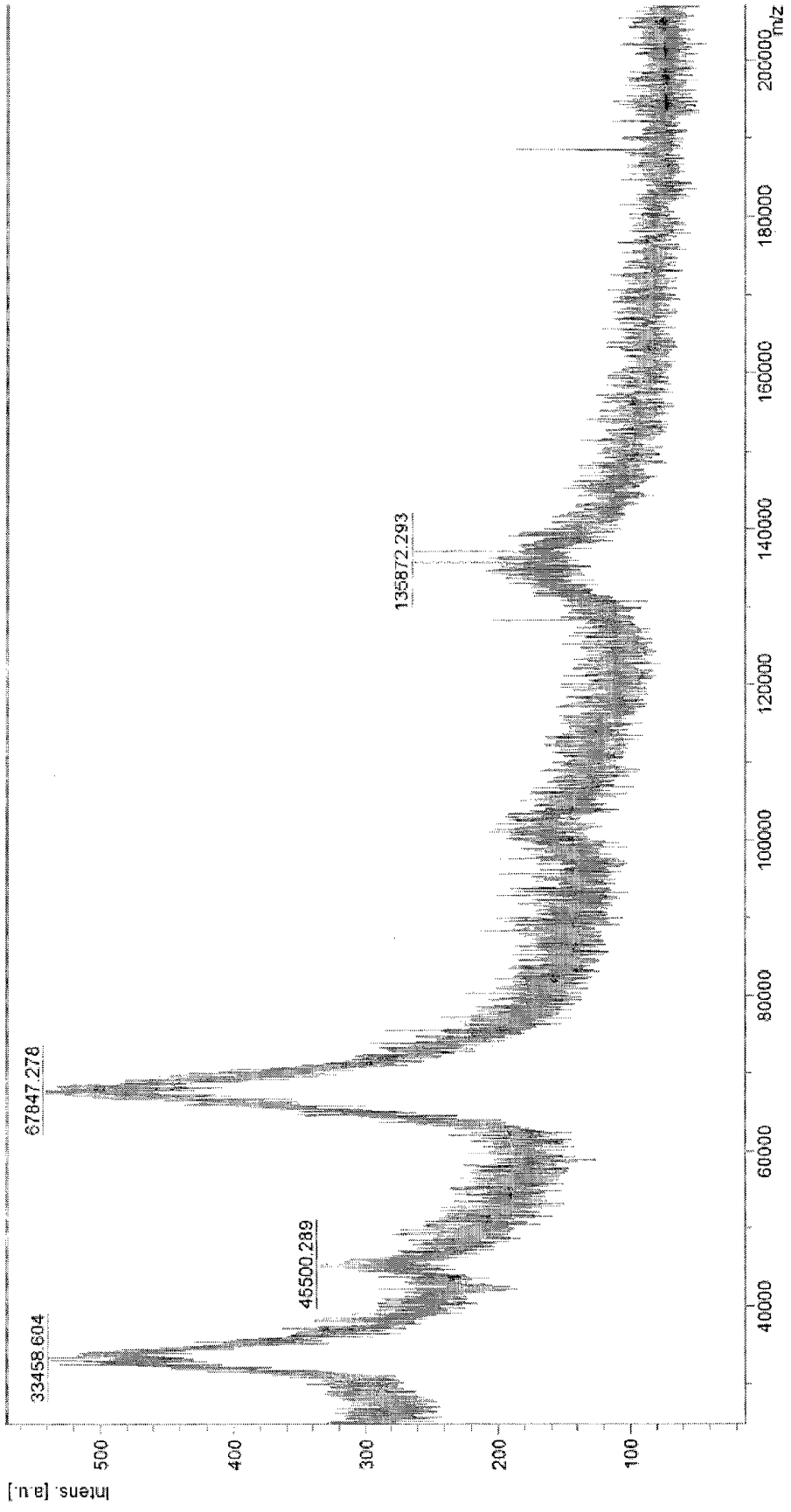


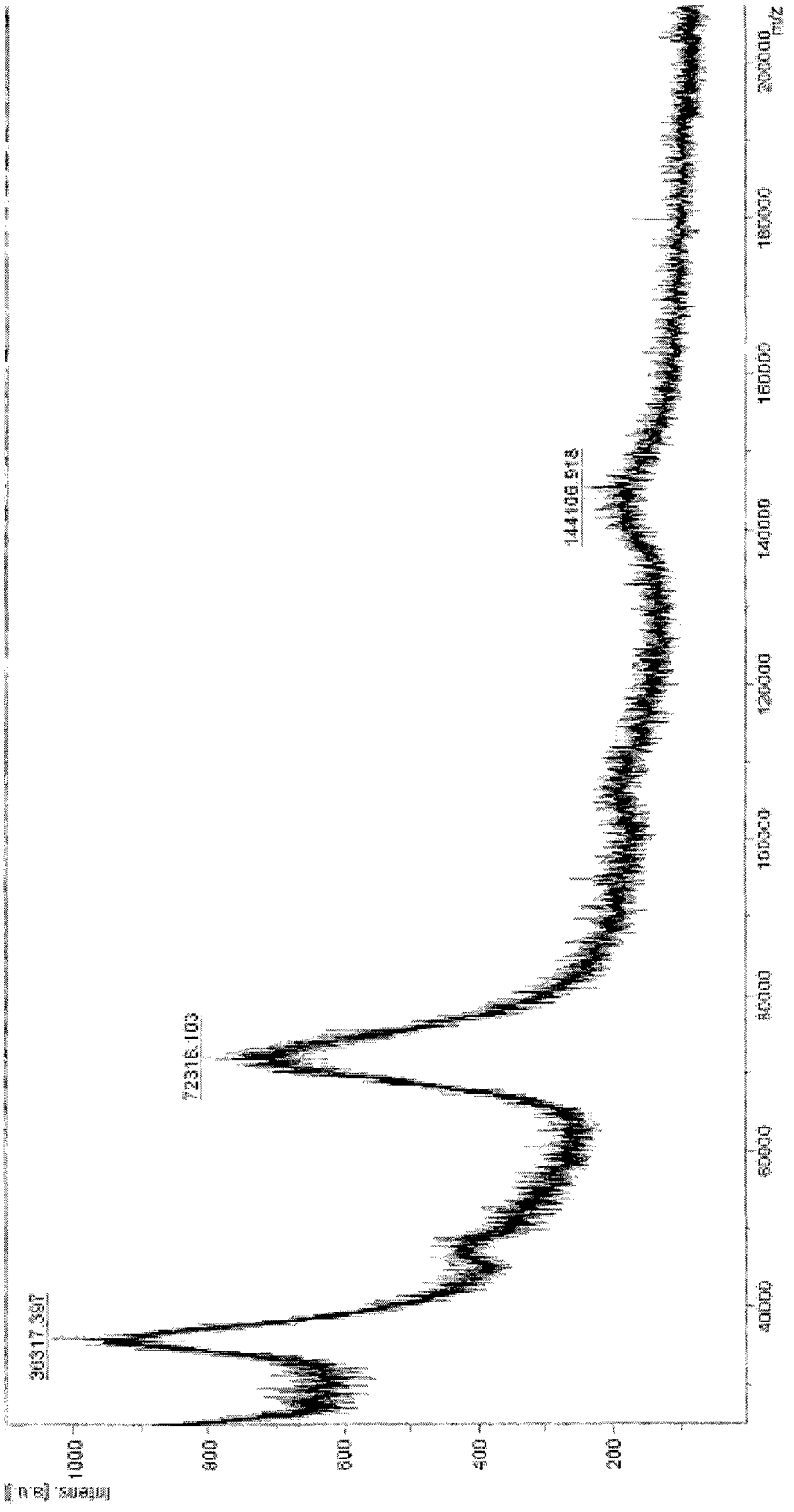
Figure 9


58490.143
Intens. [a.u.]
600
400
200
0
40000
60000
80000
100000
120000
140000
160000
180000
200000
m/z

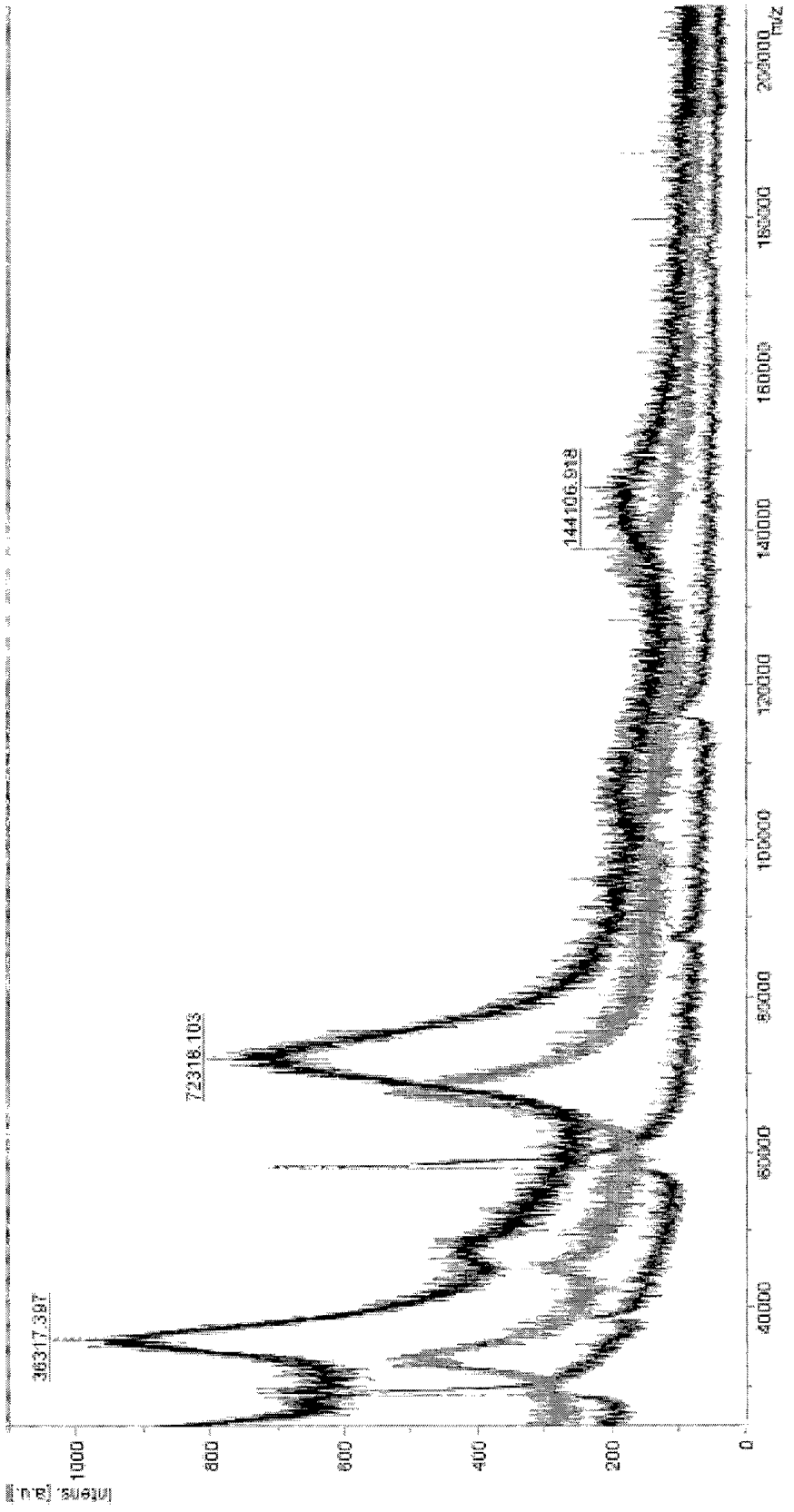
A.


Intens. [a.u.]
500
400
300
200
100
33458.604
45500.289
67847.278
135872.293
40000
60000
80000
100000
120000
140000
160000
180000
200000
m/z

B.


36317.397
72316.103
144106.918
Intens. [a.u.]
1000
800
600
400
200
40000
60000
80000
100000
120000
140000
160000
180000
200000
m/z

c.


36317.397
72318.103
144106.918
Intens. [a.u.]
1000
800
600
400
200
0
40000
60000
80000
100000
120000
140000
160000
180000
200000
m/z

D.

Figure 10

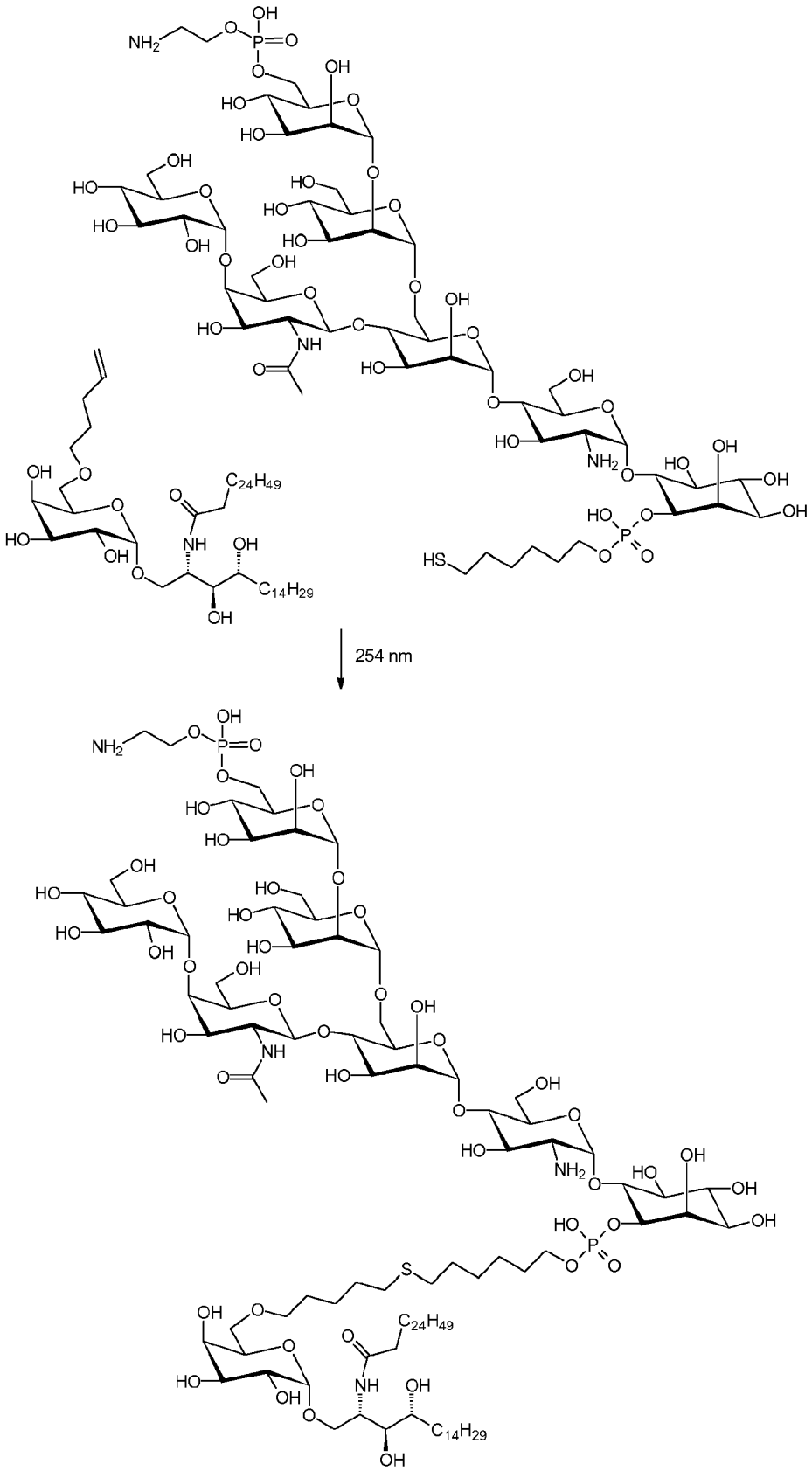


Figure 11

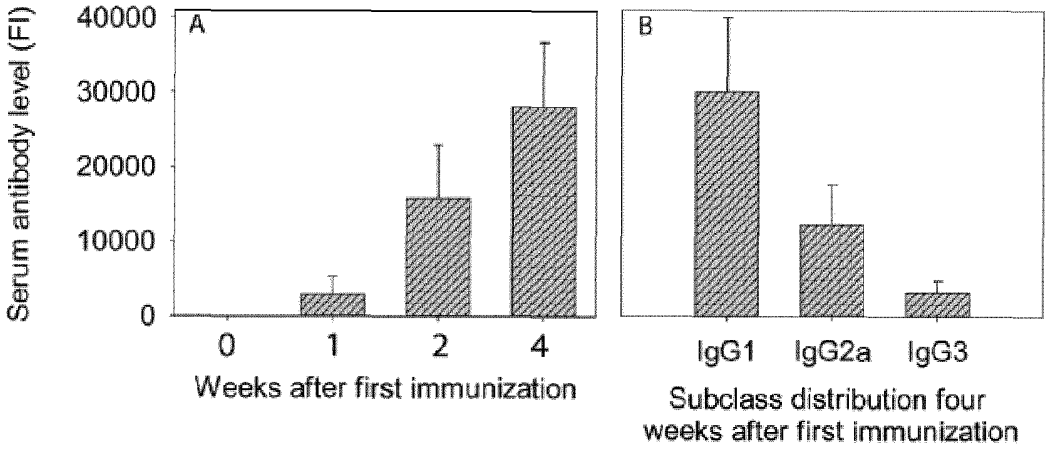

Serum antibody level (FI)
40000
30000
20000
10000
0
A
0
1
2
4
Weeks after first immunization
B
IgG1
IgG2a
IgG3
Subclass distribution four
weeks after first immunization

Figure 12

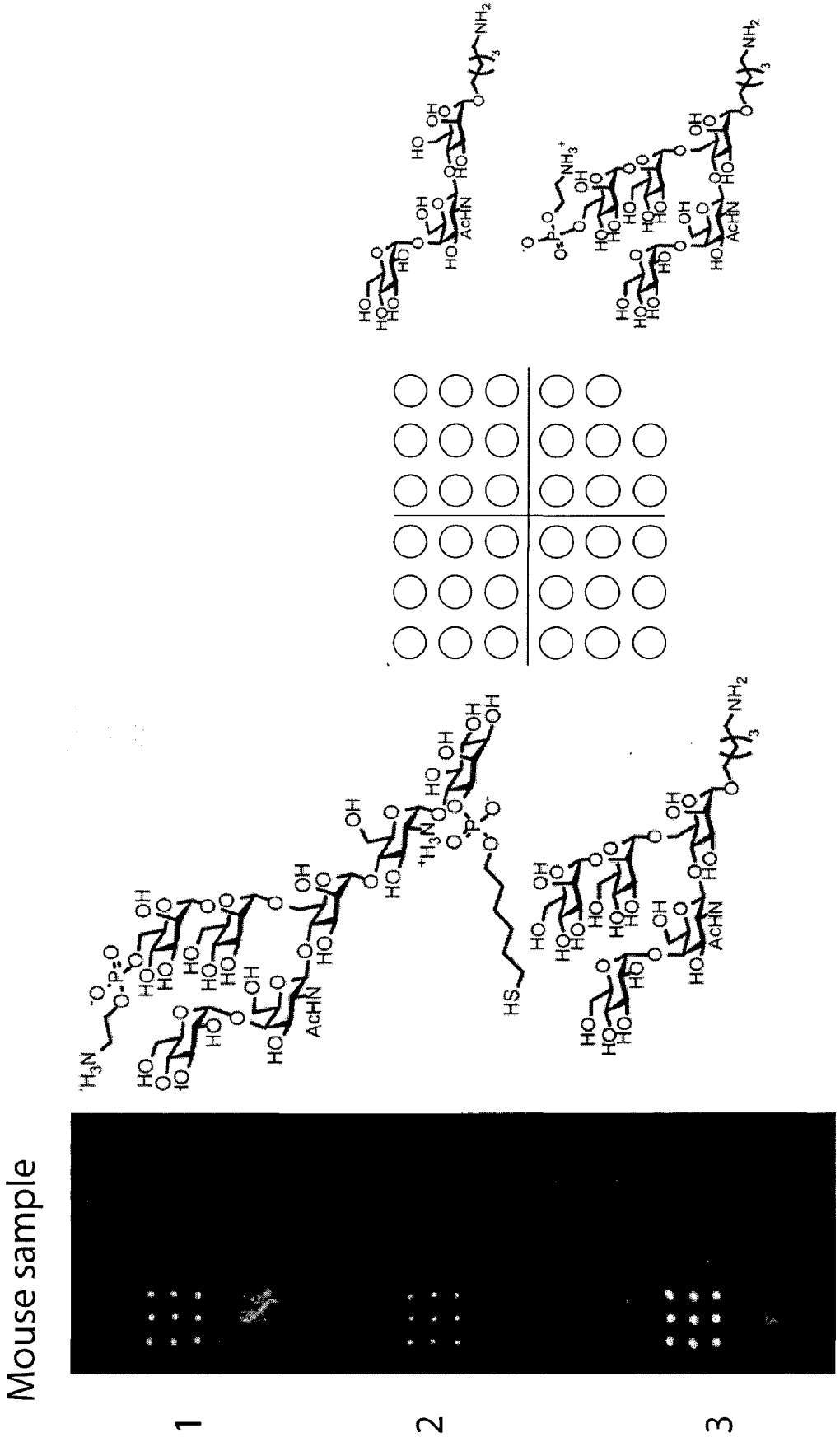
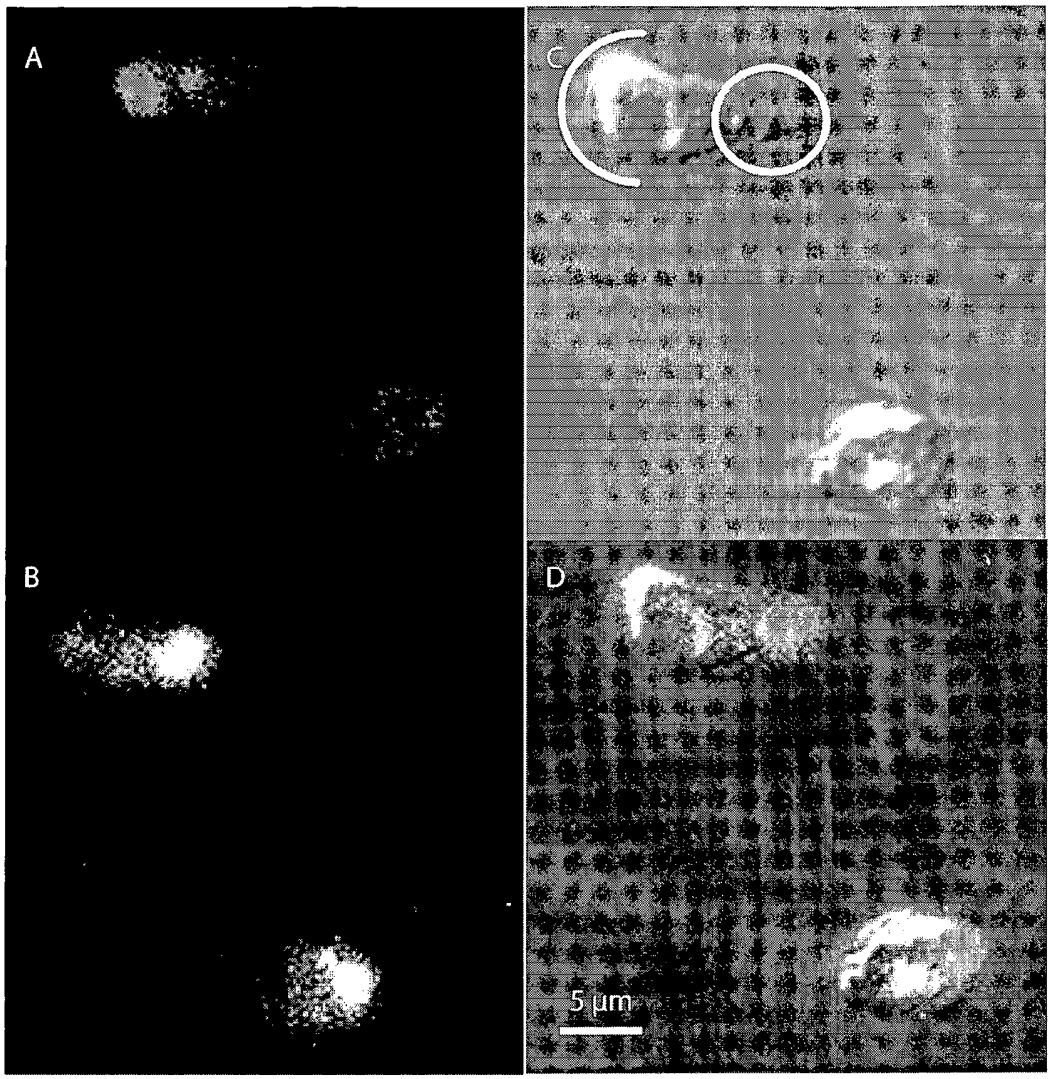


Figure 13



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/065559

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07H15/207 C08B37/00 A61K31/7034 A61P33/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07H C08B

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97/10249 A1 (AKZO NOBEL NV [NL]; KOOLEN MARCUS JOSEPHUS MARIE [NL]; DAMM JAN BAS LO) 20 March 1997 (1997-03-20) cited in the application	1-3,8-15
Y	claims 4,5,8,14-22	1-4,8-15

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 2013	24/10/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Mezzato, Stefano

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YU-HSUAN TSAI ET AL: "A General Method for Synthesis of GPI Anchors Illustrated by the Total Synthesis of the Low-Molecular-Weight Antigen from Toxoplasma gondii", ANGEWANDTE CHEMIE INTERNATIONAL EDITION, vol. 50, no. 42, 10 October 2011 (2011-10-10), pages 9961-9964, XP055039696, ISSN: 1433-7851, DOI: 10.1002/anie.201103483	1-3,8-15
Y	page 9962; compound 1 page 9963, schemes 4-5 page 9964, last paragraph	1-15
A	YONG-UK KWON ET AL: "Total syntheses of fully lipidated glycosylphosphatidylinositol anchors of Toxoplasma gondii", CHEMICAL COMMUNICATIONS, no. 17, 1 January 2005 (2005-01-01), page 2280, XP055039709, ISSN: 1359-7345, DOI: 10.1039/b501373a page 2280; figure 1; compounds 1-2 page 2281; scheme 2	1
A	Françoise Debierre-Grockiego ET AL: "Activation of TLR2 and TLR4 by Glycosylphosphatidylinositols Derived from Toxoplasma gondii", J Immunol 2007; 179, 15 July 2007 (2007-07-15), pages 1129-1137, XP055039705, Retrieved from the Internet: URL:http://www.jimmunol.org/content/179/2/1129.full.pdf [retrieved on 2012-10-01] page 1131; figure 1; compound GPIa	1
A	KLAUS PEKARI ET AL: "Synthesis of the Fully Phosphorylated GPI Anchor Pseudohexasaccharide of Toxoplasma g ondii", THE JOURNAL OF ORGANIC CHEMISTRY, vol. 66, no. 22, 1 November 2001 (2001-11-01), pages 7432-7442, XP055039716, ISSN: 0022-3263, DOI: 10.1021/jo015840q page 7433; figure 1; compounds 1a,1b	1

-/--

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/065559

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KLAUS PEKARI ET AL: "A Variable Concept for the Preparation of Branched Glycosyl Phosphatidyl Inositol Anchors", THE JOURNAL OF ORGANIC CHEMISTRY, vol. 68, no. 4, 1 February 2003 (2003-02-01), pages 1295-1308, XP055039715, ISSN: 0022-3263, DOI: 10.1021/jo026380j page 1300, scheme 7 -----	1
Y	KWON YONG-UK ET AL: "Assembly of a series of malarial glycosylphosphatidylinositol anchor oligosaccharides", CHEMISTRY - A EUROPEAN JOURNAL, WILEY - V C H VERLAG GMBH & CO. KGAA, WEINHEIM, DE, vol. 11, no. 8, 8 April 2005 (2005-04-08), pages 2493-2504, XP002463517, ISSN: 0947-6539, DOI: 10.1002/CHEM.200400934 page 2494; figure 2 page 2495; figure 3 page 2497; scheme 4 -----	1-15

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/065559

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4(completely); 1-3, 5-15(partially)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-3, 8-15(all partially)

   Compound of claim 1, wherein R2 is -SO2(R5); related derivatives, kit

   ---

2. claims: 1-3, 5-15(all partially)

   Compound of claim 1, wherein R2 is -OSO2(R5) or -OSO2(OR5); related derivatives, kit and method

   ---

3. claims: 4(completely); 1-3, 5-15(partially)

   Compound of claim 1, wherein R2 is -OP(O)(OR5)(OR6), and at least one of R5, R6 is -L-SH, -(C2H4O)r-CH2-SH or -(C2H4O)r-C2H4-SH; related derivatives, kit and method

   ---

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/065559

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9710249 A1	20-03-1997	AT 221539 T	15-08-2002
		AU 714841 B2	13-01-2000
		AU 7085296 A	01-04-1997
		CA 2232012 A1	20-03-1997
		DE 69622728 D1	05-09-2002
		EP 0850244 A1	01-07-1998
		JP 2000502244 A	29-02-2000
		US 2002058288 A1	16-05-2002
		WO 9710249 A1	20-03-1997