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(54) **Title**: CONTINUOUS FLOW PROCESSES FOR PHOTOCONJUGATION OF HIGH-MOLECULAR CHEMICAL ENTITIES

(57) **Abstract**: The invention relates to a continuous flow process for the photoconjugation of chemical entities selected from the group comprising carbohydrates, including mono-, oligo- and polysaccharides, amino acids, peptides and proteins, unbranched and branched oligomers and polymers, including dendrimers, which process comprises at least the following steps: a) providing a mixture of the reactants dissolved in an aqueous or organic solvent; b) passing said mixture through a continuous flow reactor and concomitantly irradiating the mixture with light in order to initiate and perform the photoreaction. In preferred embodiments, the photoconjugation is a thiol-ene conjugation or a [2+2] cycloaddition. A further aspect of the invention relates to the conjugates obtainable by this continuous flow process, in particular dendronized polymers such as carbohydrate-dendronized polymers.

Continuous Flow Processes for Photoconjugation Of High-molecular Chemical Entities

Background

Food contamination with *Escherichia coli*, a pathogenic bacterium, results in frequent outbreaks of infections with grave consequences. Rapid, reliable and straightforward detection of pathogenic bacteria is a key step in avoiding the spread of contamination. Developing detection systems that meet these criteria has proven challenging.

Carbohydrate binding proteins, also known as lectins, can be found on the surface of pathogenic bacteria and are a means by which these organisms recognize and bind to the cells and tissue of the organism of which they are infecting. Invasive strains of *E. coli* express high levels of mannose binding lectins which are displayed on the bacterial pili. The sugar-binding properties of these proteins have been exploited in the development of bacterial detection systems.

Supra-molecular glycopolymers, conductive glycopolymers, carbohydrate capped gold nanoparticles, and CdS quantum dots have all been used as probes to identify specific *E. coli* strains. These methods rely on the multivalent presentation of carbohydrate ligands, which is necessary because the binding affinity between an individual monosaccharide and the lectin is weak. However, these multivalent probes have structures that do not permit adjustment of the carbohydrate ligands to achieve optimal multivalent binding. This structural limitation inhibits multimodal conjugation which would also allow for optimal interaction with the pathogens.

In contrast, dendronized polymers are multivalent systems that integrate the two-dimensional structure of polymers and the three-dimensional structure of dendrimers to create a flexible polymer that can bend to adapt to the environment of the pathogen

surface and optimize binding to the bacterial carbohydrate receptors. The resulting polyvalent systems can thus adjust the carbohydrate units for the strongest and most efficient binding interaction with the lectin. Xu et al. in Journal of Polymer Science: Part A: Polymer Chemistry, Vol. 47, 4302-4313 (2009) and Rendle et al. in J. Am. Chem. Soc. 2004, 126, 4750-4751, describe such dendronized polymers and synthetic routes for preparing the same.

However, these approaches are rather time-consuming, prone to side reactions resulting in undesirable by-products and requiring expensive purification processes and are also insufficient with respect to yields.

Generally, the coupling and/or functionalization of high-molecular chemical entities, such as carbohydrates, peptides, proteins and other oligomers and polymers, often poses a severe challenge as the coupling reaction has to be selective, typically with respect to both chemoselectivity and regio- and/or stereo-selectivity, and high yielding, but must not contaminate the end product which would necessitate additional purification steps. Widely used methods for the synthesis of glycopolymers suffer from a lack of control over carbohydrate epitope density, regioselectivity, metal contamination, and low yields of the desired glycopolymer. Similar problems are encountered during the synthesis and/or functionalization of other complex molecules using conventional coupling reactions and processes as well.

In view of these drawbacks of the prior art, an object of the present invention is to provide an improved process for the conjugation of in particular high-molecular chemical entities which avoids or minimizes the problems as outlined above.

A more specific object of the invention is to provide an improved process for preparing conjugates, in particular high molecular conjugates, which represent or comprise biologically active agents, such as pharmaceuticals, including vaccines, or drugs.

A still more specific object of the invention is to provide an improved process for preparing dendronized polymers, in particular carbohydrate-dendronized polymers.

A related object is the provision of novel conjugates, in particular high molecular conjugates, which represent or comprise biologically active agents, such as pharmaceuticals, including vaccines, or drugs.

A more specific object is the provision of novel dendronized polymers, in particular carbohydrate-dendronized polymers, which may be used favorably for the detection of target bacteria.

These objects are achieved according the present invention by the continuous flow processes according to claims 1 and 2 and the conjugates, in particular dendronized polymers, according to claims 24-26. Specific embodiments and/or additional aspects of the invention are the subject of further claims.

Description of the Invention

The continuous flow process for the photoconjugation of chemical entities selected from the group comprising carbohydrates, including mono-, oligo- and polysaccharides, amino acids, peptides and proteins, unbranched and branched oligomers and polymers including dendrimers according to claim 1 comprises at least the following steps

a) providing a mixture of the reactants dissolved in an aqueous or organic solvent;

b) passing said mixture through a continuous flow reactor and concomitantly irradiating the mixture with light in order to initiate a chemical reaction leading to conjugation.

The process of the present invention may further comprise

c) recovering and optionally purifying the products of the photoreaction.

Historically, photochemical reactions have not been amenable to increasing the scale of the reaction while maintaining efficiency and relative convenience. Typically, this is related to the deficiencies of batch processes with regard to increasing the reaction scale. However, the utilization of a continuous-flow photochemical reactor renders this mode of bond formation very attractive for the coupling/functionalizing of high molecular chemical entities. The high surface-to-volume ratio of the continuous flow micro-device provides precise control over reaction conditions, including controlled radical formation as well as efficient and homogeneous mixing, thus enabling facile conjugation and greatly reduced side reactions.

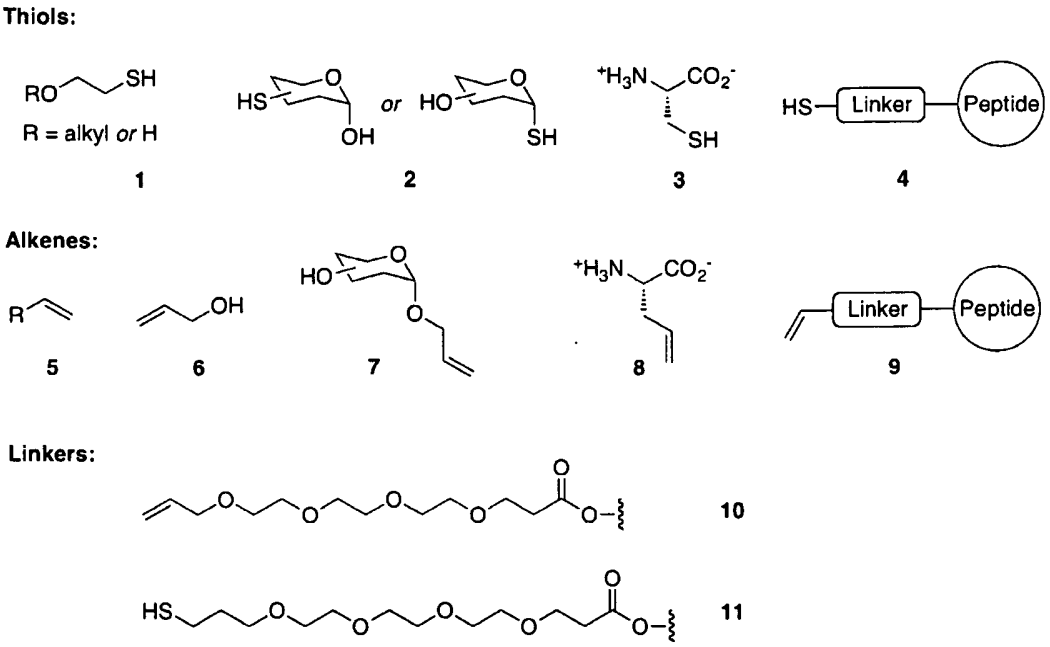
In one preferred embodiment, the process of the present invention involves a photochemical thiol-ene conjugation, also known as the thio-ene click reaction.

Principally, the thiol-containing entity may be any compound, preferable any high molecular compound, capable to participate in a thiol-ene photoconjugation. Also the second reaction component, i.e. the alkenyl double bond-containing entity, may be any compound, preferable any high molecular compound, capable to participate in a thiol-ene photoconjugation. Preferably, the alkenyl double bond will be a terminal double bond.

The following scheme 1 shows a representative but not limiting list of suitable reaction components for this specific kind of conjugation. In particular, it should be pointed out that analogous compounds with the same functional groups but higher molecular weights, e.g. in the case of alkene or alkyne units or linker units with higher chain lengths or in the case of oligo- or polysaccharides, will be suited as well and may be even preferred.

The linker can consist of any molecular entity having the requisite functionality permitting conjugation between the two

species. The linker can contain one or more of either the alkene or thiol functionalities.



Scheme 1

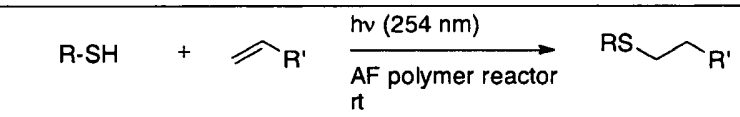
Thus, suitable thiols for this process are e.g. alkyl thiols **1**, thiosugars **2**, thiol-containing amino acids (e.g. cysteine **3**) or peptides/proteins **4** bearing a thiol linker. Suitable alkenes for this process are simple terminal alkenes **5**, allylic alcohols **6**, allyl sugars **7**, allyl amino acids **8** or peptides/proteins **9** bearing an alkene linker. The aforementioned linker **10** or **11** is typically based on a poly(ethylene glycol) structure bound to the peptide/protein *via* an ester linkage and terminated with a thiol or alkene respectively as appropriate). The peptide could also be terminated with a thiol- or allyl-bearing amino acid and be conjugated directly with the appropriate thio- or allyl sugar/carbohydrate.

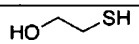
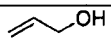
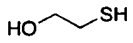
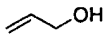
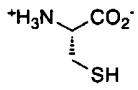
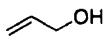
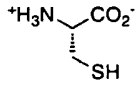
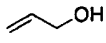
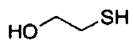
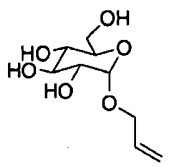
The concentration of the reactants is usually in the range of 0.001 M to 1.0 M, more specifically 0.01 M to 0.1 M or 0.02 M to 1.0 M. Relatively low concentrations such as 0.0001 to 0.0005 M are preferred for biological use. Optimally, one of the reaction

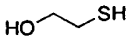
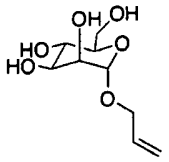
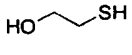
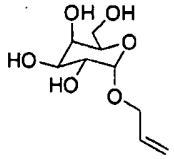
components is used in excess, although conjugations are also attainable using a 1:1 stoichiometry of reaction components.

Representative examples of conjugation reactions successfully performed are shown below in Table 1. A preferred embodiment of the invention involves the reaction of allyl sugars/carbohydrates with cysteine and reactions of peptides and proteins with allyl sugars/carbohydrates.

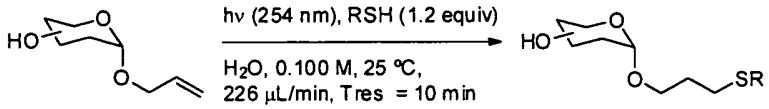
Table 1 – Representative examples of thiol–ene conjugations



Entry	Thiol (concentration)	Alkene (concentration)	Solvent	Residence time (min)	Conversion (%)
A	 (0.200 M)	 (0.200 M)	H_2O	5	>95
B	 (0.067 M)	 (0.067 M)	H_2O	15	>95
C	 (0.150 M)	 (0.050 M)	H_2O	5	>95
D	 (0.200 M)	 (0.200 M)	H_2O	10	95
E	 (0.150 M)	 (0.050 M)	MeOH	10	>95

F	 (0.150 M)	 (0.050 M)	MeOH	2.5	>95
G	 (0.150 M)	 (0.050 M)	MeOH	5	>95

A preferred embodiment of the invention involves the reaction of allyl sugars/carbohydrates with thiols such as cysteine, 2-mercaptoethanol, thioacetic acid and thioglucose according to the following scheme 2 (see Example 5).



Scheme 2

It should be noted, however, that the optimal reaction conditions for specific reactants may differ from the conditions indicated in scheme 2 above.

In an especially preferred embodiment, the reaction of allyl sugars/carbohydrates with L-cysteine is used for preparing various glycoamino acids. The glycoamino acids may be derivatives which are protected or capable to be protected and these derivatives can be used as building blocks in, e.g., solid-supported glycopeptide synthesis.

The synthesis method and system of the present invention may be advantageously used in, e.g., the functionalisation of proteins and peptides, such as biologically relevant peptides.

In particular, it has been demonstrated by the present inventors that the claimed method allows for the efficient functionalisation of peptide oligomers with multiple thiols (Example 6). Such compounds have potential application in the multiple presentation of carbohydrate antigens.

In another preferred embodiment, the process of the present invention involves a photochemical [2+2] cycloaddition reaction, which type of reaction has been previously used in a continuous flow process (J. Org. Chem. 2005, 70, 7558-7564), but not for functionalizing or coupling of polymers.

The use of [2+2] cycloaddition reactions for preparing the present dendronized polymers, in particular carbohydrate-dendronized polymers, proved to be very advantageous since they can be carried out in water using inexpensive starting materials, are not pH dependent and circumvented the use of heavy metals or other reagents that contaminate the polymer product, while avoiding the formation of side products. The photochemical transformation resulted in good yields for all the glyco-dendrimers.

Here it is reported the first use of the [2+2] cycloaddition reaction in a continuous flow system to generate a glyco-conjugated polymer that successfully bound and detected target bacteria, such as *E. coli* bacteria.

The continuous flow process for preparing dendronized polymers, according to claim 10 comprises at least the following steps
a) providing a dendrimer capped with a plurality of functional moieties and having a functional linker group comprising either ia) an alkenyl or alkynyl group or iia) a maleimido group or an electron-poor olefinic group
b) reacting said capped dendrimer with a scaffold polymer functionalized with either ib) a maleimido group or a electron-poor olefinic group or iib) an alkenyl or alkynyl group in a photochemical [2+2] cycloaddition reaction wherein the

corresponding cyclobutene adducts are formed by the reaction of the functional groups ia) and ib) or iia) and iib), respectively.

More specifically, the functional moieties are selected from the group consisting of a carbohydrate, a biologically active agent, such as a pharmaceutical or a drug, a detectable group, or a binding moiety, e.g. an antibody or an antigen-binding fragment thereof, a peptide, an oligopeptide, a protein, a nucleotide, an oligonucleotide, a polynucleotide, or combinations thereof.

In a preferred embodiment, the process of the present invention comprises a) providing a carbohydrate-capped dendrimer with a functional linker group comprising a terminal ethenyl or ethynyl group
b) reacting said carbohydrate-capped dendrimer with a scaffold polymer functionalized with a maleimido group or a electron-poor olefinic group in a photochemical [2+ 2] cycloaddition reaction wherein the corresponding cyclobuten adducts are formed.

The capped dendrimers, in particular carbohydrate-capped dendrimers, of step a) may be prepared by known methods of the prior art, e.g. as disclosed in Kikkeri et al., Chem. Commun. 2010, 46, 2197-2199, or according to Scheme 1 below. The basic coupling steps are not affected by the specific functional moieties of the caps.

The functional linker group of the carbohydrate-capped dendrimers may be an internal group or a terminal group, preferably it is a terminal group, in particular a terminal ethenyl or ethynyl group.

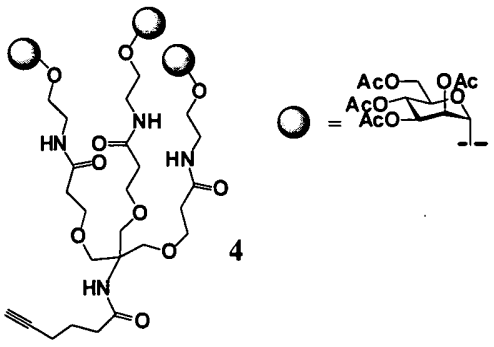
The capped dendrimer(s), in particular carbohydrate-capped dendrimer(s), may further comprise additional functional or linking groups. Such additional functional or linking groups may comprise a detectable group (label), a biologically, in particular pharmaceutically, active group, or a binding moiety, e.g. an antibody or an antigen-binding fragment thereof.

The carbohydrate(s) may be any mono-, oligo- or polysaccharide and may be unsubstituted or substituted with a desired functional group e.g. an olefinic group, a linker or binding group, including an antibody or an antigen-binding fragment thereof, and/or a detectable or biologically, e.g. pharmaceutically, active group.

Preferable the carbohydrate is capable to bind to a corresponding protein, e.g. a lectin. More specifically, the carbohydrate is a monosaccharide selected from the group consisting of galactose, N-acetylgalactosamine, mannose or fucose or a oligosaccharide comprising one or more of said monosaccharides.

In one specific embodiment of the invention, at least one of the carbohydrate moieties is replaced by another functional moiety, in particular a biologically active agent, such as a pharmaceutical or a drug, a detectable group, a binding moiety, e.g. an antibody or an antigen-binding fragment thereof, a peptide, an oligopeptide, a protein, a nucleotide, an oligonucleotide, a polynucleotide.

In another specific embodiment, the carbohydrate-capped dendrimer is a first or second generation dendrimer having one of the following structural formulae 4-11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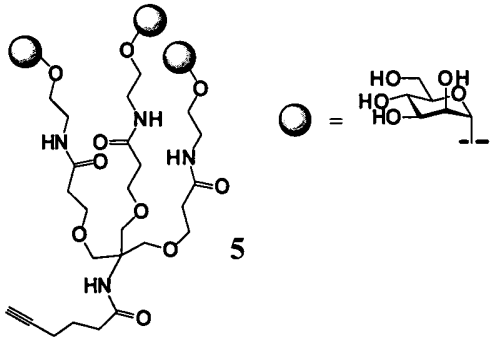
OAc
AcO
AcO
=
NH
HN
HN
HN
HN
HN
O

4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HO
HO
=
NH
HN
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HN
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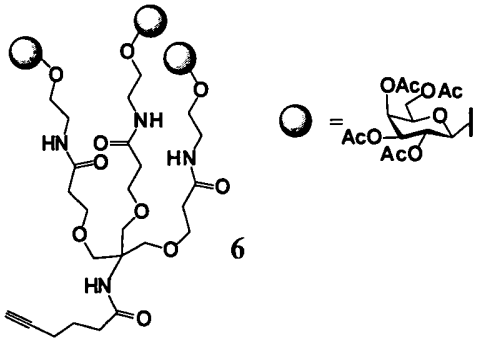
5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=
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HN
HN
HN
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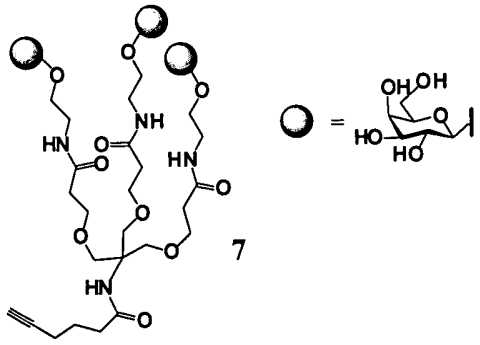
6


OH
OH
HO
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NH
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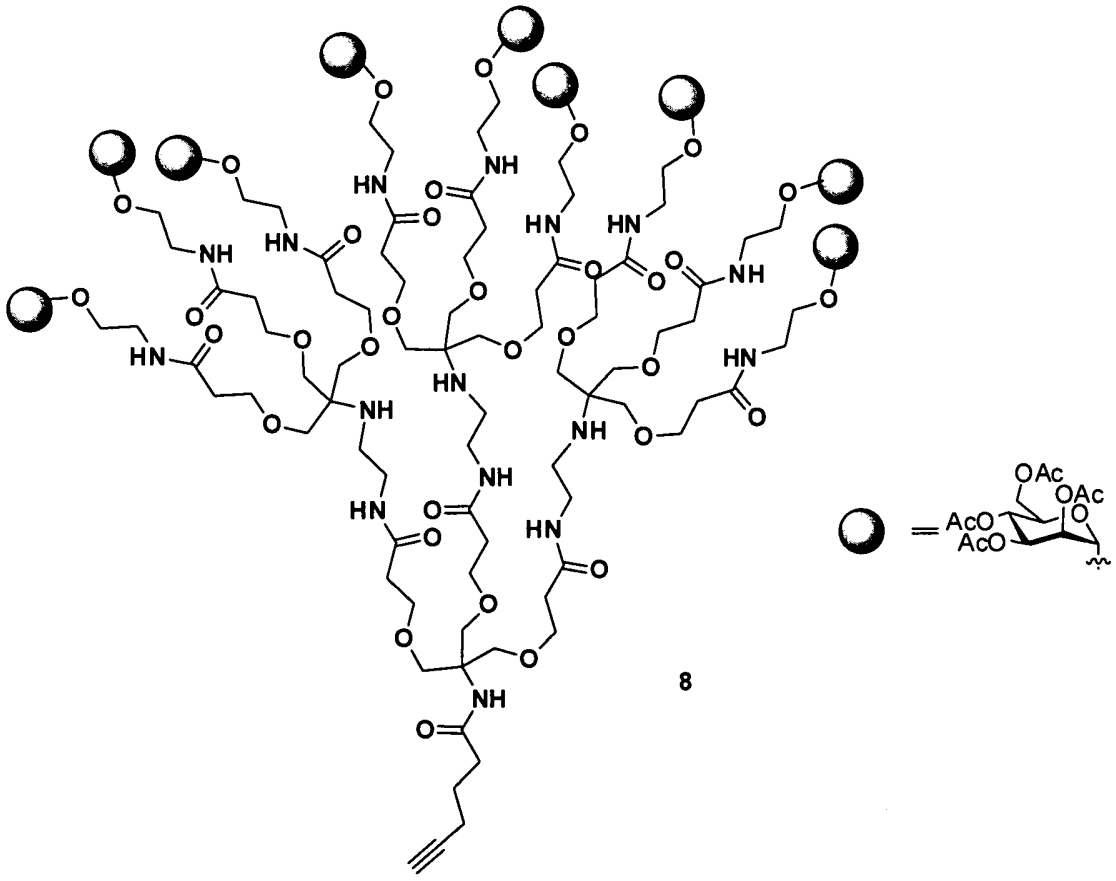
O

7



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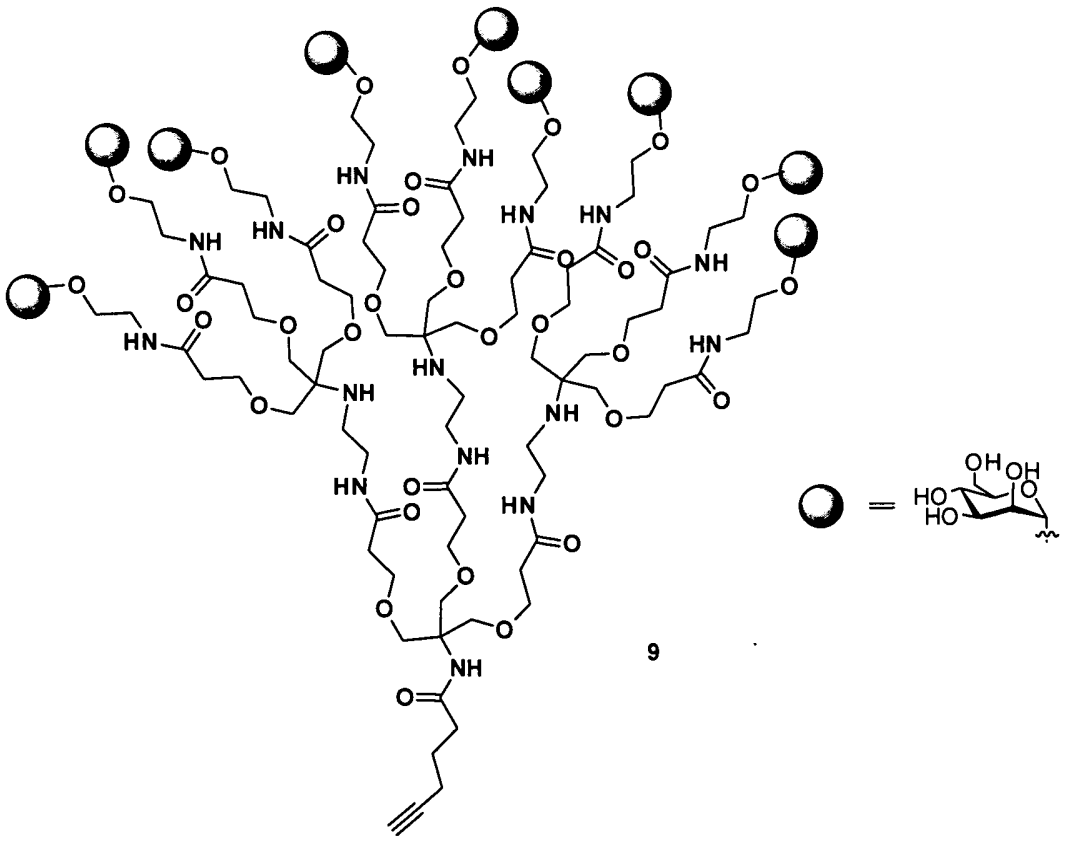

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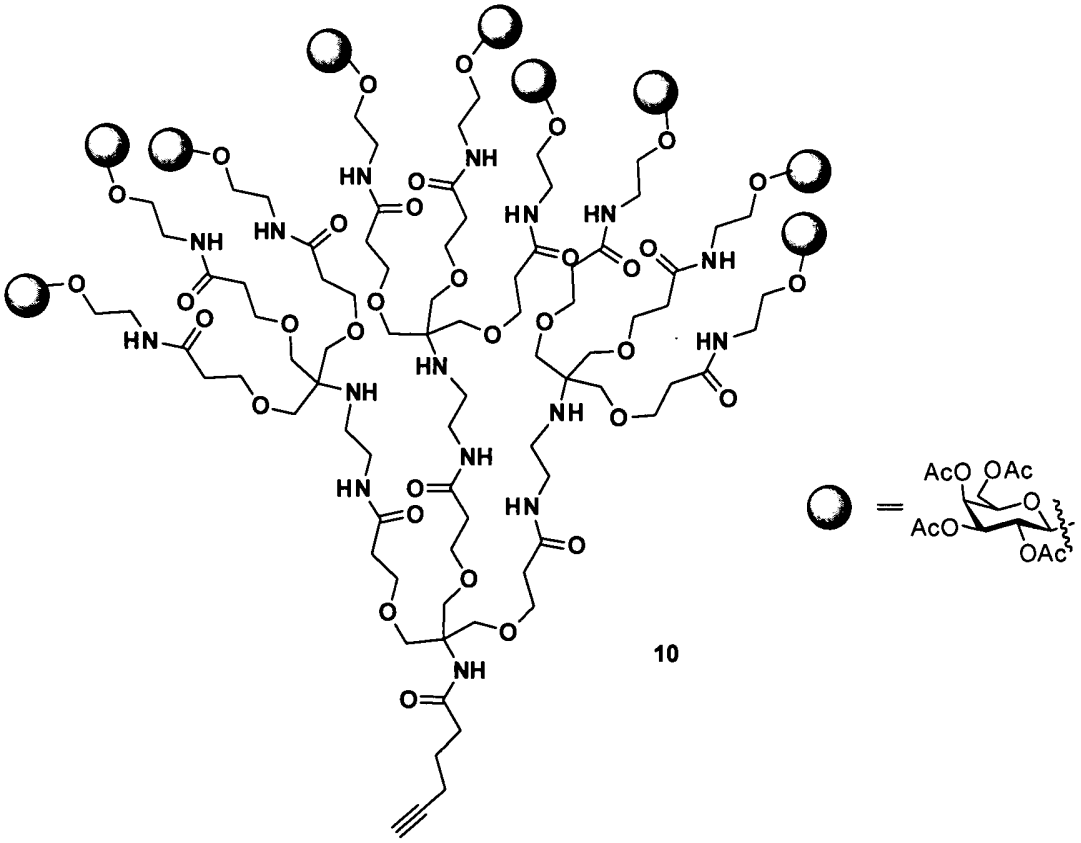

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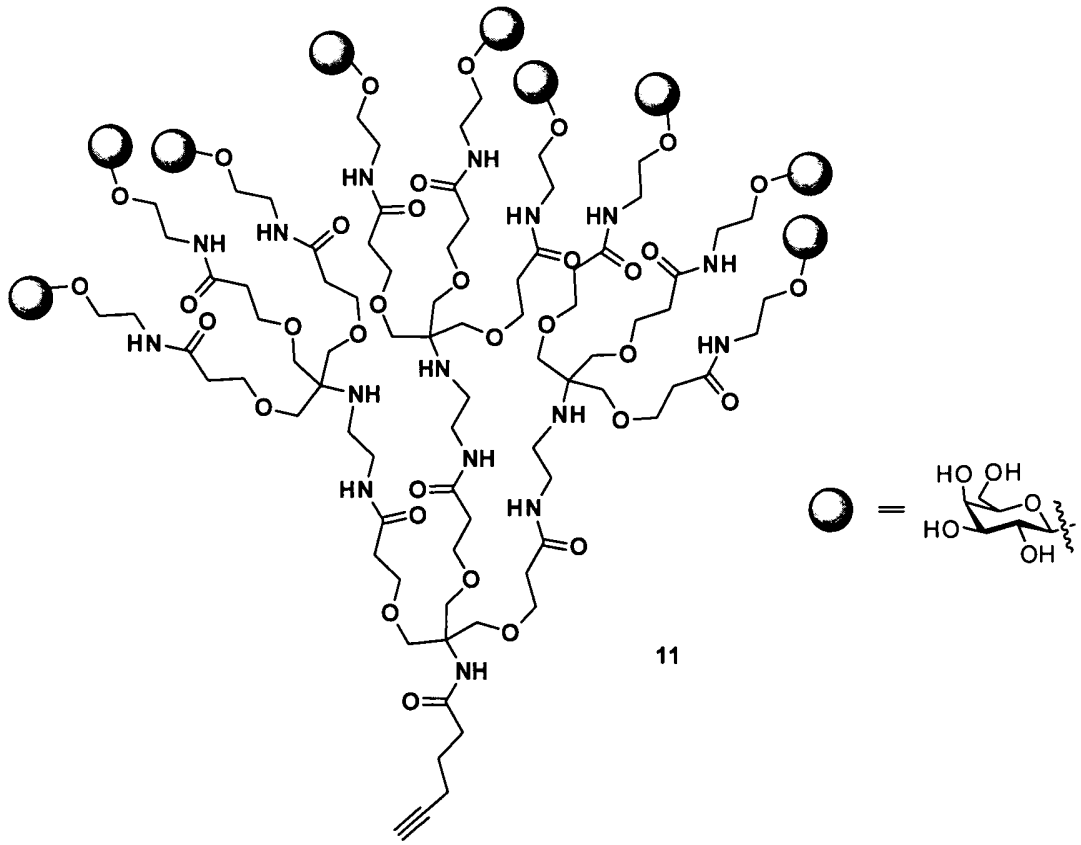


11

The polymer which is coupled to the capped dendrimer(s), in particular carbohydrate-capped dendrimer(s), may be any polymer which provides a suitable scaffold for the desired functional group(s) and capped dendrimer(s), in particular carbohydrate-capped dendrimer(s). More specifically, the polymer may be selected from the group consisting of a natural or synthetic hydrocarbon polymer, a polypeptide, including a poly(amino acid), e.g. poly-L-lysine, a polyhydroxyalkanoate, a polylactide (PLA), a polyglycolide (PGA), a polycaprolactone (PCL), a poly-saccharide, in particular derived from starch, cellulose or chitosan. The polymer, in particular hydrocarbon polymer, may comprise any aliphatic or aromatic structural units, substituted or unsubstituted, which do not interfere with the [2+2] cycloaddition reaction.

The polymer may be functionalized with a maleimido group or a electron-poor olefinic group capable to react with an alkenyl or alkynyl group of the carbohydrate-capped dendrimer(s) in a [2+2) cycloaddition reaction or, alternatively, may be functionalized with an alkenyl or alkynyl group capable to react with a maleimido group or a electron-poor olefinic group of the carbohydrate-capped dendrimer(s) in a [2+2) cycloaddition reaction.

Preferably, the polymer is functionalized with a maleimido group or a electron-poor olefinic group. The term "electron-poor olefinic group" as used herein corresponds to the common meaning of this term in the art and in particular relates to any olefinic group whose electron density is decreased due to negative inductive (-I) or mesomeric (-M) effects. Such effects are known to be produced by electron-withdrawing substituents or neighboring groups/structures and both the effects and suitable electron-withdrawing substituents, groups and structures are disclosed in any handbook of chemistry.

Additionally, the polymer may have other functional or linking groups, e.g. for attachment of a chemical moiety which for

example acts as a dectectable group, binding group (including an antibody or antigen-binding fragment thereof) or a biologically active, in particular pharmaceutically active, group.

In a specific embodiment of the invention, the polymer is poly-L-lysine. Poly-L-lysine (PLL) is a preferred polymer backbone due to its biocompatibility and biodegradability. In addition, the use of PLL is advantageous because it is commercially available in a range of molecular weights, and the lateral amino chain of PLL is readily functionalized to produce various multivalent systems.

Advantageously, the photochemical [2+2] cycloaddition reaction can be readily implemented under mild conditions with high yields. Preferably, the reaction medium is an aqueous medium, e.g. water or a mixture of water and a water-miscible organic solvent, such as an alcohol, preferably a lower alkanol having 1-9 carbon atoms, e.g. methanol, ethanol, propanol, butanol etc., or acetonitrile. Especially preferred, the reaction medium is water. Thus, the need for late stage deprotection steps is circumvented, since if the functionalization takes place in water, unprotected sugars can be used, and the reaction is environmentally friendly ("green" chemistry).

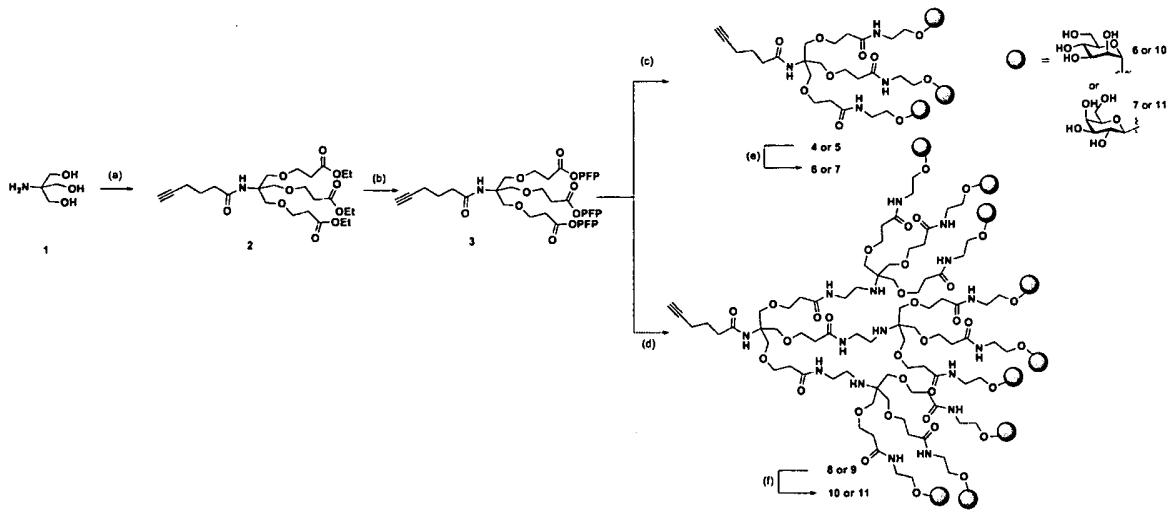
Typically, the photochemical [2+2] cycloaddition reaction of the invention is implanted effectively in water at a relative low temperature of 20-30 °C, preferable 23-28 °C or about room temperature (RT) for a time period (resident time in the flow reactor) of from 15 minutes to 60 minutes. The flow rates and concentrations of reactants can be readily assessed and optimized by the skilled artisan in dependence of the specific reaction involved. Typical ranges are flow rates in the range from 10 to 1000 microL/min, more specifically 50 to 500 microL/min, e.g. 83 microL/min to 333 microl/min, and concentrations in the range from 0.001 to 1 M, more specifically from 0.01 to 0.5 M, e.g. from 0.05 to 0.2 M. Some specific conditions are disclosed in the Examples below. The cycloaddition is typically initiated by

illumination with visible light, for example generated by a medium pressure Hg lamp having a broad wave length spectrum and a maximum at 366 nm, and having an intensity in the range of from 450 to 550 W.

The claimed method enables to produce the desired compounds in excellent yields in a very convenient, fast and efficient manner.

General synthesis of carbohydrate-capped dendrimers

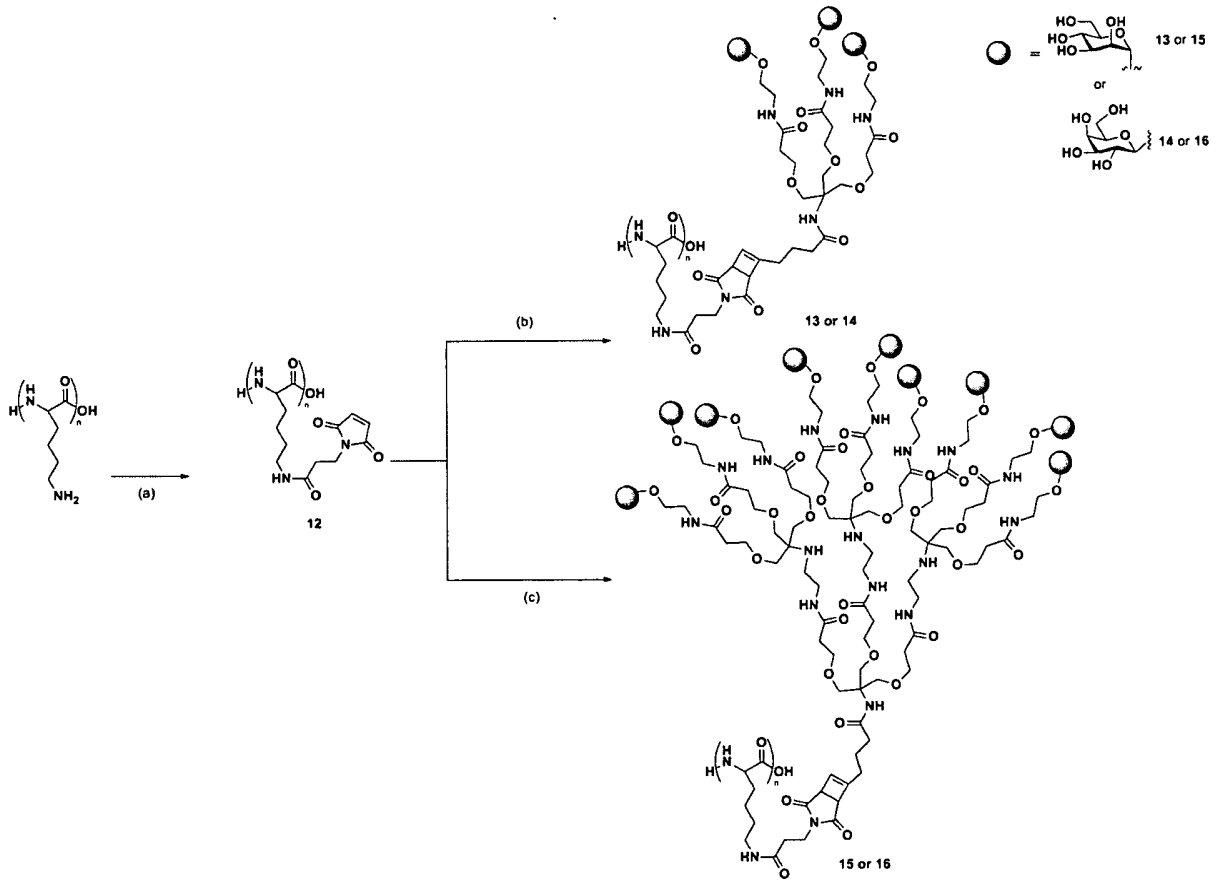
Two sets of dendrons with three and nine carbohydrates, respectively, bearing either mannose or galactose were synthesized according to Scheme 2 below. Starting from commercially available 2-amino-2-(hydroxymethyl)propane-1,3-diol **1**, treatment with acrylonitrile under basic conditions, followed by hydrolysis and esterification, prior to coupling with 5-hexynoic acid provided structure **2**. Ester hydrolysis under basic conditions, followed by coupling with pentafluorophenol yielded the active ester **3**. Reaction of **3** with peracetylated mannose or galactose yielded first generation dendrons **4** or **5**, while reaction with the peracetylated tri-pod mannose or galactose second generation dendrons **8** or **9** were obtained. Removal of the acetate groups under basic conditions yielded **6** or **7** and **10** or **11**.



Scheme 3. Synthesis of first (**6**, **7**) and second-generation dendrons (**10**, **11**). (a) Acrylonitrile/NaOH, 40%, conc HCl/ EtOH, 51%; 5-hexynoic acid/ DIC/ HOBT/ DCM, 68%; (b) NaOH/MeOH; pentafluorophenol / DIC/ HOBT/ DCM, 86%; (c) 2-(*t*-butoxy-carbonylamino)ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannoside or 2-(*t*-butoxycarbonylamino)ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-galactoside / DCM/ TEA, 71%; (d) tripod-mannose (**4**) or galactose (**5**) / DCM/ TEA, 67%; (e) MeOH/ Na quant.; (f) MeOH/ Na, quant.

Coupling of the carbohydrate-capped dendrimers to a polymer scaffold

The first and second generation dendrons were subsequently used for the functionalization of the PLL polymer backbone **12** (Scheme 4). In this way, dendronized polymers **13-16** were created by photochemical functionalization using the [2 + 2] cycloaddition reaction in a continuous flow reactor. Reactions were carried out at room temperature and in water with 40 min of irradiation using a medium pressure Hg lamp (450 W). [2+2] Cyclizations were analyzed by ^{1}H-NMR (Figure 2). Two well-separated peaks of the maleimido polymer **12** (visible at 5.8 and 6.3 ppm) disappeared after photochemical conjugation. Peaks at 3.5 ppm and 5 ppm show the complete conversion of maleimido group to cyclobuten adducts (Figure 2).



Scheme 4. Functionalization of PLL with first and second generation dendrons **5** and **7**. (a) Succinimido 3-maleidopropanoate, quant; (b) **6** or **7**, hν, 40 min, rt; (c) **10** or **11**, hν, 40 min, rt.

A second aspect of the present invention relates to the conjugates which are obtainable by the methods as disclosed above. Such a conjugate may comprise two or more chemical entities selected from the group consisting of carbohydrates, including mono-, oligo- and polysaccharides, amino acids, peptides and proteins, unbranched and branched oligomers and polymers, including dendrimers.

Preferably, the conjugates represent or comprise a biologically active agent, such as a pharmaceutical, including a vaccine, or a drug.

In a more specific embodiment of the invention, the conjugates are dendronized, in particular carbohydrate-dendronized, polymers.

The carbohydrate-dendronized polymers comprise a scaffold polymer which is preferably selected from the polymers indicated above, in particular poly-L-lysine, and carbohydrate-capped dendrons wherein the carbohydrate is selected from the group consisting of mono-, oligo- or polysacarides or functionalized derivatives thereof.

The carbohydrate(s) may be any mono-, oligo- or polysaccharide and may be unsubstituted or substituted with a desired functional group e.g. an olefinic group, a linker or binding group, including an antibody or an antigen-binding fragment thereof, and/or a detectable or biologically, e.g. pharmaceutically, active group.

Preferable the carbohydrate is capable to bind to a corresponding protein, e.g. a lectin. More specifically, the carbohydrate is a monosaccharide selected from the group consisting of galactose, N-acetylgalactosamine, mannose or fucose or a oligosaccharide comprising one or more of said monosaccharides.

The number of the functional moieties, in particular carbohydrate moieties, which a dendrimer/dendron bears can be varied as desired (e.g. depending on the target bacteria to be bound) but will be typically in range from 3 to 20, preferably in the range from 3 to 15.

In a specific embodiment, the carbohydrate-capped dendrons are derived from first or second generation dendrimers having one of the structural formulae 4-11 above.

In one specific embodiment of the invention, at least one of the carbohydrate moieties is replaced by another functional moiety, in particular a biologically active agent, such as a

pharmaceutical or a drug, a detectable group, a binding moiety, e.g. an antibody or an antigen-binding fragment thereof, a peptide, an oligopeptide, a protein, a nucleotide, an oligonucleotide, a polynucleotide.

The capped, in particular carbohydrate-capped, dendron(s) may further comprise additional functional or linking groups. Such additional functional or linking groups may comprise a detectable group (label), a biologically, in particular pharmaceutically, active group, or a binding moiety, e.g. an antibody or an antigen-binding fragment thereof.

The polymer which is coupled to the capped, in particular carbohydrate-capped, dendrimer(s) may be any polymer which provides a suitable scaffold for the desired functional group(s) and capped dendrimer(s). More specifically, the polymer may be selected from the group consisting of a natural or synthetic hydrocarbon polymer, a polypeptide, including a poly(amino acid), e.g. poly-L-lysine, a polyhydroxyalkanoate, a polylactide (PLA), a polyglycolide (PGA), a polycaprolactone (PCL), a poly-saccharide, in particular derived from starch, cellulose or chitosan. The polymer, in particular hydrocarbon polymer, may comprise any aliphatic or aromatic structural units, substituted or unsubstituted, which do not interfere with the [2+2] cycloaddition reaction.

In a specific embodiment of the invention, the polymer is poly-L-lysine.

A further aspect of the present invention relates to a method for binding or detecting a target bacterium comprising contacting a sample with a dendronized polymer as disclosed above and selectively binding the target bacterium which has at least one binding site for one or more of the functional moieties of said dendronized polymer.

More specifically, in said method the dendronized polymer is a carbohydrate-dendronized polymer and the target bacterium has at least one binding site for one or more of the carbohydrate moieties of said carbohydrate-dendronized polymer.

The invention is further illustrated by the following non-limiting Examples and Figures.

FIGURES

Fig. 1. Schematic representation of PLL functionalization; nonapod dendrimer (a); Poly-L-lysine (b); continuous flow reactor (c); dendronized polymer (d).

Fig. 2. ^{1}H-NMR spectra of compounds **15** (a), **10** (b) and **12** (c) respectively.

Fig. 3. AFM images of mannose nonapod PLL **15** on gelatin coated mica surfaces.

Fig. 4. Confocal laser scanning microscope images for the incubation of bacteria *E. coli* strain ORN178 and dendronized polymer **15** (a); and ConA-FITC (b). Strain ORN208 (negative control) in the presence of **15** (c) and ConA-FITC (d).

Fig. 5. AFM imagines in liquid media of mannose nonapod PLL **15** and ORN178 *E. coli* bacteria after incubation. Note the polymer localized on the surface of the bacterium.

Fig. 6. Detection limit aggregation of bacteria ORN178 with nanopodmannose (**15**). The number of bacteria incubated with PLL-containing solution is indicated above each imagine.

Fig. 7. Exemplary continuous flow reactor setup for the [2+2] cycloaddition process of the invention consisting of a syringe pump injection system (**1**, Harvard PHD 2000), gas tight syringe (**2**, Hamilton), multiple loops of narrow FEP tubing (**3**,

fluorinated ethylene polymer, Tub FEP Nat 1/16 in x .030 in)[i] wrapped tightly around a quartz immersion well (**4**) cooled by a thermostat (**9**), a Pyrex filter (**5**), a Hanovia medium pressure Hg lamp (**6**, 450 W), a power supply (**8**), and a collection flask (**10**).

Fig. 8. Exemplary continuous flow reactor setups for a thiol-ene photoconjugation process of the invention; A) exemplary reactor setup for a photoreaction at 254 nm; B) general schematic diagram of the photoreactor setup.

Fig. 9. Process flow diagrams; A) for a system driven by a syringe pump; B) for a system driven by an HPLC pump.

Fig. 10. Reaction of O-allyl galactose at various concentrations with cysteine (plots of fractional conversion against residence time).

Fig. 11. Reaction of various saccharides with cysteine (plots of fractional conversion against residence time).

Fig. 12. Schematic presentation of continuous flow reactor setups for the thiol-ene photoconjugation processes of the invention in the absence or presence of initiator.

Reagents and instruments used in the Examples

All chemicals were reagent grade and used as supplied except where noted. Dichloromethane (CH_2Cl_2) was purified by a Cycle-Tainer Solvent Delivery System. Triethylamine was distilled over CaH_2 prior to use. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in CAN solution followed by heating. Flash column chromatography was carried out using force flow of the indicated solvent on Fluka Kieselgel 60 (230-400 mesh).

The water was taken from a Seral purification system (PURELAB Plus) with a conductivity of 0.06 µS/cm.

^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300 (400 MHz) spectrometer. High-resolution mass spectra (HR-MALDI MS) and ESI-MS were performed by the Mass Spectrometry-service at the MPI Berlin. ESI-MS were run on an Agilent 1100 Series LC/MSD instrument. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotation measurements were conducted using a Perkin-Elmer 241 polarimeter.

Atomic Force Microscopic (AFM) was performed with multimode instrument with Nanoscope IIIa controller (Vecco Instruments) operating in the tapping mode. The samples were placed on a Mica disk and tips (Nano world TIP) with a spring constant of 42 N/m, and a resonance frequency of 285 kHz was used for air measurements and spring constant of 0.02 or 0.06 N/m, and resonance frequency of 10-20 kHz were used for liquid measurements.

Confocal laser scanning microscope imagines were performed with Carl Zeiss confocal laser scanning microscope, LSM 700.

EXAMPLE 1

Synthesis of carbohydrate-capped dendrimers

General Procedure A: Synthesis of Sugar-nonapods

The Boc-protected sugar-amine or sugar-tripod (4.0 eq) was dissolved in 10 mL dichloromethane/trifluoroacetic acid (3:1) and stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the resulting oil was dissolved in anhydrous dichloromethane (20 mL). To this mixture was added *tert*-butoxycarbonyl-3-{*N*-{tris[3-[pentafluoro-phenyl-carboxyl-ethoxy)methyl]}methyl amine}-3-β-alanine or *N*-{tris[3-[pentafluoro phenyl carboxylethoxy)methyl]}methyl amide}-5-hexynylamide (1.0 eq), adjusted to pH 8 with triethylamine (TEA) and the mixture was stirred at room temperature for 12 h. The

solvent was evaporated *in vacuo* and purified by flash silica column chromatography.

General Procedure B: Synthesis of sugar dendrons. Tripod or nonapod (1.0 eq) and sodium methoxide (10 eq) were dissolved in methanol (10 mL) and stirred at room temperature for 2 h. The solvent was then evaporated *in vacuo*, the residue was redissolved in water and dialyzed against water using 500 molecular weight cut-off resin. After two days of dialysis, the sample was lyophilized.

***N*-{Tris[3-[ethylcarboxyl-ethoxy)methyl]}methylamide}-5-hexynylamide 2.** To a solution of *N*-{tris[(3-[ethylcarboxyl-ethoxy)methyl]}methylamine (2 g, 4.7 mmol) and 5-hexynoic acid (0.53 g, 4.7 mmol) in dichloromethane (10 mL) at 0 °C, were added diisopropylcarbodiimide (0.87 mL, 5.6 mmol) and 1-hydroxybenzo-triazole (0.07 g, 0.56 mmol) The reaction mixture was stirred at room temperature for 12 h and concentrated *in vacuo*. The crude residue was purified by flash silica column chromatography to yield *N*-{tris[3-[ethylcarboxylethoxy)methyl]}methylamide}-5-hexynylamide (1.75 g, 72%). R_f = 0.5 (CH_2Cl_2/MeOH, 98:4); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86 (br.s, 1H), 4.17 (q, J = 8.0 Hz, 6H), 3.94 (br. s, 11H), 2.88 (t, J = 4.0 Hz, 6H), 2.24-2.16 (m, 4H), 1.88 (t, J = 4.0 Hz, 1H), 1.78-1.74 (m, 2H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 171.6, 69.5, 66.5, 60.3, 45.2, 37.2, 35.4, 24.3, 14.3, FTIR($CHCl_3$): 3375, 2988, 2873, 1744, 1726, 1644, 1525 cm^{-1}. HRMS (MALDI-ToF) (m/z) calcd. for $C_{24}H_{41}NO_{10}Na$ 538.2628, found: 538.2627.

***N*-{Tris[3-[pentafluoro phenyl carboxyl-ethoxy)methyl]}methyl amide}-5-hexynylamide 3.** *N*-{Tris[3-[ethylcarboxyl-ethoxy)methyl]} methylamine}-5-azido pentamide (1.5 g, 2.9 mmol) was dissolved in ethanol (10 mL) and sodium hydroxide solution (aqueous, 1 N, 2 mL) was added and the mixture was stirred at room temperature for 1 h, concentrated in *vacuo*, adjusted to pH 5 with hydrochloric acid (aqueous 1 N) and extracted with ethyl acetate. The organic layer was dried with sodium sulfate and concentrated to dryness

under reduced pressure. The residue was dissolved in dichloromethane (10 mL) and 2,3,4,5,6-pentafluorophenol (2.1 g, 11.6 mmol) was added. After cooling to 0 °C, diisopropyl carbadiazine (2.2 mL, 13.9 mmol) was added and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was concentrated in *vacuo* and purified by silica column flash chromatography to afford *tert*-butoxycarbonyl-3-{*N*-{tris[3-[pentafluorophenylcarboxylethoxy)methyl]}methylamide}-5-azido pentamide (1.56 g, 56%). R_f = 0.6 (CH_2Cl_2/EtOAc, 88:12); ^1H NMR (400 MHz, $CDCl_3$): δ 3.83 (br. s, 12H), 2.84 (t, J = 6.0 Hz, 6H), 2.22-2.17 (m, 4H), 1.71-1.62 (m, 3H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 171.1, 69.3, 66.5, 60.3, 45.2, 37.1, 35.4, 24.3, FTIR($CHCl_3$) : 3688, 3385, 1749, 1658, 1522, 1359 cm^{-1}. HRMS (MALDI-ToF) (*m/z*) calcd. for $C_{37}H_{28}F_{15}NO_{10}Na$ 952.1215, found: 952.1218.

***Tris*[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide }-5-hexynylamide 4**. General procedure A using 2-(*tert*-butoxycarbonylamino)ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (0.97 g, 1.96 mmol), *tris*[3-[pentafluoro phenyl carboxyl-ethoxy)methyl]}methyl amide}-5-hexynylamide **2** (0.5 g, 0.53 mmol) and purified by flash chromatography to yield *tris*[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]methylamide }-5-hexynylamide (0.39 g, 47%). R_f = 0.45 (CH_2Cl_2/MeOH, 93:7); $[\alpha]_D^{r.t}$ = +23.4 (c = 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 6.79 (br.s, 2H), 6.33 (br.s, 1H), 5.27-5.20 (m, 9H), 4.80 (d, J = 9.0 Hz, 3H), 4.25 (dd, J = 9.0, 5.1 Hz, 3H), 4.10 (dd, J = 2.1, 9.9 Hz, 3H), 3.90 (br.s, 3H), 3.76 (dd, J = 4.5, 5.4 Hz, 3H), 3.68 (dd, J = 5.4, 6.0 Hz, 6H), 3.64 (m, 6H), 3.54-3.52 (m, 6H), 3.37 (br.s, 8H), 2.72 (t, J = 5.4 Hz, 6H), 2.39 (t, J = 9.0 Hz, 4H), 2.28 (t, J = 5.4 Hz, 3H), 2.12 (t, J = 5.4 Hz, 2H), 2.07 (s, 9H), 2.02 (s, 9H), 1.96 (s, 9H), 1.71-1.62 (m, 2H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4, 170.5, 170.0, 169.5, 155.8, 97.6, 69.3, 69.2, 68.6, 67.3, 65.1, 62.2, 60.4, 53.8, 39.0, 35.9, 34.5, 23.5, 20.3; FTIR($CHCl_3$): 3376, 2918, 1751, 1663, 1515, 1457, 1250 cm^{-1}; HRMS (MALDI-ToF) (*m/z*) calcd. for $C_{67}H_{98}N_9O_{37}Na$ 1573.5808; found: 1573.5810.

***Tris*-[3-4-ethoxy-α-D-mannosepyranosyl-ethoxy}methyl]-5-hexynylamide 6.** General procedure B with t*ris*[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]-methyl-amide}-5-hexynyl amide **3** (100 mg, 97.5 μmol) sodium methoxide (10 mg, 0.18 mmol) yielded 45 mg, (65%) of *cis*-ruthenium(II)bis-(bipyridine){1,1'-(2,2'-bipyridine-4,4'-diyl)bis-3-β-beta-propane-{tris-[3-4-ethoxy-β-D-galactopyranosyl-ethoxy}-methyl] methylamide. $[\alpha]_D^{r.t}$ = +1.7 (c = 1.0, H_2O); ^1H NMR (400 MHz, D_2O/MeOD): δ 4.72 (s, 3H), 3.96-3.94 (m, 4H), 3.90 (d, *J* = 2.7 Hz, 12H), 3.75-3.65 (m, 34H), 3.61 (br.s, 24H), 3.49-3.46 (m, 12H), 3.73-3.21 (m, 44H), 2.37 (t, *J* = 6.0 Hz, 6H), 2.35 (t, *J* = 6.0 Hz, 3H), 2.12 (t, *J* = 6.0 Hz, 2H), 1.71-1.62 (m, 2H), ^{13}C NMR (100 MHz, CD_3OD): δ 173.2, 172.6, 101.3, 74.8, 71.8, 71.5, 68.5, 67.6, 66.6, 62.4, 61.4, 40.0, 37.3, 34.9,27.3, 23.1, 21.2, 18.5. MALDI-ToF (*m/z*): $[M-1]^+$ Calcd for $C_{43}H_{174}N_4O_2$ 1046.4642; Found: 1046.4645.

***Tris*[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl]methylamide}-5-hexynylamide 5.** General procedure A using 2-(*tert*-butoxycarbonylamino)ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (0.42 g, 1.06 mmol), *tert*-butoxycarbonyl-3-{*N*-{tris[3-[pentafluoro-phenyl-carboxyl-ethoxy)methyl]}methyl amine}-5-hexynylamide **2** (0.25 g, 0.26 mmol) and purified by flash chromatography to yield *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methyl]-methylamide}-3-β-alanine (0.27 g, 57%). R_f = 0.45 (CH_2Cl_2/MeOH, 93:7); $[\alpha]_D^{r.t}$ = +17.4 (c = 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 6.47 (br.s, 2H), 5.37 (d, *J* = 4.0 Hz, 4H), 5.14 (t, *J* = 8.0 Hz, 3H), 4.99 (dd, *J* = 4.0 Hz, 3H), 4.49 (d, *J* = 8.0 Hz, 3H), 4.12-4.10 (m, 6H), 3.92 (t, *J* = 6.0 Hz, 3H), 3.84-3.83 (m, 3H), 3.70 (t, *J* = 6.0 Hz, 8H), 3.65 (s, 9H), 3.42 (t, *J* = 6.0 Hz, 6H), 3.33 (q, *J* = 6.0 Hz, 2H), 2.39 (t, *J* = 9.0 Hz, 4H), 2.28 (t, *J* = 5.4 Hz, 3H), 2.12 (t, *J* = 6.0 Hz, 2H), 2.07 (s, 9H), 2.02 (s, 9H), 1.96 (s, 9H), 1.71-1.62 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 170.0, 169.8, 169.7, 155.7, 101.1, 70.6, 69.1, 68.7, 67.2,

67.0, 66.9, 61.2, 59.7, 45.65, 41.67, 39.1, 37.0, 36.4, 28.3, 20.6; FTIR($CHCl_3$): 3376, 2918, 1751, 1663, 1515, 1457, 1250 cm^{-1}; HRMS (MALDI-ToF) (*m/z*) calcd. for $C_{67}H_{98}N_9O_{37}Na$ 1573.5808; found: 1573.5810.

***Tris*[3-4-ethoxy- β-D-galactopyranoside -ethoxy}methyl]-5-hexynylamide 7**. General procedure D with *cis*-ruthenium(II)bis-(bipyridine){1,1′-(2,2′-bipyridine-4,4′-diyl)bis-3-β-propane-{tris-[3-4-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy}methyl]methylamide **5** (100 mg, 97.7 µmol) sodium methoxide (10 mg, 2.2 µmol) yielded 51 mg, (76%) of *tris*[3-4-ethoxy- β-D-galactopyranoside -ethoxy}methyl]-5-hexynylamide . $[\alpha]_D^{r.t}$ = +1.8 (c = 1.0, H_2O); ^1H NMR (400 MHz, D_2O/MeOD): δ 4.36 (d, *J* = 8.0 Hz, 3H), 3.96-3.94 (m, 4H), 3.90 (d, *J* = 4.0 Hz, 12H), 3.75-3.65 (m, 17H), 3.61 (br.s, 12H), 3.49-3.46 (m, 6H), 3.43-3.41 (m, 2H), 2.37 (t, *J* = 6.0 Hz, 6H), 2.35 (t, *J* = 6.0 Hz, 3H), 2.12 (t, *J* = 6.0 Hz, 2H), 1.71-1.62 (m, 2H), ^{13}C NMR (100 MHz, CD_3OD): δ 174.9, 173.2, 104.3, 77.8, 75.3, 73.5, 71.8, 71.8, 68.5, 67.6, 66.6, 62.4, 61.4, 40.0, 37.3, 30.1, 26.4. MALDI-ToF (*m/z*): $[M-1]^+$ Calcd for $C_{43}H1_{74}N_4O_2$ 1046.4642; Found: 1046.4645.

3-{Tris[3-carboxylethoxy]methyl]3′-{tris-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-manno pyranoside -ethoxy]methyl] methylamide}-5-hexynylamide 8. General procedure A using *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]methylamide}-3-β-alanine (0.34 g, 0.35 mmol), {*N*-{tris[3-[pentafluoro phenyl carboxyl-ethoxy)methyl]}methyl amide}-5-hexynlamide (50 mg, 0.053 mmol) and purified by flash chromatography to yield 3-{tris[3-carboxylethoxy]methyl]3′-{tris-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy] methyl] methylamide}-5-azido pentamide (0.22g, 57 %). $[\alpha]_D^{r.t}$ = +19.4 (c =1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 6.84 (br.s, 3H), 6.13 (br.s, 1H), 4.87-5.8 (m, 27H), 4.25 (s, 9H), 3.81 (dd, *J* = 6.0, 4.0 Hz, 9H), 3.77 (d, *J* = 6.0 Hz, 9H), 3.55 (br.s, 9H), 3.31- 3.01 (m, 57H), 3.06 (m, *J* = 4.0 Hz, 34H), 2.47 (br, 32H), 2.32 (br.s, 2H), 2.21 (br.s, 2H), 2.13 (s, 27H), 2.06 (s, 27H),

2.04 (s, 27H), 1.96 (s, 27H), 1.38-1.32 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.92, 172.2, 171.4, 102.7, 70.7, 69.6, 67.3, 63.4, 61.8, 54.5, 45.2, 39.7, 35.6, 20.6, 9.6. FTIR($CHCl_3$): 3332, 2734, 1745,1365,cm^{-1};HRMS-MALDI (m/z): $[M+ Na]^+$ Calcd for $C_{211}H_{314}N_{16}O_{118}$ 4989.9061; Found : 4989.9071.

***Tris*-[3-carboxyl-ethoxy]methyl]3′-{tris[2′-ethoxy-β-D-mannose-pyranosyl-ethoxy]methyl]-5-hexynylamide 10**. General procedure D with 3-{tris[3-carboxylethoxy]methyl]3′-{tris-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-manno pyranoside -ethoxy]methyl] methylamide}-5-hexynylamide **7** (200 mg, 40.0 μmol) and sodium methoxide (22 mg, 0.4 mmol) gave 108 mg (78%) of *tris*-[3-carboxyl-ethoxy]methyl]3′-{tris[2′-ethoxy-β-D-mannosepyranosyl-ethoxy]methyl]-5-hexynyl-amide. $[\alpha]_D^{r.t}$ = +22.3 (c =1.0, H_2O); ^{1}H NMR (400MHz, MeOD): δ 4.79 (s, 9H), 3.78-3.6 (m, 98H), 3.54 (m, 24H), 3.41 (m, 24H), 2.46 (t, *J* = 4.0 Hz, 32H), 2.25 (t, *J* = 6.0 Hz, 3H), 2.16 (t, *J* = 6.0 Hz, 2H), 1.71-1.62 (m, 2H); ^{13}C NMR (125MHz, CD_3OD): δ 173.4, 104.3, 73.8, 71.5, 70.8, 68.8, 67.5, 65.6, 62.6, 60.4, 37.4, 35.1, 25.3, 16.4. MALDI-HRMS (m/z): $[M+1]^+$ Calcd for $C_{139}H_{243}N_{16}O_{82}$ 3448.5258; Found: 3448.5251.

3-{Tris[3-carboxylethoxy]methyl]3′-{tris-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl] methylamide}-5-hexynylamide 9. General procedure A using *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl- β-D-galactopyranoside-ethoxy]methylamide}-3-β-alanine (0.15 g, 0.09 mmol), {*N*-{tris[3-[pentafluoro phenyl carboxyl-ethoxy)methyl]}methyl amide}-5-hexynlamide (21mg, 0.021 mmol) and purified by flash chromato-graphy to yield 3-{tris[3-carboxylethoxy]methyl]3′-{tris-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy] methyl] methylamide}-5-azido pentamide (95 g, 54%). $[\alpha]_D^{r.t}$ = +2.4 (c =1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.64 (br.s, 3H), 5.37 (d, *J* = 3.3 Hz, 9H), 5.12 (t, *J* = 8.0 Hz, 9H), 4.49 (d, *J* = 4.0 Hz, 9H), 4.31-4.15 (m, 9H), 4.14-4.08 (m, 18H), 3.95-3.92 (m, 9H), 3.84-3.78 (m, 9H), 3.66-3.56 (m, 64H), 3.41-3.36 (m, 34H), 2.41 (t, *J* = 5.4 Hz, 32H), 2.32 (br.s, 2H), 2.21 (br.s, 2H), 2.13 (s, 27H), 2.06 (s, 27H), 2.04 (s, 27H), 1.96 (s, 27H), 1.38-1.32

(m, 2H), ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.92, 172.2, 171.4, 102.7, 70.7, 69.6, 67.3, 63.4, 61.8, 40.1, 35.6, 20.6, FTIR($CHCl_3$): 3332, 2734, 1745,1365,cm^{-1};HRMS-MALDI (m/z): $[M+ Na]^+$ Calcd for $C_{211}H_{314}N_{16}O_{118}$ 4989.9061; Found : 4989.9071.

***Tris*-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-β-D-galactopyranoside-ethoxy]methyl]-5-hexynylamide 11**. General procedure D with 3-{tris[3-carboxylethoxy]methyl]3'-{tris-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]-methyl] methylamide}-5-hexynylamide 7 (90 mg, 18.0 µmol) and sodium methoxide (10 mg, 0.22 mmol) gave 41 mg (76%) of *tris*-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-β-D-galactopyranoside-ethoxy]methyl]-5-hexynylamide. $[\alpha]_D^{r.t}$ = + 1.3 (c =1.0, H_2O); ^{1}H NMR (400MHz, MeOD): δ 4.77 (s, 9H), 4.39 (d, *J* = 7.8 Hz, 9H), 4.15 (q, *J* =4.0 Hz, 8H), 3.91-3.85 (m, 18H), 3.76-3.60 (m, 93H), 3.47-3.45 (m, 24H), 3.41-3.35 (m, 26H), 2.47-2.42 (m, 32H); 2.25 (t, *J* = 6.0 Hz, 3H), 2.16 (t, *J* = 6.0 Hz, 2H), 1.71-1.62 (m, 2H), ^{13}C NMR (125 MHz, CD_3OD): δ 173.4, 104.3, 73.8, 71.5, 70.8, 68.8, 67.5, 65.6, 62.6, 60.4, 37.4, 35.1, 25.3, 16.4. MALDI-HRMS (m/z): $[M+1]^+$ Calcd for $C_{139}H_{243}N_{16}O_{82}$ 3448.5258; Found: 3448.5251.

EXAMPLE 2

Synthesis of carbohydrate-dendronized PLL polymers by [2+2] photocycloaddition

PLL-maleimido 12. Poly-L-Lysine hydrobromide (10 mg, 0.5 pmol) was added to 3- maleimidopropionic acid-*N*-hydrosuccinimide ester (133 mg, 0.5 mmol) in 6 mL of nano pure water. The solution was basified with 1 N KOH solution and shaken at room temperature. The aqueous layer was washed with dichloromethane (3 x 7 mL) to remove unreacted maleimido derivative gave 19 mg of pure yellow oil (98%). [α]D 25= -1.2 (c=1.5, MeOH/H2O 1:1); ^{1}H NMR (300 MHz, MeOD) δ 2.2-2.4 (m, 10 H); 3.5 (t, J = 7 Hz, 2H); 5.8 (d, J = 13 Hz, 1H); 6.3 (d, J = 13 Hz, 1H); ^{13}C NMR (300 MHz, MeOD) δ26.2. 37.8, 38.5, 125.5, 138.4, 167.5, 174.6, 178.6, 179.9.

General Procedure C: Synthesis of PLL-dendrone and nonapod. 3-Maleimidopropionic acid-N-poly-L-Lysine (0.2 μmol) and tripod or nonapod (0.4 mmol or 0.2 mmol)) were dissolved in water (3 mL) and flushed in a continuous flow reactor for 40 min under irradiation (medium pressure Hg Lamp, Hanovia, 450W). The crude product was further purified with sephadex G-10 and finally the sample was lyophilized.

PLL-tripodmannose 13. General procedure C with *tris*-[3-4-ethoxy-α-D-mannosepyranosyl-ethoxy}methyl]-5-hexynylamide **6** (30 mg, 0.4 mmol) and maleimido-PLL (2 mg, 0.2 μmol) to yield 20 mg of PLL-tripodmannose (75%). ^{1}H NMR (300 MHz, D_2O) δ 0.97 (d, *J* = 6.6 Hz, 1H); 1.69 - 1.59 (m, 3H); 1.78 (s, 1H); 1.87 (s, 1H); 2.11 (m, 3H); 2.21 (m, 3H); 2.39 (m, 6H); 3.24-3.29 (m, 2H); 3.31 - 3.28 (m, 2H); 3.33-3.36 (m, 2H); 3.37-3.39 (m, 1H); 3.48 - 3.45 (m, 2H); 3.51 - 3.47 (m, 6H); 3.52 (s, 2H); 3.52 (s, 2H); 3.64 - 3.59 (m, 10H); 3.68 - 3.64 (m, 7H); 3.73 (d, *J* = 1.8 Hz, 2H); 3.76 (d, *J* = 2.0 Hz, 1H); 3.81 (m, 3H); 4.74 (m, 3H); ^{13}C NMR (300 MHz, D_2O) δ 175.90, 174.19, 99.63, 72.79, 70.48, 69.97, 68.42, 67.43, 66.65, 65.76, 60.85, 60.13, 38.95, 36.08, 35.19, 24.06, 21.80, 16.97.

PLL-tripodgalactose 14. General procedure C with *Tris*[3-4-ethoxy-β-D-galactopyranoside -ethoxy}methyl]-5-hexynylamide **7** (30 mg, 0.4 mmol) and PLL-maleimido (2 mg, 0.2 μmol) to yield 14 mg of PLL-tripodgalactose (60%). ^{1}H NMR (300 MHz, D_2O) δ 0.93 (d, *J* = 6.4 Hz, 1H); 1.01 (t, *J* = 7.1 Hz, 1H); 1.11 (t, *J* = 7.2 Hz, 1H); 1.66 - 1.55 (m, 3H); 1.74 (s, 4H); 2.08 (t, *J* = 7.0 Hz, 3H); 2.18 (t, *J* = 7.4 Hz, 3H); 2.31-2.20 (m, 2H); 2.51 - 2.47 (m, 1H); 3.33 - 3.24 (m, 6H); 3.37-3.39 (m, 2H); 3.49 - 3.46 (m, 1H); 3.55 - 3.49 (m, 9H); 3.65 - 3.55 (m, 16H); 3.76 (d, *J* = 3.4 Hz, 3H); 3.85 - 3.78 (m, 4H); 3.91 (m, 1H); 4.24 (m, 3H); 4.84 (s, 1H); ^{13}C NMR (300 MHz, D_2O) δ 175.89, 174.22, 107.23, 102.94, 82.84, 80.92, 76.58, 75.10, 72.64, 70.70, 68.54, 68.32, 67.41, 66.17, 62.67, 60.91, 60.13, 39.33, 39.18, 36.08, 35.18, 24.05, 16.96.

PLL-nonapodmannose 15. General procedure C with *tris*-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-β-D-mannosepyranosyl ethoxy]-methyl]-5-hexynylamide **10** (30 mg, 0.2 mmol) and PLL-maleimido (2 mg, 0.2 μmol) to yield 14 mg of PLL-nonapodmannose (50%). ^{1}H NMR (300 MHz, D_2O) δ 2.12 - 2.00 (m, 1H); 2.20 (s, 1H); 2.53 (t, *J* = 6.6 Hz, 1H); 2.59-2.64 (m, H); 2.69 (s, 1H); 2.86 - 2.72 (m, 6H); 2.95-3.00 (m, H); 3.74 - 3.65 (m, 3H); 3.83 - 3.73 (m, 3H); 3.90-3.97 (m, H); 3.99-4.07 (m, H); 4.26 - 4.13 (m, 5H); 5.20 - 5.13 (m, 2H); ^{13}C NMR (300 MHz, D_2O) δ 174.06, 173.79, 173.39, 129.11, 125.26, 99.59, 72.77, 70.45, 69.95, 68.39, 67.44, 66.62, 65.72, 60.82, 60.17, 60.06, 38.90, 36.02, 35.79, 35.60, 17.01.

PLL-nonapodgalactose 16. General procedure C with *tris*-[3-carboxyl-ethoxy]methyl]3'-{tris[2'-ethoxy-β-D-galactopyranoside-ethoxy]methyl]-5-hexynylamide **11** (30 mg, 0.2 mmol) and PLL-maleimido (2 mg, 0.2 μmol) to yield 18 mg of PLL-nonapodgalactose (50%). ^{1}H NMR (300 MHz, D_2O) δ 1.70 (dt, *J* = 14.6, 7.3 Hz, 1H); 1.84 (s, 1H); 1.91 (s, 1H); 2.17 (t, *J* = 7.0 Hz, 1H); 2.29 - 2.23 (m, 1H); 2.49 - 2.32 (m, 8H); 2.64 - 2.57 (m, 1H); 2.87 - 2.80 (m, 1H); 3.28 (t, *J* = 5.6 Hz, 1H); 3.33 - 3.44 (m, H); 3.78 - 3.53 (m, 25H); 3.86 (d, *J* = 3.3 Hz, 2H); 3.96 - 3.87 (m, 3H); 3.96 - 3.87 (m, 3H); 4.34 (d, *J* = 7.8 Hz, 1H); 4.94 (s, H); ^{13}C NMR (300 MHz, D_2O) δ 174.10, 173.80, 173.39, 107.21, 104.98, 102.92, 82.82, 80.92, 76.57, 75.08, 72.62, 70.69, 68.52, 68.29, 67.43, 66.14, 62.64, 60.90, 60.19, 41.43, 39.31, 39.16, 36.04, 35.78, 35.57, 17.01.

PLL-mono-mannose 17. 3-Maleimidopropionic acid-*N*-poly-L-lysine (10 mg, 0.2 μmol) and allyl-galactose (88 mg, 0.4 mmol) were dissolved in H_2O and flushed in a continuous flow reactor for 20 min under irradiation (medium pressure Hg Lamp, Hanovia, 450W). The crude product was further purified with sephadex G-10 to yield 10 mg of PLL-mannose (90%). ^{1}H NMR (300 MHz, D_2O) δ 1.14 (t, *J* = 7.1 Hz, 1H); 1.24 (t, *J* = 7.5 Hz, 2H); 1.97-1.78 (m, 10H); 2.40-2.36 (m, 1H); 2.65-2.61 (m, 8H); 2.68-2.71 (m, 8H); 2.86 (t, *J* = 6 Hz, 5H); 3.19-3.13 (m, 1H); 3.33-3.37 (m, 2H);

3.52-3.63 (m, 23H); 3.65-3.78 (m, 29H); 3.79-3.95 (m, 28H); 4.63 (s, 1H); 4.83 (s, 1H); 5.29 (dd, *J* = 25.0, 13.9 Hz, 2H); 5.84-6.02 (m, 1H); ^{13}C NMR (300 MHz, MeOD) δ 44.80, 45.50, 50.23, 56.83, 71.69, 76.06, 77.09, 83.62, 87.01, 116.44, 116.48.

EXAMPLE 3

Characterization and testing of the novel dendronized polymers of the invention

The size and shape of the dendronized polymers was characterized by Atomic Force Microscopy (AFM) analysis of samples that were immobilized on a gelatin coated mica surface.

Preparation of Mica Surface. Mica surfaces were prepared according to the procedure reported by Doktycz, M. J., Sullivan C. J., Hoyt, P. R.; Pelletier, D. A.; Wu, S.; Allison D. P. *Ultramicroscopy* **2003**, *97*, 209-216. Gelatin solution was prepared dissolving 0.5 g of gelatin (Sigma #G6144) and 10 mg of chromium ammonium sulphate in 100 ml of nanopure water at 60°C. After cooling to 40°C a mica disk was vertically dipped into the solution and dried overnight.

The nonapod-mannose-functionalized polymer **15** adopts a globular structure, with a diameter of approximate 4 Å (Figure 3). The globular shape likely results from polymer and dendrimer integration.

In order to demonstrate the clustering capability of the dendronized polymers, bacterial detection assays were performed. The dendronized polymers were incubated with either the mannose-binding, invasive strain of *E. coli*, ORN178 (positive control), or with the mutant *E. coli* strain, ORN208 that does not bind mannose (negative control). Binding, and any resultant clustering was detected by confocal microscopy using DAPI (4′,6-diamidino-2-phenylindole) a fluorescent dye that stains bacterial DNA.

Different numbers of *E. coli* bacteria were suspended in buffer and incubated with polymer **15** and a single mannose containing polymer **17**. After centrifugation and washing, the cells were re-suspended and stain with DAPI (4´,6-diamidino-2-phenylindole). Confocal images revealed no aggregation in case of single mannose polymer **17** (data not shown). Figure 4 shows that incubation of the nona-mannose functionalized polymer **15** with strain ORN178 resulted in large clusters of bacteria (Figure 4a), while strain ORN208 did not aggregate with the polymer (Figure 4c and 4d). Specific multivalent interactions between specific *E. coli* strains and polymer **15** were confirmed by adding ConA-FITC (Concavanalin A-fluorescein isothiocyanate conjugate). ConA is a lectin well known for its strong interaction with α-mannose. Solutions containing different numbers of *E. coli* bacteria (10^5-10^7) were incubated with polymer **15**. The cells were then incubated with ConA-FITC for approximately 10 min, centrifuged and washed. ConA-FITC staining successfully localized polymer **15** within a cluster of *E. coli* strain ORN178 (Figure 4b). Neither clustering nor significant ConA-FITC staining were observed in samples of *E. coli* strain ORN208 incubated with compound **15** (Figure 4d). Therefore, it is concluded that the mannose-binding *E. coli* was aggregated by a matrix of the nona-mannose dendronized polymer **15**, which was localized by fluorescently labeled ConA. *E. coli* bacteria that do not bind to mannose were not bound by the flexible polymer and aggregated only in a non-specific manner.

Finally, high resolution Atomic Force Microscopy (AFM) was employed to confirm the presence of dendronized polymers around the mannose-binding *E. coli*. AFM images of a single ORN178 *E. coli* cell were obtained before and after incubation with dendronized polymer **15**. The surface topology of a single cell of strain ORN178 after incubation with polymer **15** is shown in Figure 5. To assess the lower limit for detecting bacterial aggregates in the presence of polymer **15**, serially diluted solutions of *E. coli* ORN178 were incubated with polymer **15** for 1 h. Samples were

centrifuged, the pellets were washed to remove unbound polymer, and the bacteria were visualized using confocal microscopy. Polymer **15** displays optimal binding with as little as 10^5 bacteria (Fig. 6). Using confocal microscopy we found that the detection limit of the aggregates is similar to that observed using other multivalent biosensors. However, using AFM allows for detection of an individual bacterium that binds the glyco-dendronized polymer.

Cell Growth and Incubation with Polymer. The strains used in this study were kindly donated by Prof. Orndorff (College of Veterinary Medicine, Raleigh, NC United States), ORN178 for the mannose binding strain and ORN208 for the mutant strain that does not bind mannose. Cells were grown in LB media. The inoculated culture was incubated overnight at 37°C by shaking on an incubator shaker at 250 rpm. Intermittently, aliquots of bacteria were removed from the batch culture to monitor growth until they reached an approximate OD600 of 1.0. Aliquot of 2 ml cultures were then centrifuged for 10 min at approximately 1,600 x g. The resulting pellets were then washed twice with PBS buffer and resuspended in 100 µL PBS. A 10 µg of the polymer was added in each incubation mixture or omitted from the control incubations. Each suspension was then incubated for 30 min at room temperature with gentle shaking and centrifuged to pellet the cells. To remove excess of polymer pellets were softly washed twice with PBS and centrifuged. This procedure was repeated twice for each culture.

Atomic Force Microscopy. After incubation with polymer, the suspension of bacteria was centrifuged to pellet the cell and washed four times with PBS buffer (4 x 400 µL of buffer) to remove excess polymer. An aliquot of 20 µL was pipetted onto gelatine treated mica disk. The sample was allowed to stand for 30 min, rinsed with nanopure water (2 x 50 µL) and allowed to dry for air imaging or covered with nanopure water for liquid imaging.

Confocal Fluorescence Microscopy. The washed polymer-treated and non-treated bacteria were fixed with 100 µL with 4% (w/v) paraformaldehyde for 15 min. Bacteria were then centrifuged and the pellet resuspended in 100 µL of PBS containing the organic dye 4',6-diamidino-2-phenylindole dihydrochloride, (DAPI) at dilution of 1/1000 and incubated for 15 min. DAPI forms fluorescent complexes with double-stranded DNA. Alternatively to image the polymers, following DAPI treatment bacteria were centrifuged and the pellet incubated for additional 15 min in the presence of 100 µL PBS containing fluorescein-labelled Concanavalin A (ConA-FITC) at a dilution of 1/1000. Bacteria were then centrifuged, washed twice as described above prior to analysis. Bacteria were imaged using a Carl Zeiss confocal laser scanning microscope (LSM 700) using a 63 x oil immersion or a 40 x objectives.

In conclusion, the present inventors have developed an efficient, fast and readily scalable synthetic route to synthesize dendronized polymers in an aqueuos medium, preferably water, employing a photochemical continuous flow reactor. These glyco-dendronized polymers constitute a flexible platform to study carbohydrate-pathogen interactions. Using fluorescent microscopy techniques and AFM, it was demonstrated that these glyco-dendronized polymers can be used as a sensitive and selective biosensor for mannose-binding *E. coli*.

EXAMPLE 4

Conjugation of an allyl alcohol with L-cysteine.

1. Reactor design

The reactor design (Fig. 8A) is adapted from the design of Booker-Milburn *et al.* (*J. Org. Chem.* **2005**, *70*, 7558-7564) and principally involves a length of polymer tubing coiled around a low-pressure mercury lamp in a quartz shield. The reactant

solution is passed through the tubing and is exposed to ultraviolet light, initiating reaction. This setup is similar to that described above for cycloadditions.

The important difference with this system is the ability to perform reactions at wavelengths below 300 nm; in this case at 254 nm. This is achieved by using tubing made of Teflon AF-2400 polymer (other polymer grades are available and are expected to work as well), manufactured by DuPont, which is transparent to light of this wavelength. The lamp used is typically a low-pressure mercury lamp with a maximum emission at 254 nm (e.g. a Spectroline 11SC-1 quartz pen low-pressure mercury lamp with an intensity of 4500 $\mu W/cm^2$ at 254 nm). The tubing is wrapped around an inner jacket made of quartz glass, and the jacket and tubing are submerged in a thermal bath which may or may not be temperature regulated, itself contained within the shielded outer jacket. Ideal solvents for the photochemical processes occurring within the Teflon AF-2400 tubing are water, methanol or cyclohexane. Use of acetonitrile should also be possible, although this solvent absorbs ~2% of incident radiation at 254 nm.

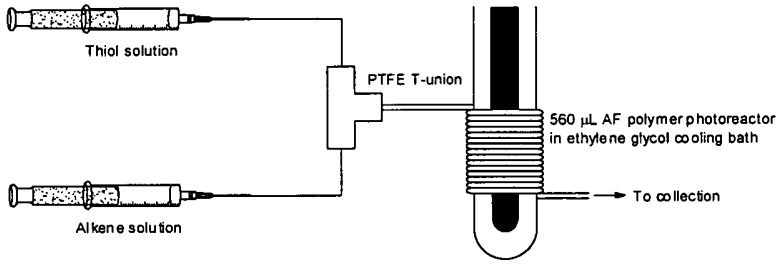
The reactor volume can be altered by the use of different length tubing and is optimum whenever the coiled length appropriately fits with the lamp in use. The reaction mixture is moved through the system *via* syringe pump, although other fluid pumping systems would also work. The thermal bath liquid can be any appropriate agent provided they are not rapidly degraded by the 254 nm UV light. For example ethylene glycol can be used for the thermal bath.

The input components were either premixed in a batch process into a single solution which was then placed into the photoreactor system, or the input components could also be mixed together in continuous flow which would then directly enter the photoreactor tubing via connectors in the same process. In either case the

components were shielded from all UV and Visible light while they were mixed.

2. Representative procedures for the thiol-ene conjugation:

General experimental.



For the following exemplary experiments, a 560 µL reactor was constructed as shown above from 2 m of 0.6/0.8 mm ID/OD AF-2400 tubing wrapped directly around the outside of a quartz shield for the SpectroLine short wave (254 nm) lamp. The reactor was clamped inside a graduated cylinder that was filled with ethylene glycol (for temperature moderation with the tubing immersed in the ethylene glycol) and then covered with aluminum foil. The system was rinsed thoroughly with solvent, and the lamp was allowed to warm for 30 min before use. HPLC-grade solvents were used in all cases. Syringes containing a solution of each component (delivered using a KD Scientific KDS-101-CE syringe pump) were mixed in a T-union before direct subsequent delivery to the 254 nm lamp reactor. From the T-union to the exit point the tubing was wrapped in aluminum foil.

(*R*)-2-amino-3-((3-hydroxypropyl)thio)propanoic acid.

A solution of allyl alcohol (68 µL, 1.0 mmol, 1.0 equiv) in water (2.5 mL) was mixed with a solution of L-cysteine (121 mg, 1.0 mmol, 1.0 equiv) in water (2.5 mL) and passed through the photoreactor described above at a flow rate of 28 µL/min (residence time 10 min). The reactor was then flushed with water (1.5 mL) and the resulting solution concentrated under reduced

pressure to give (*R*)-2-amino-3-((3-hydroxypropyl)thio)propanoic acid (147 mg, 82%, 100% conversion by 1H-NMR) as a white solid: δH (400 MHz, CD_3OD) 3.72 (1H, dd, *J* 8.0, 4.0, CH), 3.53 (2H, t, *J* 6.0, CH_2OH), 3.14 (1H, dd, *J* 14.0, 4.0, CHC*H*HS), 2.95 (1H, dd, *J* 14.0, 8.0 CHCH*H*S), 2.66 (2H, dt, J, 8.0, 3.0, $HOCH_2CH_2CH_2S$), 1.70 (2H, m, $HOCH_2CH_2CH_2S$); δC (100 MHz, CD_3OD) 173.3, 61.3, 55.1, 33.7, 33.2, 29.2; *m/z* (ESI) 180.0 $[MH]^+$.

EXAMPLE 5

Conjugation of allyl sugars with L-cysteine and other thiols

1. General reactor design

The reactor design was similar to that described in Example 4 and Fig. 8 and consisted of loops of transparent tubing wrapped around a suitable light source and cooling jacket, following the design of Booker-Milburn. Teflon AF-2400, transparent in the deep-UV region was chosen as the tubing material (note FEP tubing decomposes) and was wrapped around a quartz cooling jacket containing a 100 W indium amalgam lamp. A temperature of 25 °C was maintained for all experiments with a cryostat, using spectroscopically pure water as the coolant. Separate solutions of the thiol and olefin components were delivered by syringe pump, and mixed with a T-union. All optimisation reactions were performed using a 566 mL loop of Teflon AF2400, while a 2266 mL loop was used for scaleup. Although the reaction is tolerant to oxygen, all solvents were degassed before use.

2. Procedures for performing the thiol-ene reaction in continuous flow

2.1 - General procedure **A**, *for the optimization of the reaction.*

A photoreactor was set up according to Fig 8B, using a 566 µL loop of Teflon AF-2400 polymer tubing (0.8 mm outer diameter, 0.6 mm internal diameter) wrapped around a quartz cooling jacket containing a NNIQ low pressure In/Hg lamp. A recirculating chiller (Julabo FL-460, filled with spectroscopically pure water

as coolant) was used to maintain a reactor temperature of 25 °C. Using a syringe pump (Harvard PHD2000), separate solutions of the thiol (1.2 equiv) in degassed water and allyl glycoside (1.0 equiv) in degassed water were mixed using a T-mixer and passed into the photoreactor at the specified rate. After two reactor volumes of product had eluted from the reactor, an aliquot of the output solution was collected and analysed directly by HPLC/MS (0-20% MeCN/H_2O over 40 min, 0.1% formic acid) to assess the fractional conversion of the allyl glycoside into the desired product.

2.2 General procedure **B**, *for the thiol-ene reaction of allyl glycosides.*

A photoreactor was set up according to the diagram in Fig 8B, using a 2266 µL loop of Teflon AF-2400 polymer tubing (0.8 mm outer diameter, 0.6 mm internal diameter) wrapped around a quartz cooling jacket containing a NNIQ low pressure In/Hg lamp. A recirculating chiller (Julabo FL-460, filled with spectroscopically pure water as coolant) was used to maintain a reactor temperature of 25 °C. Using a syringe pump (Harvard PHD2000), a solution of the allyl glycoside (100 mg, 0.454 mmol, 1.0 equiv) in degassed water (2.27 mL) was mixed with a solution of the thiol (0.545 mmol, 1.2 equiv) *via* a T-mixer at a rate of 113 µL/min per syringe (combined flow rate 226 µL/min, residence time 10 min) and passed into the photoreactor. After discarding the first reactor volume of output, the entire product solution was collected and freeze-dried under reduced pressure to afford the crude products.

2.3 - General procedure **C**, *for the derivatisation of glycoaminoacids to obtain Fmoc-protected analytical samples.*

To a solution of the crude glycoamino acid (30 mg, 0.088 mmol assuming 100% purity, 1.0 equiv) in water (0.5 mL) and acetone (0.5 mL) was added *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (36 mg, 0.105 mmol, 1.2 equiv) then $NaHCO_3$ (11 mg, 0.132 mmol, 1.5 equiv) at rt. After stirring for 12 h at rt, the reaction

mixture was diluted with water (10 mL) and washed with dichloromethane (10 mL). The aqueous phase was freeze-dried under reduced pressure and purified by preparative HPLC to afford the Fmoc protected products.

2.4 - Procedure for the large-scale preparation of OAc protected glycoamino acid building blocks.

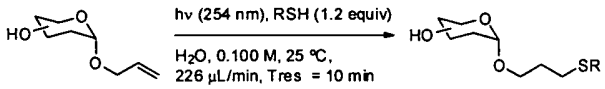
A photoreactor was set up according to the diagram in Fig. 8B, using a 2266 µL loop of Teflon AF-2400 polymer tubing (0.8 mm outer diameter, 0.6 mm internal diameter) wrapped around a quartz cooling jacket containing a NNIQ low pressure In/Hg lamp. A recirculating chiller (Julabo FL-460, filled with spectroscopically pure water as coolant) was used to maintain a reactor temperature of 25 °C. Separate solutions of the reactants were prepared: the *O*-allyl glycoside (4.33 g, 11.2 mmol, 1.0 equiv) in methanol (60 mL) and L-cysteine (2.03 g, 16.7 mmol, 1.5 equiv) in water (60 mL). Using a dual-channel HPLC pumping module (Vapourtec R2), the two solutions were mixed using a T-mixer and passed into the photoreactor (113 µL/min, 10 min residence time). The reactor output was concentrated under reduced pressure, taken up in MeOH (100 mL) and filtered over Celite to remove excess L-cysteine. The filtrate was concentrated under reduced pressure and triturated with cold diethyl ether (3 x 30 mL) to afford the glycoaminoacid building block as a white solid (2.67 g, 44%).

3. General experimental setup and results

Firstly, the reaction of various *O*-allyl glycosides, easily accessible from the parent carbohydrate, with L-cysteine in water was investigated. A slight excess (1.2 equiv) of cysteine was used. The relationship between residence time and conversion for reaction of *O*-allyl galactose with L-cysteine was studied at three different concentrations (Fig. 10). Almost complete conversion was observed within a residence time of 15 min, even at the lowest concentration studied. No significant thermal background reaction was observed at a concentration of 0.1 M.

Plots of fractional conversion against residence time for *O*-allyl glycosides of Table 2 below showed little relationship between carbohydrate structure and reactivity under these con-ditions, although alpha glycosides reacted faster than their beta isomers (Fig. 11). The reactions were repeated at 0.1 M to afford glycoamino acids with complete conversion and in good yields (Table 2). The products were more simply purified as their *N*-Fmoc derivatives by preparative HPLC. The process was not limited to cysteine thiols: 2-mercaptoethanol, thioacetic acid and thioglucose also react with a-*O*-allyl galactose in good yield.

Table 2. Thiol-ene reactions of *O*-allyl glycosides



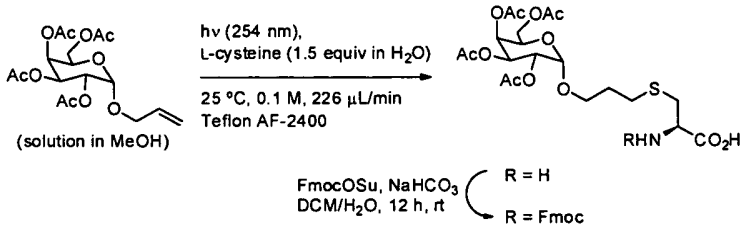
Entry	Glycoside	Thiol	% Yield
A	α-Gal	L-cysteine	77[a]
B	ß-Gal	L-cysteine	70 [a]
C	α-Glu	L-cysteine	80 [a]
D	ß-Glu	L-cysteine	71 [a]
E	α-Man	L-cysteine	75 [a]
F	α-Gal	$HSCH_2CH_2OH$	74

[a]By NMR of the crude mixture with maleic acid as an internal standard. Analytical samples were obtained as *N*Fmoc derivates. Purified as the *O*-acetylated derivative.

Adaptation of the process allowed the gram-scale preparation of fully-protected glycoamino acid building blocks from acetylated *O*-allyl glycosides. Initial optimisation determined that high conversion (>90%) could be attained mixing separate 0.1 M solutions of the carbohydrate in methanol and L-cysteine (1.5 equiv)in water and passing the solutions, mixed by a T-mixer, through the same 2.26 mL irradiated loop as used above.

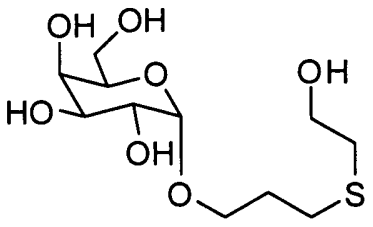
Conversion was comparably slow when performing the reaction in MeCN-H_2O. Scaleup of these conditions, using of a commercially available Vapourtec R2 pumping module for continuous delivery of reagents affords the free glycoamino acid following precipitation of excess L-cysteine with MeOH, concentration and trituration of the resulting solid with diethyl ether. The glycoamino acids can then be used as their Fmoc derivatives in solid phase peptide synthesis.

Scheme 5. Gram-scale preparation of glycoamino acid building blocks



4. Exemplary compounds prepared *via* continuous flow thiol-ene reaction

(2S,3R,4S,5R,6R)-2-(3-((2-hydroxyethyl)thio)propoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol.

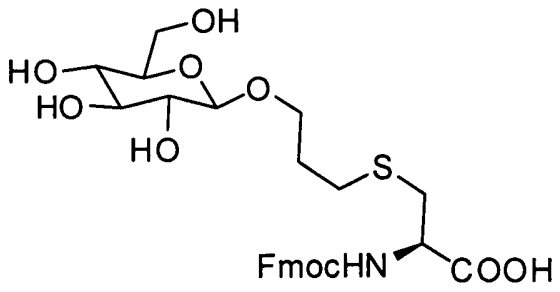


a) α-*O*-allyl galactose (100 mg, 0.454 mmol, 1.0 equiv) was reacted with 2-mercaptoethanol (38.0 µL, 0.545 mmol, 1.2 equiv) according to general procedure **B**. The reactor output was concentrated by freeze-drying under reduced pressure and purified over silica gel (EtOAc, then EtOAc/*i*PrOH/H_2O 9:4:2) to give (2S,3R,4S,5R,6R)-2-(3-((2-hydroxyethyl)thio)propoxy)-6-

(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (100 mg, 74%) as a colourless oil: δ_H (400 MHz, D_2O) 4.95 (1H, d, *J* 3.5), 3.99 (1H, d, *J* 3.5), 3.96 (1H, t, *J* 6.5), 3.88-3.80 (3H, m), 3.78-3.75 (4H, m), 3.61 (1H, dt, *J* 10.0, 6.0), 2.76 (2H, t, *J* 6.5), 2.71 (2H, t, *J* 7.5), 1.95 (2H, sext, *J* 6.5); δ_C (100 MHz, D_2O) 98.2, 70.9, 69.4, 69.2, 68.2, 66.5, 61.1, 60.2, 33.2, 28.6, 27.9; *m/z* (ESI) 321 $[MNH_4]^+$ 137, 121.

b) Alternatively, a solution of α-*O*-allyl galactose (66 mg, 0.3 mmol, 1.0 equiv) in methanol (3.0 mL) was mixed with a solution of 2-mercaptoethanol (63 µL, 0.9 mmol, 3.0 equiv) in methanol (3.0 mL) and passed through the photoreactor described in Example 4 above at a flow rate of 37.5 µL/min (residence time 15 min). Concentration of the reactor output under reduced pressure afforded (2*S*,3*R*,4*S*,5*R*,6*R*)-2-(3-((2-hydroxyethyl)thio)propoxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (100 % conversion by 1H-NMR) as a white solid: δH (400 MHz, CD_3OD) 4.79 (1H, d, *J* 3.5, H-1), 3.87 (1H, dd, *J* 3.5, 1.5, H-3), 3.82-3.63 (7H, m), 3.43 (1H, ddd, *J* 12.0, 10.0, 6.0, $OCH\mathit{H}CH_2CH_2S$), 2.70 (1H, dd, *J* 6.0, 6.0), 2.60-2.54 (4H, m), 1.80-1.72 (2H, m, $OCH_2C\mathit{H}_2CH_2S$); δC (100 MHz, CD_3OD) 100.3, 72.4, 71.5, 71.0, 70.2, 67.6, 62.6, 62.4, 35.1, 30.7, 29.8; *m/z* (ESI) 321 $[MNa]^+$.

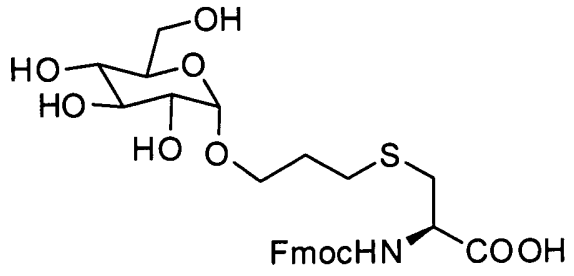
(R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)propyl)thio)propanoic acid.



A solution of β-*O*-allyl glucose (100 mg, 0.454 mmol, 1.0 equiv) was reacted with L-cysteine (66 mg, 0.545 mmol, 1.2 equiv)

according to general procedure **B** and concentrated by freeze-drying under reduced pressure to give the crude glycoamino acid (171 mg, 64% purity, 71% yield) as an off-white solid. A sample of the crude glycoamino acid (30 mg) was reacted according to general procedure **C**. Purification by preparative HPLC (Nucleodur C18 Pyramid column, 20-50% MeCN/H_2O, 0.1% TFA over 30 mins T_R = 26.4 min) afforded an analytical sample of (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-propyl)thio)propanoic acid as a white solid: $\nu_{(max)}$ 3315, 1703, 1541, 1450, 1284, 1041, 757, 739 cm^{-1}; δH (400 MHz, CD_3OD) 7.82 (2H, d, *J* 7.5), 7.71 (2H, d, *J* 7.5), 7.41 (2H, t, *J* 7.5), 7.34 (2H, t, *J* 7.5), 4.42-4.33 (3H, m), 4.29-4.25 (2H, m), 3.99 (1H, dt, *J* 10.0, 6.5), 3.86 (1H, dd, *J* 12.0, 2.0), 3.67 (2H, dd, *J* 12.0, 5.5), 3.39-3.35 (1H, m), 3.30-3.26 (2H, m), 3.20 (1H, dd, *J* 9.0, 8.0), 3.08 (1H, dd, *J* 14.0, 4.5), 2.88 (1H, dd, *J* 14.0, 8.5), 2.73 (2H, t, *J* 7.0), 1.94-1.87 (2H, m); δC (100 MHz, CD_3OD) 174.2, 158.5, 145.3, 145.2, 128.8, 128.2, 126.3, 120.9, 104.4, 78.0, 77.9, 75.1, 71.6, 69.1, 68.2, 62.7, 55.4, 34.7, 30.8, 29.8; *m/z* (ESI) 1149 $[M_2Na]^+$, 586 $[MNa]^+$, 413, 301, 179 (Found: $[MNa]^+$, 586.1711. $C_{27}H_{33}NO_{10}S$ requires $[MNa]^+$, 586.1723).

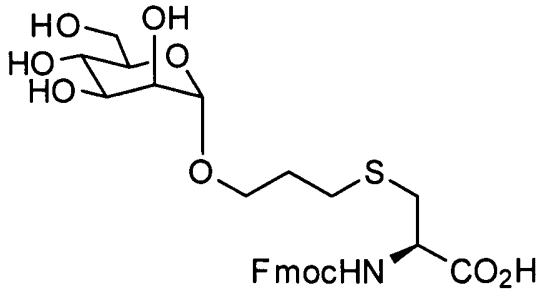
(R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)propyl)thio)propanoic acid.



A solution of α-*O*-allyl glucose (100 mg, 0.454 mmol, 1.0 equiv) was reacted with L-cysteine (66 mg, 0.545 mmol, 1.2 equiv) according to general procedure **B** and concentrated by freeze-drying under reduced pressure to give the crude glycoamino acid

(194 mg, 64% purity, 80% yield) as an off-white solid. A sample of the crude glycoamino acid (30 mg) was reacted according to general procedure **C**. Purification by preparative HPLC (Nucleodur C18 Pyramid column, 20-50% MeCN/H_2O, 0.1% TFA over 30 mins T_R = 27.1 min) afforded an analytical sample of (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-propyl)thio)propanoic acid as a white solid: $\nu_{(max)}$ 3327, 1732, 1687, 1536, 1275, 1024, 950, 758, 737 cm^{-1}; δH (400 MHz, CD_3OD) 7.80 (2H, d, *J* 7.5), 7.70 (2H, d, *J* 7.5), 7.40 (2H, t, *J* 7.5), 7.32 (2H, t, *J* 7.5), 4.78 (1H, d, *J* 3.5), 4.41-4.35 (3H, m), 4.25 (1H, t, *J* 7.0), 3.86-3.83 (1H, m), 3.81 (1H, dd, *J* 12.0, 3.5), 3.69 (1H, dd, *J* 9.5, 6.5), 3.65 (1H, d, *J* 6.5), 3.61-3.56 (1H, m), 3.53 (1H, dt, *J* 10.0, 6.0), 3.39 (1H, dd, *J* 10.0, 3.5) 3.31-3.28 (2H, m), 3.06 (1H, dd, *J* 14.0, 5.0), 2.88 (1H, dd, *J* 14.0, 8.0), 2.78-2.68 (2H, m), 1.95-1.86 (2H, m); δC (100 MHz, CD_3OD) 174.2, 158.5, 145.3, 145.2, 142.6, 128.8, 128.2, 126.3, 120.9, 100.2, 75.1, 73.7, 73.6, 71.8, 68.2, 67.3, 62.7, 55.4, 34.6, 30.5, 29.9; *m/z* (ESI) 1149 $[M_2Na]^+$, 586 $[MNa]^+$, 413, 179 (Found: $[MNa]^+$, 586.1720. $C_{27}H_{33}NO_{10}S$ requires $[MNa]^+$, 586.1723).

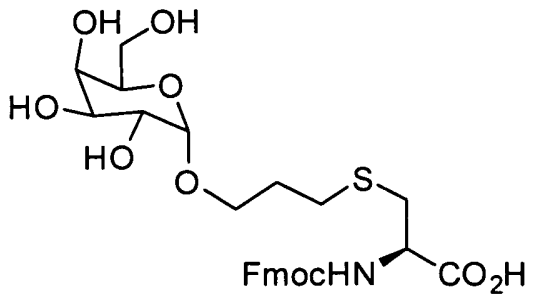
(R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-(((2S,3S,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)propyl)thio)propanoic acid.



A solution of α-*O*-allyl mannose (100 mg, 0.454 mmol, 1.0 equiv) was reacted with L-cysteine (66 mg, 0.545 mmol, 1.2 equiv) according to general procedure **B** and concentrated by freeze-drying under reduced pressure to give the crude glycoamino acid

(163 mg, 71% purity, 75% yield) as an off-white solid. A sample of the crude glycoamino acid (30 mg) was reacted according to general procedure **C**. Purification by preparative HPLC (Nucleodur C18 Pyramid column, 20-50% MeCN/H_2O, 0.1% TFA over 30 mins T_R = 27.4 min) afforded an analytical sample of (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-(((2S,3S,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-propyl)thio)propanoic acid as a white solid: $\nu_{(max)}$ 3394, 2930, 1702, 1517, 1450, 1204, 1133, 1056, 760, 740 cm^{-1}; δH (400 MHz, CD_3OD) 7.82 (2H, d, *J* 7.5), 7.71 (2H, d, *J* 7.5), 7.41 (2H, t, *J* 7.5), 7.33 (2H, t, *J* 7.5), 4.76 (1H, d, *J* 1.5), 4.41-4.37 (3H, m), 4.27 (1H, t, *J* 6.0), 3.87-3.81 (3H, m), 3.75-3.70 (2H, m), 3.63 (1H, t, *J* 9.5), 3.57-3.49 (2H, m), 3.07 (1H, dd, *J* 14.0, 4.5), 2.88 (1H, dd, *J* 14.0, 9.0), 2.69 (2H, t, *J* 7.0), 1.88 (2H, pent, *J* 6.5); δC (100 MHz, CD_3OD) 172.7, 157.1, 143.9, 143.8, 141.1, 127.4, 126.8, 124.9, 119.5, 100.2, 73.2, 71.2, 70.8, 67.2, 66.7, 65.4, 61.5, 54.0, 33.3, 29.1, 28.6; *m/z* (ESI) 1149 $[M_2Na]^+$, 586 $[MNa]^+$, 440, 179 (Found: $[MNa]^+$, 586.1698. $C_{27}H_{33}NO_{10}S$ requires $[MNa]^+$, 586.1723).

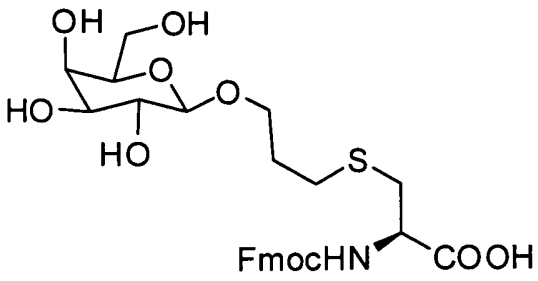
(R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-(((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)propyl)thio)propanoic acid.



A solution of α-*O*-allyl galactose (100 mg, 0.454 mmol, 1.0 equiv) was reacted with L-cysteine (66 mg, 0.545 mmol, 1.2 equiv) according to general procedure **B** and concentrated by freeze-drying under reduced pressure to give the crude glycoamino acid (168 mg, 71% purity, 77% yield) as an off-white solid. A sample

of the crude glycoamino acid (30 mg) was reacted according to general procedure **C**. Purification by preparative HPLC (Nucleodur C18 Pyramid column, 20-50% MeCN/H_2O, 0.1% TFA over 30 mins T_R = 26.1 min) afforded an analytical sample of (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-(((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-propyl)thio)propanoic acid as a white solid: $[a]_D^{20}$: + 64.4 (c = 0.25 in MeOH); $\nu_{(max)}$ 3221, 2926, 1694, 1586, 1145, 1031, 972, 738 cm^{-1}; δ_H (400 MHz, CD_3OD) 7.82 (2H, d, *J* 7.5), 7.71 (2H, d, *J* 7.5), 7.41 (2H, t, *J* 7.5), 7.41 (2H, t, *J* 7.5), 7.33 (2H, t, *J* 7.5), 4.82 (1H, d, *J* 3.0), 4.42-4.33 (3H, m), 4.27 (1H, t, *J* 7.0), 3.90 (1H, br s), 3.86-3.80 (2H, m), 3.78-3.76 (2H, m), 3.72 (2H, dd, *J* 6.5, 3.0), 3.54 (1H, dt, *J* 10.0, 6.0), 3.07 (1H, dd, *J* 14.0, 4.0), 2.88 (1H, dd, *J* 14.0, 8.0), 2.73 (2H, t, *J* 7.5), 1.98 - 1.87 (2H, m); δ_C (100 MHz, d_6-DMSO) δ 171.9, 155.3, 143.9, 143.9, 140.7, 127.6, 127.1, 125.3, 120.1, 98.8, 71.2, 69.6, 68.7, 68.5, 65.50, 65.45, 60.36, 55.0, 46.7, 34.2, 29.4, 28.3; *m/z* (ESI) 1149 $[M_2Na]^+$, 586 $[MNa]^+$, 440, 179 (Found: $[MNa]^+$, 586.1716. $C_{27}H_{33}NO_{10}S$ requires $[MNa]^+$, 586.1723).

(R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-(((2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)propyl)thio)propanoic acid.



A solution of ß-*O*-allyl galactose (100 mg, 0.454 mmol, 1.0 equiv) was reacted with L-cysteine (66 mg, 0.545 mmol, 1.2 equiv) according to general procedure **B** and concentrated by freeze-drying under reduced pressure to give the crude glycoamino acid (162 mg, 67% purity, 70% yield) as an off-white solid. A sample

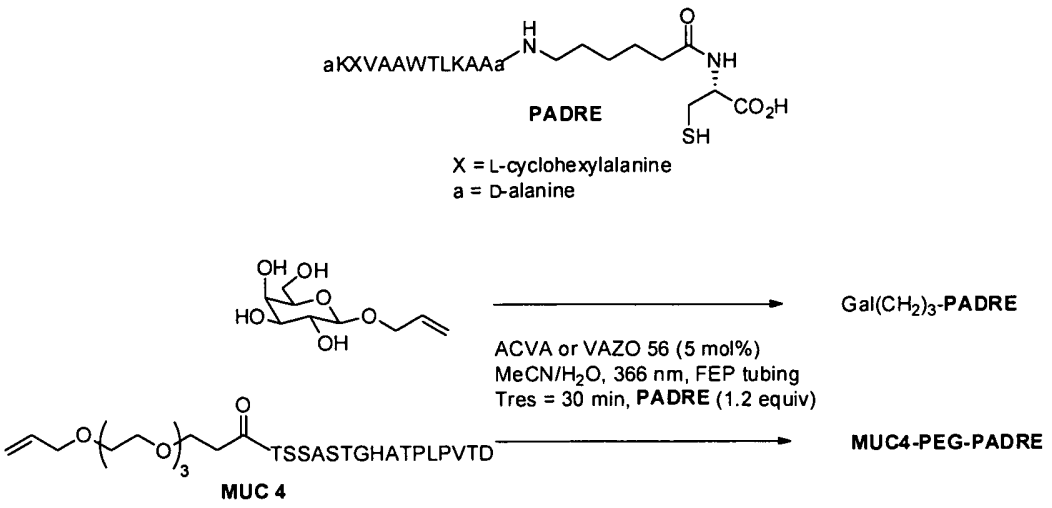
of the crude glycoamino acid (30 mg) was reacted according to general procedure **C**. Purification by preparative HPLC (Nucleodur C18 Pyramid column, 20-50% MeCN/H_2O, 0.1% TFA over 30 mins T_R = 26.6 min) afforded an analytical sample of (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-(((2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-propyl)thio)propanoic acid as a white solid: δ_H (400 MHz, CD_3OD) 7.82 (2H, d, *J* 7.5), 7.71 (2H, d, *J* 7.5), 7.41 (2H, t, *J* 7.5), 7.73 (2H, t, *J* 7.5) 4.42-4.33 (3H, m), 4.25-4.21 (2H, m), 3.98 (1H, dt, *J* 10.0, 6.0), 3.84 (1H, dd, *J* 3.0, 1.0), 3.71-3.69 (2H, m), 3.66 (1H, dt, *J* 10.0, 6.0), 3.55-3.45 (3H, m), 3.07 (1H, dd, *J* 14.0, 5.0), 2.88 (1H, dd, *J* 14.0, 8.5), 2.72 (2H, t, *J* 7.0), 1.90 (2H, pent, *J* 6.5); δ_C (100 MHz, CD_3OD) 174.2, 158.5, 145.3, 145.2, 142.6, 128.8, 128.2, 126.3, 120.9, 105.0, 76.6, 75.0, 72.6, 70.3, 69.1, 68.1, 62.5, 55.4, 34.7, 30.9, 29.8.

EXAMPLE 6

Functionalization of peptides and proteins

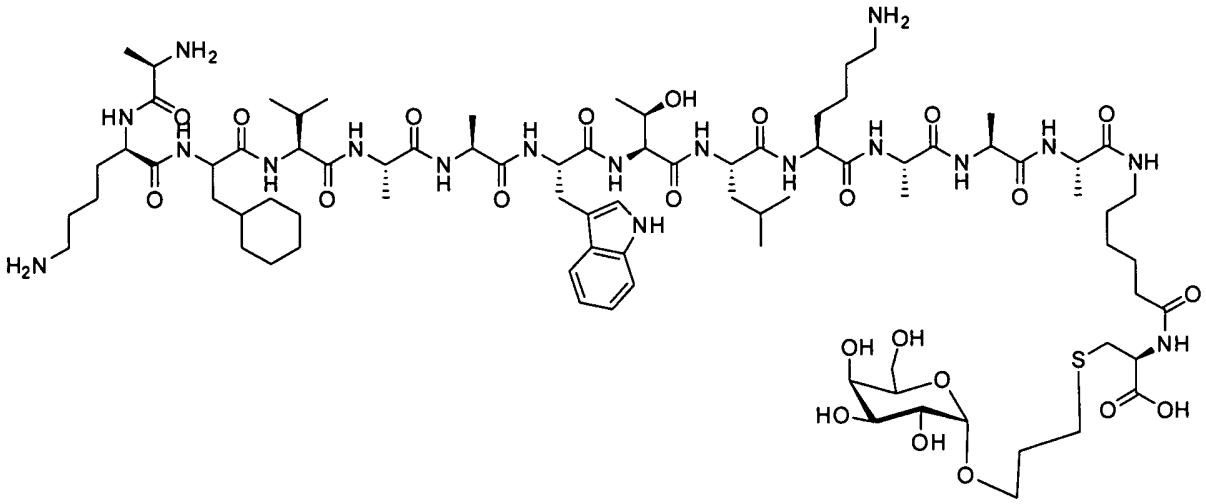
The system also has application for the functionalization of biologically relevant peptides. Since it was expected that 254 nm light would be incompatible with compounds containing aromatic amino acid residues, the reactor design was changed accordingly to a loop of FEP tubing irradiated with tubing irradiated with a medium-pressure mercury lamp (λ_{max} = 366 nm) shielded with a pyrex filter. The reaction requires an initiator to proceed effectively at this wavelength, and the water-soluble AIBN derivatives VAZO56 and ACVA were selected for the present studies. Other water-soluble radical initiators can be used. The Pan-DR helper epitope **PADRE**, recently reported as an immunogenic carrier peptide for conjugate vaccines, was prepared by standard solid phase peptide synthesis. **PADRE**, which bears a terminal cysteine residue was selected due to its ease of analysis and purification by HPLC and its potential application in vaccine development. The reaction of PADRE with both *O*-allyl galactose, as a model for carbohydrate

antigens, and the allyl-tagged immunogenic peptide MUC-4 was studied.



Scheme 6. Continuous flow conjugation of carbohydrate and peptide fragments

Procedure for the conjugation of allyl-linked carbohydrates and peptides with the exemplary thiol-bearing peptide PADRE:



A photoreactor was set up according to the diagram in Fig 8B, using a 565 µL loop of FEP tubing (0.8 mm outer diameter, 0.6 mm internal diameter) wrapped around a quartz cooling jacket containing a 400W medium pressure mercury lamp. A recirculating chiller (Julabo FL-460, filled with spectroscopically pure

ethylene glycol) was used to maintain a reactor temperature of 25 °C. A solution was prepared of PADRE (2.5 mg, 1.59 μmol, 1.0 equiv), α-*O*-allyl galactose (5.3 mg, 0.024 mmol, 15.0 equiv), (*E*)-4,4'-(diazene-1,2-diyl)bis(4-cyanopentanoic acid) (447 mL of a 3.56 μM solution in water, 1.59 μmol, 1.0 equiv) and trifluoroacetic acid (1.23 μL, 0.016 mmol, 10 equiv) in water (52 μL). The solution was injected through the photoreactor *via* syringe pump (18.8 mL/min, 30 min residence time), washing with water (1 mL). After removal of the excess α-*O*-allyl galactose by dialysis, the reactor output was lyophilized to give the peptide conjugate as a white solid.

The system can also be used for the functionalisation of peptide oligomers with multiple thiols. The products of this reaction have potential application in the multiple presentation of carbohydrate antigens. Oligomers bearing multiple olefins or thiols can be reacted with the corresponding thiol- or allyl-group respectively.

Summarizing, the present invention provides in one embodiment a continuous flow photochemical system for the production of glycoamino acid building blocks and peptide conjugates *via* the thiol-ene reaction. The system is efficient enough to allow multiple couplings to be performed along an oligomeric backbone.

CLAIMS

1. A continuous flow process for the photoconjugation of chemical entities selected from the group comprising carbohydrates, including mono-, oligo- and poly-saccharides, amino acids, peptides and proteins, unbranched and branched oligomers and polymers, including dendrimers, which process comprises at least the following steps:
a) providing a mixture of the reactants dissolved in an aqueous or organic solvent;
b) passing said mixture through a continuous flow reactor and concomitantly irradiating the mixture with light in order to initiate and perform the photoreaction.

2. The process according to claim 1, wherein the photoconjugation is a thiol-ene conjugation or a [2+2] cycloaddition.

3. The process according to claim 2, wherein the photoconjugation is a thiol-ene conjugation and wherein the thiol is selected from the group consisting of alkyl thiols, thiosugars, thiol-containing amino acids, and peptides/proteins bearing a thiol group, in particular a thiol linker.

4. The process according to claim 2 or 3, wherein the photoconjugation is a thiol-ene conjugation and the chemical entity reacting with the thiol and comprising the alkenyl double bond is selected from the group consisting of terminal alkenes, allylic alcohols, allyl sugars, allyl amino acids or peptides/proteins bearing an alkene linker.

5. The process according to claim 3 or 4, wherein the photoconjugation is a multiple thiol-ene conjugation along a peptide backbone.

6. The process according to claim 5, wherein the thiol is a thiosugar reacting with peptides/proteins bearing an alkene linker or the thiol is a peptide/protein bearing a thiol group, in particular a thiol linker, reacting with an allyl sugar.

7. The process according to claim 2, wherein the solvent is selected from the group consisting of water, a C_1-C_6-alkanol, preferably methanol, hexanes, e.g. cyclohexane, n-hexane, and acetonitrile.

8. The process according to claim 2, wherein the photoconjugation is a thiol-ene conjugation which is initiated by UV light having a wavelength of less than 300 nm, preferably about 254 nm.

9. The process according to claim 3, wherein the linker is based on a poly(ethylene glycol) structure bound to the peptide/protein *via* an ester linkage and terminated with a thiol.

10. The process according to claim 4, wherein the linker is based on a poly(ethylene glycol) structure bound to the peptide/protein *via* an ester linkage and terminated with an alkene.

11. The process according to claim 2, wherein the photoconjugation is a [2+2] cycloaddition and wherein one chemical entity has a functional linker group comprising an alkenyl or alkynyl group and the other chemical entity has a functional linker group comprising a maleimido group or an electron-poor olefinic group.

12. The process according to claim 11 which is a process for preparing dendronized polymers and comprises

a) providing a dendrimer capped with a plurality of functional moieties and having a functional linker group comprising either ia) an alkenyl or alkynyl group or iia) a maleimido group or an electron-poor olefinic group

b) reacting said capped dendrimer with a scaffold polymer functionalized with either ib) a maleimido group or a electron-poor olefinic group or iib) an alkenyl or alkynyl group in a photochemical [2+2] cycloaddition reaction wherein the corresponding cyclobuten adducts are formed by the reaction of the functional groups ia) and ib) or iia) and iib), respectively.

13. The process according to claim 12, wherein the functional moieties are selected from the group consisting of a carbohydrate, a biologically active agent, such as a pharmaceutical or a drug, a detectable group, or a binding moiety, e.g. an antibody or an antigen-binding fragment thereof, a peptide, an oligopeptide, a protein, a nucleotide, an oligonucleotide, a polynucleotide, or combinations thereof.

14. The process according to claim 13, which comprises

a) providing a carbohydrate-capped dendrimer with a functional linker group comprising a terminal ethenyl or ethynyl group

b) reacting said carbohydrate-capped dendrimer with a scaffold polymer functionalized with a maleimido group or a electron-poor olefinic group in a photochemical [2+2] cycloaddition reaction wherein the corresponding cyclobuten adducts are formed.

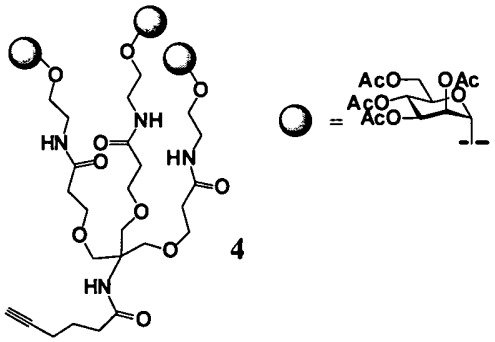
15. The process according to claim 12, wherein the acaffold polymer is selected from the group consisting of a hydrocarbon polymer, a polypeptide, including a poly(amino acid), e.g. poly-L-lysine, a polyhydroxyalkanoate, a polylactide (PLA), a polyglycolide (PGA), a polycaprolactone

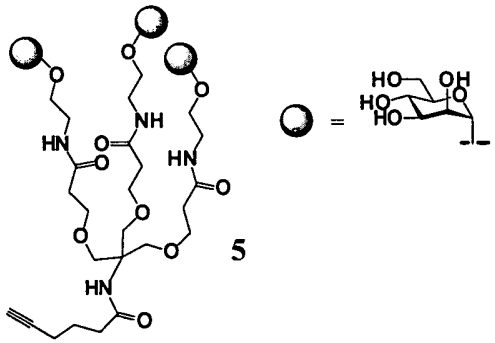
(PCL), a polysaccharide, in particular derived from starch, cellulose or chitosan.

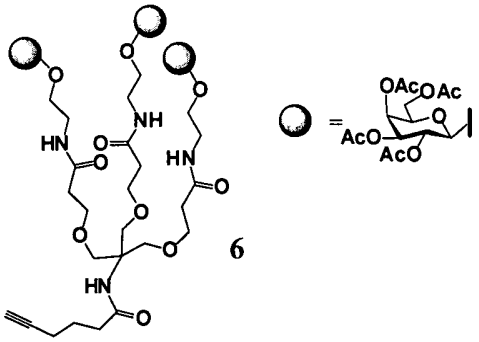
16. The process according to claim 13, wherein the carbohydrate is selected from the group consisting of bacterial lectin binding mono-, oligo- or polysaccharides.

17. The process according to claim 16, wherein the carbohydrate is a monosaccharide selected from the group consisting of galactose, N-acetylgalactosamine, mannose or fucose or a oligosaccharide comprising one or more of said monosaccharides.

18. The process according to claim 13, wherein the carbohydrate-capped dendrimer is a first or second generation dendrimer having one of the following structural formulae 4-11







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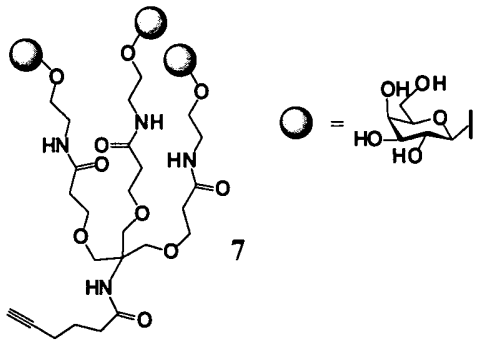

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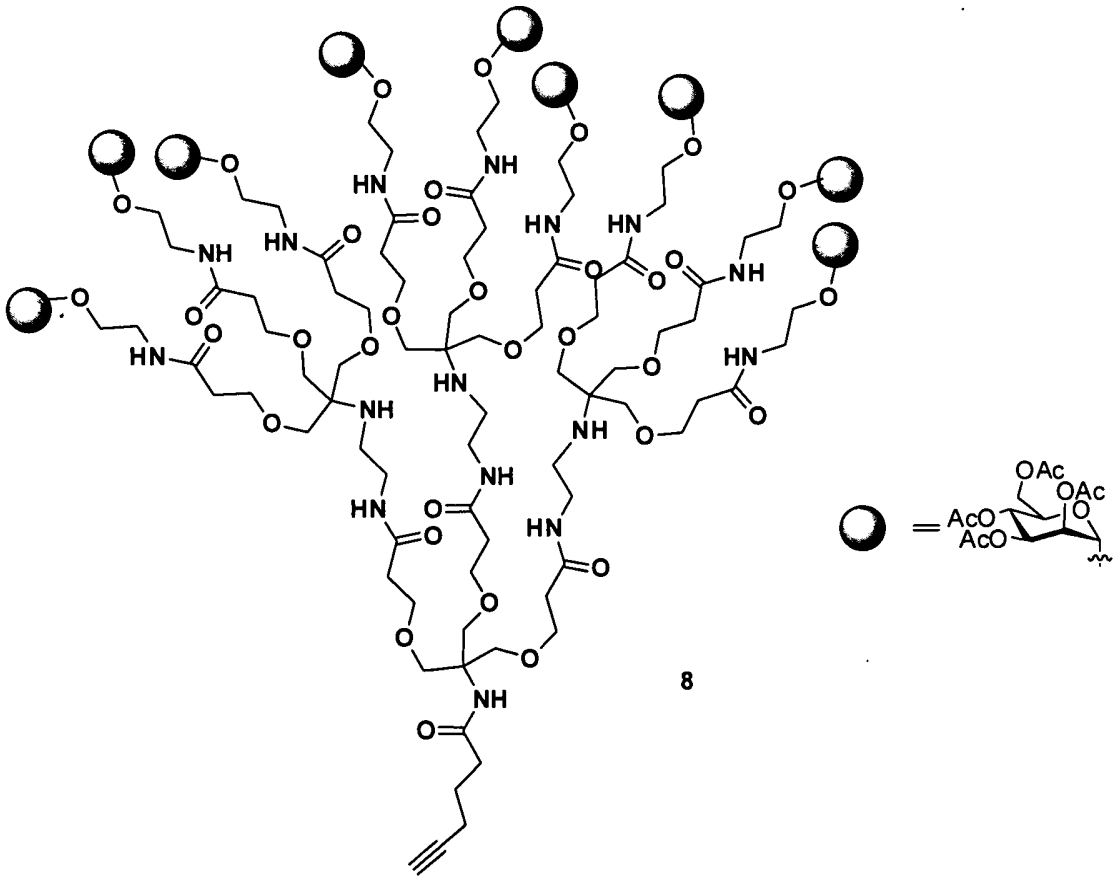

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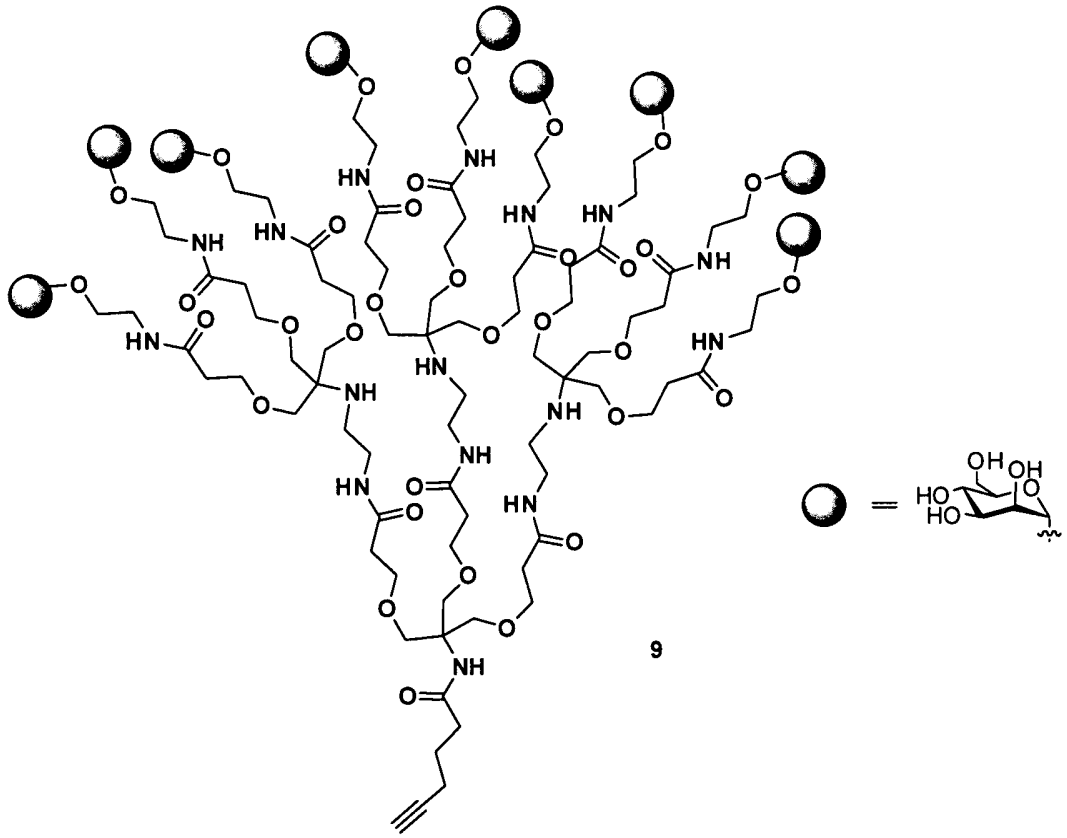

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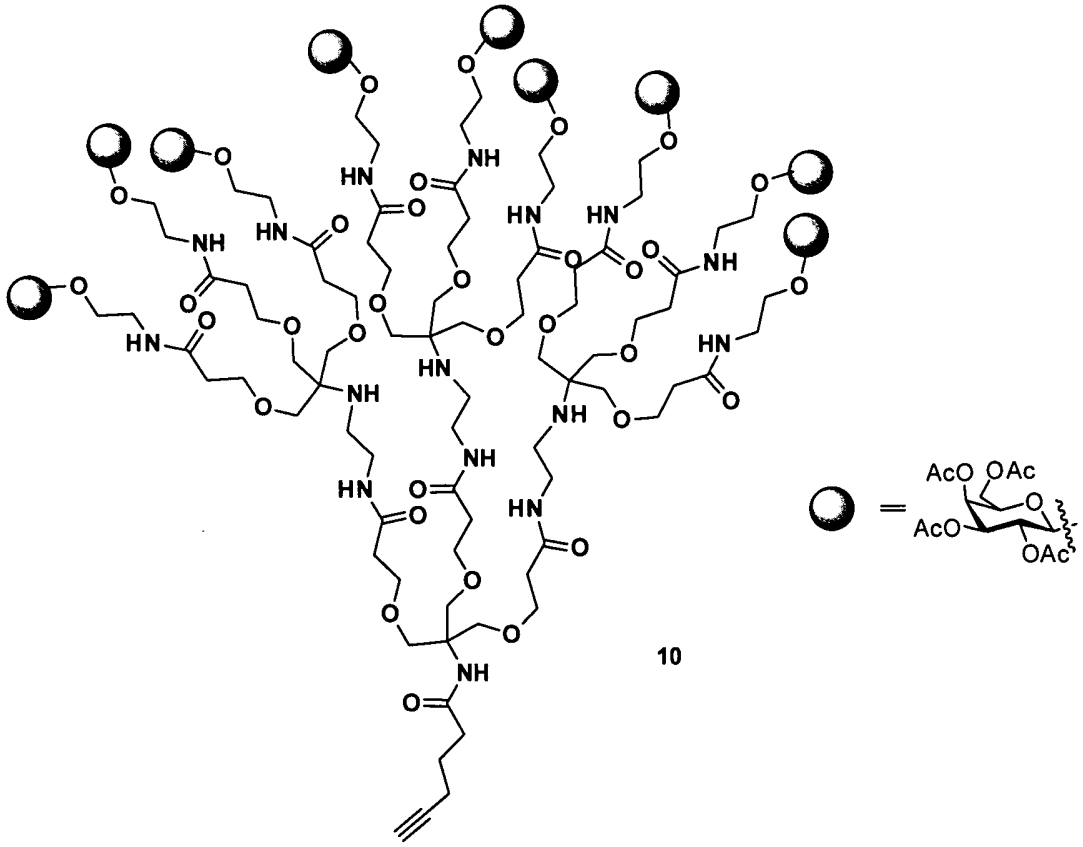

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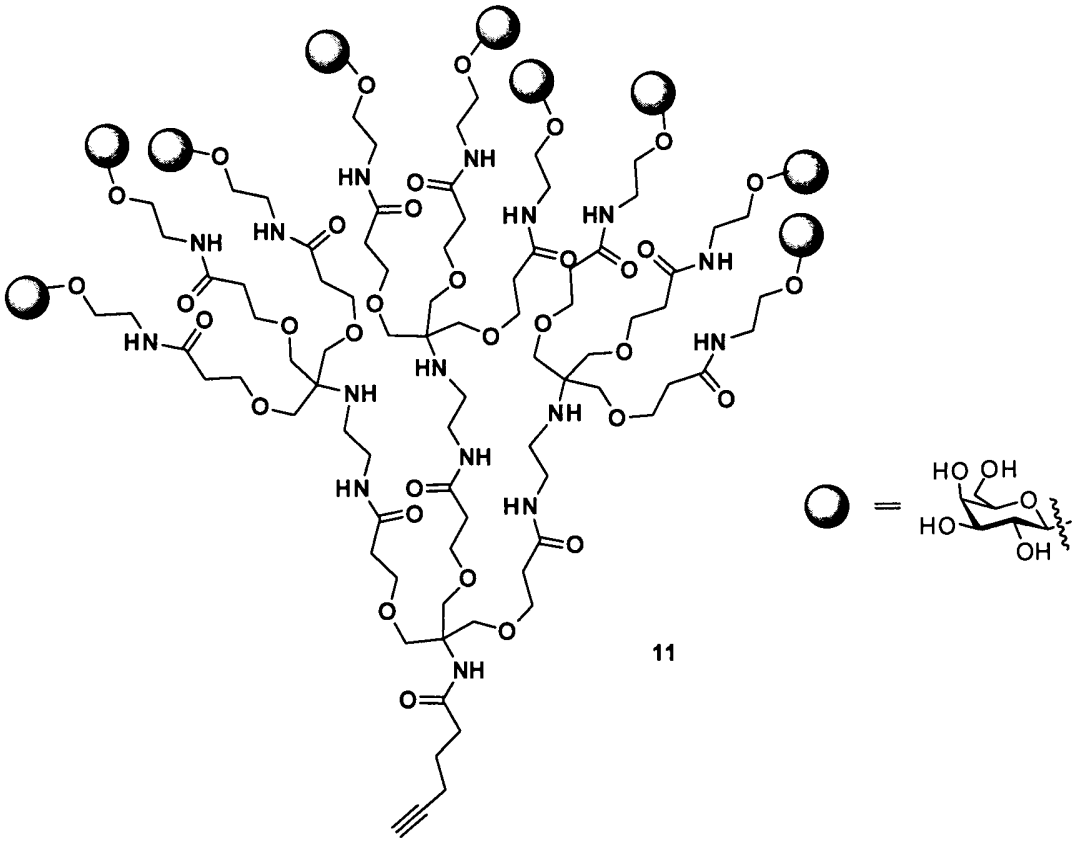

11

19. The process according to claim 12, wherein the scaffold polymer and/or the capped dendron(s) comprise additional functional or linking groups.

20. The process according to claim 12, wherein the additional functional or linking groups comprise a detectable group (label), a biologically, in particular pharmaceutically, active group, or a binding moiety, e.g. an antibody or an antigen-binding fragment thereof.

21. The process according to claim 14, wherein at least one of the carbohydrate moieties is replaced by another functional moiety, in particular a biologically active agent, such as a pharmaceutical or a drug, a detectable group, or a binding moiety, e.g. an antibody or an antigen-binding fragment thereof.

22. The process according to claim 12, wherein the photochemical [2+2] cycloaddition is effected in an aqueous medium and at a temperature in the range of from 20-30 °C for 30-60 minutes.

23. The process according to claim 22, wherein the aqueous medium is water and the reaction temperature is in the range of from 22 to 28 °C.

24. A conjugate obtainable by the method of any one of claims 1-23 and comprising two or more chemical entities selected from the group comprising or consisting of carbohydrates, including mono-, oligo- and polysaccharides, amino acids, peptides and proteins, unbranched and branched oligomers and polymers, including dendrimers.

25. The conjugate according to claim 24 which represents or comprises a biologically active agent, such as a pharmaceutical, including a vaccine, or a drug.

26. The conjugate according to claim 24 which is a dendronized polymer and comprises a scaffold polymer and dendrons capped with a plurality of functional moieties.

27. The dendronized polymer according to claim 26, wherein the functional moieties are selected from the group consisting of a carbohydrate, a biologically active agent, such as a pharmaceutical or a drug, a detectable group, or a binding moiety, e.g. an antibody or an antigen-binding fragment thereof, a peptide, an oligo-peptide, a protein, a nucleotide, an oligonucleotide, a polynucleotide, or combinations thereof.

28. The dendronized polymer according to claim 26 which is a carbohydrate-dendronized polymer.

29. The carbohydrate-dendronized polymer according to claim 28, wherein the carbohydrate is selected from the group consisting of bacterial lectin binding mono-, oligo- or polysaccharides.

30. The carbohydrate-dendronized polymer according to claim 29 wherein the carbohydrate is a monosaccharide selected from the group consisting of galactose, N-acetyl-galactosamine, mannose or fucose or a oligosaccharide comprising one or more of said monosaccharides.

31. The carbohydrate-dendronized polymer according to claim 30 wherein the dendrons have a structure derived from one or more of the structures 4-11 of claim 18.

32. The carbohydrate-dendronized polymer according to claim 28, wherein the scaffold polymer is selected from the group consisting of a hydrocarbon polymer, a polypeptide, including a poly(amino acid), e.g. poly-L-lysine, a polyhydroxyalkanoate, a polylactide (PLA), a polyglycolide (PGA), a polycaprolactone (PCL), a polysaccharide, in particular derived from starch, cellulose or chitosan.

33. The carbohydrate-dendronized polymer according to claim 28, wherein the scaffold polymer and/or the carbohydrate-capped dendron(s) comprise additional functional or linking groups.

34. The carbohydrate-dendronized polymer according to claim 33, wherein the additional functional or linking groups comprise a detectable group (label), a biologically, in particular pharmaceutically, active group, or a binding moiety, e.g. an antibody or an antigen-binding fragment thereof.

35. The carbohydrate-dendronized polymer according to claim 28, wherein at least one of the carbohydrate moieties is replaced by another functional moiety, in particular a biologically active agent, such as a pharmaceutical or a

drug, a detectable group, or a binding moiety, e.g. an antibody or an antigen-binding fragment thereof, a peptide, an oligopeptide, a protein, a nucleotide, an oligonucleotide, a polynucleotide.

36. A method for binding or detecting a target bacterium comprising contacting a sample with a dendronized polymer of claims 27-35 and selectively binding the target bacterium which has at least one binding site for one or more of the functional moieties of said dendronized polymer.

37. The method according to claim 36, wherein the dendronized polymer is a carbohydrate-dendronized polymer and the target bacterium has at least one binding site for one or more of the carbohydrate moieties of said carbohydrate-dendronized polymer.

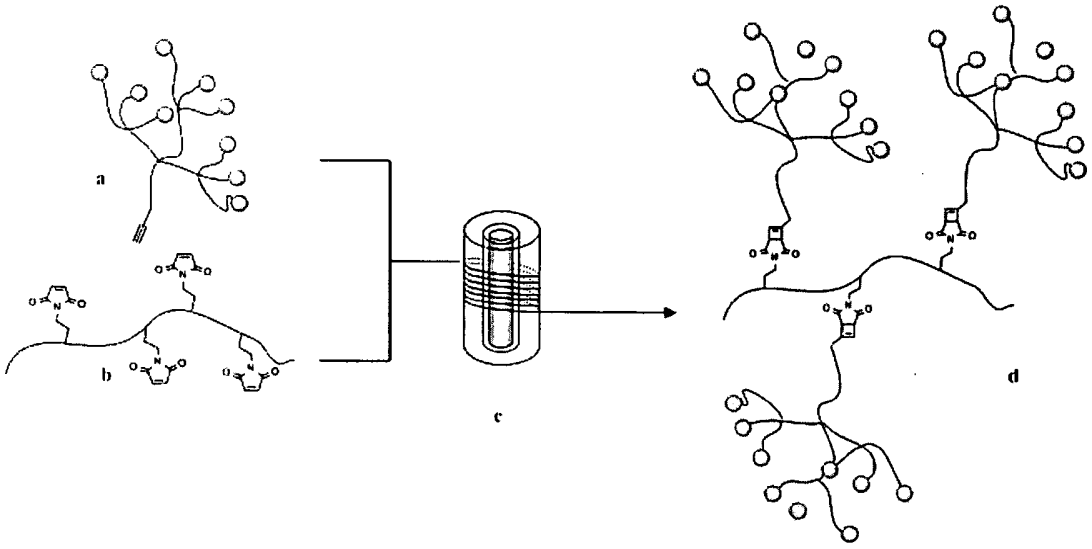

Fig. 1

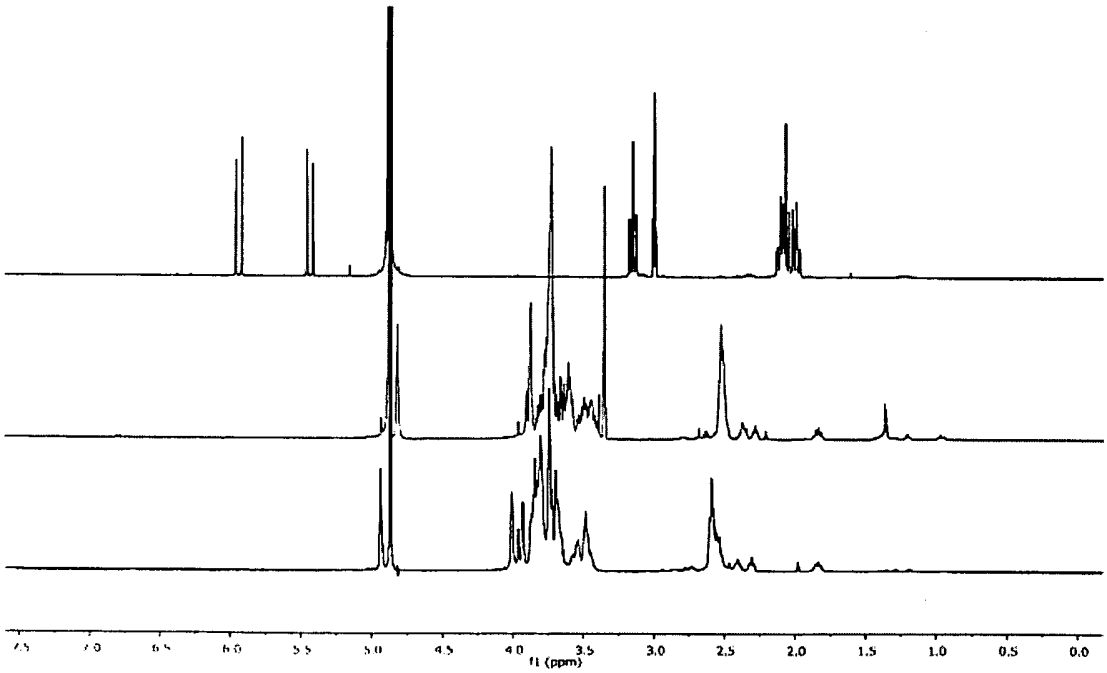

Fig. 2

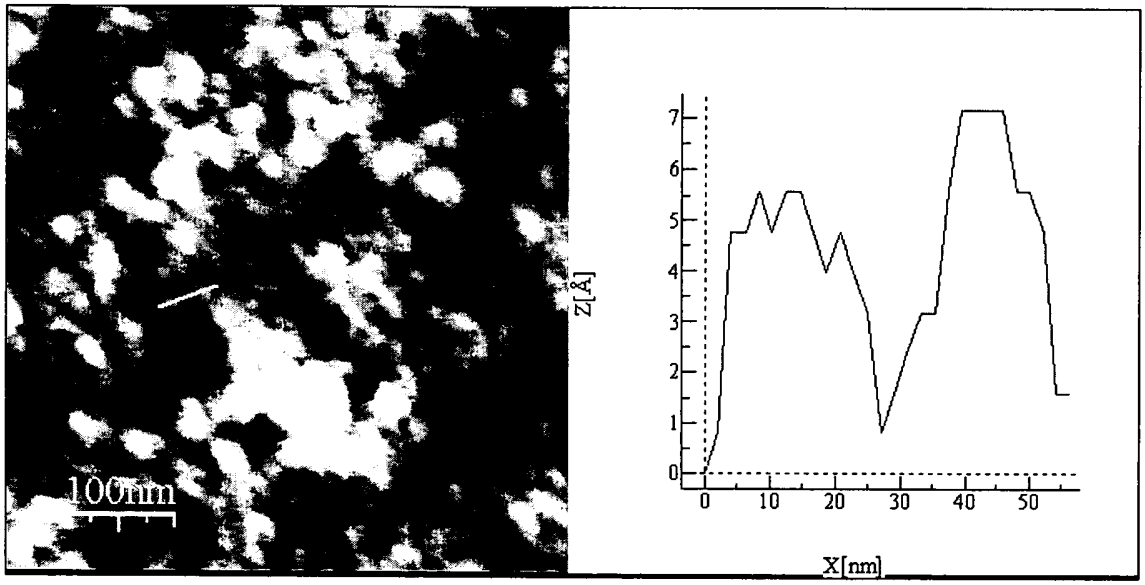

Fig. 3

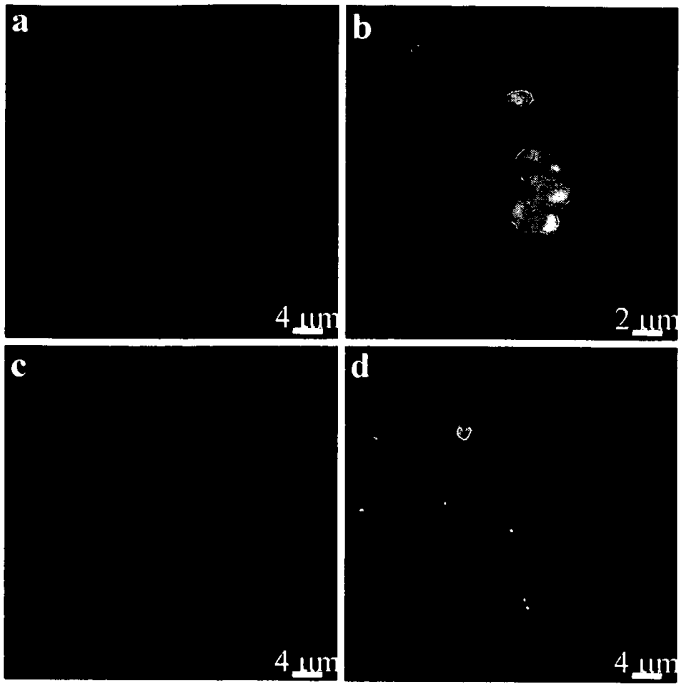

Fig. 4

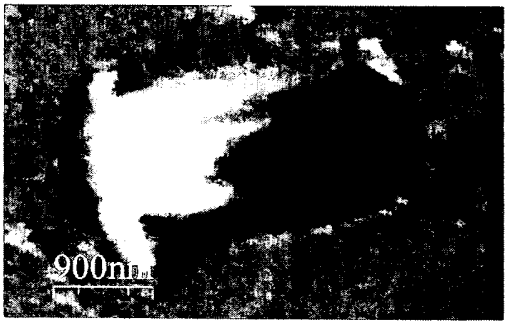

Fig. 5

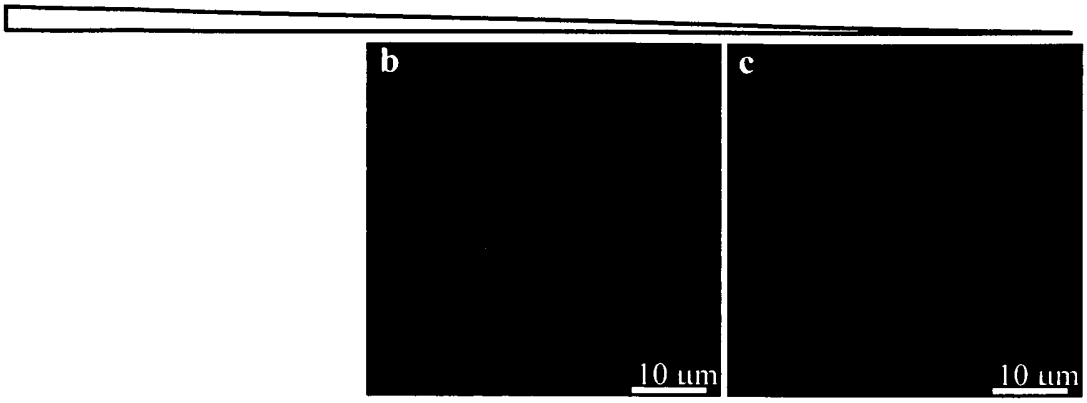

Fig. 6

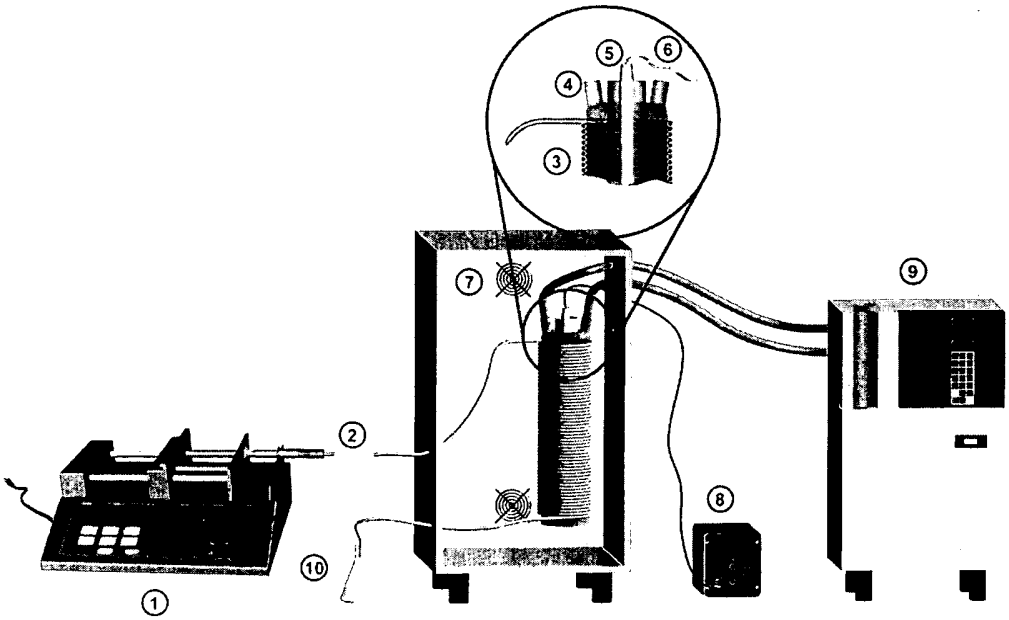

Fig. 7

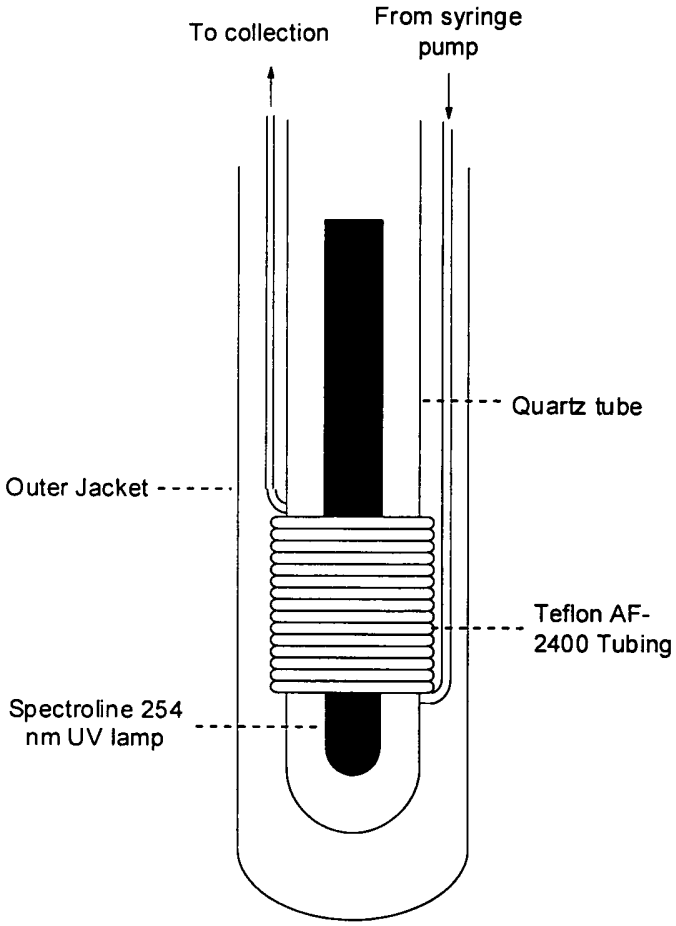
To collection
From syringe pump
Quartz tube
Outer Jacket
Teflon AF-2400 Tubing
Spectroline 254 nm UV lamp

Fig. 8A

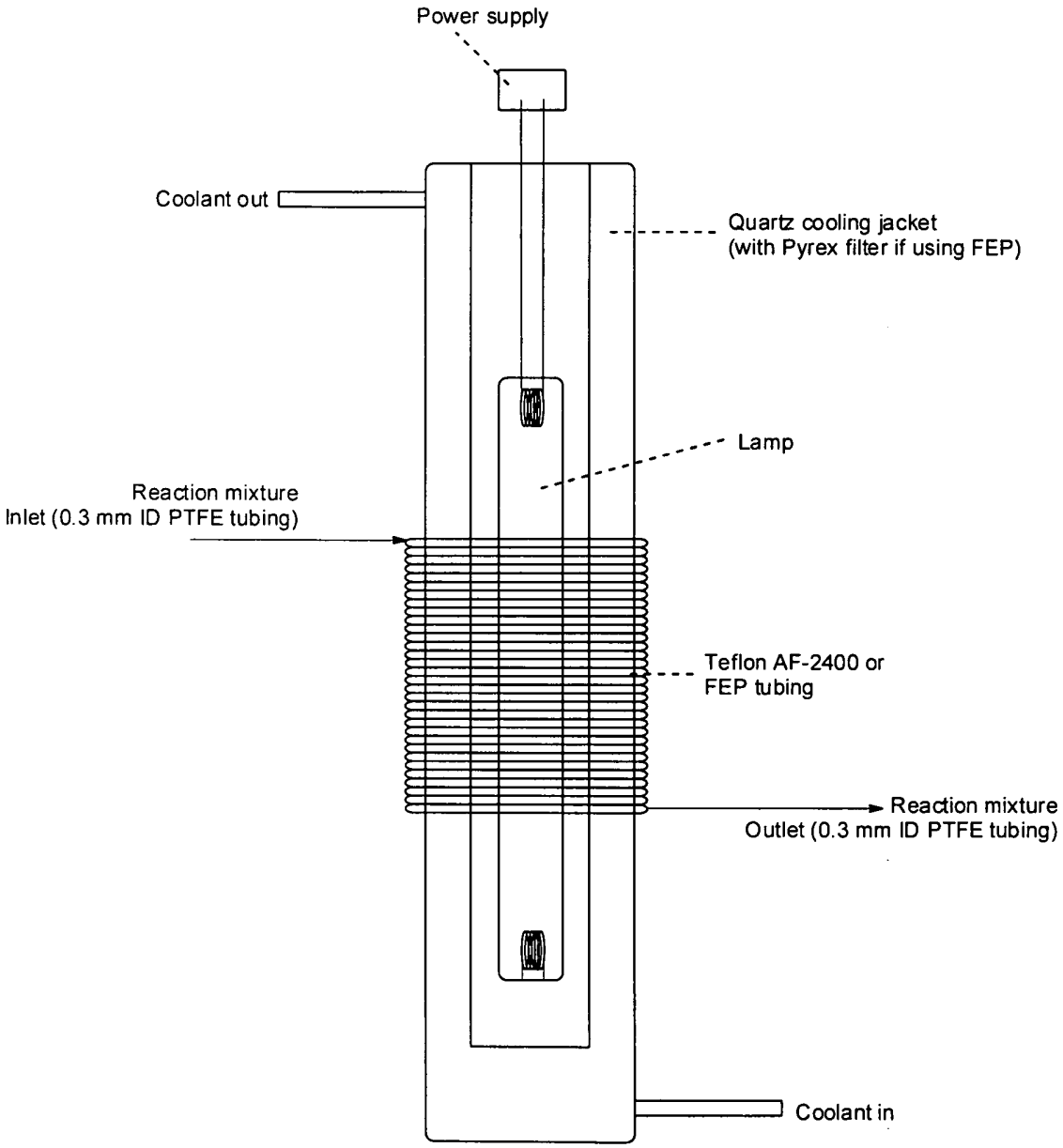
Power supply
Coolant out
Quartz cooling jacket
(with Pyrex filter if using FEP)
Lamp
Reaction mixture
Inlet (0.3 mm ID PTFE tubing)
Teflon AF-2400 or
FEP tubing
Reaction mixture
Outlet (0.3 mm ID PTFE tubing)
Coolant in

Fig. 8B

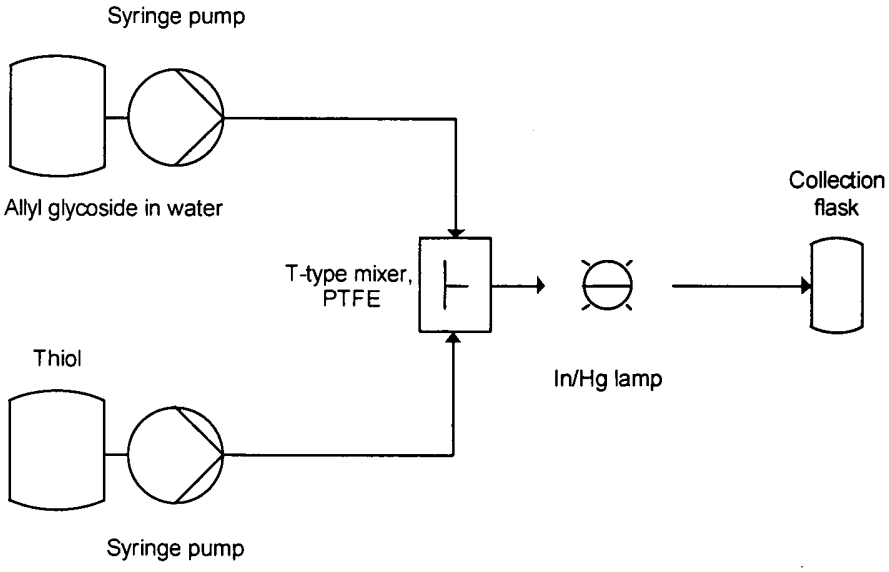
Syringe pump
Allyl glycoside in water
Collection flask
T-type mixer, PTFE
Thiol
In/Hg lamp
Syringe pump

Fig. 9A

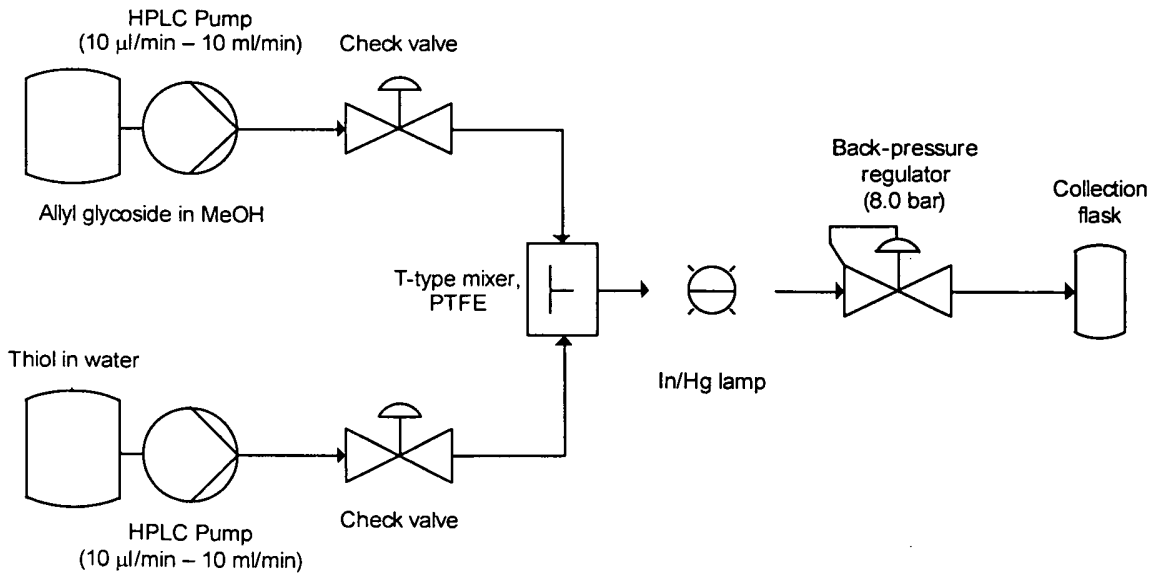
HPLC Pump (10 µl/min – 10 ml/min)
Check valve
Back-pressure regulator (8.0 bar)
Collection flask
Allyl glycoside in MeOH
T-type mixer, PTFE
Thiol in water
In/Hg lamp
Check valve
HPLC Pump (10 µl/min – 10 ml/min)

Fig. 9B

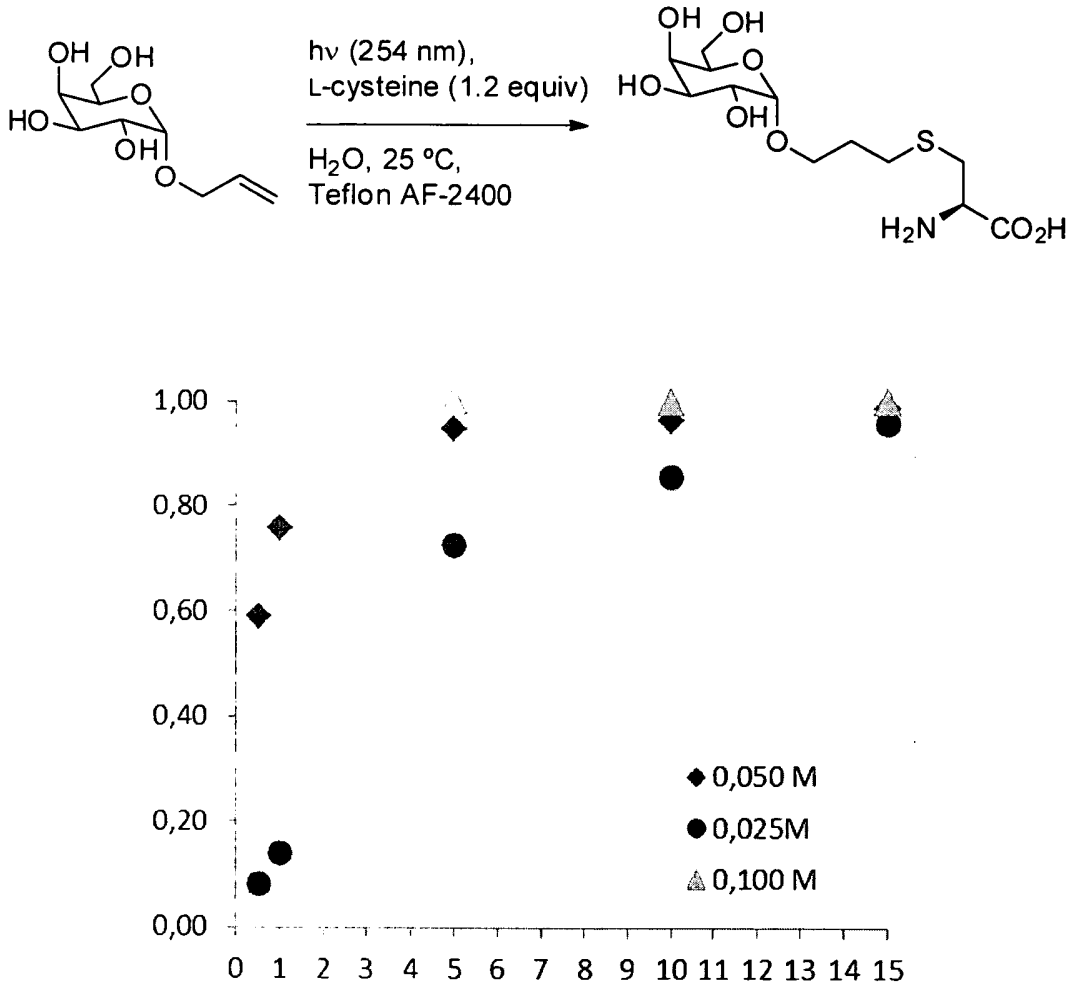
hν (254 nm),
L-cysteine (1.2 equiv)
H2O, 25 °C,
Teflon AF-2400
OH
HO
O
S
H2N
CO2H
1,00
0,80
0,60
0,40
0,20
0,00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
0,050 M
0,025M
0,100 M

Fig. 10

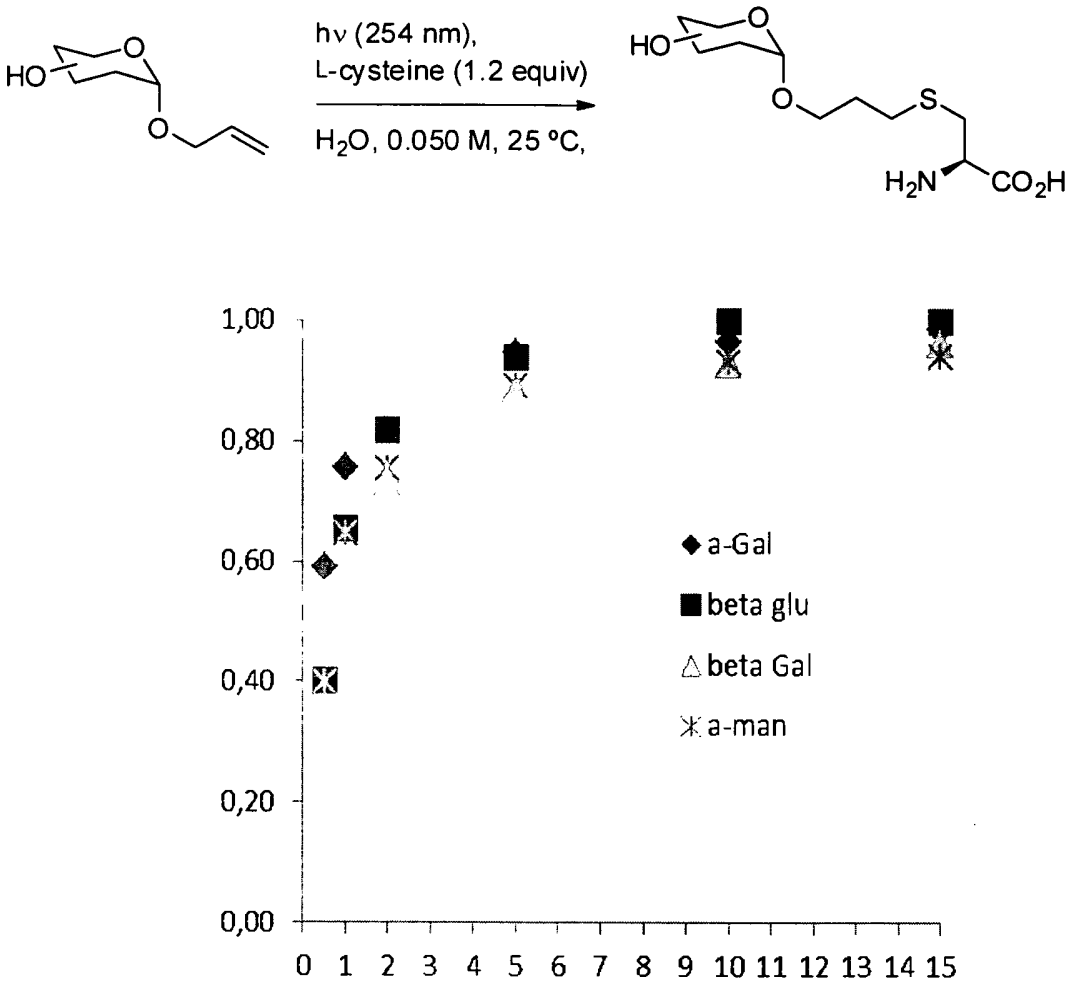
hν (254 nm),
L-cysteine (1.2 equiv)
H2O, 0.050 M, 25 °C,
HO
O
S
H2N
CO2H
1,00
0,80
0,60
0,40
0,20
0,00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
a-Gal
beta glu
beta Gal
a-man

Fig. 11

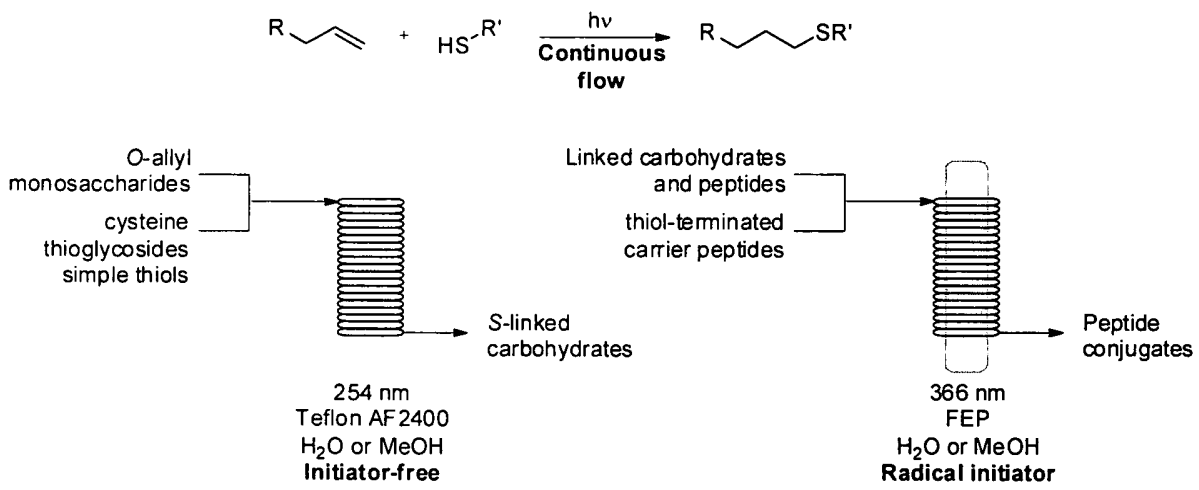
R
HS
R'
hν
Continuous flow
R
SR'
O-allyl monosaccharides
cysteine thioglycosides simple thiols
S-linked carbohydrates
254 nm
Teflon AF2400
H2O or MeOH
Initiator-free
Linked carbohydrates and peptides
thiol-terminated carrier peptides
Peptide conjugates
366 nm
FEP
H2O or MeOH
Radical initiator

Fig. 12

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/005898

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K47/48
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)
A61K

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 practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. ORG. CHEM., vol. 70, 2005, pages 7558-7564, XP007919839, cited in the application	1,2,7, 11,24
Y	figures 2,3	1-17, 19-37

A	KIKKERI ET AL.: CHEM. COMMUN., vol. 46, 24 February 2010 (2010-02-24), pages 2197-2199, XP002672543, cited in the application Scheme 1	18

	-/--	

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