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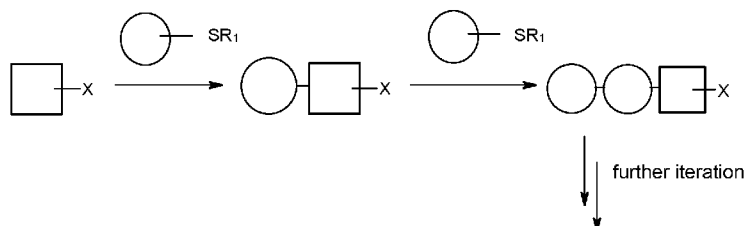
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(54) Title: PROCESS FOR THE PREPARATION OF POLYSACCHARIDES

Figure 2

(57) **Abstract:** The present invention relates to a process for the preparation of polysaccharides of 8 or more monosaccharide residues in length and polysaccharides accessible by these processes, the process comprising coupling a chain extending polysaccharide of four monosaccharide residues in length with a first polysaccharide of four or more monosaccharide residues in length. The coupling proceeds by substitution of a thio leaving group (such as -SR₁) bonded to the anomeric carbon of a monosaccharide residue in either the chain extending polysaccharide or the first polysaccharide.



Process for the preparation of polysaccharides

Field of invention

The present invention relates to processes for the production of polysaccharides and polysaccharides accessible by these methods.

Background

Polysaccharides are an important class of molecules that have a number of roles in the human body. For example, glycosaminoglycans (GAGs) such as heparin (H) and heparin sulphate (HS) are a family of naturally occurring linear polysaccharides that are heavily sulphated. These compounds have proven to be useful in bonding and regulation of a number of proteins and are known to regulate a variety of cell signalling pathways through modulation of interactions between cytokines and their receptors. For instance, Fibroblast Growth Factor 2 (FGF2) and Vascular Endothelial Growth Factor (VEGF₁₆₅) are potent pro-angiogenic cytokines which require H and HS to bind and activate their respective receptors.

Thus, there is considerable interest in providing structurally defined polysaccharide sequences and, in particular, glycosaminoglycans such as H/HS sequences as tools to further probe such biological pathways and for further structural interaction studies, which may lead to new drugs. Whilst research developments so far have mainly delivered shorter chain-length polysaccharide mimics (e.g. Fondaparinux sodium salt, a pentasaccharide presently marketed by GSK), evidence suggests that longer chain lengths should provide more effective biological activity. For instance, studies by the present inventors (e.g. Gardiner, J. *et al. PLoS ONE* 5, e11644, 2010) have indicated that longer chain H/HS mimics provide more effective FGF2 inhibition. Accordingly, there is a desire to provide long chain polysaccharides for further studies, such as polysaccharides of eight monosaccharide residues or more in length, and especially polysaccharides of twenty monosaccharide residues or more in length.

Due to the chemical complexity of saccharides, a vast number of theoretical monosaccharide combinations and derivatizations are possible and it is therefore essential that efficient and flexible synthetic strategies are developed to provide access to a diverse range of long chain polysaccharides. It has however so far proven to be extremely difficult to provide synthetic access to structurally defined polysaccharide chains of long chain lengths. These challenges are compounded when H/HS mimics are desired, since these require the provision of iduronate residues, which are notoriously difficult to access synthetically.

It is understood that the difficulty in accessing long chain lengths efficiently is at least in part the result of the decreased reactivity of longer chains in glycosidic coupling reactions.

Prior art processes have therefore principally focussed on iterative methods keeping at least one of the reacting residues short so as to mitigate the decreased reactivity of longer chain lengths. For instance, processes using mono- and disaccharide building blocks are known to provide access to heparin-like polysaccharide chains of modest chain lengths, i.e. up to twelve monosaccharide residues in length (see WO02/058633, CA1265132, and WO2006/129075). However, such prior art processes involving iterative extensions using small building blocks have so far proven to be a relatively impractical way of accessing longer chain lengths, not least because of the many iterative steps required to build suitably long chains from small fragments.

Some limited success in reaching modest chain lengths by combining chains longer than two monosaccharide residues in length has been achieved using reactive trichloroacetimidate glycosylation chemistry to compensate for the decreased reactivity of longer chain lengths. For instance, the use of tri-saccharide building blocks has been attempted as reported in WO02/058633, giving access to a polysaccharide of six monosaccharide residues in length and in modest yield (62%). Furthermore, the synthesis of polysaccharides of twelve monosaccharide residues in length has also been performed involving trichloroacetimidate-mediated 4+4 and 4+8 coupling of polysaccharide building blocks (see Bonnaffe, D. *et al Nature Chemical Biology*, **2009**, 5(10), 743 and Bonnaffe, D. *et al. C. R. Chimie*, **2011**, 14, 59-73). However, whilst these processes appear to have been successful in accessing modest chain lengths, these methods have not been proven to be useful for efficiently accessing longer chains (e.g. 20+ monosaccharide residues in length), which is in keeping with the general understanding that longer chain lengths are less efficient coupling partners. Moreover, whilst these reported methods have shown limited success in combining larger building blocks using reactive trichloroacetimidate chemistry to overcome the typically inefficient glycosidic bond formation at longer chain lengths, the trichloroacetimidate building blocks themselves are not readily amenable to storage and so do not provide commercially attractive synthetic intermediates.

As a result, in order for research into the pharmacological utility of long chain polysaccharides, such as heparin-type compounds to progress, new commercially viable synthetic strategies are needed that can deliver flexible access to long chain polysaccharides cleanly, with high anomeric selectivity and in high yields. The present invention addresses these problems.

Furthermore, conventional pharmacological evaluation of heparin-type compounds has relied on their anticoagulant properties, which can be measured in patients using universally available tests of the clotting cascade. However, many potential therapeutic applications of glycosaminoglycans are unrelated to the anti-coagulation pathways acted on by heparin. In such therapeutic areas, it is of course desirable to avoid anticoagulant side effects. This can be done by providing structurally-defined synthetic heparin-type polysaccharides, such as HS-type polysaccharides. Such compounds present a critical drug developmental problem, however, because if such compounds no longer possess anticoagulant properties, it is no longer possible to evaluate their physiological effect *in vivo* using the conventional clotting cascade tests. It is therefore desirable to provide an alternative method

by which the pharmacokinetic properties of polysaccharide therapeutics, especially glycosaminoglycans, can be assessed quantitatively *in vivo*.

Summary of invention

5 The present inventors have conceived a new synthetic approach that is generally applicable to the synthesis of polysaccharides of eight or more monosaccharide residues in length, and especially polysaccharides of twenty or more monosaccharide residues in length. In its broadest sense, the process of the present invention involves the elongation of polysaccharides of at least four residues in length by installation of polysaccharide building blocks of four monosaccharide residues in length
10 using thioglycoside coupling-chemistry.

As explained above, the resulting polysaccharide products of these processes represent valuable synthetic scaffolds, which may be easily manipulated and derivatised, e.g. for further investigation in drug discovery research, such as in activity assays and pharmacokinetic studies.

15 The inventors have also developed a new radiolabelling strategy that suitably allows for the quantification of tissue distribution and metabolic stability of polysaccharides *in vivo* thus providing a valuable tool to assist the pre-clinical and clinical development of potential polysaccharide therapeutics.

20

Description of the Invention

In a first aspect, the present invention provides a process for preparing a polysaccharide of 8 or more monosaccharide residues in length, the process comprising coupling a chain extending polysaccharide of four monosaccharide residues in length with a first polysaccharide of four or more
25 monosaccharide residues in length by substitution of a thio leaving group bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue of either the chain extending polysaccharide or the first polysaccharide to form a chain extended polysaccharide of 8 or more residues in length. Suitably, the thio leaving group may be an -SR₁ group wherein R₁ is selected from the group consisting of optionally substituted C₁₋₁₀alkyl, optionally substituted C₂₋₁₀alkenyl, optionally substituted
30 C₂₋₁₀alkynyl, optionally substituted C₃₋₁₀cycloalkyl, optionally substituted C₃₋₁₀cycloalkenyl, optionally substituted C₃₋₁₀heterocycloalkenyl, optionally substituted C₆₋₁₄aryl, optionally substituted C₅₋₁₄heteroaryl, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support.

35 Accordingly, the coupling reaction involves the substitution of a thio group at a polysaccharide terminus to form a new glycosidic bond.

The process of the present invention provides a powerful new synthetic approach which allows flexible and efficient access to a wide variety of polysaccharides. The examples show that the present
40 process can viably access polysaccharides up to forty monosaccharide residues in length, which is a

ground-breaking development. Even with chains as long as forty monosaccharide residues in length, the reactions proceed in unexpectedly good yield (64% for the 40-mer) and purity (the remaining material recovered is mostly starting material) and provide excellent anomeric stereoselectivity in the coupling step. Indeed, the inventors have produced a fully-synthetic sulphonated heparin 20-mer at
5 exceptionally high purity, demonstrating that the provision of longer synthically pure heparins is viable with this methodology. Furthermore, the present process also has the additional benefit that the thioglycoside building blocks are stable and thus can be stored for long periods of time.

It is surprising that longer chain fragments (i.e. of at least four monosaccharide residues in length) can
10 be combined cleanly and with excellent efficiency to provide access to polysaccharide chains as long as forty residues in length, particularly using the relatively less reactive thioglycoside coupling chemistry presently adopted. The result is a more scaleable and commercially viable route using readily storable building blocks. Thus, the present invention represents a valuable contribution to the art in the field of polysaccharide chemistry and has particular value in the field of glycosaminoglycan
15 synthesis (such as to access H- and HS-type sequences), where reaction efficiency is particularly important due to the difficulty in obtaining synthetic iduronate building blocks.

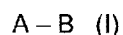
The resulting products are thus valuable synthetic tools that can be accessed in meaningful quantities and further derivatised if necessary, e.g. to investigate pharmacological properties or perform
20 structure / activity assays.

Various embodiments of the processes and synthetic polysaccharides of the present application are described in this application. The skilled person will recognise that features specified in each of these embodiments may be combined with features specified in other embodiments to provide further
25 embodiments of the invention.

Polysaccharide

As described above, the process of the present invention relates to a process for preparing a polysaccharide of 8 or more monosaccharide residues in length, especially a polysaccharide of 20 or
30 more monosaccharide residues in length. The process is generally applicable to the preparation of any polysaccharide and thus the polysaccharide produced by the present invention may be any suitable polysaccharide as defined herein.

Suitably, the polysaccharide may be a compound of formula (I):



wherein

A is a chain of 8 or more monosaccharide residues in length; and

B is bonded to the anomeric carbon of a monosaccharide residue in the chain, and is selected from a thio leaving group as defined above, $-OR_2$ and $-SeR_2$, wherein R_2 is H or an end-capping group

R_{ec} , preferably wherein B is selected from a thio leaving group as defined above and $-OR_2$, more preferably $-OR_2$.

Length

- 5 In the process of the present invention, the polysaccharide is of 8 or more monosaccharide residues in length. Suitably, the polysaccharide is 9 or more monosaccharide residues in length, such as 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, e.g. 40 monosaccharide residues in length.

- The polysaccharide may be up to 70 monosaccharide residues in length, suitably up to 60 residues, up to 50 residues, or up to 40 residues. In further embodiments, the process is for preparing a polysaccharide of 9 to 40 monosaccharide residues in length, suitably 10 to 40, 11 to 40, 12 to 40, 13 to 40, 14 to 40, 15 to 40, 16 to 40, 17 to 40, 18 to 40, 19 to 40, 20 to 40, 21 to 40, 22 to 40, 23 to 40, 24 to 40, 25 to 40, 26 to 40, 27 to 40, 28 to 40, 29 to 40, 30 to 40, 31 to 40, 32 to 40, 33 to 40, 34 to 40, 35 to 40, 36 to 40, 37 to 40, 38 to 40, or 39 to 40 monosaccharide residues in length. Preferably, the process is for preparing a polysaccharide of 12 to 40 monosaccharide residues in length, more preferably 16 to 40 monosaccharide residues in length, 20 to 40 or 24 to 40 monosaccharide residues in length.

Monosaccharide residues

- In the above aspect, the polysaccharide may comprise any combination of monosaccharide residues.
- 25 Suitable monosaccharide residues include aldoses and ketoses, which may suitably be in furanose or pyranose forms. Exemplary monosaccharides are optionally substituted hexoses, including hexosamines, hexoses and hexuronic acids. Hexuronic acid residues include optionally substituted hexuronic acid residues (such as optionally substituted glucuronic acid residues and optionally substituted iduronic acid residues) and suitable hexoses include optionally substituted glucose residues and optionally substituted galactose residues, such as optionally substituted glucosamines and optionally substituted galactosamines and derivatives thereof (e.g. azide derivatives).

- In some embodiments, one or more monosaccharide residues in the polysaccharide is a pyranose.
- 35 Preferably, at least half and preferably more than half of the residues are pyranoses, more preferably substantially all of the monosaccharide residues are pyranoses, and most preferably each monosaccharide residue is a pyranose.

- In embodiments, one or more monosaccharide residues is selected from the group consisting of optionally substituted hexuronic acid residues and optionally substituted glucose residues. Suitably, at

least half and preferably more than half of the residues may be selected from the group consisting of optionally substituted hexuronic acid residues and optionally substituted glucose residues, more preferably substantially all of the monosaccharide residues, and most preferably each monosaccharide residue is selected from the group consisting of optionally substituted hexuronic acid residues and optionally substituted glucose residues. In embodiments, the polysaccharide comprises an alternating sequence of optionally substituted hexuronic acid residues and optionally substituted glucose residues.

The optionally substituted hexuronic acid residues may suitably be selected from optionally substituted glucuronic acid residues (such as β -D-glucuronic acid (GlcUA) and 2-O-sulfo- β -D-glucuronic acid GlcUA(2S)) and optionally substituted iduronic acid residues (such as α -L-iduronic acid (IdoUA), and 2-O-sulfo- α -L-iduronic acid (IdoUA(2S))). In embodiments, each optionally substituted hexuronic acid residue is a glucuronic acid residue. In alternative embodiments, each optionally substituted hexuronic acid residue is an iduronic acid residue.

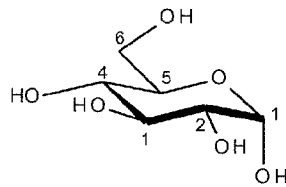
The optionally substituted glucose residues may be optionally substituted glucosamine residues (such as α -D-N-acetylglucosamine (GlcNAc), α -D-N-sulfoglucosamine (GlcNS) and α -D-N-sulfoglucosamine-6-O-sulfate (GlcNS(6S))).

Typically, one or more residues is selected from optionally substituted iduronic acid residues and optionally substituted glucosamine residues. Suitably, at least half and preferably more than half of the monosaccharide residues may be selected from optionally substituted iduronic acid residues and optionally substituted glucosamine residues, more preferably substantially all residues are selected from optionally substituted iduronic acid residues and optionally substituted glucosamine residues, and most preferably each monosaccharide residue is selected from the group consisting of optionally substituted iduronic acid residues and optionally substituted glucosamine residues.

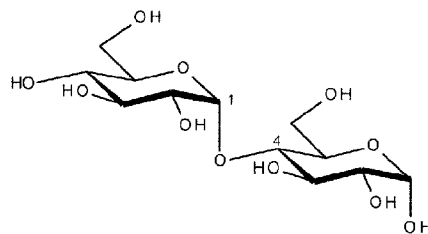
In embodiments, the term "substantially all of the monosaccharide residues" means at least 90% of the number of monosaccharide residues in the polysaccharide, such as at least 95% of the number of monosaccharide residues in the polysaccharide, e.g. at least 98%.

The monosaccharide residues are suitably linked in a chain by glycosidic bonds. The glycosidic bonds bridge the anomeric (C1) position of one residue and any suitable position of the adjoining monosaccharide residue.

Conventionally, glycosidic bonds between monosaccharide residues may be further defined by the position of the carbon atoms attached via glycosidic bond within each monosaccharide scaffold. For instance, the conventional numbering of a pyranose is as follows:



Thus, a glycosidic bond formed by reaction of the anomeric carbon (1-position) on one monosaccharide residue and a hydroxyl group at the 4-position of another monosaccharide residue results in the formation of a C1-C4 O-glycosidic bond as illustrated below.



For instance, where glucose is the nucleophile participating in a glycosidic bond, any of C1-C2, C1-C3, C1-C4 and C1-C6 O-glycosidic bonds may be formed.

- 10 Accordingly, in embodiments, the polysaccharide comprises monosaccharide residues that are coupled by a C1-C3 or C1-C4 glycosidic bonds, preferably C1-C4 glycosidic bonds. Suitably, at least half and preferably more than half of the monosaccharide residues may be coupled by a C1-C4 glycosidic bond, more preferably substantially all monosaccharide residues are coupled by a C1-C4 glycosidic bond, and most preferably each monosaccharide residue is coupled by a C1-C4 glycosidic bond.
- 15

- The skilled person will also understand that a glycosidic bond may be an α - or β -glycosidic bond depending on the stereochemical configuration at the anomeric carbon participating in the bond. Thus the polysaccharide may contain α -glycosidic bonds, β -glycosidic bonds or both α - and β -glycosidic bonds. In embodiments, one or more glycosidic bonds is an α -glycosidic bond. In further
- 20
- embodiments, one or more glycosidic bonds is a β -glycosidic bond. In embodiments each bond is an α -glycosidic bond.

- When a pendant hydroxyl group bonds with an anomeric carbon to form a glycosidic bond, the bond is conventionally referred to as an "O-glycosidic bond". Similarly, when an amino group forms a bond with an anomeric carbon, the bond is conventionally referred to as an "N-glycosidic bond". The glycosidic bonds in the polysaccharide may be selected from O-glycosidic bonds (e.g. C1-C4 O-glycosidic bonds) and N-glycosidic bonds (e.g. C1-C2 N-glycosidic bonds). Thus the polysaccharide may comprise O-glycosidic bonds, N-glycosidic bonds or both O- and N-glycosidic bonds. In typical
- 25
- embodiments, the glycosidic bonds are O-glycosidic bonds. In other embodiments, one or more glycosidic bonds may be an N-glycosidic bond.
- 30

In embodiments, the polysaccharide is a glycosaminoglycan. Suitably, the glycosaminoglycan may be unbranched. Preferably, the glycosaminoglycan chain comprises a plurality of repeating disaccharide units consisting of an aminosaccharide (e.g. glucosamines or galactosamines, such as *N*-acetylglucose amine or *N*-acetylgalactose amine) and either a uronic acid (e.g. glucuronic acid or iduronic acid) or galactose.

Protecting groups

The polysaccharide may be protected by any respective protecting group as defined herein. Any nucleophilic moiety present in the polysaccharide may be protected, such as hydroxy and / or amino groups. The skilled person will appreciate that the number of protecting groups in the polysaccharide can vary depending on the desired functionality and also the number of nucleophilic groups present, which may further depend on the number of monosaccharide residues in the polysaccharide.

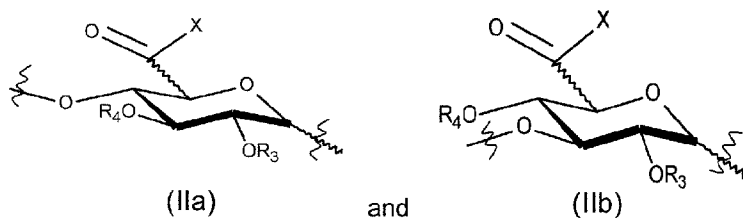
Typically, at least one nucleophilic group (such as hydroxy and / or amino groups) is protected. In embodiments, at least half and preferably more than half of the nucleophilic groups (such as hydroxyl and / or amine groups) in the polysaccharide are protected. Suitably, substantially all nucleophilic groups (such as hydroxyl and / or amine groups) may be protected, for example, all nucleophilic groups in the polysaccharide (such as hydroxyl and / or amine groups) may be protected.

Typically, when a plurality of protecting groups is present, the protecting groups may be the same or at least two or more of the protecting groups may be different and, optionally, orthogonal protecting groups are used. Advantageously, the use of orthogonal protecting groups allows for selective deprotection of one or more nucleophilic groups (e.g. a hydroxy / amino protecting group), thus facilitating the selective derivatization (e.g. sulfation such as in the synthesis of heparin-type compounds) and / or glycosidic bond formation in a further synthetic step, if desired.

Thus, the polysaccharide may be "orthogonally protected" meaning that it contains at least two different protecting groups that are orthogonal to each other. The skilled person is familiar with such orthogonal protecting group strategies, which are reviewed in many textbooks, such as T. W. Greene and G. M. Wicks, *Protecting Groups in Organic Synthesis*, Third Edition, Wiley, New York, 1999, and Philip J. Kocienski, *Protecting Groups*, Third Edition, Thieme, New York, 2004.

Hexuronic acid residues

In embodiments, the optionally substituted hexuronic acid residues are independently selected from residues of the following formulae:

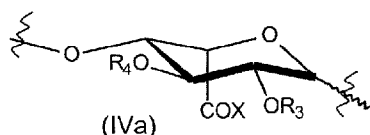


wherein

each R_3 and R_4 is independently H, or a hydroxyl protecting group wherein R_3 and R_4 are optionally taken together to form a cyclic protecting group when OR_3 and OR_4 are attached to adjacent carbon atoms; and

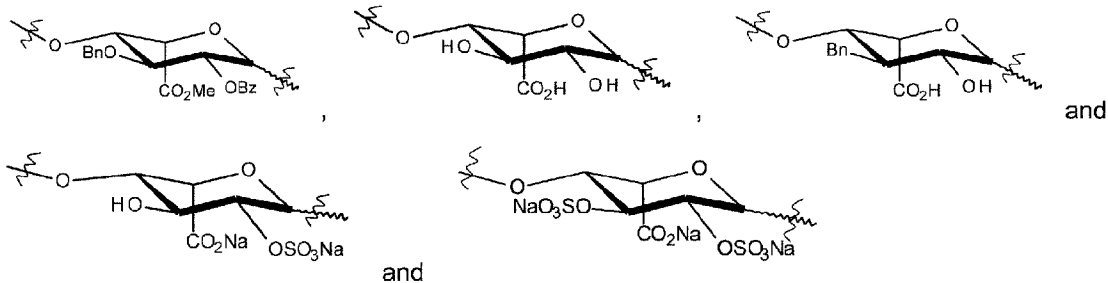
X is OR_7 or $N(R^a)_2$, wherein R_7 is H, a cation or optionally substituted C_{1-10} alkyl.

In embodiments, the optionally substituted hexuronic acid residues are independently selected from residues of formula (IIa), such as formula (IVa):



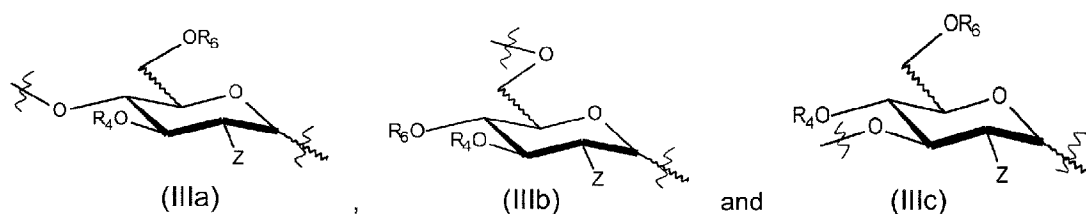
wherein X , R_3 and R_4 are as defined above.

Suitably, each optionally substituted hexuronic acid residue may be independently selected from residues of the following formulae:



Glucose residues

In embodiments, the optionally substituted glucose residues are independently selected from residues of the following formulae (IIIa-c):

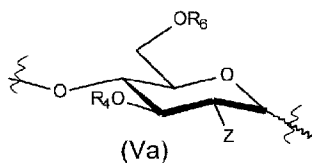


wherein

Z is OR_3 , N_3 or $N(R^a)_2$; wherein each R^a is independently H or an amino protecting group; and each R_3 , R_4 and R_6 is independently H, or a hydroxyl protecting group, wherein R_3 and R_4 are

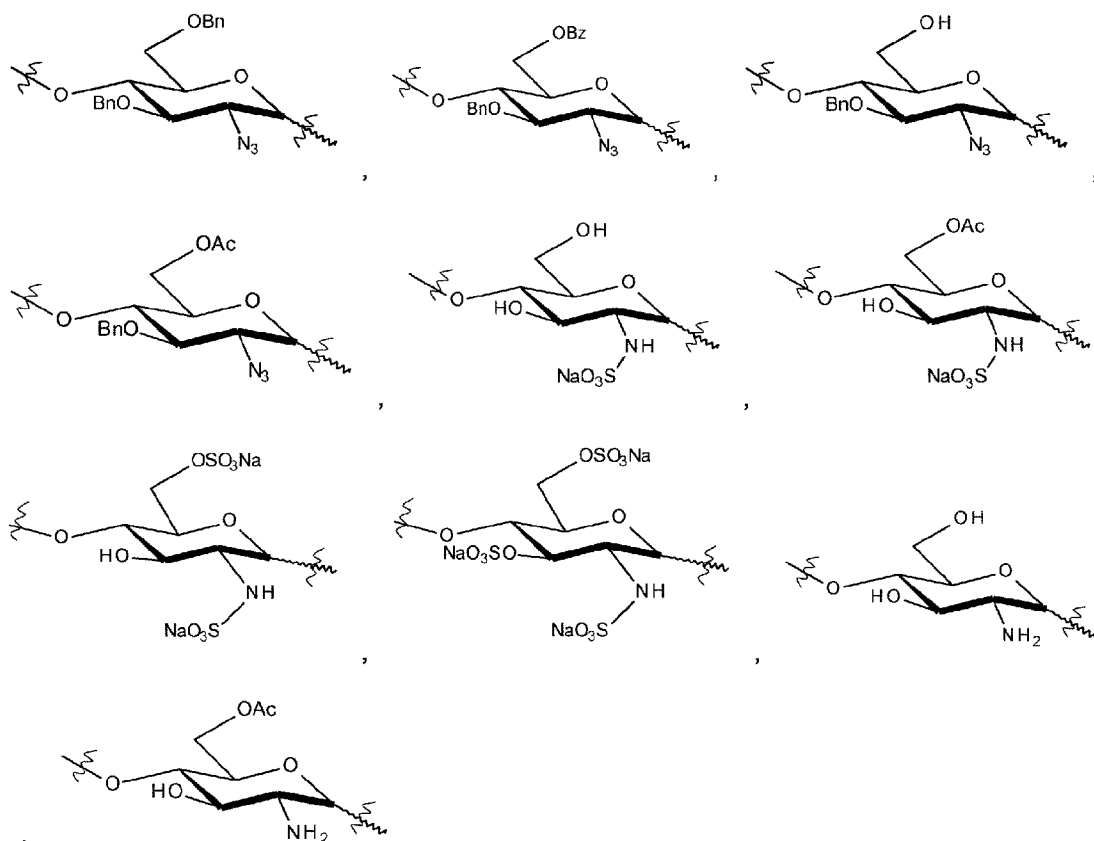
optionally taken together to form a cyclic protecting group when OR_3 and OR_4 are bonded to adjacent carbon atoms.

In embodiments, each optionally substituted glucose residue is independently selected from residues of formula (IIIa), such as formula (Va):



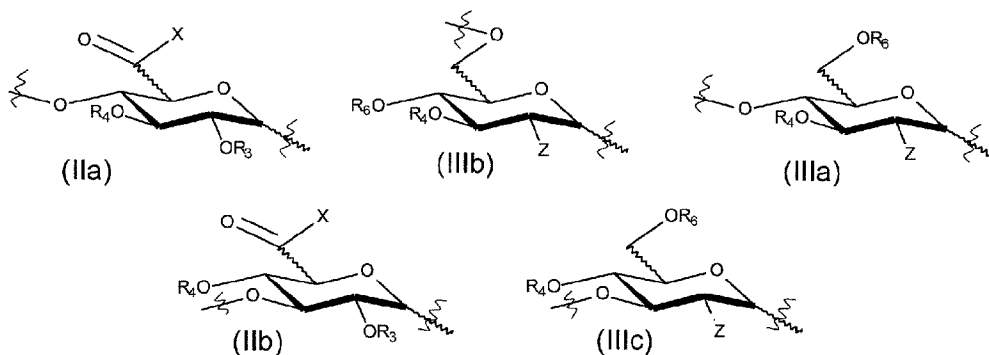
wherein Z, R_4 and R_6 are as defined above.

Suitably, each optionally substituted glucose residue may be independently selected from residues of the following formulae:



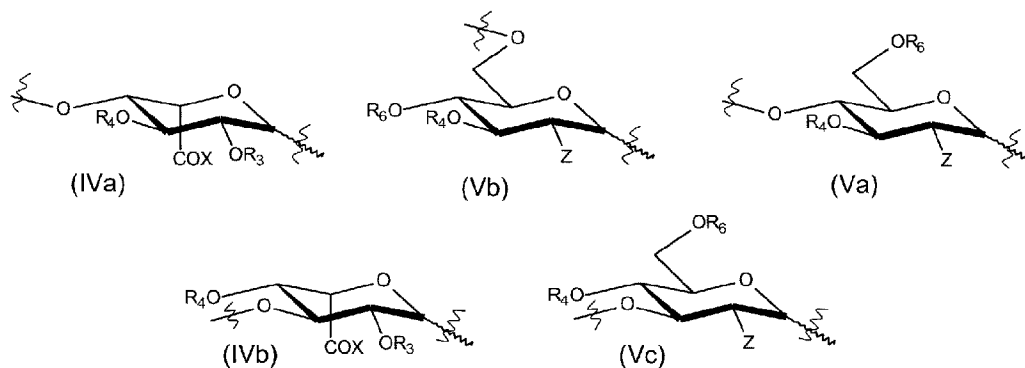
Preferred hexuronic acid and glucose residues

In embodiments, the polysaccharide comprises one or more monosaccharide residues independently selected from formulae (IIa), (IIb), and (IIIa-c):



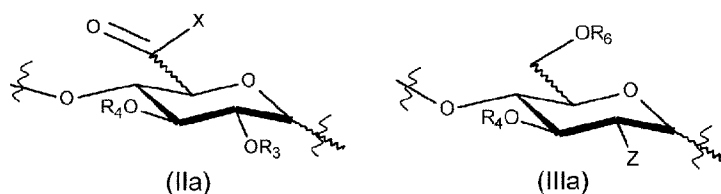
wherein R_3 , R_4 , R_6 , X and Z are each independently as defined above.

- 5 In embodiments, the polysaccharide comprises one or more monosaccharide residues independently selected from formulae (IVa), (IVb) and (Va-c):



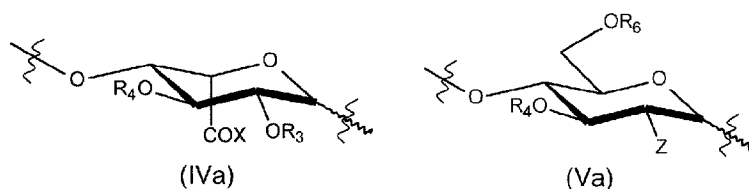
wherein R_3 , R_4 , R_6 , X and Z are independently as defined above.

- 10 Preferably, the polysaccharide comprises one or more monosaccharide residues independently selected from the group consisting of formulae (IIa) and (IIIa):



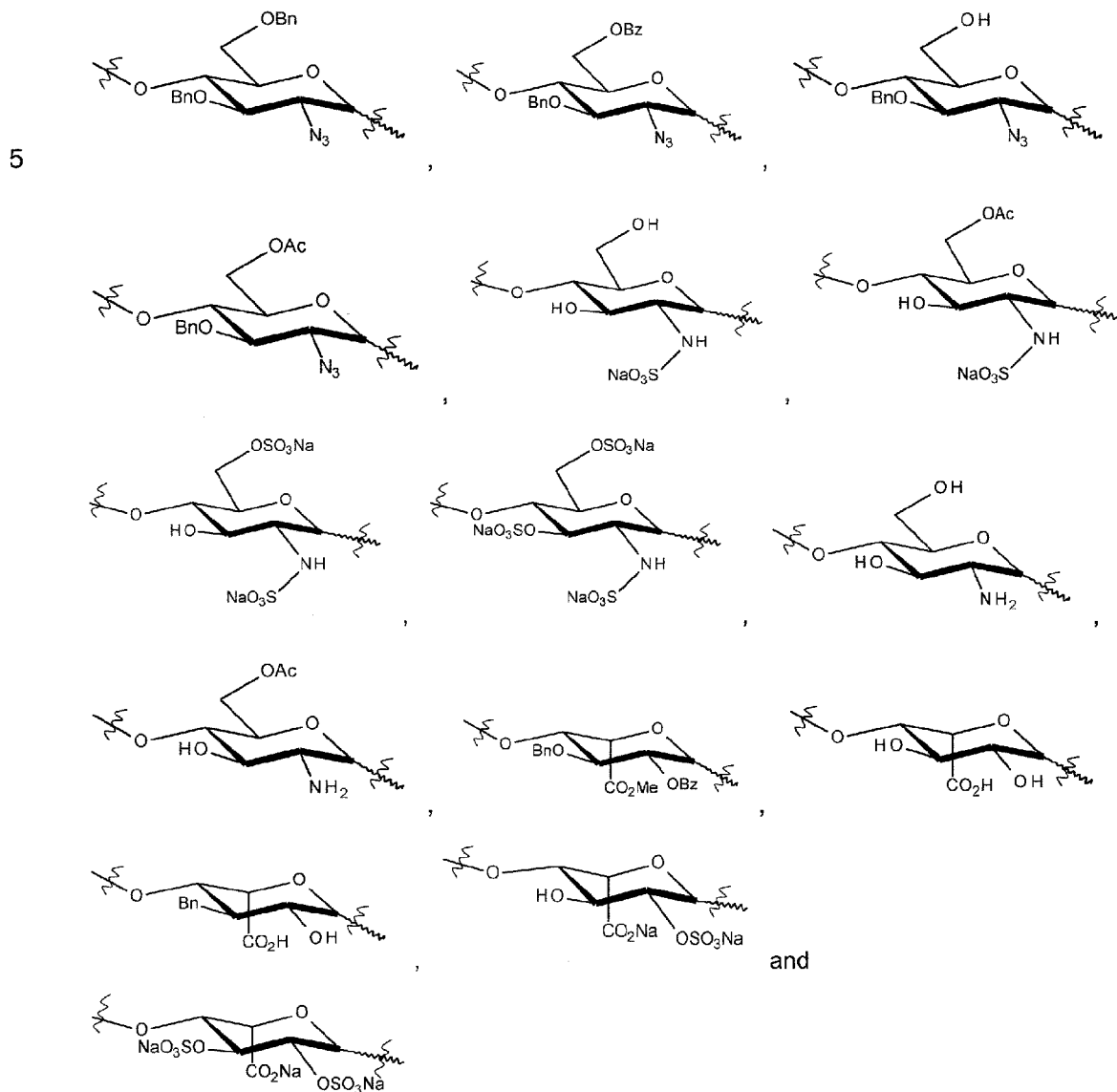
wherein R_3 , R_4 , R_6 , X and Z are independently as defined above.

Suitably, the residues of formula (IIa) and (IIIa) may be residues of formulae (IVa) and (Va):

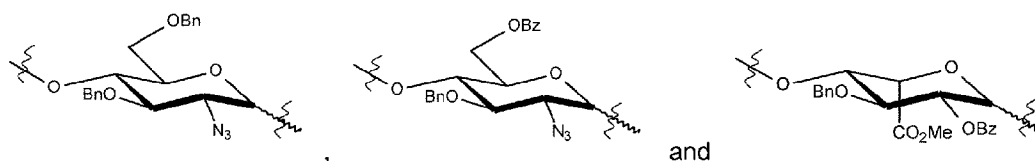


wherein R_3 , R_4 , R_6 , X and Z are independently as defined above.

In embodiments, the polysaccharide comprises one or more monosaccharide residues independently selected from the group consisting of:



Suitably, the monosaccharide residues may be independently selected from the group consisting of:

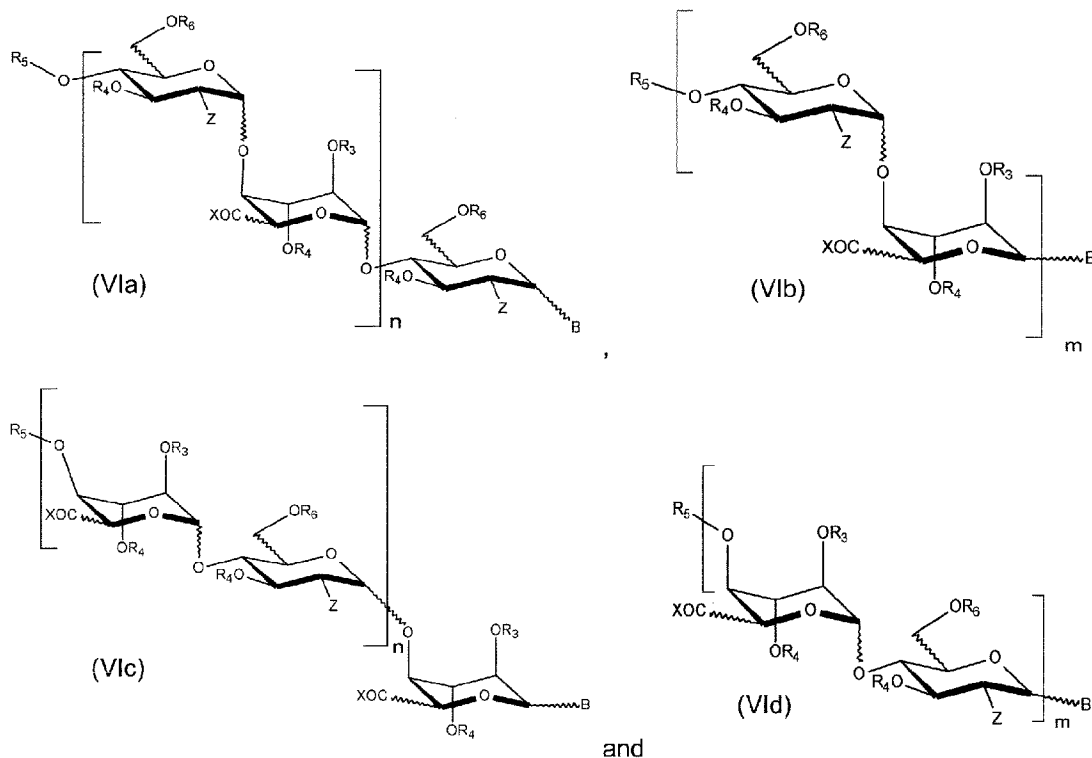


Suitably, at least half and preferably more than half of the monosaccharide residues in the polysaccharide may be independently selected from formulae (IIa) and (IIIa), e.g. formulae (IVa) and (Va), more preferably substantially all and most preferably each of the monosaccharide residues in

the polysaccharide is independently selected from formulae (IIa) and (IIIa), e.g. formulae (IVa) and (Va).

Polysaccharides comprising hexuronic acid and glucose residues

- 5 In embodiments, the polysaccharide comprises an alternating sequence of optionally substituted hexuronic acid residues and optionally substituted glucose residues. In embodiments, the polysaccharide comprises an alternating sequence of residues of formulae (IIa) and (IIIa), and in preferred embodiments the polysaccharide is selected from formulae (VIa-d):



wherein

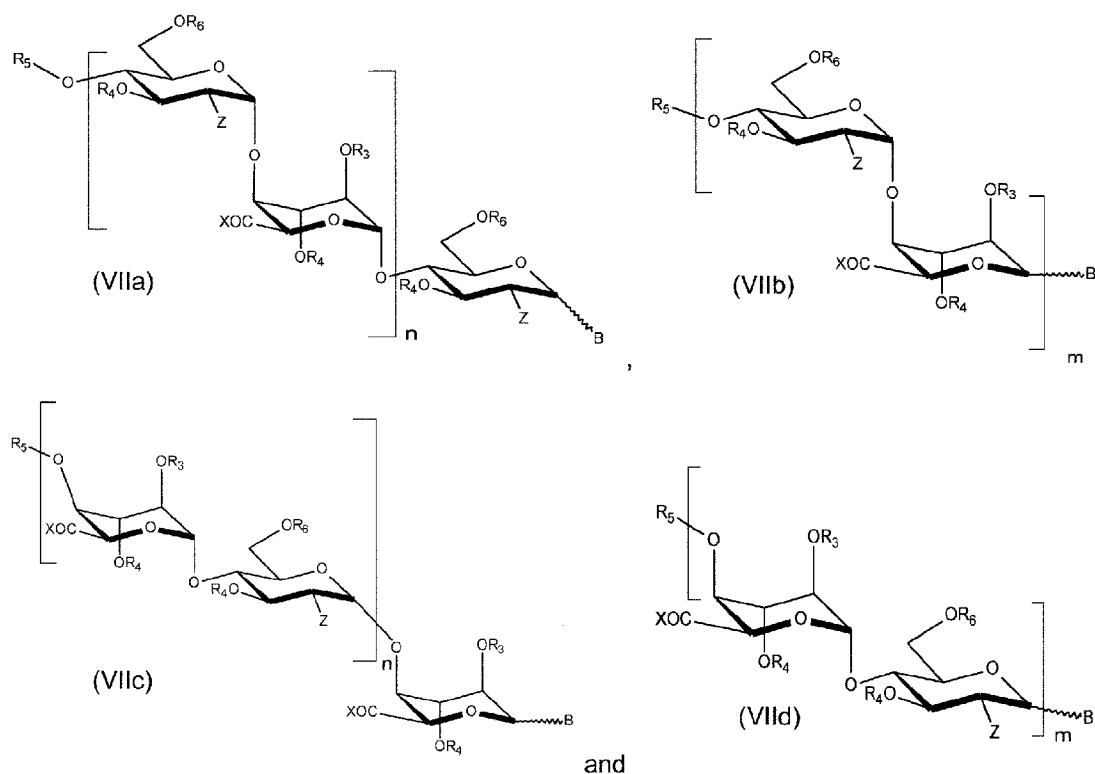
R₅ is H or a hydroxy protecting group;

B, R₃, R₄, R₆, X and Z are as defined above;

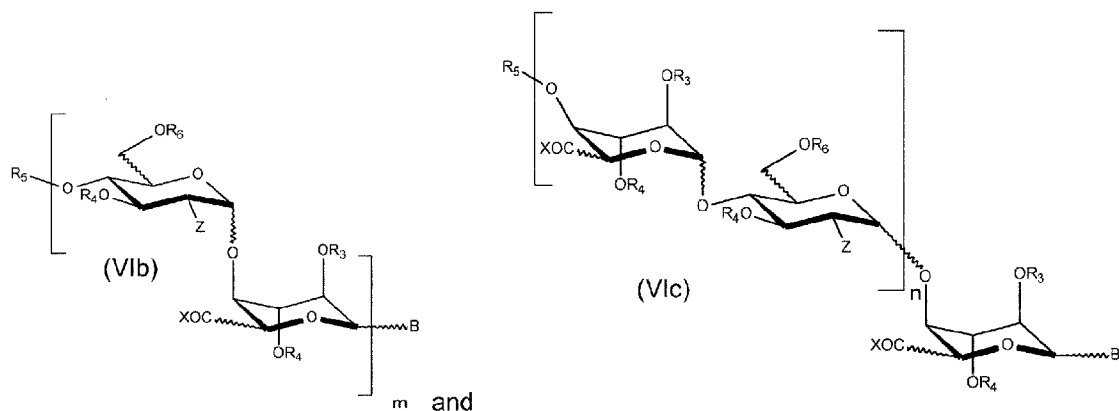
n is an integer of 4 or more, such as 4 to 19; and

m is an integer of 4 or more, such as 4 to 20.

In embodiments, the polysaccharide comprises an alternating sequence of residues of formulae (IVa) and (Va) and preferably the polysaccharide is selected from formulae (VIIa-d):

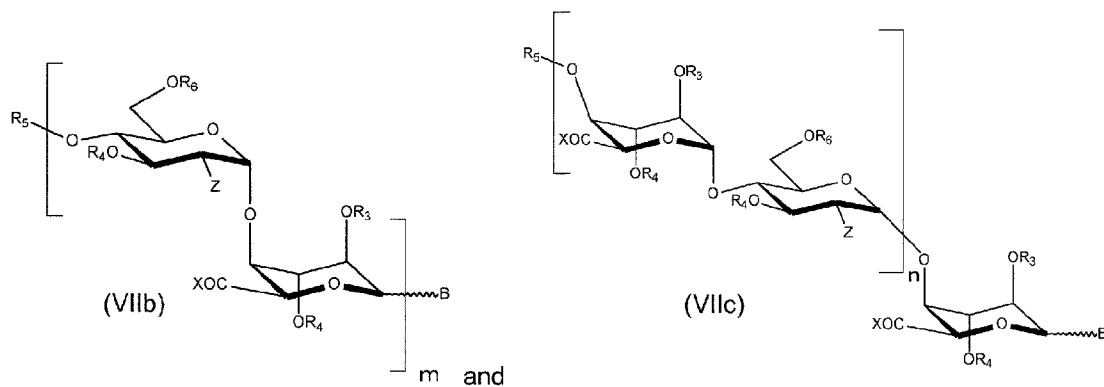


In embodiments, the polysaccharide is a compound selected from the following formulae:



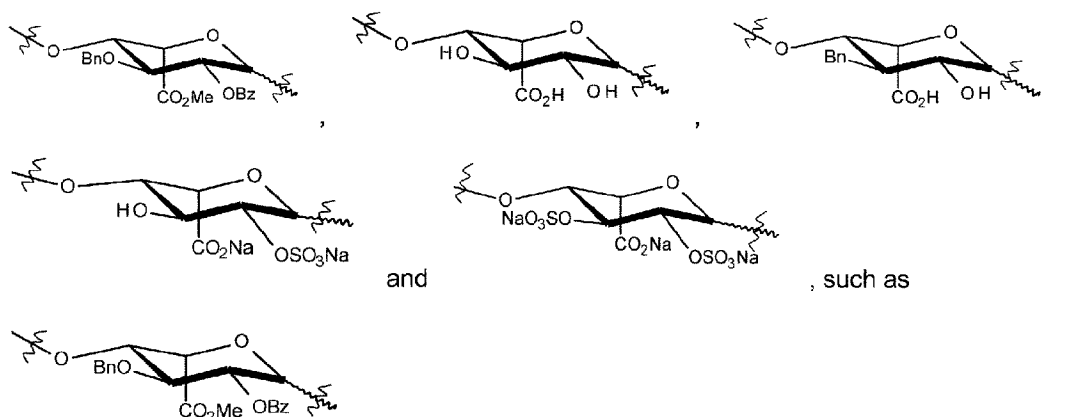
- 5 wherein B, R_3 - R_6 , X and Z are as defined above, n is an integer of 4 or more, such as 4 to 19; and m is an integer of 4 or more, such as 4 to 20.

Preferably, the polysaccharide is a compound selected from the following formulae:



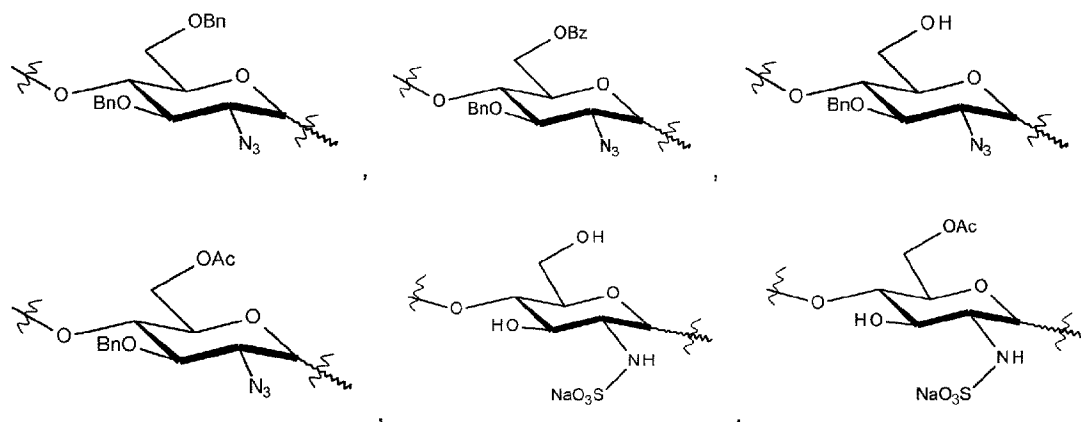
wherein B, R_3 - R_6 , X and Z are as defined above, n is an integer of 4 or more, such as 4 to 19; and m is an integer of 4 or more, such as 4 to 20.

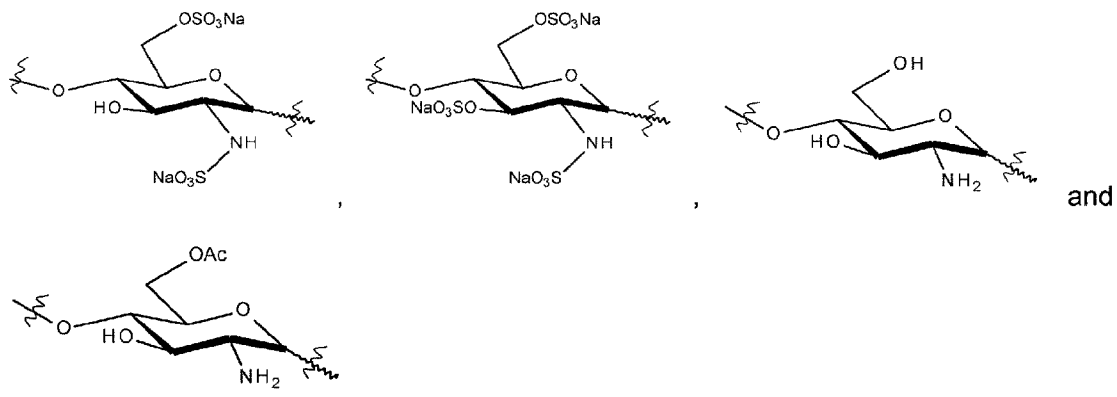
- 5 In embodiments, each optionally substituted hexuronic acid residue in the compounds of formulae (VIa-d) and (VIIa-d) is selected from the following formulae:



10

In embodiments, each optionally substituted glucose residue in the compounds of formulae (VIa-d) and (VIIa-d) is selected from the following formulae:

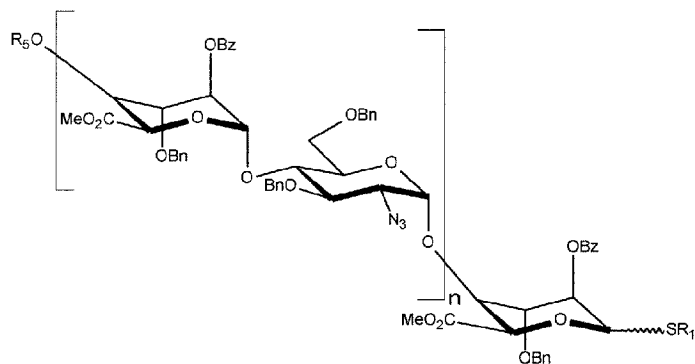




In embodiments of polysaccharides of formula (I), B is in an axial configuration on the pyranose ring.

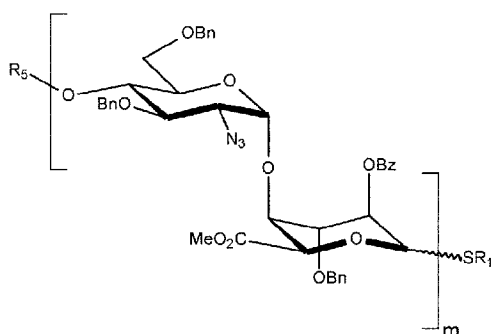
5

Suitably, the polysaccharide may be selected from formulae (VIa-d) and (VIIa-d) wherein B is a thio-leaving group as defined above. In some embodiments, the polysaccharide is selected from formulae (VIb) and (VIc) (such as from formulae (VIIb) and (VIIc)) and B is a thio leaving group as defined above. Thus, in embodiments, the polysaccharide is selected from the following formulae:



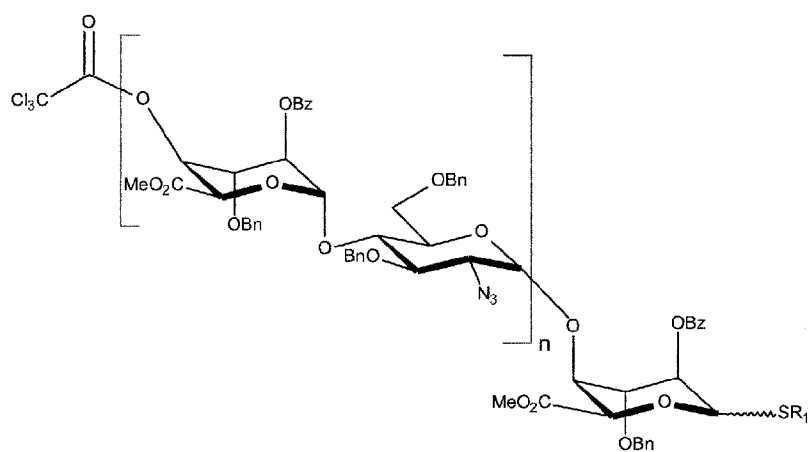
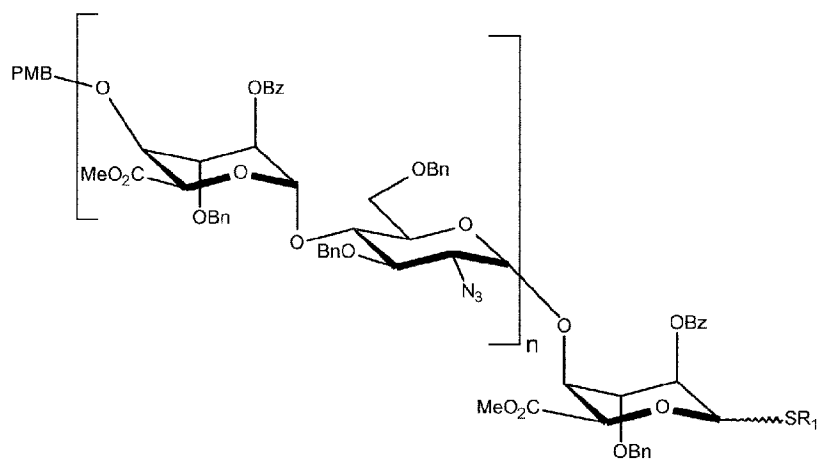
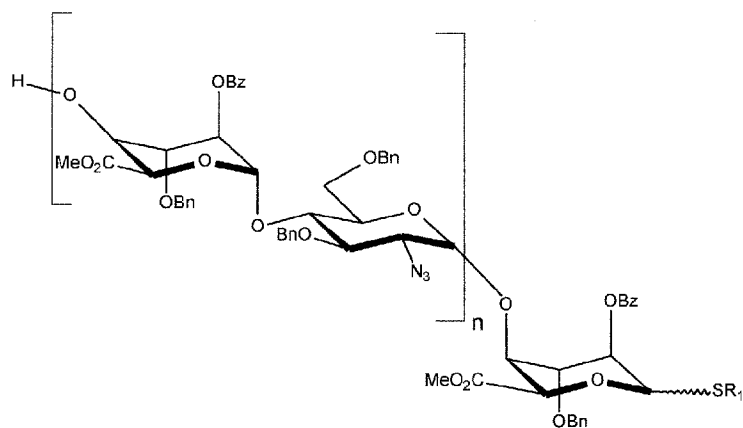
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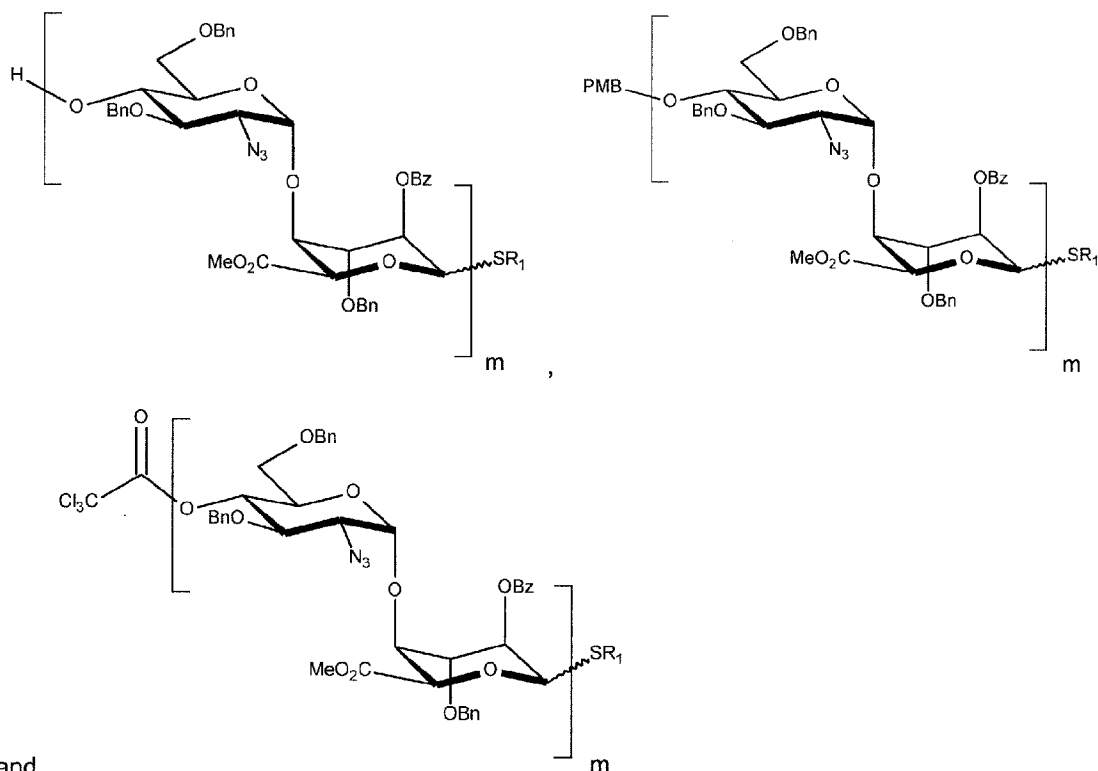
and



wherein R_1 , R_5 , n and m are as defined in the embodiment above.

In embodiments, the polysaccharide is selected from the following formulae:



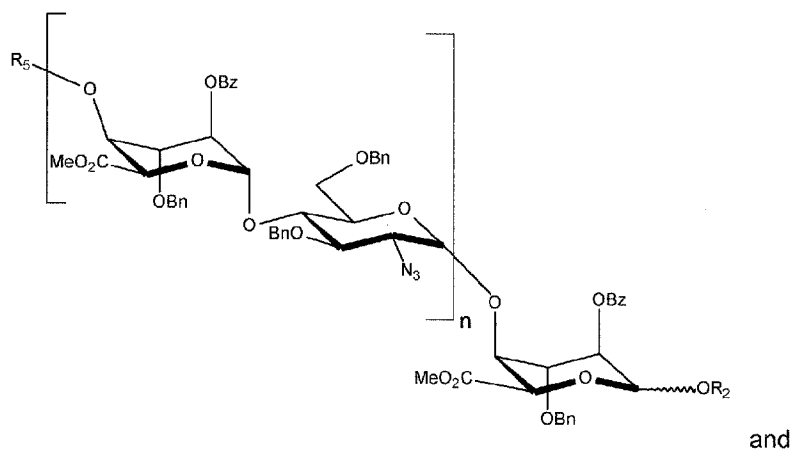


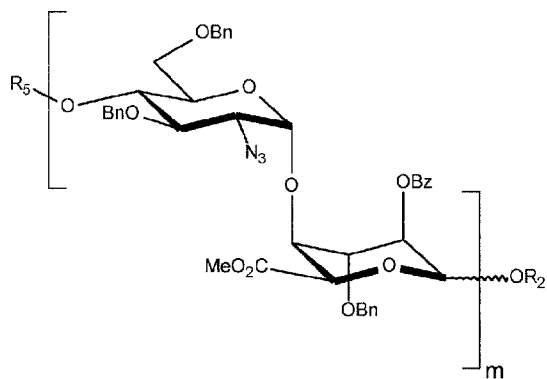
wherein R_1 , m and n are as defined above.

- 5 In embodiments, the polysaccharide is selected from formulae (VIa-d) (preferably (VIIa-d)) and B is OR_2 , wherein R_2 is as defined above.

In embodiments, the polysaccharide is selected from formulae (VIb) and (VIc) (preferably (VIIb) and (VIIc)) and B is OR_2 .

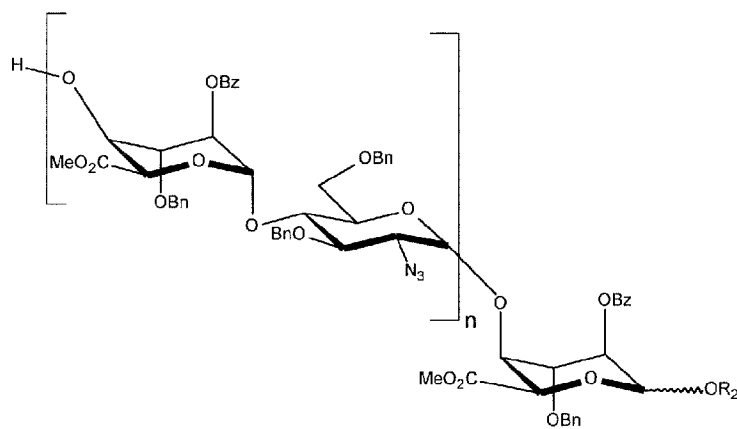
- 10 In embodiments, the polysaccharide is selected from the following formulae:



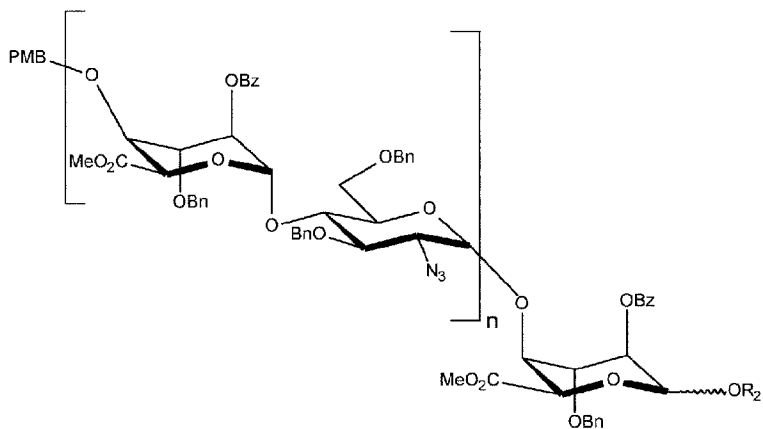


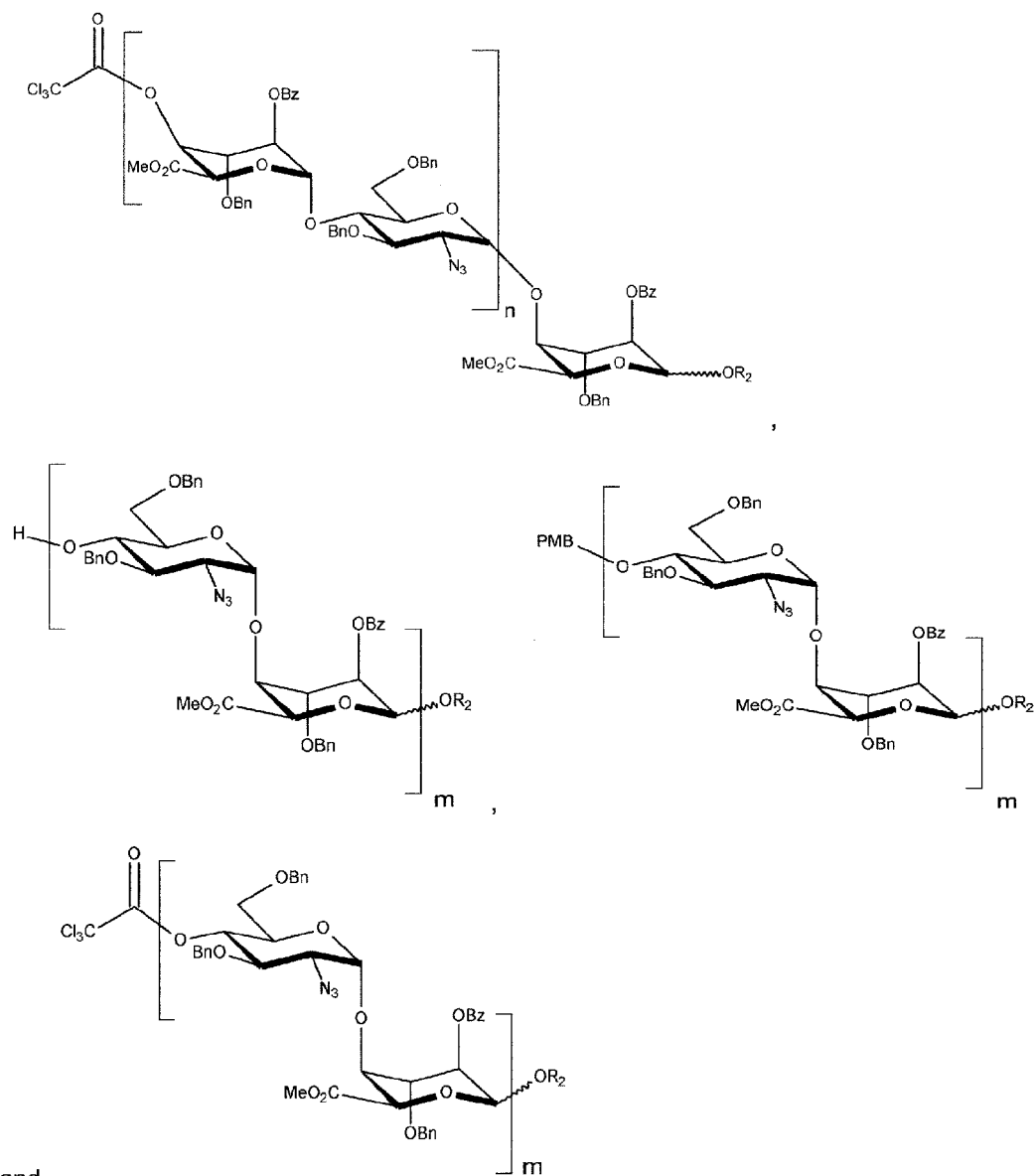
wherein R_2 , R_5 , n and m are as defined in the embodiment above.

Suitably, the polysaccharide may be selected from the following formulae:



5

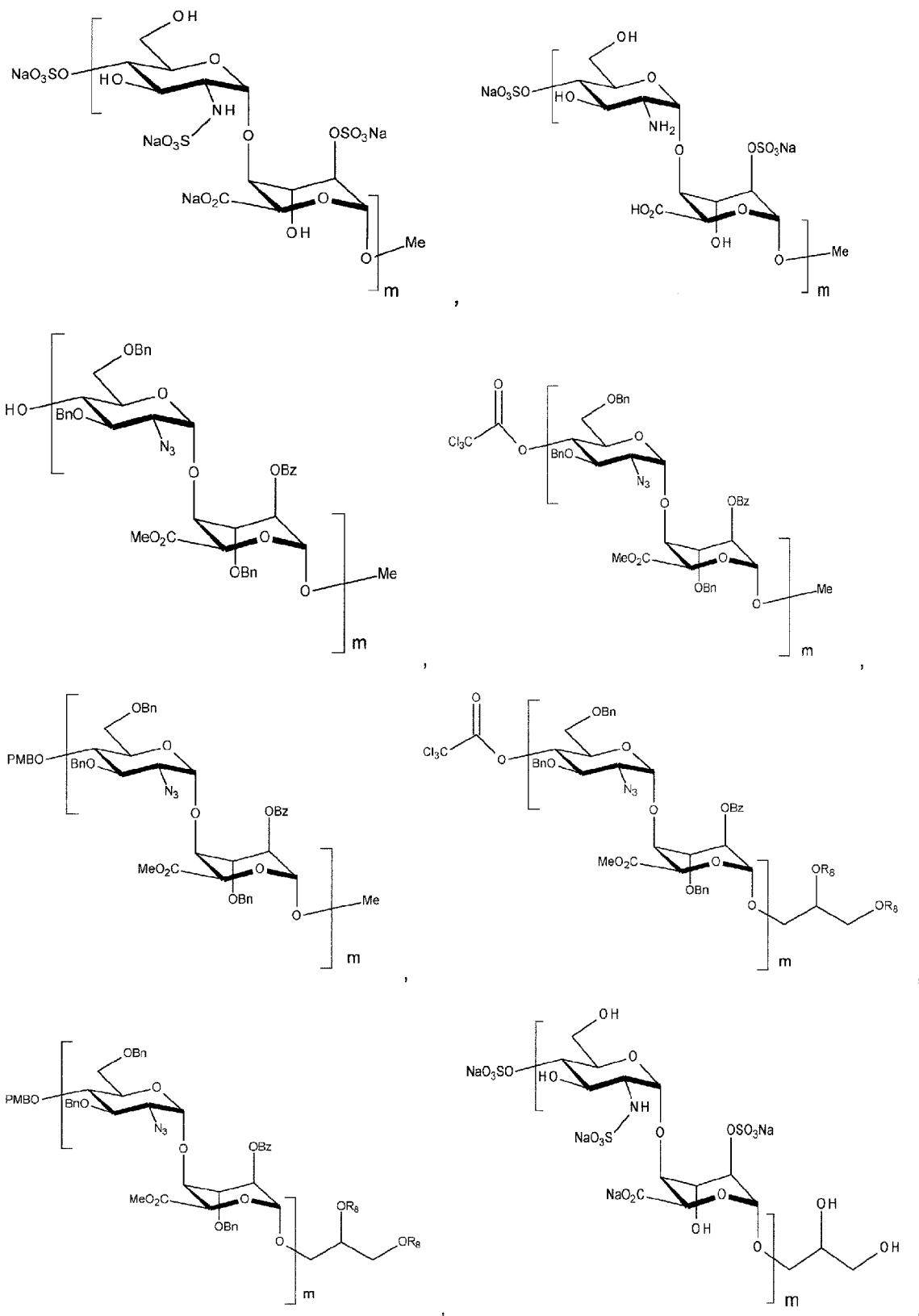


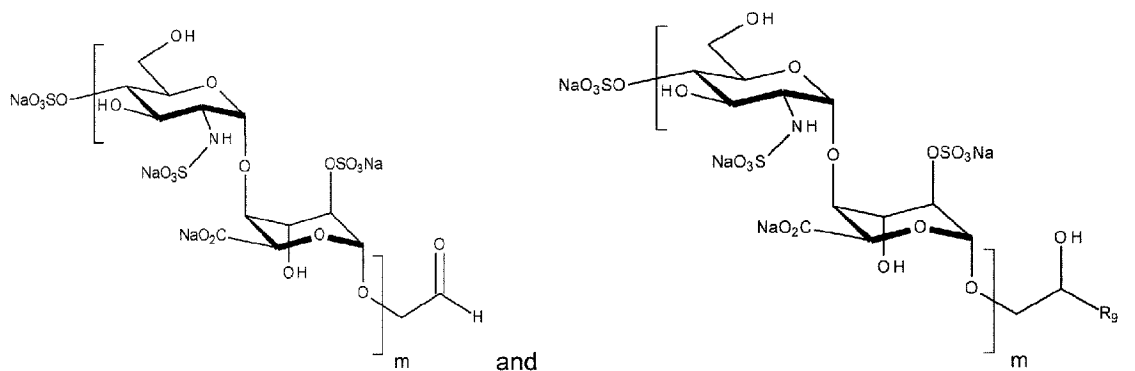


5

Typically, the OR_2 group is in an axial position.

In embodiments, the polysaccharide is selected from the following formulae:





wherein R_8 is H or a hydroxyl protecting group;

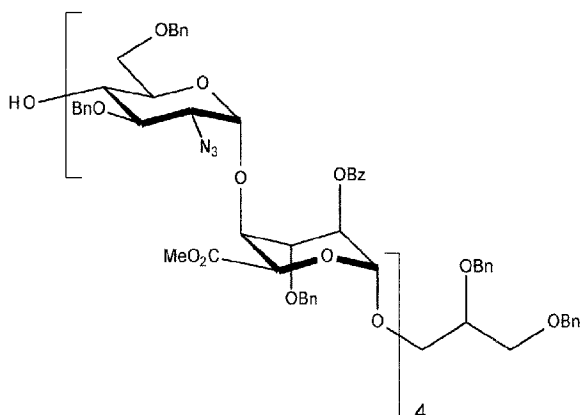
R_9 is H, optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} heteroalkyl, a radiolabel, a fluorogenic tag, a fluorescent label, a tether for attachment to a carrier molecule or solid support or a
 5 tether attached to a carrier molecule or solid support, preferably H, optionally substituted C_{1-10} alkyl or a radiolabel; and

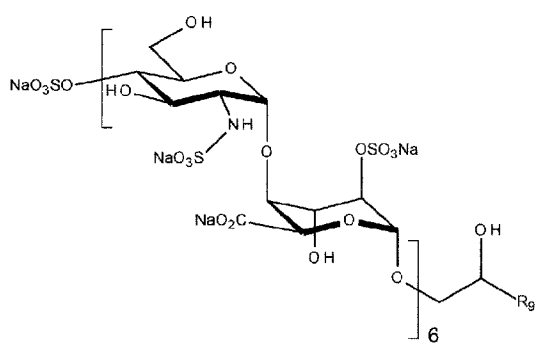
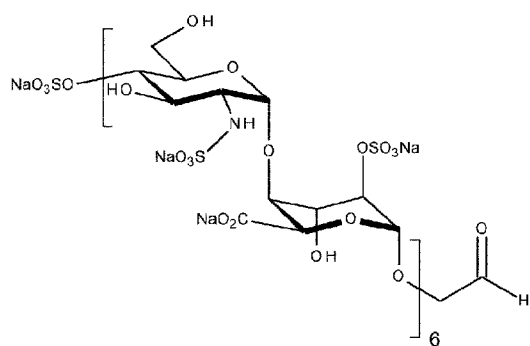
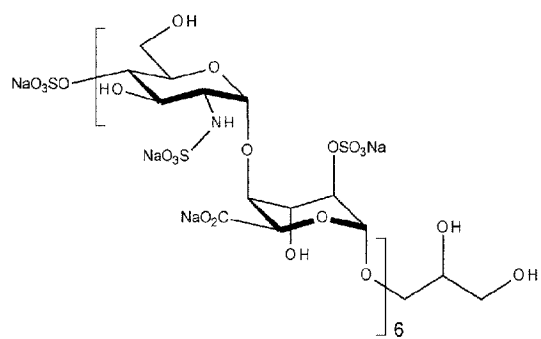
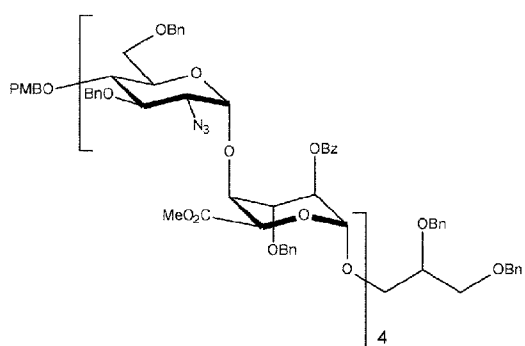
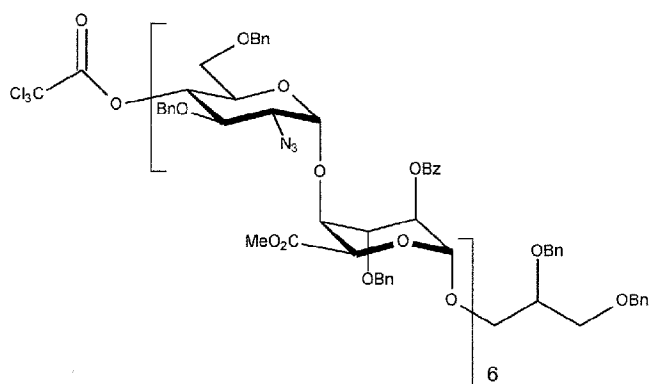
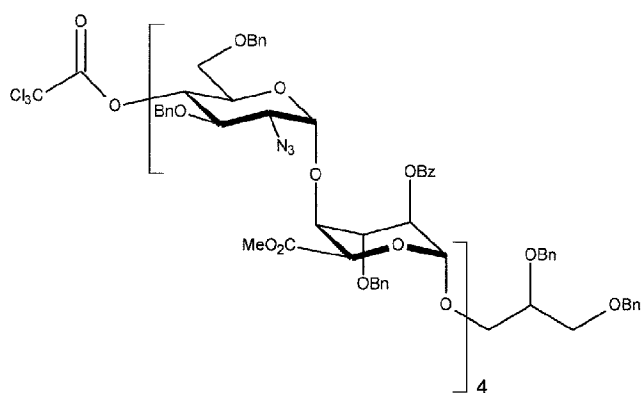
m is an integer of 4 or more, such as 4 to 20.

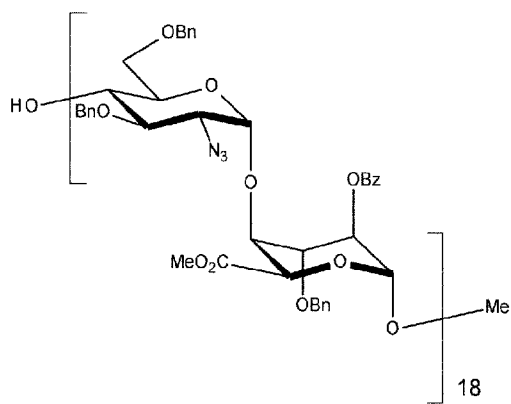
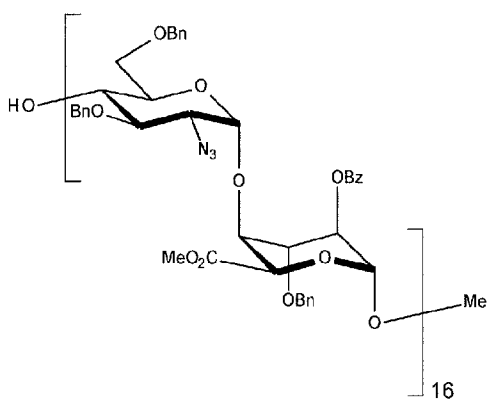
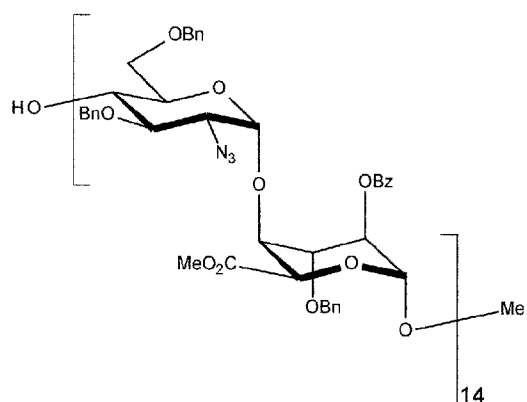
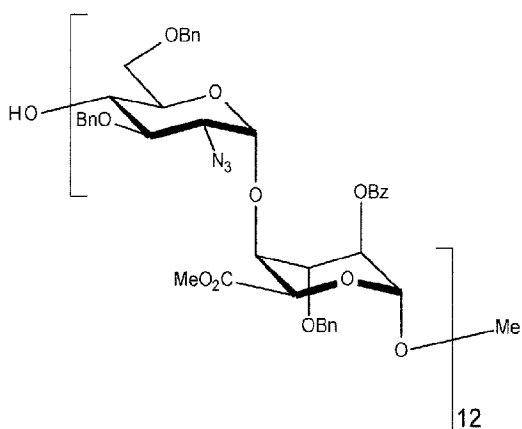
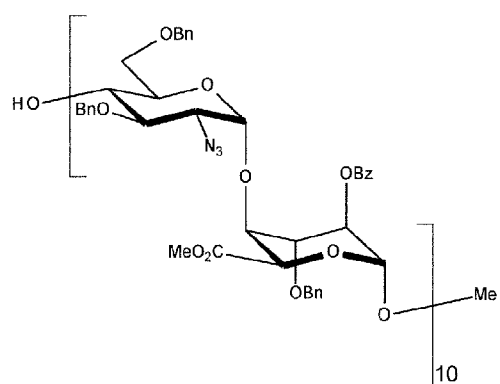
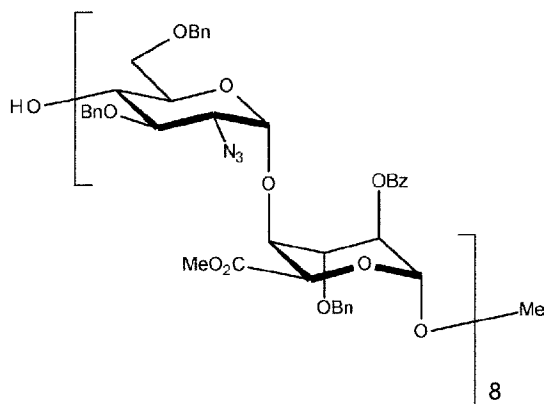
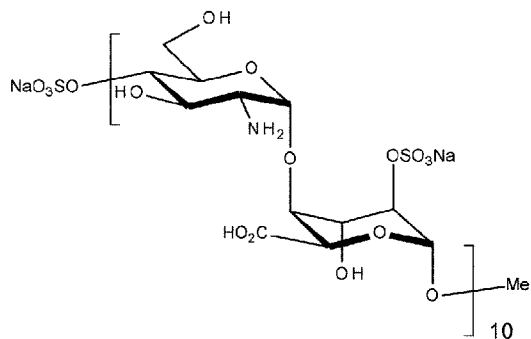
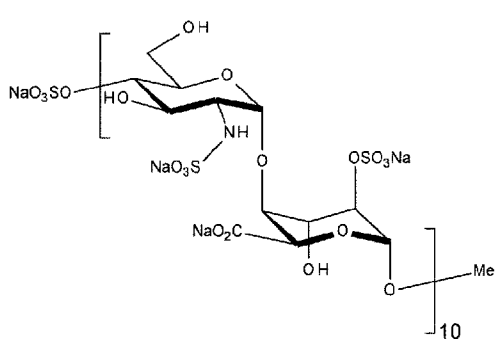
10 In the compounds of formulae (VIa-d) and (VIIa-d), m is an integer of 4 or more, suitably 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, or 16 or more, 17 or more, 18 or more, 19 or more, or 20 or more. In preferred embodiments, m is an integer of 4 to 20, more preferably 5 to 20, 6 to 20, 7 to 20, 8 to 20, 9 to 20, 10 to 20, 11 to 20, 12 to 20, 13 to 20, 14 to 20, 15 to 20, 16 to 20, or 17 to 20, 18 to 20 or 19 to 20, e.g. 20.

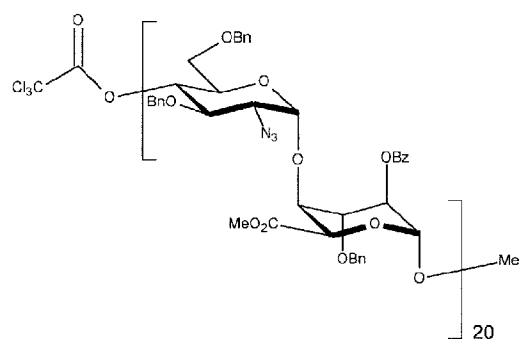
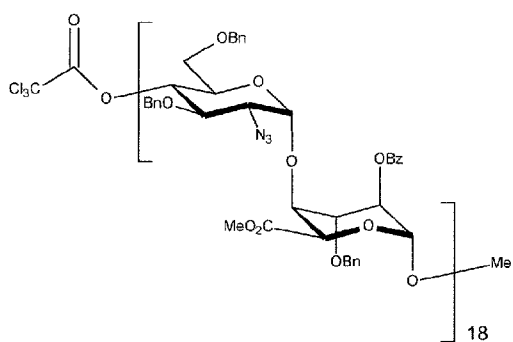
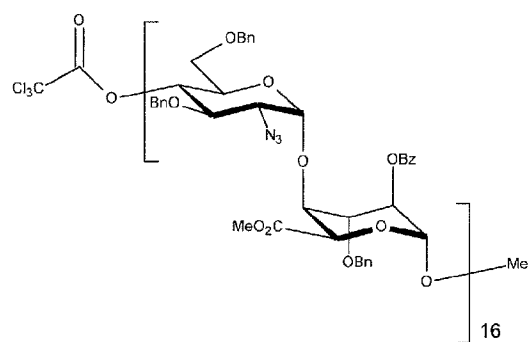
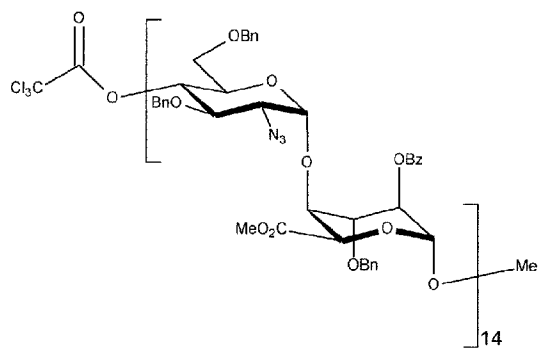
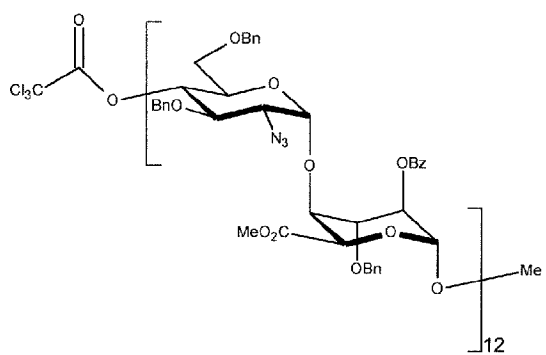
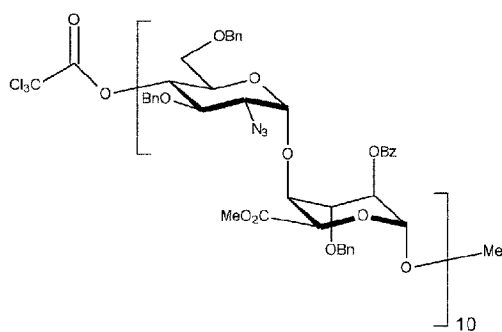
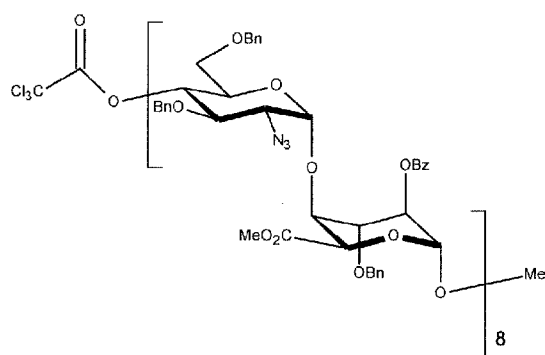
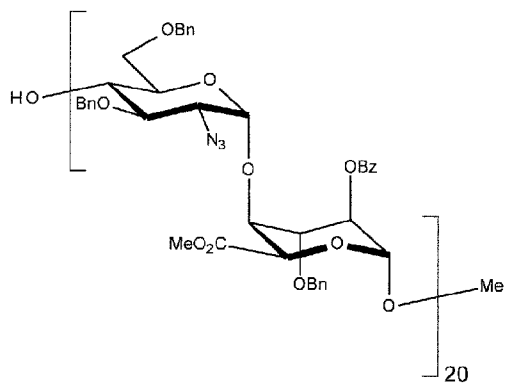
15 In the above compounds of formulae (VIa-d) and (VIIa-d), n is an integer of 4 or more, suitably 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, or 19 or more or 20 or more. Preferably, n is an integer of 4 to 20, more preferably 5 to 20, 6 to 20, 7 to 20, 8 to 20, 9 to 20, 10 to 20, 11 to 20, 12 to 20, 13 to 20, 14 to 20, 15 to 20, or 16 to 20, 17 to 20, 18 to 20, or 19 to 20, e.g. 20.
 20

In embodiments, the polysaccharide is selected from the following compounds:









and

R-group definitions:***B***

In polysaccharide compounds of formula (I), group B is bonded to the anomeric carbon of a monosaccharide residue in the polysaccharide chain (i.e. at a chain terminus) and is selected from a thio leaving group bonded by a sulphur atom to the anomeric carbon, -OR₂ and -SeR₂, preferably a thio leaving group and -OR₂, wherein R₂ is defined below. In embodiments the B is a thio leaving group, preferably an SR₁ group, wherein R₁ is as defined below. In other embodiments, B is -OR₂ wherein R₂ is as defined below

- Group B may be in an axial or equatorial configuration with respect to the monosaccharide ring to which it is bonded. Suitably, B may be in an axial position. The axial position usually provides the most stable configuration by virtue of the well-known "anomeric effect".

R₁

- R₁ is selected from the group consisting of optionally substituted C₁₋₁₀alkyl, optionally substituted C₂₋₁₀alkenyl, optionally substituted C₂₋₁₀alkynyl, optionally substituted C₃₋₁₀cycloalkyl, optionally substituted C₃₋₁₀cycloalkenyl, optionally substituted C₃₋₁₀heterocycloalkenyl, optionally substituted C₆₋₁₄aryl, optionally substituted C₅₋₁₄heteroaryl, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support.

- In embodiments, R₁ is selected from the group consisting of optionally substituted C₁₋₁₀alkyl, optionally substituted C₃₋₁₀heterocycloalkenyl, optionally substituted C₆₋₁₄aryl, optionally substituted C₅₋₁₄heteroaryl and a tether to a solid support. Preferably, R₁ is selected from the group consisting of C₁₋₁₀alkyl, C₃₋₁₀heterocycloalkenyl, C₆₋₁₄aryl and C₅₋₁₄heteroaryl, more preferably C₁₋₄alkyl, C₅₋₁₀heterocycloalkenyl, C₆₋₁₄aryl and C₅₋₁₄heteroaryl, most preferably methyl, ethyl and phenyl.

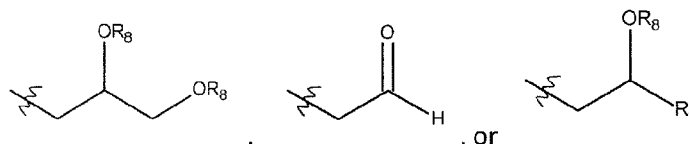
R₂

In the polysaccharides of the present invention, R₂ is H or an end-capping group R_{ec}. In embodiments, R₂ is H. Preferably R₂ is an end-capping group R_{ec}.

R_{ec}

Suitably, R_{ec} may be any group other than H that may suitably bond to the anomeric carbon (or the C-4 carbon) via an oxygen atom. The skilled person will appreciate that the choice of group at the anomeric carbon (or the C-4 carbon) will depend on the required functionality of the compound. In typical embodiments of the invention, the end capping group R_{ec} will be suitably stable under the conditions used to couple the chain extending and first polysaccharides (or chain extended polysaccharide as the case may be). It is also desirable that the end-capping group is stable to one or more deprotection steps such that the end-cap remains intact during any functional manipulation of the polysaccharide.

- In embodiments, R_{ec} is a hydroxyl protecting group as suitably defined herein, preferably selected from the group consisting of optionally substituted C_{1-10} alkyl, optionally substituted C_{3-10} cycloalkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} alkynyl, optionally substituted C_{3-10} cycloalkenyl, optionally substituted C_{2-10} heteroalkyl, optionally substituted C_{3-10} heterocycloalkyl, optionally substituted C_{2-10} heteroalkenyl, optionally substituted C_{3-10} heterocycloalkenyl, optionally substituted C_{6-14} aryl, optionally substituted silyl, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support. Suitably, the R_{ec} group may be selected from the group consisting of optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} heteroalkyl, optionally substituted C_{3-10} heterocycloalkyl, optionally substituted C_{2-10} heteroalkenyl and optionally substituted C_{3-10} heterocycloalkenyl, more preferably optionally substituted C_{1-10} alkyl and optionally substituted C_{2-10} alkenyl, and most preferably optionally substituted C_{1-10} alkyl. Suitably, the optionally substituted C_{1-10} alkyl may be optionally substituted C_{1-4} alkyl, such as C_{1-4} alkyl.
- In embodiments where the R_{ec} group is substituted as defined above, the respective substituents may be selected from OR_8 , $N(R^a)_2$, $=O$, $-C(=O)H$, C_{6-14} aryl, halo, halo C_{1-4} alkyl, $=N(R^b)$, a fluorogenic tag, a fluorescent label, a biotin residue, polyethyleneglycol and a radiolabel, wherein C_{6-14} aryl may be further substituted with one to three substituents independently selected from halo, C_{1-4} alkoxy and C_{1-4} alkyl, wherein R^a and R_8 are as defined below and each R^b is independently H, C_{1-4} alkyl, or $-C(=O)C_{1-4}$ alkyl. More preferably, when the R_{ec} group is substituted as defined above, the respective substituents may be selected from OR_8 , $=O$, halo, and a radiolabel, most preferably OR_8 , wherein R_8 is as defined below.



- In embodiments, R_{ec} is methyl, wherein R_8 and R_9 are as defined below. Most preferably R_{ec} is methyl. Suitably R_{ec} includes an amine functionality, suitably a $-NH_2-$ group.

R_3 , R_4 , R_5 and R_6

- In the polysaccharides of formula (I), each R_3 , R_4 , R_5 and R_6 is independently H or a hydroxyl protecting group, wherein R_3 and R_4 are optionally taken together to form a cyclic protecting group when OR_3 and OR_4 are bonded to adjacent carbons in the monosaccharide ring. In some embodiments, each of R_3 to R_6 is independently a hydroxyl protecting group as suitably defined herein.

- In embodiments, each hydroxyl protecting group is independently selected from the group consisting of optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{1-10} heteroalkyl, optionally substituted C_{1-10} heterocycloalkyl, optionally substituted C_{1-10} heteroalkenyl, optionally substituted C_{1-10} heterocycloalkenyl, optionally substituted silyl, $-PO(R^x)_2$, $-SO_2R^x$, a tether

for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support, wherein each R^x is independently selected from OR^y , $C_{1-4}alkyl$, $C_{6-14}aryl$, $C_{1-4}alkylC_{6-14}aryl$, $haloC_{1-4}alkyl$ and $N(R^y)_2$, wherein each R^y is independently selected from H, a cation and $C_{1-4}alkyl$.

5

In preferred embodiments, each hydroxyl protecting group is independently selected from the group consisting of optionally substituted $C_{1-10}alkyl$, optionally substituted $C_{2-10}alkenyl$, optionally substituted $C_{1-10}heteroalkyl$, optionally substituted $C_{1-10}heterocycloalkyl$, optionally substituted $C_{1-10}heteroalkenyl$, optionally substituted $C_{1-10}heterocycloalkenyl$, $-SO_2R^x$, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support, wherein each R^x is independently selected from OR^y , $C_{1-4}alkyl$, $C_{6-14}aryl$, $C_{1-4}alkylC_{6-14}aryl$, $haloC_{1-4}alkyl$ and $N(R^y)_2$, wherein each R^y is independently selected from H, a cation and $C_{1-4}alkyl$.

10

More preferably, the hydroxyl protecting group is selected from the group consisting of optionally substituted $C_{1-10}alkyl$, optionally substituted $C_{2-10}alkenyl$, optionally substituted $C_{1-10}heteroalkyl$, optionally substituted $C_{1-10}heterocycloalkyl$, optionally substituted $C_{1-10}heteroalkenyl$, optionally substituted $C_{1-10}heterocycloalkenyl$, and $-SO_2R^x$, wherein each R^x is independently selected from OR^y , $C_{1-4}alkyl$, $C_{6-14}aryl$, $C_{1-4}alkylC_{6-14}aryl$, $haloC_{1-4}alkyl$ and $N(R^y)_2$, wherein each R^y is independently selected from H, a cation and $C_{1-4}alkyl$. Typically, when R^y is a cation, only one of the R^y groups is a cation.

15

20

Suitably, the hydroxyl protecting group may be selected from optionally substituted $C_{1-10}alkyl$, optionally substituted $C_{2-10}alkenyl$, optionally substituted $C_{1-10}heteroalkyl$, optionally substituted $C_{1-10}heterocycloalkyl$, optionally substituted $C_{1-10}heteroalkenyl$ and $-SO_3R^y$, wherein y is as defined above.

25

In embodiments, each R_3 , R_4 , R_5 and R_6 , is independently selected from H, optionally substituted $C_{1-10}alkyl$ and $-S(O_2)OR^y$, wherein y is as defined above, optionally wherein the cation is sodium.

30

Suitably, when the hydroxyl protecting group is substituted as defined above, the respective substituents may be selected from $=O$, $C_{6-14}aryl$, halo, $haloC_{1-4}alkyl$, $=N(R^b)$ and a radiolabel wherein $C_{6-14}aryl$ may be further substituted with one to three substituents independently selected from halo, $C_{1-4}alkoxy$ and $C_{1-4}alkyl$, wherein each R^b is independently H, $C_{1-4}alkyl$, or $-C(=O)C_{1-4}alkyl$. In a preferred embodiment, where one R_3 , R_4 , R_5 and R_6 is substituted by radio label, the radiolabel is at R_5 .

35

In embodiments, when the hydroxyl protecting group is substituted as defined above, the respective substituents may be selected from $=O$, $C_{6-14}aryl$, halo, $haloC_{1-4}alkyl$, and $=N(R^b)$, wherein $C_{6-14}aryl$ may be further substituted with one to three substituents independently selected from halo, $C_{1-4}alkoxy$ and $C_{1-4}alkyl$, wherein each R^b is independently H, $C_{1-4}alkyl$, or $-C(=O)C_{1-4}alkyl$.

40

In embodiments, the hydroxyl protecting group is selected from groups which combine with the O atom to form:

- a) esters, such as acetyl (Ac), benzoyl (Bz), chloroacetyl (ClAc), trichloroacetyl (TCA), bromoacetyl (BrAc), pivaloyl (Piv), levulinoyl (Lev), difluorobenzoyl (dfBz);
- b) alkoxyalkyl ethers, such as methoxymethyl (MOM), (2-Methoxyethoxy) methyl (MEM) benzyloxymethyl (BOM), p-methyl benzyloxymethyl (pMBOM), Trimethylsilylethoxymethyl (SEM);
- c) carbonates such as 9-Fluorenylmethyl carbonyl (Fmoc), [2,2,2-Trichloroethoxycarbonyl] (Troc), allyloxycarbonyl (Alloc);
- d) ethers, such as naphthylmethyl (Nap) (including both 2-naphthylmethyl, NAP, and 1-naphthylmethyl, 1-NAP), benzyl (Bn), p-methoxybenzyl (PMB), trityl (Tr), tetrahydro-2-pyranyl (THP), methoxytrityl (MTr), dimethoxytrityl (DMTr), allyl (All);
- e) tosylates, such as p-toluene sulfonyl (Ts), methanesulfonyl (Ms) and trifluoromethane sulfonyl (Tf); and
- f) silyl ethers such as t-butyldimethylsilyl (TBS), hexyldimethylsilyl (TDS), t-butyldiphenyl silyl (TBDPS), triisopropylsilyl (TIPS), trimethylsilyl (TMS), triethylsilyl (TES), triphenylsilyl (TPS), di-tert-butylmethylsilyl (DTBMS), diethylisopropylsilyl (DEIPS), dimethylisopropylsilyl (DMIPS),

Suitably, optionally substituted C₁₋₁₀alkyl groups may be selected from acetyl (Ac), benzoyl (Bz), chloroacetyl (ClAc), trichloroacetyl (TCA), 9-Fluorenylmethyl carbonyl (Fmoc), [2,2,2-Trichloroethoxycarbonyl] (Troc), allyloxycarbonyl (Alloc), naphthylmethyl (Nap) (including both 2-naphthylmethyl and 1-naphthylmethyl), benzyl (Bn), p-methoxybenzyl (PMB), trityl (Tr), methoxytrityl (MTr), dimethoxytrityl (DMTr) and allyl (All), optionally selected from acetyl (Ac), benzoyl (Bz), trichloroacetyl (TCA), benzyl (Bn), and p-methoxybenzyl (PMB).

Suitably, the optionally substituted C₁₋₁₀heteroalkyl groups may be selected from alkoxyalkyl ethers, such as methoxymethyl (MOM), (2-Methoxyethoxy) methyl (MEM) benzyloxymethyl (BOM), p-methyl benzyloxymethyl (pMBOM) and Trimethylsilylethoxymethyl (SEM).

In embodiments, when R₃ and R₄ are taken together to form a ring, the protecting group may be selected from an acetonide, di-tert-butylsilylene (DTBS), tetra-tert-butoxydisiloxan-1,3-diyl (TBDS), and 1,1,3,3,-tetra-isopropylidisiloxane (TIPDS).

In preferred embodiments, each of R₃, R₄ and R₆ is independently selected from H, Ac, Bz, Bn and -S(O)₂OR^y, wherein R^y is selected from H, C₁₋₄alkyl and a cation. Suitably, R₅ may be selected from H, -C(O)CCl₃ and PMB, more preferably C(O)CCl₃ and PMB, most preferably -C(O)CCl₃.

Suitably, the cation may be a counter-ion of any base addition salt as defined herein, preferably a sodium cation.

X

In polysaccharides of the present invention defined above, each X group where present is independently OR_7 or $N(R^a)_2$, wherein R_7 and R^a are defined below. Thus, the polysaccharide may comprise OR_7 groups at X, $N(R^a)_2$ groups at X, or both OR_7 and $N(R^a)_2$ groups. Preferably X is OR_7 . In embodiments, at least one X is $N(R^a)_2$.

R_7

Each R_7 is independently H, a cation or optionally substituted C_{1-10} alkyl, such as C_{1-10} alkyl. Preferably, the optionally substituted C_{1-10} alkyl group is optionally substituted C_{1-4} alkyl, such as a C_{1-4} alkyl, more preferably methyl, ethyl or propyl, most preferably methyl.

In embodiments, each R_7 is H or a cation, such as H. The cation may be a counter-ion of any base addition salt as defined herein, preferably a sodium cation.

Z

In polysaccharides of the present invention defined above, each Z group is independently OR_3 , N_3 or $N(R^a)_2$ wherein each R_3 is independently as defined above and each R^a is as defined below. In typical embodiments, each Z is independently N_3 or $N(R^a)_2$, such as N_3 .

R^a

Each R^a is independently H or an amino protecting group. Accordingly, each $N(R^a)_2$ group may be independently selected from NH_2 , NH (amino protecting group) and N (amino protecting group) $_2$, typically NH_2 or NH (amino protecting group). In embodiments, each R^a is H, (i.e. NH_2). In

embodiments wherein R^a is an amino protecting group, the amino protecting group may be any amino protecting group as defined herein. Optionally, the two R^a groups are taken together with the nitrogen atom to which they are attached to form a cyclic nitrogen protecting group (such as a phthalimide group).

In embodiments, the amino protecting group is selected from the group consisting of optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} heteroalkyl, optionally substituted C_{3-10} heterocycloalkyl, optionally substituted C_{2-10} heteroalkenyl, optionally substituted C_{3-10} heterocycloalkenyl, $-PO(R^x)_2$, $-SO_2R^x$, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support, wherein each R^x is independently selected from OR^y , C_{1-4} alkyl, C_{6-14} aryl, C_{1-4} alkyl C_{6-14} aryl, halo C_{1-4} alkyl and $N(R^y)_2$, wherein each R^y is independently selected from H, a cation and C_{1-4} alkyl.

More preferably, the amino protecting group is selected from the group consisting of optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} heteroalkyl, optionally substituted C_{3-10} heterocycloalkyl, optionally substituted C_{2-10} heteroalkenyl, optionally

substituted C₃₋₁₀heterocycloalkenyl, -SO₂R^x, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support, wherein each R^x is independently selected from OR^y, C₁₋₄alkyl, C₆₋₁₄aryl, C₁₋₄alkylC₆₋₁₄aryl, haloC₁₋₄alkyl and N(R^y)₂, wherein each R^y is independently selected from H, a cation and C₁₋₄alkyl.

5

More preferably, the amino protecting group is selected from the group consisting of optionally substituted C₁₋₁₀alkyl, optionally substituted C₂₋₁₀alkenyl, optionally substituted C₂₋₁₀heteroalkyl, optionally substituted C₃₋₁₀heterocycloalkyl, optionally substituted C₂₋₁₀heteroalkenyl, optionally substituted C₃₋₁₀heterocycloalkenyl, and -SO₂R^x, wherein each R^x is independently selected from OR^y, C₁₋₄alkyl, C₆₋₁₄aryl, C₁₋₄alkylC₆₋₁₄aryl, haloC₁₋₄alkyl and N(R^y)₂, and most preferably optionally substituted C₁₋₁₀alkyl, optionally substituted C₂₋₁₀alkenyl, optionally substituted C₂₋₁₀heteroalkyl, optionally substituted C₃₋₁₀heterocycloalkyl, optionally substituted C₂₋₁₀heteroalkenyl and -S(O)₂OR^y, wherein y is as defined above.

10

15 In embodiments, each R^a is independently selected from H, optionally substituted C₁₋₁₀alkyl and -S(O)₂OR^y, preferably H, Ac, Boc, Cbz, Bz, and -S(O)₂OR^y, most preferably H and S(O)₂OR^y, wherein R^y is selected from H, C₁₋₄alkyl and a cation, optionally wherein the cation is sodium.

20 Suitably, when the respective amino protecting group is substituted as defined above, the respective substituents may be selected from =O, C₆₋₁₄aryl, halo, haloC₁₋₄alkyl; and =N(R^b); wherein C₆₋₁₄aryl may be further substituted with one to three substituents independently selected from halo, C₁₋₄alkoxy and C₁₋₄alkyl, wherein each R^b is independently H, C₁₋₄alkyl, or -C(=O)C₁₋₄alkyl.

25 Preferably, the amino protecting group is selected from acetyl (Ac), chloroacetyl, trichloroacetyl (TCA), tert-butyloxycarbonyl (Boc), benzyloxy carbonyl (Cbz), phthalimido (Phthal), tetrachlorophthaloyl (TCP), N-dithiasuccinyl (Dts) (cleavable orthogonally in the presence of an azide using propane-1,3-dithiol (PDT) and DIPEA), [2,2,2-Trichloroethoxycarbonyl] (Troc), levulinoyl (Lev), tosyl (Tos), nosyl (Nos), allyloxycarbonyl (Alloc), trifluoroacetyl (TFA), trityl (Tr), benzylideneamine, oxazolidine, diglycolyl (DG) and dimethylglutamyl (DMG).

30

The cation may be a counter-ion of any base addition salt as defined herein, preferably a sodium cation.

R₈

35 Each R₈ is independently H or a hydroxyl protecting group. Accordingly each R₈ may be independently defined as above for the R₃-R₆ groups.

R₉

40 In embodiments comprising an R₉ group bonded to the substituent at the R₂ position, R₉ is selected from H, optionally substituted C₁₋₁₀alkyl, optionally substituted C₂₋₁₀heteroalkyl, a radiolabel, a

fluorogenic tag, a fluorescent label, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support.

In embodiments, R_9 is selected from H, optionally substituted C_{1-10} alkyl and a radiolabel. Suitably, R^9 may be H. In embodiments, R^9 is optionally substituted C_{1-10} alkyl. In embodiments, R^9 is a radiolabel as defined herein. R^9 may be added by suitable nucleophilic addition to a precursor aldehyde. Thus, any suitable radiolabel may be added at the relevant position using simple aldehyde chemistry, as illustrated in the examples.

10 *m*

In embodiments of the above *m* is an integer of 4 or more, preferably 5 or more, more preferably 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, or 16 or more, 17 or more, 18 or more, 19 or more, or 20 or more. In embodiments, *m* is an integer of 4 to 20, preferably 5 to 20, most preferably 6 to 20, 7 to 20, 8 to 20, 9 to 20, 10 to 20, 11 to 20, 12 to 20, 13 to 20, 14 to 20, 15 to 20, 16 to 20, or 17 to 20, 18 to 20 or 19 to 20, e.g. 20.

n

In embodiments of the above aspect wherein *n* is present, *n* is an integer of 4 or more, such as 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, or 19 or more or 20 or more. For example, in some embodiments, *n* is an integer of 4 to 20, such as 5 to 20, 6 to 20, 7 to 20, 8 to 20, 9 to 20, 10 to 20, 11 to 20, 12 to 20, 13 to 20, 14 to 20, 15 to 20, or 16 to 20, 17 to 20, 18 to 20, or 19 to 20, e.g. 20.

25 **The coupling reaction**

In the above aspect and embodiments, the coupling of the first and chain extending polysaccharides is by substitution of a thio leaving group (such as $-SR^1$) bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue in the chain extending polysaccharide or the first polysaccharide. The process provides chain elongation by virtue of either the first or the chain extending polysaccharide substituting the thio leaving group (e.g. $-SR^1$) at the anomeric position of the other respective polysaccharide, resulting in a glycosidic bond between the first and chain extending polysaccharides to form a chain extended polysaccharide. In other words, the process of the present invention involves the coupling of a glycoside acceptor with a thioglycoside donor to form a chain extended polysaccharide.

Thus, in the process of the present invention at least one of the chain extending polysaccharide and the first polysaccharide (or chain extended polysaccharide as the case may be) must contain a thio leaving group (such as $-SR^1$) bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue in the respective chain.

In embodiments, both of the chain extending polysaccharide and the first polysaccharide (or chain extended polysaccharide as the case may be) contains a thio leaving group (such as $-SR^1$) bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue in each polysaccharide. In such reactions, the resulting chain extended polysaccharide will thus contain a thio leaving group (such as $-SR^1$) bonded by a sulphur atom to an anomeric carbon, which may then participate in a further reaction with another chain extending polysaccharide (see figure 1, where the circle depicts the chain extending polysaccharide building block and the square depicts the first polysaccharide building block). In such processes, care should be taken if further iteration of the chain extending step is not desired.

In typical embodiments, only one of the chain extending and first polysaccharides (or chain extended polysaccharide as the case may be) contains a thio leaving group (such as $-SR^1$) bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue. Any suitable group may then be bonded to the anomeric carbon at the terminus of the other respective polysaccharide chain. As the resulting chain extended polysaccharide would not then contain a thio leaving group bonded by a sulphur atom to an anomeric carbon (i.e. the only thio group present is substituted in the bond formation), this embodiment allows the synthetic chemist to control the chain extension, thus preventing runaway iteration. For instance, the anomeric end of one of the polysaccharides may be capped with a relatively unreactive group or a linker to a solid phase support to prevent further iterative addition to the resulting chain extended polysaccharide (see figure 2, wherein the first polysaccharide contains a capping group X bonded to the anomeric carbon at its terminus). In embodiments, the group bonded to the anomeric carbon of a monosaccharide residue in the other reacting polysaccharide (i.e. at its terminus) is selected from $-OR_2$ and $-SeR_2$, preferably $-OR_2$, wherein R_2 is selected from H and an end-capping group R_{ec} .

Thus, in embodiments, the chain extending polysaccharide contains the thio leaving group, and the group bonded to the anomeric carbon of a monosaccharide in the first polysaccharide (i.e. at a chain terminus) is selected from $-OR_2$ and $-SeR_2$, preferably $-OR_2$, wherein R_2 is selected from H and an end-capping group R_{ec} . In other embodiments, the first polysaccharide contains the thio leaving group and the group bonded to the anomeric carbon of a monosaccharide in the chain extending polysaccharide (i.e. at the anomeric terminus) is selected from $-OR_2$ and $-SeR_2$, preferably $-OR_2$, wherein R_2 is selected from H and an end-capping group R_{ec} .

In embodiments, the thio leaving group (e.g. $-SR^1$) is bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue in the first polysaccharide (or chain extended polysaccharide as the case may be) and the chain extending polysaccharide is a compound of formula (Ia):



wherein

A^1 is the chain of monosaccharide residues; and

-OR₂ is bonded to the anomeric carbon of a monosaccharide residue in the , wherein R₂ is H or an end-capping group R_{ec}.

Preferably, the coupling is by substitution of a thio leaving group (e.g. -SR¹) bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue of the chain extending polysaccharide, most preferably wherein the thio leaving group is bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue of the chain extending polysaccharide and the first polysaccharide (or chain extended polysaccharide as the case may be) is a compound of formula (Ib):



wherein

A² is the chain of monosaccharide residues; and

-OR₂ is bonded to the anomeric carbon of a monosaccharide residue, wherein R₂ is H or an end-capping group R_{ec}.

15 Nucleophilic substitution of the thio leaving group

In the processes of the invention, any pendant nucleophilic group attached to the reacting polysaccharide may perform the nucleophilic substitution of the thio leaving group. In embodiments, the nucleophilic group that displaces the thio leaving group is a pendant hydroxyl or amino group on a monosaccharide residue in the polysaccharide chain, preferably a hydroxyl group.

The nucleophilic group in the reaction may suitably be attached to any monosaccharide residue along the respective acceptor polysaccharide chain provided a polysaccharide of 8 or more monosaccharide residues in length is produced. Preferably, the nucleophilic group that displaces the thio leaving group in the coupling reaction is attached to a monosaccharide residue located at the terminus of the polysaccharide chain furthest from the anomeric carbon terminus of the incoming polysaccharide. This has the advantage of ensuring end-to-end bonding of the polysaccharide building blocks, which provides most efficient access to longer chain lengths.

In embodiments, the coupling in the process of the invention is by formation of a glycosidic bond formed between the chain extending polysaccharide and first polysaccharide (or chain extended polysaccharide as the case may be). The glycosidic bond may be an O-glycosidic bond (preferably a C1-C4 O-glycosidic bond) or N-glycosidic bond (e.g. preferably a C1-C2 N-glycosidic bond). Thus, in embodiments comprising more than one chain extending step, O-glycosidic bonds, N-glycosidic bonds or both O- and N-glycosidic bonds may be formed in different chain extending steps. The glycosidic bonds formed may be C1-C2, C1-C3, C1-C4 or C1-C6 glycosidic bonds, preferably C1-C4 glycosidic bonds, most preferably C1-C4 O-glycosidic bonds.

The glycosidic bond may be an α- or β-glycosidic bond. In embodiments, the glycosidic bond formed is an α-glycosidic bond, preferably an α-glycosidic bond. Typically, the processes of the present

invention allow for highly stereoselective couplings resulting in products that are substantially anomerically pure.

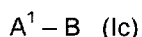
Chain extending polysaccharide

5 *Length*

In the process of the invention, the chain extending polysaccharide is a polysaccharide of four monosaccharide residues in length. The phrase "of four monosaccharide residues in length" refers to a polysaccharide wherein the longest chain of monosaccharides from an anomeric donor terminus is four monosaccharide residues in length.

10

In some embodiments, the chain extending polysaccharide is a compound of formula (I):



wherein

A^1 is a chain of 4 monosaccharide residues in length; and

15

B is bonded to the anomeric carbon of a monosaccharide residue in the chain (i.e. at the anomeric terminus), wherein B is a thio leaving group as defined above, $-OR_2$ or SeR_2 , preferably a thio leaving group as defined above or $-OR_2$, more preferably a thio leaving group as defined above, wherein R_2 is as defined above.

20

Monosaccharide residues

Any combination of monosaccharides residues may be present in the chain extending polysaccharides of the present invention. The polysaccharide as defined in the preceding section is at least in part the product of reaction of a chain extending polysaccharide and a first polysaccharide. Accordingly, the monosaccharide residues in the chain extending polysaccharide may be as defined as above for the polysaccharide.

25

Protecting groups

The chain extending polysaccharide may be suitably protected by any respective protecting group as defined herein. Any nucleophilic moiety present in the chain extending polysaccharide may be protected, such as hydroxy and / or amino groups.

30

The polysaccharide as defined in the preceding section is at least in part the product of reaction between a chain extending polysaccharide and a first saccharide. Accordingly, the protecting groups of the chain extending polysaccharide may be defined as in any of the above embodiments for the polysaccharide.

35

Typically, at least one hydroxyl and / or amino moiety in the chain extending polysaccharide is protected. Optionally, orthogonal protecting groups are used. Thus, by using protecting groups, particularly orthogonal protecting groups, the skilled person can control which position will react in the coupling of the present process to substitute the thio group (e.g. SR^1) in the glycoside donor.

40

Furthermore, by selective removal of temporary protecting groups in the chain extended polysaccharide, it is possible to control future derivatization, such as by revealing specific hydroxyl and / or amino groups for further sulfation (to form selectively sulphated analogues) and / or for further glycosidic bonding, such as using the methods herein.

5

Suitably, in embodiments wherein the chain extending polysaccharide is the nucleophile (i.e. glycoside acceptor) in the coupling step, at least one nucleophilic moiety will be unprotected, such as one. In that case, typically all other nucleophilic groups in the chain extending polysaccharide may be protected to provide complete control over which nucleophilic group reacts in the glycosidic bond

10

formation.

Alternatively, in embodiments wherein the chain extending polysaccharide is the glycoside donor in the reaction, i.e. wherein the coupling is by substitution of the thio leaving group from the chain extending polysaccharide, the chain extending polysaccharide may be suitably protected to prevent self-condensation and runaway iterative extension of the chain extended product. Thus, in

15

embodiments wherein the chain extending polysaccharide is the glycoside donor, typically all of the nucleophilic groups in the chain extended polysaccharide may be protected.

In embodiments wherein the resulting chain extended polysaccharide is required to participate as a nucleophile in a further chain extending step, one or more of the protecting groups in the chain extended polysaccharide may thus be deprotected to reveal the respective hydroxyl / amino nucleophile prior to reaction with a further chain extending saccharide having a thio leaving group bonded to the anomeric carbon.

20

Advantageously, the use of an orthogonal protecting group strategy can allow selective deprotection of one or more hydroxy and / or amino groups, such as a single hydroxy or amino protecting group, thus facilitating selective glycosidic bond formation in a further chain extension step and / or selective derivatization, e.g. sulfation. Thus, the chain extending polysaccharide may be "orthogonally protected", meaning that it contains at least two different protecting groups that are orthogonal to each other.

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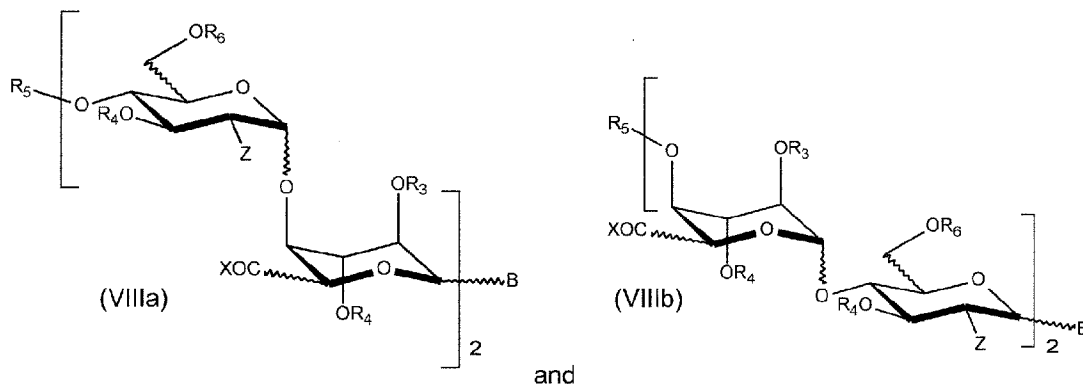
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Suitably, the protecting groups may be the same or different. Preferably at least one protecting group is different in order to facilitate selective removal of protecting groups later, as described above.

35

Preferred chain extending polysaccharides

In embodiments the chain extending polysaccharide may comprise an alternating sequence of residues of formulae (IIa) and (IIIa) as defined above, preferably wherein the chain extending polysaccharide is a compound of formula (VIIIa) or (VIIIb):



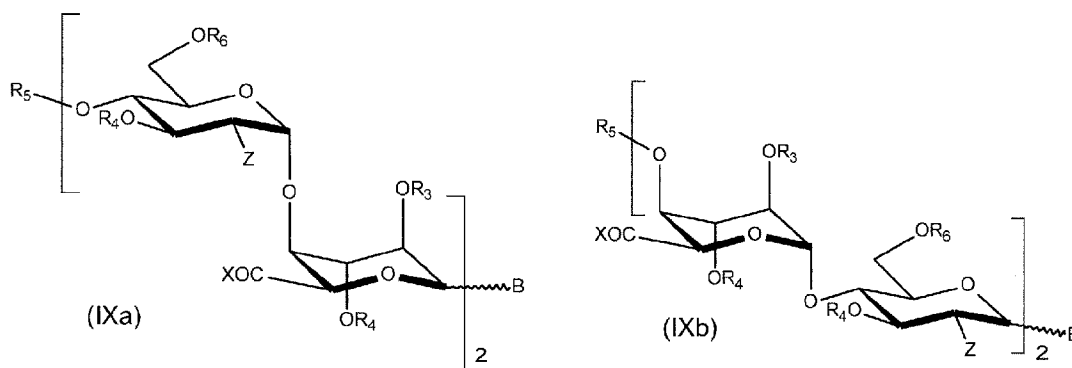
wherein

R_5 is H or a hydroxyl protecting group.

B, R_3 - R_4 , R_6 , X and Z are as defined in any of the above embodiments.

5

Preferably, the compounds of formulae (VIIIa) and (VIIIb) are compounds of formulae (IXa) and (IXb):



In preferred embodiments, the chain extending polysaccharide is a compound of formula (VIIIa), most preferably formula (IXa). Alternatively, the chain extending polysaccharide may be a compound of formula (VIIIb), such as (IXb).

10

Preferred protecting groups

As described above under "protecting groups" the skilled person will understand that when the chain extending polysaccharide is the nucleophile in the glycosidic bond forming reaction, (e.g. wherein B is OR_2) at least one of R_3 - R_6 in the compounds of formulae (VIIIa), (VIIIb), (IXa) and (IXb) must be H in order to facilitate the glycosidic bond formation with the first polysaccharide (or chain extended polysaccharide as the case may be). Preferably only one of the R_3 - R_6 groups is H, such as R_3 , R_4 , R_5 or R_6 , preferably R_5 .

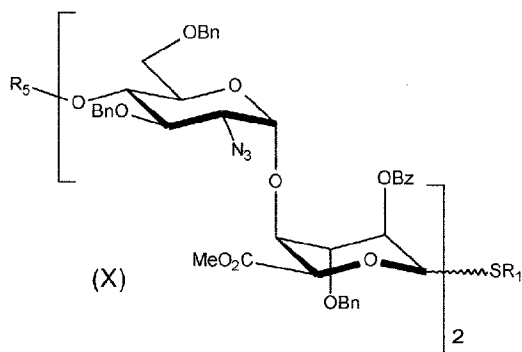
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Furthermore, where the chain extending polysaccharide is the thioglycoside donor, one or more of R_3 - R_6 in the compounds of formula (VIIIa), (VIIIb), (IXa) and (IXb) may be a protecting group in order to control the glycosidic bond formation. In embodiments where the chain extending polysaccharide is

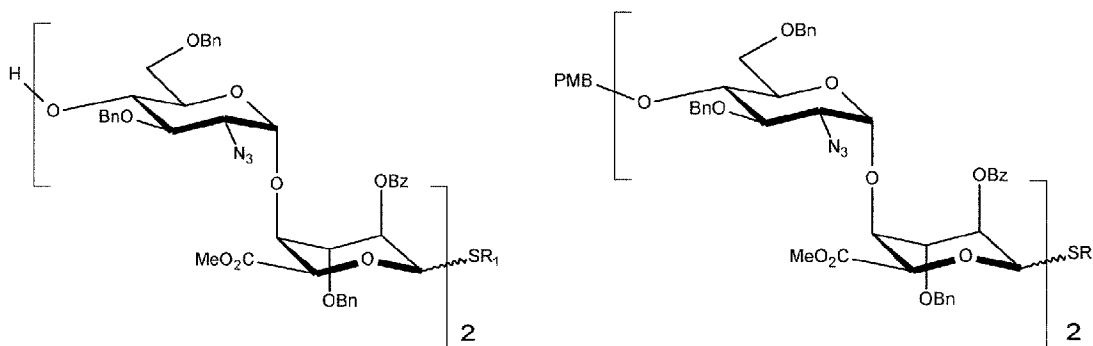
the thioglycoside donor, preferably each of R_3 - R_6 in the compounds of formulae (VIIIa), (VIIIb), (IXa) and (IXb) is a protecting group.

- In preferred embodiments B is a thio leaving group as defined above (such as SR_1). In preferred
 5 embodiments, the chain extending polysaccharide is a compound of formula (X):

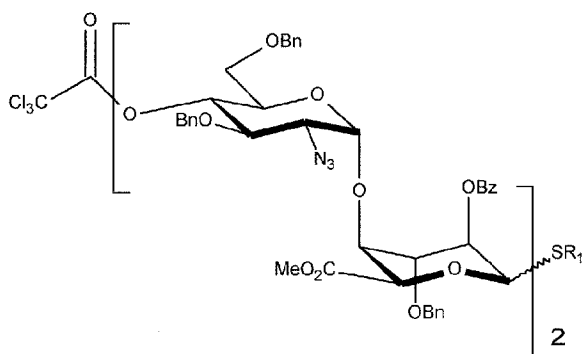


wherein R_5 is as defined above.

Suitably, the chain extending polysaccharide may be selected from the following formulae:

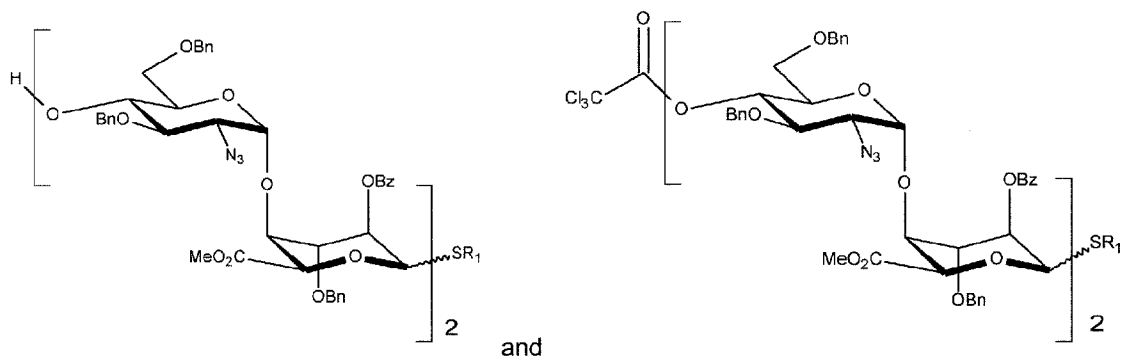


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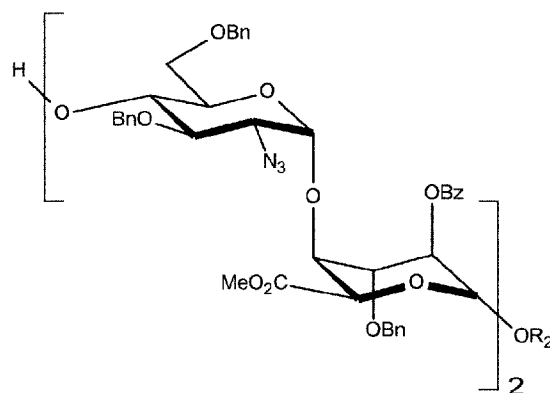
and

, such as selected from



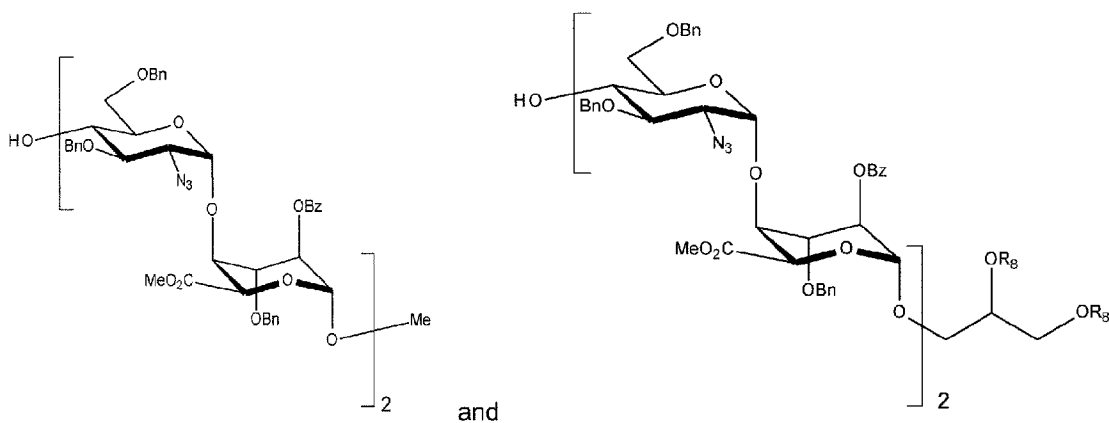
In other embodiments, B is OR_2 . Preferably the compound of formula (Ia) is a compound of formula (VIIIa) or (VIIIb), more preferably (VIIIa), wherein B is OR_2 .

- 5 In preferred embodiments of formulae (VIIIa), (VIIIb), (IXa) and (IXb), when B is OR_2 , R_5 is H. Suitably, the chain extending polysaccharide is selected from compounds of the following formula:



wherein R_2 is as defined in any embodiment above.

- 10 In embodiments, the chain extending polysaccharide is a compound selected from the following formulae:



wherein R_8 is H or a hydroxyl protecting group.

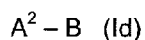
Unless otherwise specified above, the respective R-group definitions for the chain extending polysaccharide is as defined above for the polysaccharide.

First polysaccharide

5 Length

The first polysaccharide is of four or more monosaccharide residues in length. Thus, this refers to polysaccharide wherein the longest chain of monosaccharides from an anomeric donor terminus is four or more monosaccharide residues in length, such as 4 to 36 residues in length.

10 In embodiments, the first polysaccharide is a compound of formula (Id):



wherein

A^2 is a chain of 4 or more monosaccharide residues in length; and

15 B is bonded to the anomeric carbon of a monosaccharide residue in the chain, wherein B is a thio leaving group as defined above, $-OR_2$, or $-SeR_2$, preferably a thio leaving group as defined above or $-OR_2$, more preferably $-OR_2$, wherein R_2 is as defined above.

In embodiments, the first polysaccharide is of 5 or more monosaccharide residues in length, preferably 6 or more monosaccharide residues in length, more preferably 7 or more, 8 or more, 9 or
20 more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, or 35 or more (e.g. 36) monosaccharide residues in length. The first polysaccharide may be up to 70 monosaccharide residues in length, such as up to 60 residues, e.g.
25 up to 50 residues.

In embodiments, the first polysaccharide is of 5 to 36 monosaccharide residues in length, preferably 6 to 36 monosaccharide residues in length, more preferably 7 to 36, 8 to 36, 9 to 36, 10 to 36, 11 to 36, 12 to 36, 13 to 36, 14 to 36, 15 to 36, 16 to 36, 17 to 36, 18 to 36, 19 to 36, 20 to 36, 21 to 36, 22 to
30 36, 23 to 36, 24 to 36, 25 to 36, 26 to 36, 27 to 36, 28 to 36, 29 to 36, 30 to 36, 31 to 36, 32 to 36, 33 to 36, 34 to 36, or 35 to 36 monosaccharide residues in length. Typically, the first polysaccharide is of 8 or more monosaccharide residues in length.

Aside from the difference in chain length as set out above, the first polysaccharide may be suitably
35 defined as set out above for the polysaccharide and chain extending polysaccharide provided at least one of the chain extending and first polysaccharides has a thio leaving group bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue in the chain.. Accordingly, the limitations described above may also be applied analogously to the first polysaccharide. For the avoidance of doubt, the first polysaccharide may be defined independently of the chain extending polysaccharide

such that the monosaccharide components of the first and chain extending polysaccharides may be the same or different.

Monosaccharide residues

- 5 Any combination of monosaccharide residues may be used in the first polysaccharides of the present invention. Suitably, the monosaccharide residues in the first polysaccharide chain may be as defined above for the polysaccharide.

Protecting groups

- 10 The first polysaccharide may be suitably protected by any respective protecting group as defined herein. Any nucleophilic moiety present in the first polysaccharide may be protected, such as hydroxy and / or amino groups.

- 15 The polysaccharide as defined above is at least in part the product of reaction between a chain extending polysaccharide and a first saccharide. Accordingly, the protecting groups of the first polysaccharide may be defined above for the polysaccharide and / or chain extending polysaccharide.

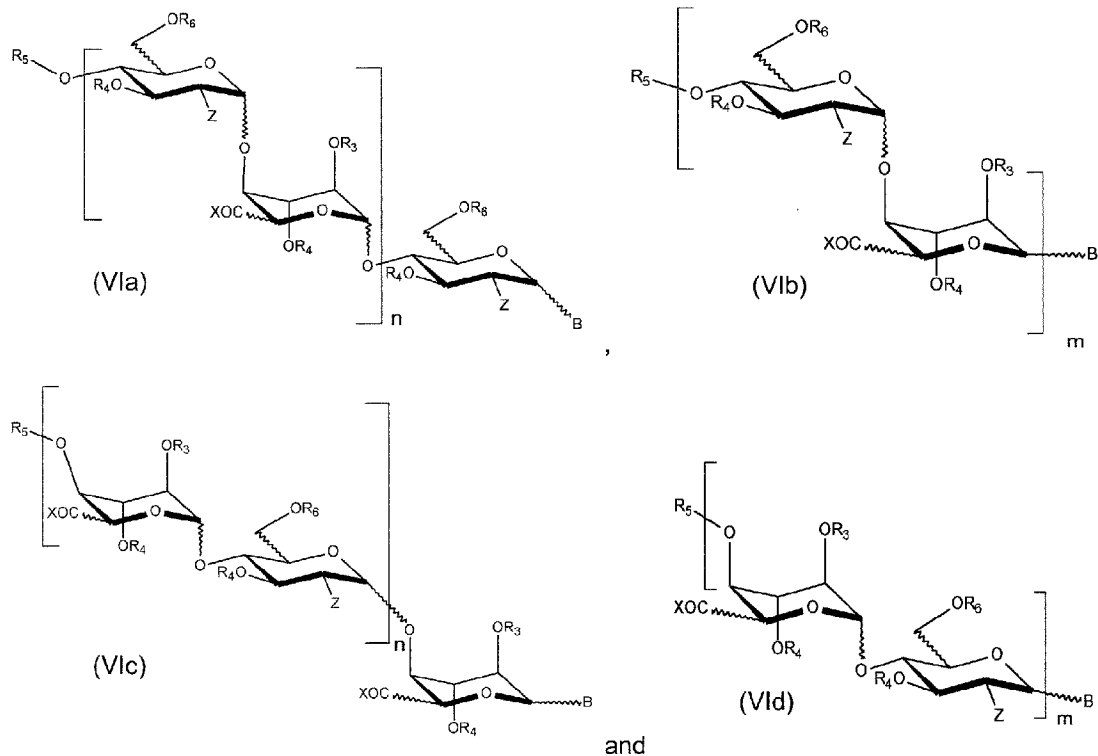
- 20 Suitably, when the first polysaccharide is the nucleophile (i.e. glycoside acceptor) in the coupling step, at least one nucleophilic moiety (e.g. hydroxyl and / or amino groups) is unprotected. Thus, typically all other potentially nucleophilic groups in the first polysaccharide may be protected to provide complete control over which nucleophilic group reacts in the glycosidic bond formation.

- Alternatively, in embodiments wherein the first polysaccharide is the glycoside donor in the reaction, i.e. wherein the coupling is by substitution of the thio leaving group from the first polysaccharide, the first polysaccharide may be suitably protected to prevent self-condensation and runaway iterative extension of the chain extended product, preferably wherein each nucleophilic group in the first polysaccharide is protected, optionally by orthogonal protecting groups. In embodiments wherein the resulting chain extended polysaccharide is required to participate as a nucleophile in a further chain extending step, one or more protecting groups in the chain extended polysaccharide may thus be deprotected selectively to reveal the respective nucleophile (e.g. hydroxyl / amino groups) prior to reaction with a further chain extending thioglycoside donor.
- 25
- 30

Preferred embodiments of the first polysaccharide

- Where the first polysaccharide is of four monosaccharide residues in length, the first polysaccharide may be independently defined in accordance with embodiments of the chain extended polysaccharide as described above.
- 35

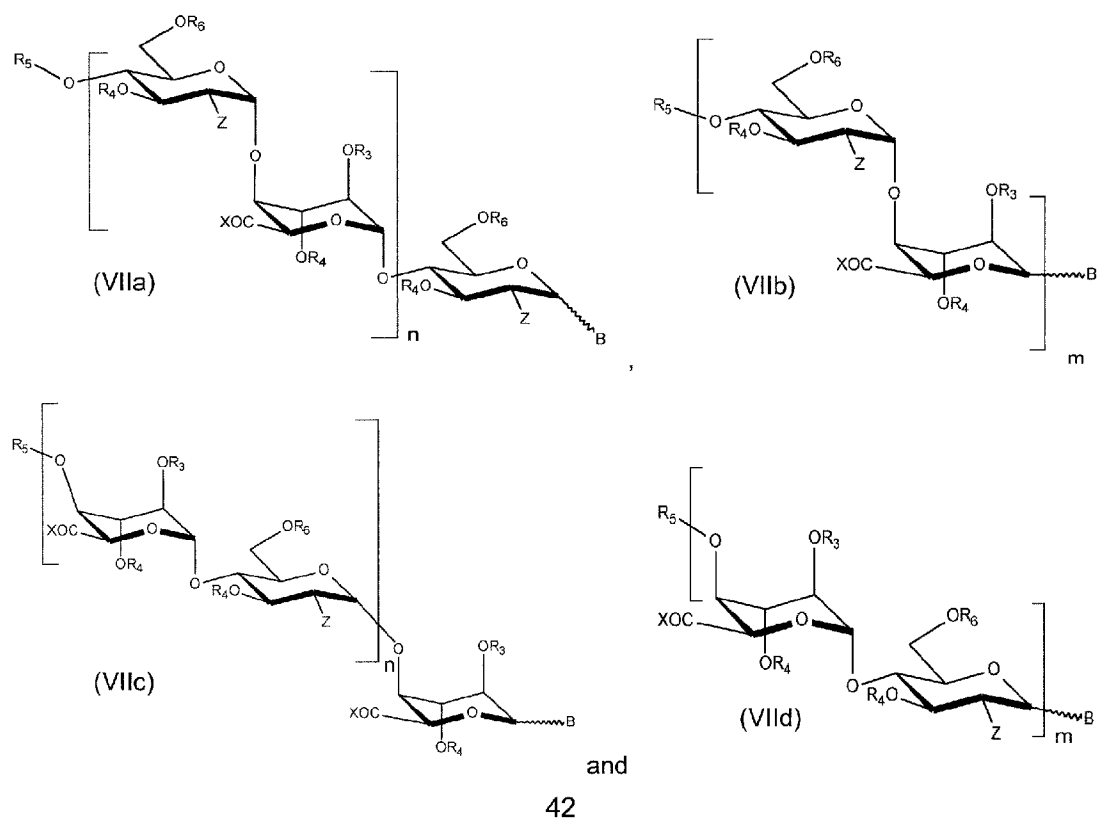
In embodiments, the first polysaccharide comprises an alternating sequence of residues of formulae (IIa) and (IIIa), preferably the first polysaccharide is a compound selected from formulae (VIa-d):



wherein B, R₃-R₆, X and Z are as defined above, n is an integer of 2 or more, such as 2 to 17; and m is an integer of 2 or more, such as 2 to 18.

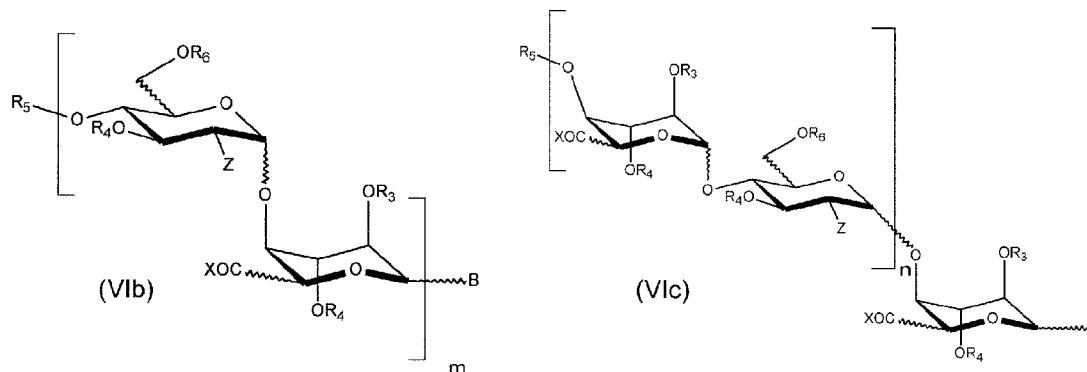
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In preferred embodiments, the first polysaccharide may be selected from formulae (VIIa-d):



wherein B, R₃-R₆, X and Z are as defined according to any of the above embodiments, n is an integer of 2 or more, such as 2 to 17; and m is an integer of 2 or more, such as 2 to 18.

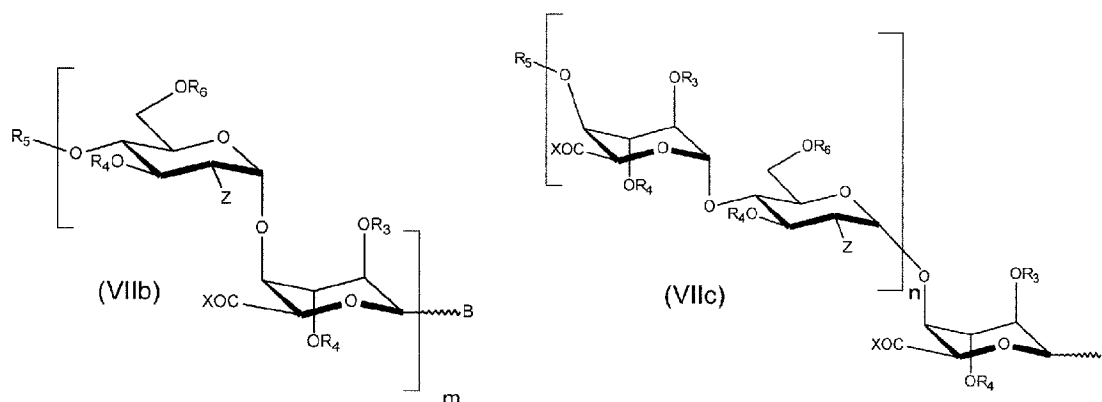
In embodiments, the first polysaccharide is a compound of formula (VIb) or (VIc):



5

wherein B, R₃-R₆, X and Z are as defined according to any of the above embodiments, n is an integer of 2 or more, such as 2 to 17; and m is an integer of 2 or more, such as 2 to 18.

Preferably, the first polysaccharide is a compound of formula (VIIb) or (VIIc):



10

wherein B, R₃-R₆, X and Z are as defined according to any of the above embodiments, n is an integer of 2 or more, such as 2 to 17; and m is an integer of 2 or more, such as 2 to 18.

Preferred protecting groups

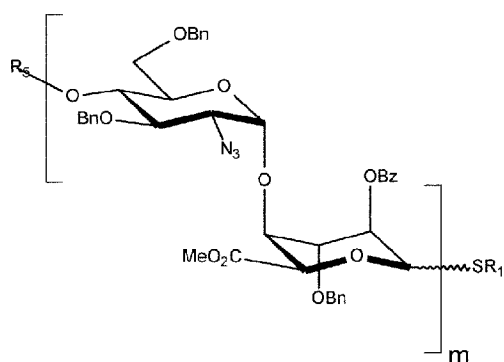
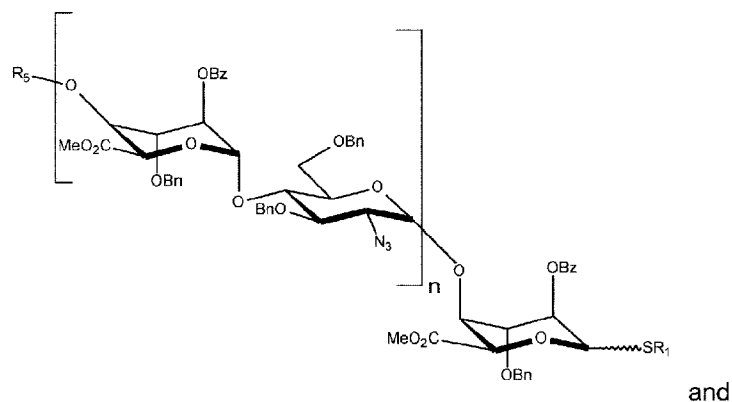
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As described above under "protecting groups" the skilled person will understand that when the first polysaccharide is the nucleophile in the glycosidic bond forming reaction, at least one of R₃-R₆ in the first polysaccharide must be H in order to facilitate the glycosidic bond formation with the chain extending polysaccharide. In such embodiments, typically only one of R₃-R₆ is H. Preferably, in such embodiments R₅ is H.

20

Furthermore, where the first polysaccharide is the thioglycoside donor, one or more of R₃-R₆ in the compounds of formula (VIa-d) and (VIIa-d) may be a protecting group in order to control the glycosidic bond formation. In typical embodiments, where the first polysaccharide is the thioglycoside donor, preferably each of R₃-R₆ in the compounds of formulae (VIa-d) and (VIIa-d) is a protecting group.

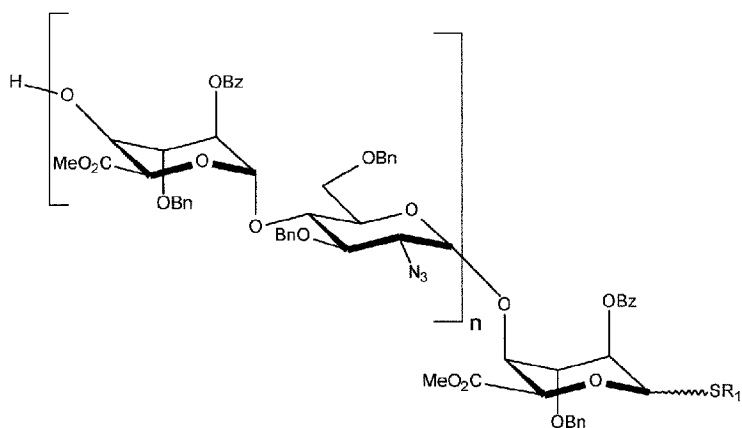
In embodiments, B is a thio leaving group (e.g. SR_1). Thus, suitably the first polysaccharide is a compound of the following formulae:

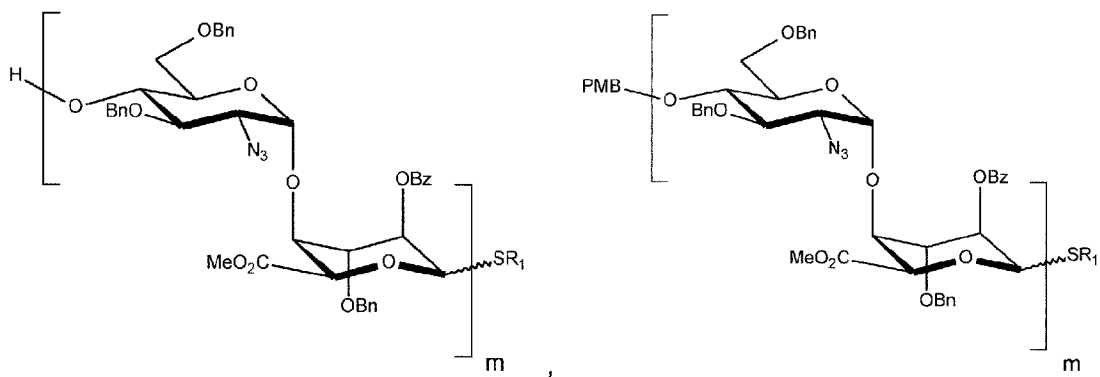
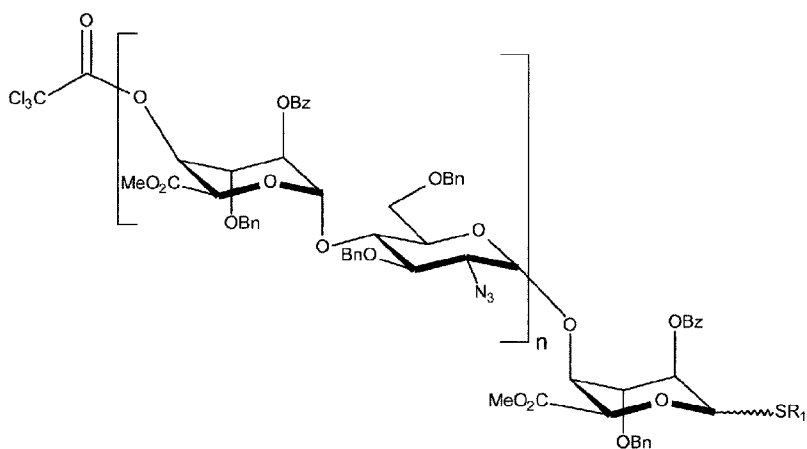
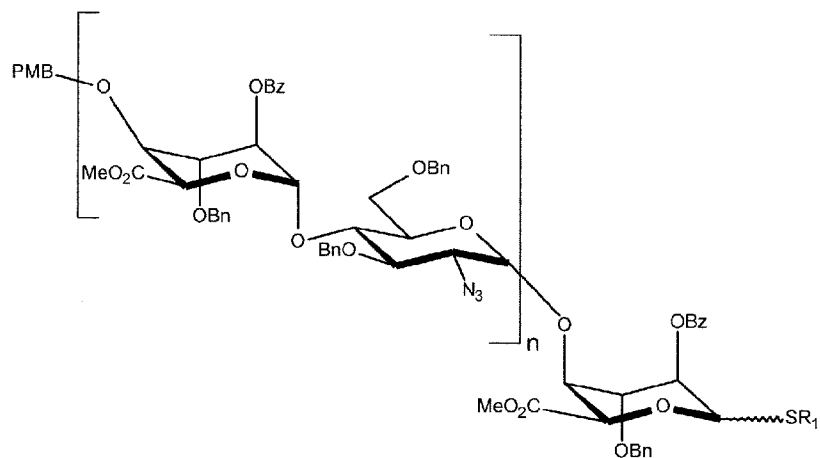


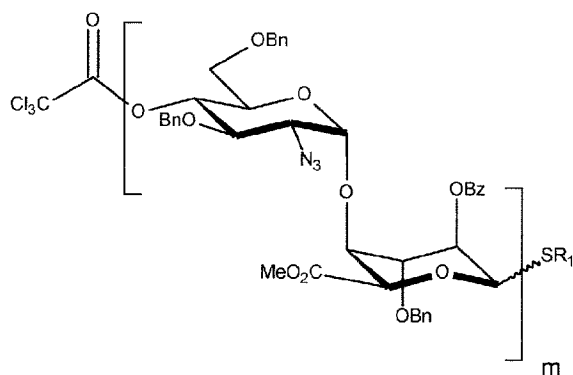
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wherein R_5 is as defined above; n is an integer of 2 or more, such as 2 to 17; and m is an integer of 2 or more, such as 2 to 18.

Preferably, the first polysaccharide is selected from the following formulae:





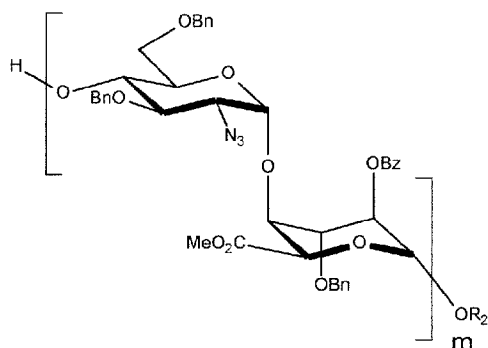
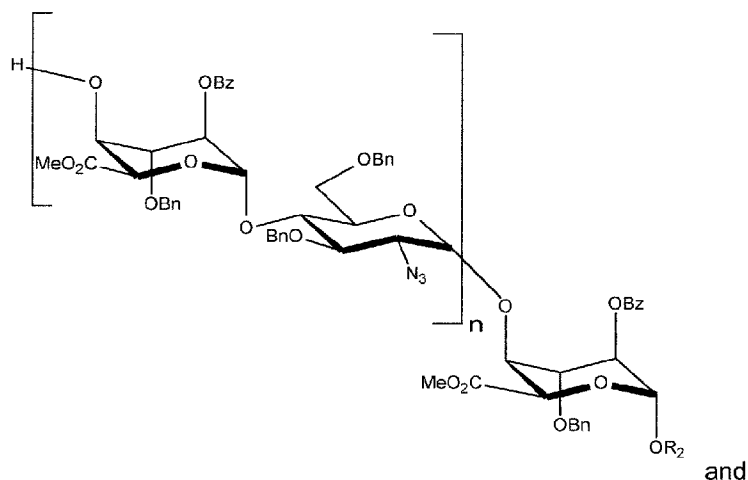


and

wherein R_1 , m and n are as defined above.

- 5 In preferred embodiments, B is OR_2 (i.e. wherein the chain extending polysaccharide is the thioglycoside donor). For instance, where the first polysaccharide is a compound of formula (Id) as described above, B is preferably OR_2 , more preferably wherein R_5 is H.

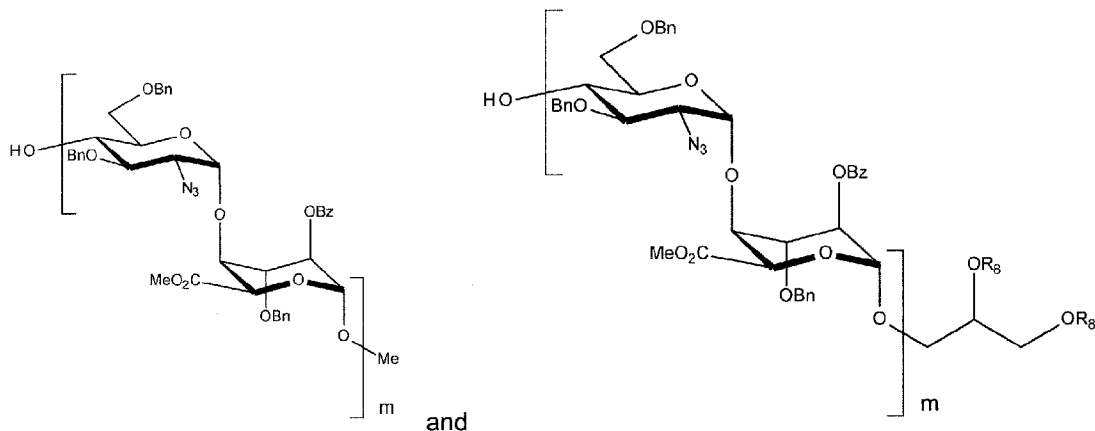
Suitably, the first polysaccharide is selected from compounds of the following formula:



10

wherein R_2 is as defined in any embodiment above; n is an integer of 2 or more, such as 2 to 17; and m is an integer of 2 or more, such as 2 to 18.

Preferably, the first polysaccharide is a compound selected from the following formulae:



wherein R_8 is H or a hydroxyl protecting group;
 m is 2 or more, such as 2 to 18.

- 5 In the above embodiments m is an integer of 2 or more, preferably 3 or more, more preferably 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, or 16 or more, 17 or more, 18 or more, or 19 or more. In embodiments, m is an integer of 2 to 18, preferably 3 to 18, more preferably 4 to 18, 5 to 18, 6 to 18, 7 to 18, 8 to 18, 9 to 18, 10 to 18, 11 to 18, 12 to 18, 13 to 18, 14 to 18, 15 to 18, 16 to 18, or 17 to 18, e.g. 18.

- 10 In the above embodiments, n is an integer of 2 or more, preferably 3 or more, more preferably 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, or 16 or more. In embodiments, n is an integer of 2 to 17, preferably 3 to 17, more preferably 4 to 17, 5 to 17, 6 to 17, 7 to 17, 8 to 17, 9 to 17, 10 to 17, 11 to 17, 12 to 17, 13 to 17, 14 to 17, 15 to 17, or 16 to 17, e.g. 17.

The respective R-group definitions for the first polysaccharide are as defined above for the polysaccharide.

Chain extended polysaccharide

The process of the present invention comprises the preparation of a chain extended polysaccharide resulting from the coupling of a first polysaccharide and chain extending polysaccharide as defined herein.

25 The chain extended polysaccharide is a product prepared by combination of chain extending and first polysaccharides. Accordingly, the skilled person will understand that the structure of the chain extended polysaccharide will depend on the chain extending and first polysaccharides. The chain extending polysaccharide may therefore be suitably defined as above for the chain extending polysaccharide, first polysaccharide or polysaccharide.

Length

The chain extended polysaccharide according to the present invention is of eight or more monosaccharide residues in length. This refers to a polysaccharide wherein the longest chain of monosaccharides from an anomeric donor terminus is eight or more monosaccharide residues in length, preferably 8 to 40 residues in length. Typically, the chain extended polysaccharide is not branched.

In embodiments, the chain extended polysaccharide is of 9 or more monosaccharide residues in length, preferably 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, or 39 or more (e.g. 40) monosaccharide residues in length. Suitably, the chain extended polysaccharide may be up to 70 monosaccharide residues in length, preferably 60 residues, 50 residues and most preferably 40 residues.

In embodiments, the chain extended polysaccharide is of 9 to 40 monosaccharide residues in length, preferably 10 to 40, 11 to 40, 12 to 40, 13 to 40, 14 to 40, 15 to 40, 16 to 40, 17 to 40, 18 to 40, 19 to 40, 20 to 40, 21 to 40, 22 to 40, 23 to 40, 24 to 40, 25 to 40, 26 to 40, 27 to 40, 28 to 40, 29 to 40, 30 to 40, 31 to 40, 32 to 40, 33 to 40, 34 to 40, 35 to 40, 36 to 40, 37 to 40, or 38 to 40 or 39 to 40 monosaccharide residues in length. Preferably, the chain extended polysaccharide is of 12 or more monosaccharide residues in length.

Monosaccharide residues

Any combination of monosaccharides residues may be present in the chain extended polysaccharides of the present invention. Suitably, the monosaccharide residues may be as defined above for the polysaccharide.

Protecting groups

In the chain extended polysaccharides of the present invention, any hydroxyl and / or amine moieties in the monosaccharide residues may be protected by respective hydroxyl and / or amino protecting groups as defined herein. Suitably, the protecting groups may be as defined above for the chain extending and first polysaccharides.

As explained above, it is often desirable to provide at least one protecting group in the reacting chain extending and first polysaccharides in order to provide a greater degree of control in the glycosidic bond formation and also in further functionalization of the chain extended polysaccharide. In preferred embodiments, a single nucleophilic group will be present in the coupling reaction to form the chain extended polysaccharide and other groups will be protected as this allows for the greatest degree of

regioselective control in the glycosidic bond formation. Accordingly, the direct product of such glycosidic bond formation will typically be fully protected. Thus, in some embodiments, each nucleophilic group in the chain extended polysaccharide is protected, for instance each hydroxyl and / or amino group is protected by suitable hydroxyl and amino protecting groups described herein.

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In embodiments wherein the resulting chain extended polysaccharide is required to participate as a nucleophile in a further chain extending step (see the "further process steps" section below), the process typically further comprises the deprotection of one or more protecting groups, preferably one protecting group, to reveal a nucleophilic group that can then participate in a further chain extending step by coupling with a further chain extending thioglycoside donor. Deprotection of a single nucleophile in the chain extended polysaccharide may be achieved by employing suitable orthogonal protecting groups in the chain extending and first polysaccharides.

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Orthogonal protecting group strategies such as described above would therefore be particularly suitable for this process in order to allow selective deprotection of the chain extended polysaccharide prior to further reaction, thus controlling the site of further chain extension and / or derivatization.

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Preferred chain extended polysaccharides

The chain extended polysaccharide is the product of coupling the chain extending and first polysaccharides and thus the preferred embodiments described above for the chain extended and first polysaccharides may be applied analogously to the chain extended polysaccharide.

20

Further process steps

In embodiments, the process comprises a further chain extending step, the further chain extending step comprising coupling a further chain extending polysaccharide of four monosaccharide residues in length with the chain extended polysaccharide by substitution of a thio leaving group (e.g. $-SR^1$) bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue in either the chain extending polysaccharide or the further chain extended polysaccharide to form a chain extended polysaccharide of 12 or more monosaccharide residues in length, e.g. 12 to 40 monosaccharide residues in length.

25

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Thus, the process in other words comprises a first chain extending step comprising the coupling of a chain extending polysaccharide and a first polysaccharide followed by a second chain extending step comprising coupling a second chain extending polysaccharide with the chain extended polysaccharide to form a second chain extended polysaccharide.

35

The chain extending and chain extended polysaccharides may be as defined above and the statements above in the "coupling reaction" and "nucleophilic substitution", "chain extending" and "first polysaccharides" sections apply analogously here.

In some embodiments, the process is for preparing a polysaccharide of 12 or more monosaccharide residues in length, preferably 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, e.g. 40 monosaccharide residues in length. In
5 embodiments, the polysaccharide may be up to 70 monosaccharide residues in length, suitably up to 60 residues, such as up to 50 residues, preferably up to 40 residues. In embodiments, the process is for preparing a polysaccharide of 13 to 40 monosaccharide residues in length, preferably 14 to 40, 15 to 40, 16 to 40, 17 to 40, 18 to 40, 19 to 40, 20 to 40, 21 to 40, 22 to 40, 23 to 40, 24 to 40, 25 to 40,
10 26 to 40, 27 to 40, 28 to 40, 29 to 40, 30 to 40, 31 to 40, 32 to 40, 33 to 40, 34 to 40, 35 to 40, 36 to 40, 37 to 40, 38 to 40 or 39 to 40 monosaccharide residues in length. In preferred embodiments, the process is for preparing a polysaccharide of 12 to 40 monosaccharide residues in length.

In embodiments, the process comprises two or more further chain extending steps, such as two to
15 seven further chain extending steps, wherein each further chain extending step comprises coupling a further chain extending polysaccharide of four monosaccharide residues in length with a chain extended polysaccharide by substitution of a thio leaving group (e.g. $-SR^1$) bonded to the anomeric carbon of a monosaccharide residue of either the chain extending polysaccharide or the chain extended polysaccharide to form a chain extended polysaccharide of 16 or more monosaccharide
20 residues in length, e.g. 16 to 40 monosaccharide residues in length. Thus, the process comprises a third, fourth, fifth, sixth, seventh, eighth, ninth and tenth, etc. chain extending step wherein a respective third, fourth, fifth etc. chain extending polysaccharide is coupled with a respective second, third, fourth, fifth, etc. chain extended polysaccharide. Suitably, the respective chain extending and chain extended polysaccharides are as defined above. The process is for preparing a polysaccharide
25 of 16 or more monosaccharide residues in length, preferably 17 or more, more preferably 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, or 38 or more, e.g. 40 monosaccharide residues in length. In
30 embodiments, the polysaccharide may be up to 70 monosaccharide residues in length, suitably up to 60 residues, such as up to 50 residues, preferably up to 40 residues. Suitably, the process may be for preparing a polysaccharide of 16 to 40 monosaccharide residues in length, preferably 17 to 40 monosaccharide residues in length, more preferably 18 to 40, 19 to 40, 20 to 40, 21 to 40, 22 to 40, 23 to 40, 24 to 40, 25 to 40, 26 to 40, 27 to 40, 28 to 40, 29 to 40, 30 to 40, 31 to 40, 32 to 40, 33 to 40, 34 to 40, 35 to 40, 36 to 40, 37 to 40, 38 to 40, or 39 to 40 monosaccharide residues in length.

35 Accordingly, the present invention provides the skilled person with means to access long chain polysaccharides cleanly and efficiently by iterative addition of chain extending polysaccharide building blocks of four monosaccharide residues in length. A skilled person can use the process of the present invention to access to any conceivable sequence of monosaccharides by controlling the
40 monosaccharide sequence within the chain extending and first polysaccharides.

Suitably, each chain extending polysaccharides used in a chain extension step may be the same or different to one or more chain extending polysaccharides used in one or more previous steps. In other words, the first, second, third and fourth, etc. chain extending polysaccharides may be the same or different to one or more previous chain extending polysaccharides. In some embodiments, one or more chain extending polysaccharides are the same, e.g. wherein each is identical. In other embodiments, one or more chain extending polysaccharides is different, e.g. wherein each chain extending polysaccharide used is different. Thus, by varying the chain extending polysaccharides, a diverse array of polysaccharides can be accessed using this chemistry (see, e.g. figure 3).

The skilled person may suitably manipulate the chain extended polysaccharide at any point in the process. In embodiments, the process further comprises the step of coupling the chain extended polysaccharide with a further saccharide, which may be a monosaccharide, or a polysaccharide of two or three monosaccharide residues in length to form a chain extended polysaccharide of 9 or more monosaccharide residues in length, e.g. 9 to 40 monosaccharide residues in length. Any suitable further saccharide may be used. In embodiments, the process is for preparing a chain extended polysaccharide of 10 or more, preferably 11 or more, more preferably 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, e.g. 40 monosaccharide residues in length. Suitably, the polysaccharide may be up to 70 monosaccharide residues in length, such as up to 60 residues, e.g. up to 50 residues, preferably up to 40 residues. In embodiments, the process is for preparing a polysaccharide of 10 to 40 monosaccharide residues in length, preferably 11 to 40, 12 to 40, 13 to 40, 14 to 40, 15 to 40, 16 to 40, 17 to 40, 18 to 40, 19 to 40, 20 to 40, 21 to 40, 22 to 40, 23 to 40, 24 to 40, 25 to 40, 26 to 40, 27 to 40, 28 to 40, 29 to 40, 30 to 40, 31 to 40, 32 to 40, 33 to 40, 34 to 40, 35 to 40, 36 to 40, 37 to 40, 38 to 40, or 39 to 40 monosaccharide residues in length. Typically, the process is for preparing a polysaccharide of 12 to 40 monosaccharide residues in length, preferably 16 to 40 monosaccharide residues in length, more preferably 20 to 40, most preferably 24 to 40 monosaccharide residues in length.

Where the relevant chain extending, first and / or chain extended polysaccharide comprises one or more protecting groups (such as hydroxyl and / or amino protecting groups), the process may include one or more deprotection steps at any stage in the process. For instance, as explained above, one or more protecting groups of the chain extended polysaccharide may be deprotected in order to reveal a suitable nucleophilic moiety that may be used in a further chain extension step with a further chain extending polysaccharide. Typically, a suitable orthogonal protecting group strategy may be adopted which will allow the selective removal of one or more protecting groups in the chain extended polysaccharide.

In some embodiments, the process may also comprise one or more derivatization steps, such as a functional group protection step. In embodiments, the further derivatization step includes conjugation of the polysaccharide to a non-saccharide moiety, sulfation, sulphonation, phosphorylation and / or phosphonation of one or more hydroxyl and / or amino groups in the chain extended polysaccharide.

- 5 Preferably, the process includes the step of sulfation of the chain extended polysaccharide to provide a sulfated polysaccharide, such as a heparin-type polysaccharide. In embodiments, the process also comprises the step of forming the respective acid or base addition salts of any basic or acidic functionality in the polysaccharide. Exemplary basic / acidic functionalities include amino groups, carboxylic acid groups and sulphate groups. Appropriate salts are defined herein.

10

Further aspects of the invention

Second aspect

- 15 In a second aspect, the invention provides a polysaccharide of 8 or more monosaccharide residues in length, e.g. 8 to 40, wherein a thio leaving group is bonded to the anomeric carbon of a monosaccharide residue in the polysaccharide. Suitably, the thio leaving group is an SR₁ group as defined above.

- 20 As explained above, evidence suggests that longer polysaccharide chain lengths are more attractive targets from a biological perspective. Accordingly, the synthetic polysaccharides of the following aspects of the invention represent flexible and thus valuable synthetic scaffolds that can be accessed by the novel processes taught herein and which can be easily manipulated and derivatised, e.g. for further investigation in drug discovery research, such as in activity assays and pharmacokinetic studies. The compounds described in the second aspect (which may be accessed by the process of the present invention as described herein) have particular utility because they suitably contain a thio
- 25 leaving group bonded to the anomeric carbon of a monosaccharide residue in the polysaccharide chain, which can facilitate further derivatization at the anomeric terminus, such as by adding biological fluorescent/ radio labels, further saccharide units (such as using the methods taught herein), and / or other anomeric end-capping groups.

- 30 Preferably the polysaccharide is isolated. Suitably, the polysaccharide may be substantially pure. In embodiments, "substantially pure" means that at least 80mol% of the total polysaccharide content of a sample of the polysaccharide consists of a polysaccharide of the second aspect of the invention, such as at least 85 mol%, 90 mol%, 91 mol%, 92 mol%, 93 mol%, 94 mol%, 95 mol%, 96 mol%, 97 mol%, 98 mol% or more preferably at least 99mol%. In preferred embodiments, "substantially pure" means
- 35 that at least 80mol% of the total content of a sample of the polysaccharide consists of a polysaccharide of the second aspect of the invention, such as at least 85 mol%, 90 mol%, 91 mol%, 92 mol%, 93 mol%, 94 mol%, 95 mol%, 96 mol%, 97 mol%, 98 mol% or more preferably at least 99mol%.

In embodiments, the isolated polysaccharide is in solid form. Preferably, the isolated solid form of the polysaccharide comprises at least 80mol% of a polysaccharide of the second aspect; more preferably 85mol%, 90mol%, 91mol%, 92mol%, 93mol%, 94mol%, 95mol%, 96mol%, 97mol%, 98mol% or most preferably at least 99mol% of a polysaccharide of the second aspect.

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In embodiments, the polysaccharide is a synthetic polysaccharide.

In a further aspect, the invention provides a composition comprising the polysaccharide of the second aspect of the invention, for instance a solid composition comprising an isolated solid form as defined above.

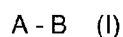
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The process of the first aspect and embodiments is generally applicable to the synthesis of any type of polysaccharide. The synthetic polysaccharide of the second aspect may be of any polysaccharide structure provided it is of 8 or more monosaccharide residues in length and has a thio leaving group bonded to the anomeric carbon of a monosaccharide residue in the polysaccharide chain. In some embodiments, the polysaccharide of the second aspect is structurally defined in accordance with the polysaccharide of the first aspect and embodiments of the invention, provided that a thio leaving group bonded to the anomeric carbon of a monosaccharide residue in the polysaccharide chain.

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Thus, the polysaccharide of the second aspect may be a compound of formula (I):



wherein B is bonded to the anomeric carbon of a monosaccharide residue in the polysaccharide chain and B is a thio leaving group as defined above for the first aspect.

25

Length

The term "of 8 or more" in this context refers to a synthetic polysaccharide wherein the longest chain of monosaccharides is 8 or more monosaccharide residues in length from an anomeric terminus bearing the thio leaving group, such as 8 to 40 residues in length. In typical embodiments, the synthetic polysaccharide is not branched.

30

In embodiments, the synthetic polysaccharide of the second aspect is of 9 or more monosaccharide residues in length, preferably 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, or 39 or more (e.g. 40) monosaccharide residues in length. Suitably, the polysaccharide may be up to 70 monosaccharide residues in length, such as up to 60 residues, preferably up to 50 residues, more preferably 40 residues. In embodiments, the synthetic polysaccharide is of 9 to 40 monosaccharide residues in length, preferably 10 to 40, 11 to 40, 12 to 40, 13 to 40, 14 to 40, 15 to 40, 16 to 40, 17 to 40, 18 to 40, 19 to 40, 20 to 40, 21 to 40, 22 to 40, 23 to 40, 24 to 40, 25 to 40, 26 to 40, 27 to 40, 28

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to 40, 29 to 40, 30 to 40, 31 to 40, 32 to 40, 33 to 40, 34 to 40, 35 to 40, 36 to 40, 37 to 40, or 38 to 40 or 39 to 40 monosaccharide residues in length. Suitably, the synthetic polysaccharide is of 12 or more monosaccharide residues in length.

5 Monosaccharides

As explained above, any combination of monosaccharide residues may be present in the polysaccharides of the second aspect. Suitably, the monosaccharide residues in the polysaccharide of the second aspect may be as defined above for the polysaccharide, chain extending polysaccharide or first polysaccharide in the first aspect and embodiments of the invention.

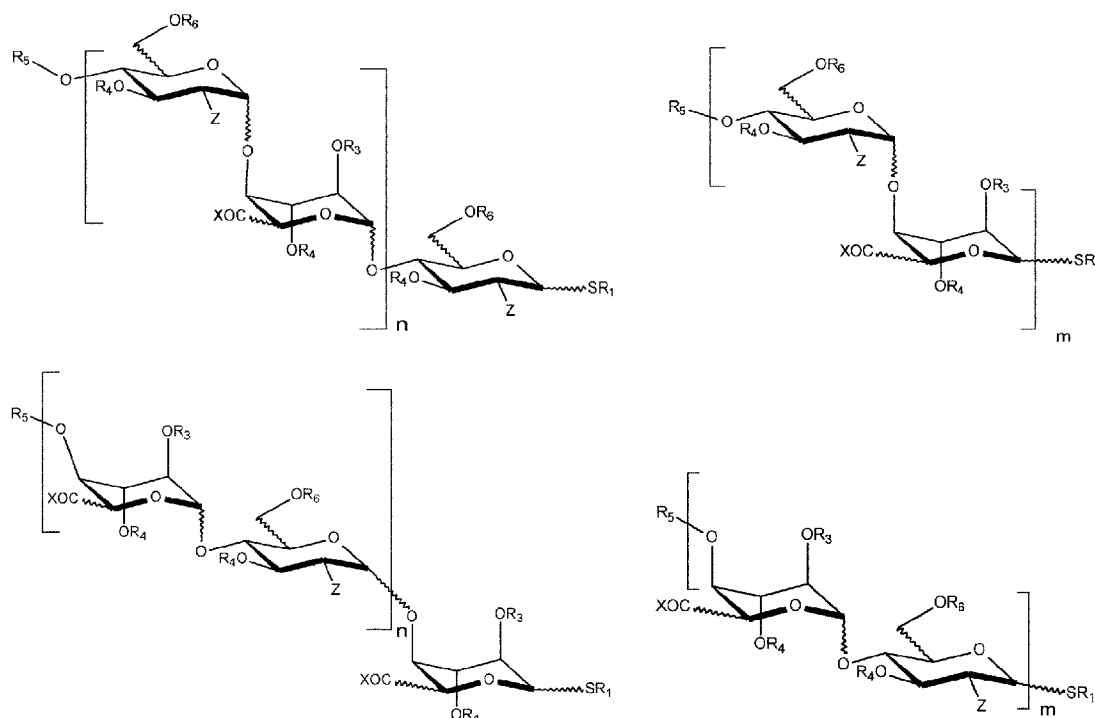
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Protecting groups

In the polysaccharide of the second aspect, any nucleophilic group may be suitably protected, e.g. any of the hydroxyl and / or amine moieties in the monosaccharide residues may be protected by respective hydroxyl and / or amino protecting groups as defined herein. Suitably, the protecting groups may be as defined above for the polysaccharide of the first aspect and embodiments of the invention.

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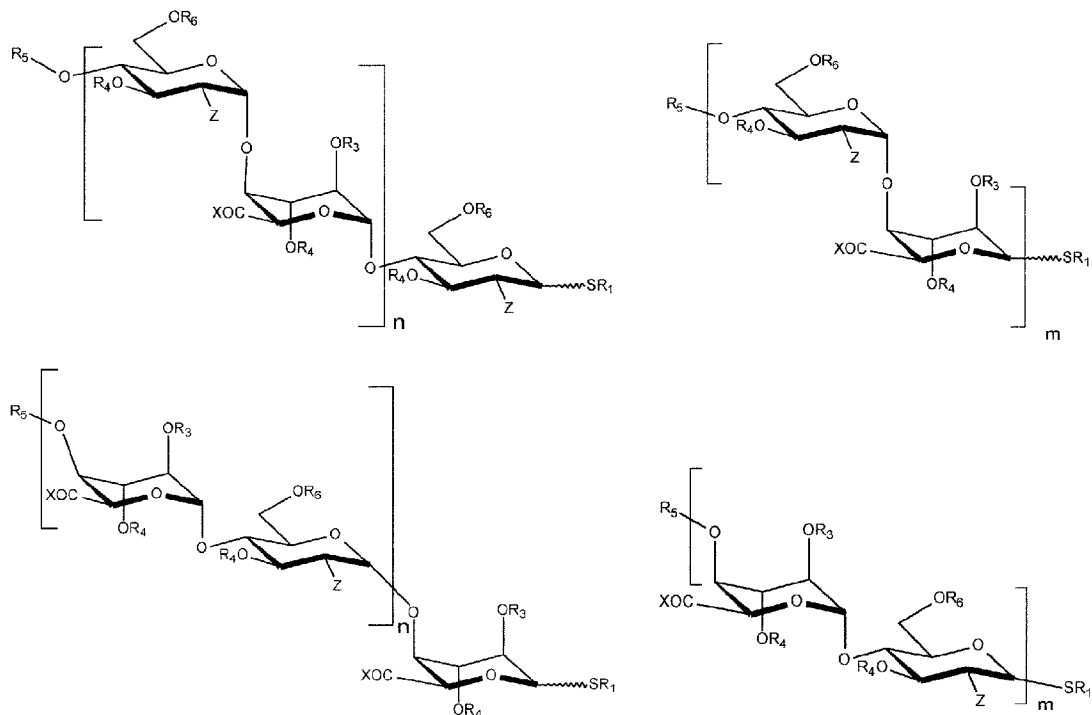
In embodiments, the polysaccharide is selected from the following formulae:



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wherein R_1 , R_3 - R_6 , X and Z are as defined according to any embodiment above, n is an integer of 4 or more, such as 4 to 19; and m is an integer from 4 or more, such as 4 to 20.

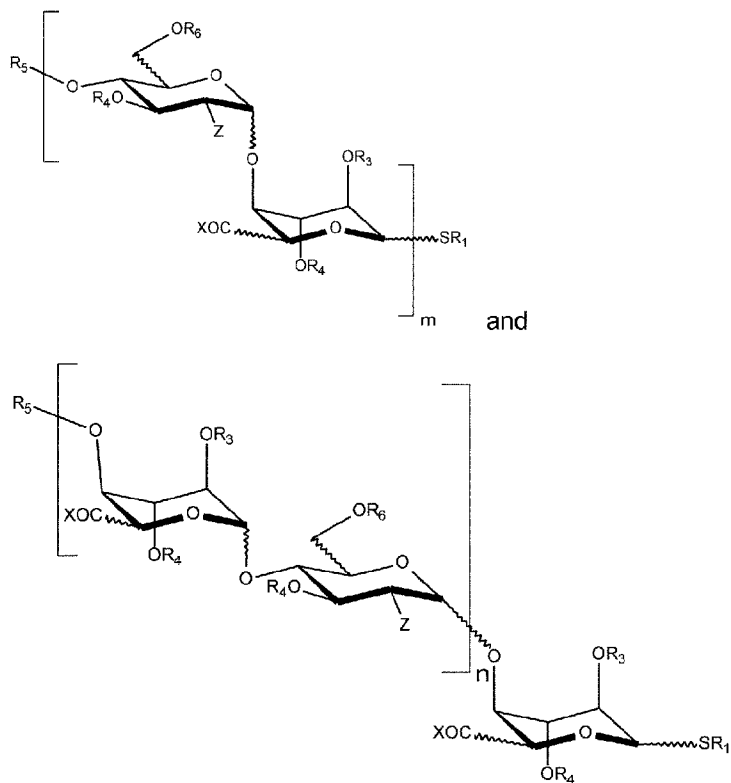
In embodiments, the polysaccharide may be selected from the following formulae:



wherein R_1 , R_3 - R_6 , X and Z are as defined according to any embodiment above, n is an integer of 4 or more, such as 4 to 19, and m is an integer from 4 or more, such as 4 to 20.

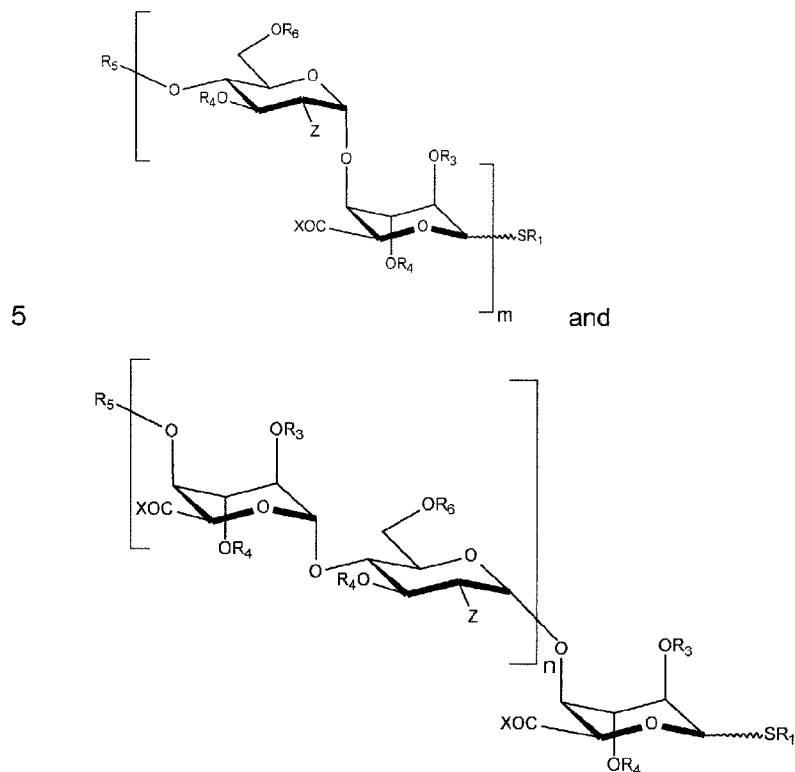
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In preferred embodiments, the polysaccharide is selected from the following formulae:



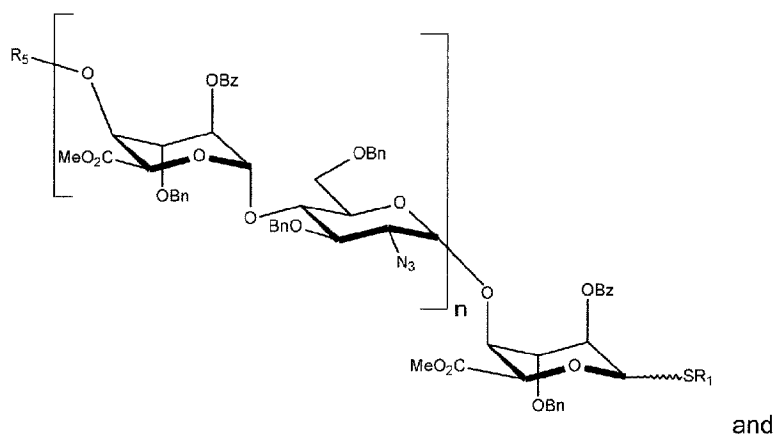
wherein R_3 - R_6 , X and Z are as defined according to any embodiment above, n is an integer of 4 or more, such as 4 to 19; and m is an integer from 4 or more, such as 4 to 20.

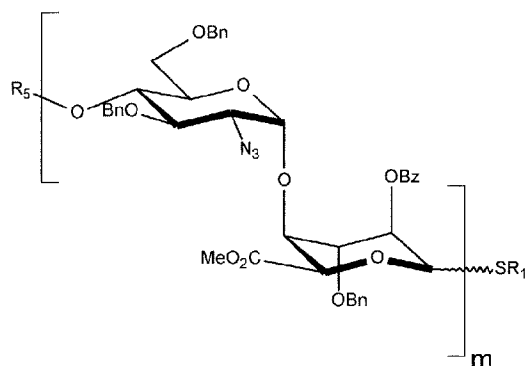
In embodiments, the polysaccharide may be selected from the following formulae:



wherein R_1 , R_3 - R_6 , X and Z are as defined according to any embodiment above, n is an integer of 4 or more, such as 4 to 19; and m is an integer from 4 or more, such as 4 to 20.

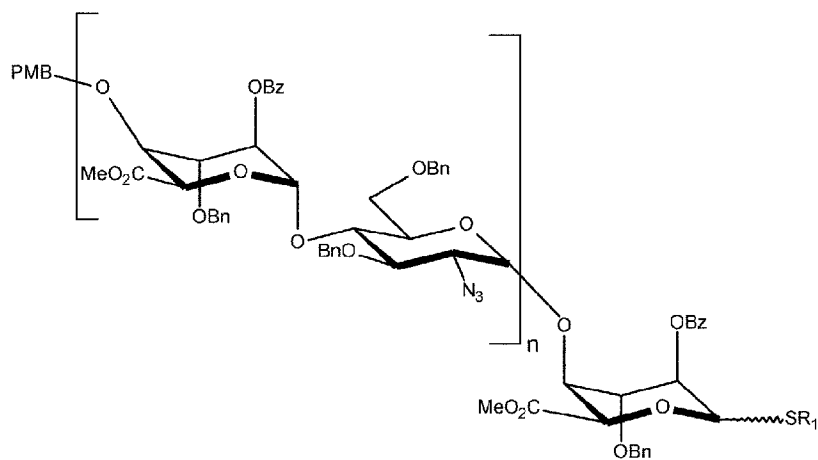
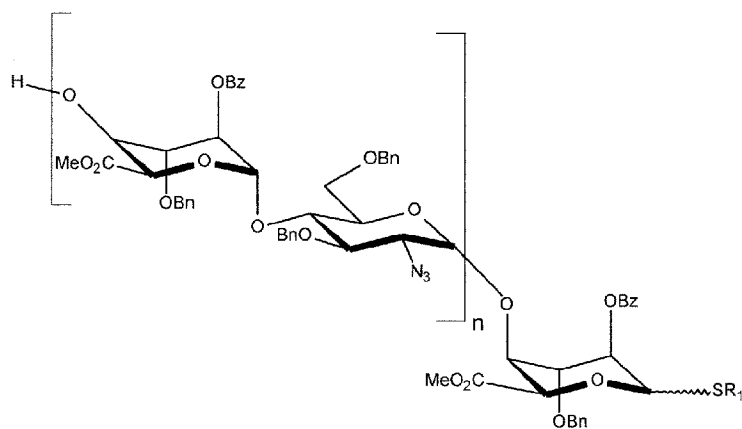
10 In further embodiments, the polysaccharide may be selected from the following formulae:

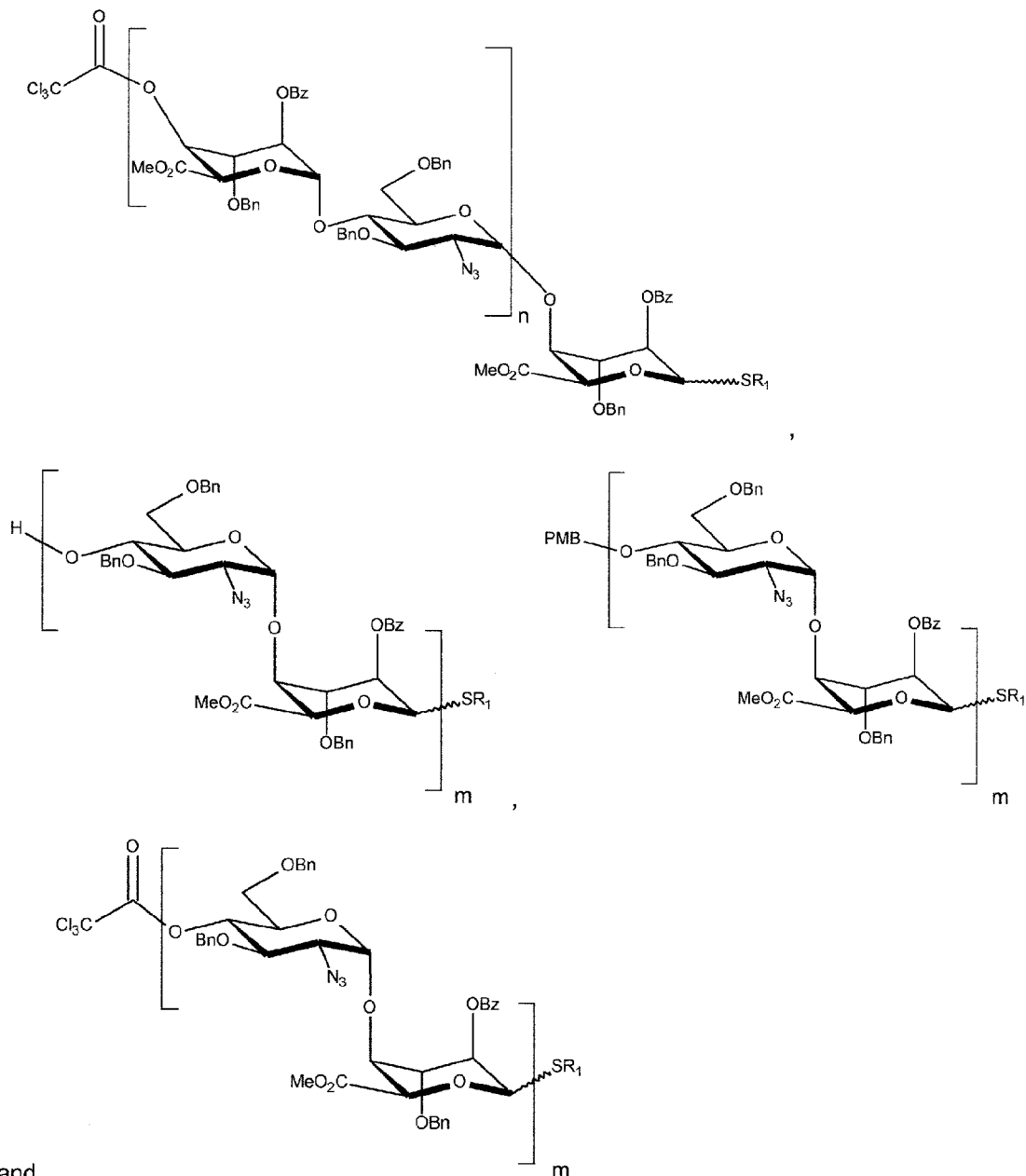




wherein R_1 and R_5 are as defined according to any embodiment above, n is an integer of 4 or more, such as 4 to 19; and m is an integer from 4 or more, such as 4 to 20.

5 Preferably, the polysaccharide of the second aspect is selected from the following formulae:





5

Suitably, m is an integer of 4 or more, preferably 5 or more, such as 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more or 19 or more, e.g. 20. In embodiments, m is an integer of 4 to 20, such as 5 to 20, 6 to 20, 7 to 20, 8 to 20, 9 to 20, 10 to 20, 11 to 20, 12 to 20, 13 to 20, 14 to 20, 15 to 20, 16 to 20, 17 to 20, 18 to 20, or 19-20, e.g. 20.

10

Suitably, n is an integer of 4 or more, preferably 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, or

18 or more, e.g. 19. In embodiments, n is an integer of 5 to 19, such as 6 to 19, 7 to 19, 8 to 19, 9 to 19, 10 to 19, 11 to 19, 12 to 19, 13 to 19, 14 to 19, 15 to 19, 16 to 19, 17 to 19, or 18 to 19, e.g. 19.

Third aspect

- 5 In a third aspect, the invention provides a polysaccharide of 17 or more monosaccharide residues in length, such as 17 to 40. The term "of 17 to 40" in this context refers to a polysaccharide wherein the longest chain of monosaccharides is 17 or more monosaccharide residues in length, such as 17 to 40 residues in length. In some embodiments, the polysaccharide is not branched.
- 10 Preferably the polysaccharide of the third aspect of the invention is an isolated polysaccharide. Suitably, the polysaccharide may be substantially pure. In embodiments, "substantially pure" means that at least 80mol% of the total polysaccharide content of a sample of the polysaccharide is a polysaccharide of the third aspect of the invention, such as at least 85 mol%, 90 mol%, 91 mol%, 92 mol%, 93 mol%, 94 mol%, 95 mol%, 96 mol%, 97 mol%, 98 mol% or more preferably at least 99mol%.
- 15 In preferred embodiments, "substantially pure" means that at least 80mol% of the total content of a sample of the polysaccharide consists of a polysaccharide of the third aspect of the invention, such as at least 85 mol%, 90 mol%, 91 mol%, 92 mol%, 93 mol%, 94 mol%, 95 mol%, 96 mol%, 97 mol%, 98 mol% or more preferably at least 99mol%.
- 20 In embodiments, the isolated polysaccharide is in solid form. Preferably, the isolated solid form of the polysaccharide comprises at least 80mol% of a polysaccharide of the third aspect; more preferably 85mol%, 90mol%, 91mol%, 92mol%, 93mol%, 94mol%, 95mol%, 96mol%, 97mol%, 98mol% or most preferably at least 99mol% of a polysaccharide of the third aspect.
- 25 In embodiments, the polysaccharide is a synthetic polysaccharide.

In a further aspect, the invention provides a composition comprising a polysaccharide of the third aspect of the invention, for instance a solid composition comprising an isolated solid form as defined above.

- 30 The novel processes described herein in relation to the first aspect and embodiments are generally applicable to the synthesis of any type of polysaccharide. The synthetic polysaccharide of the third aspect above may be of any polysaccharide structure, provided it is of 17 or more monosaccharide residues in length. In some embodiments, the synthetic polysaccharide of the third aspect is
- 35 structurally defined in accordance with the polysaccharide of the first aspect and embodiments of the invention, provided the synthetic polysaccharide is of 17 or more monosaccharide residues in length.

In embodiments, the polysaccharide is a compound of formula (Ie):



- 40 wherein

E is a chain of 17 or more monosaccharide residues in length;

B is an $-OR_2$ group bonded to the anomeric carbon of a monosaccharide residue in the polysaccharide chain, wherein R_2 is H or an end-capping group R_{ec} , wherein R_2 and R_{ec} are as defined above for the first aspect and embodiments.

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Length

The polysaccharide of the third aspect of the invention is of 17 or more monosaccharide residues in length, preferably 18 or more, more preferably 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, or 39 or more (e.g. 40) monosaccharide residues in length. The polysaccharide may be up to 70 monosaccharide residues in length, such as up to 60 monosaccharide residues, preferably 50 monosaccharide residues, more preferably 40 monosaccharide residues in length.

10

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In embodiments, the polysaccharide is of 18 to 40 monosaccharide residues in length, such as 19 to 40, 20 to 40, 21 to 40, 22 to 40, 23 to 40, 24 to 40, 25 to 40, 26 to 40, 27 to 40, 28 to 40, 29 to 40, 30 to 40, 31 to 40, 32 to 40, 33 to 40, 34 to 40, 35 to 40, 36 to 40, 37 to 40, or 38 to 40 or 39 to 40 monosaccharide residues in length. Suitably, the polysaccharide is of 20 to 40 monosaccharide residues in length, preferably 24 to 40, more preferably 25 to 40, e.g. 28 to 40.

20

Monosaccharides

As explained above, any combination of monosaccharide residues may be present in the polysaccharide of the third aspect of the invention. Suitably, the monosaccharide residues in the polysaccharide of the third aspect may be as defined above for the polysaccharide in the first aspect and embodiments of the invention.

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Protecting groups

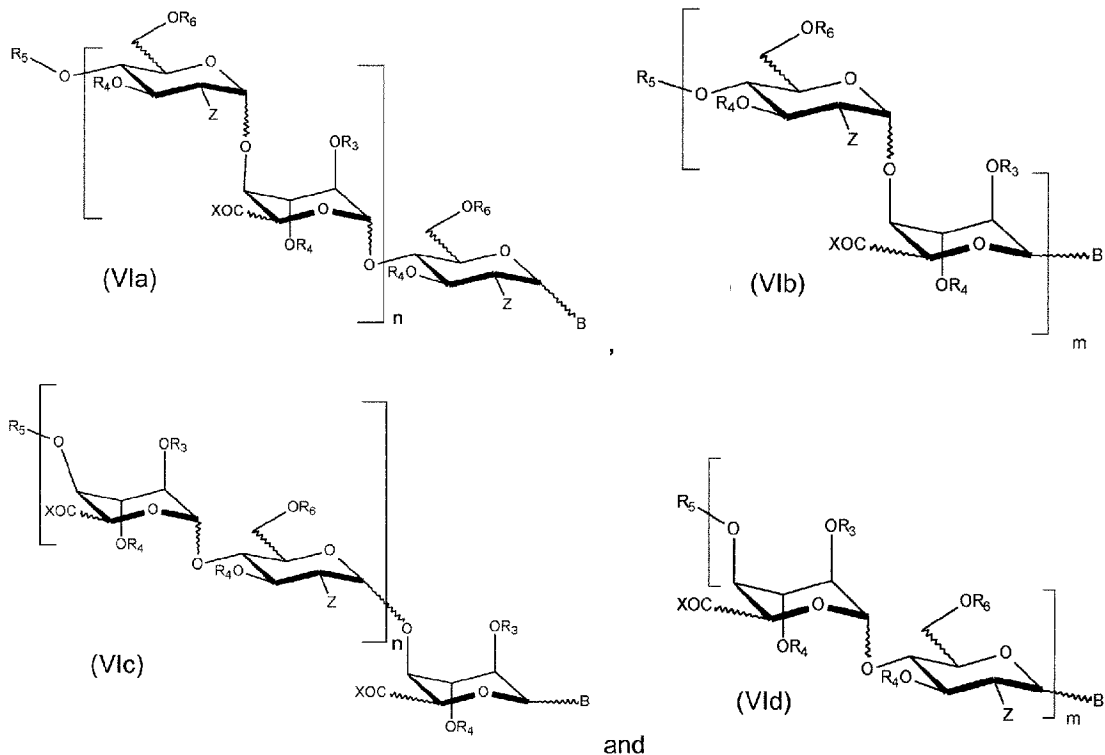
In the polysaccharide of the present invention, any nucleophilic group may be suitably protected, e.g. any of the hydroxyl and / or amine moieties in the monosaccharide residues may be protected by respective hydroxyl and / or amino protecting groups as defined herein. Suitably, the protecting groups may be as defined above for the polysaccharide of the first aspect and embodiments of the invention.

30

Preferred polysaccharides

In embodiments the polysaccharide comprises one or more glucosamine residues. In embodiments, the polysaccharide of the third aspect is selected from formulae (VIa-d):

35



R_5 is H or a hydroxyl protecting group;

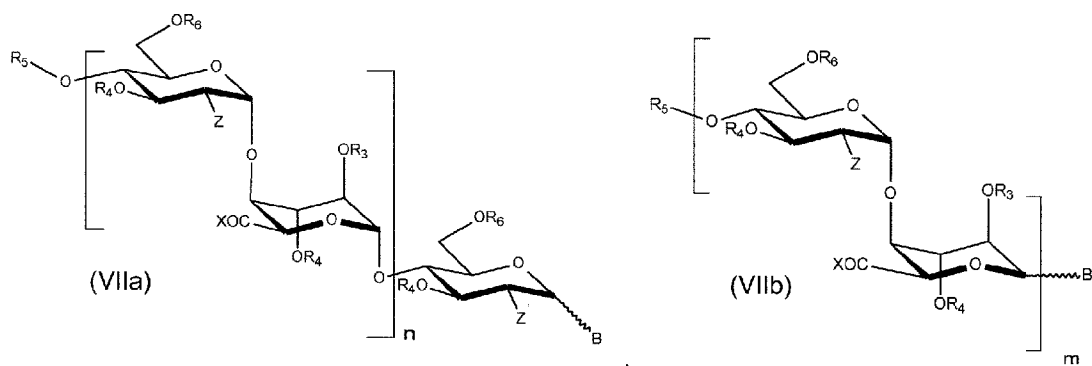
B, R_3 , R_4 , R_6 , X and Z are as defined above;

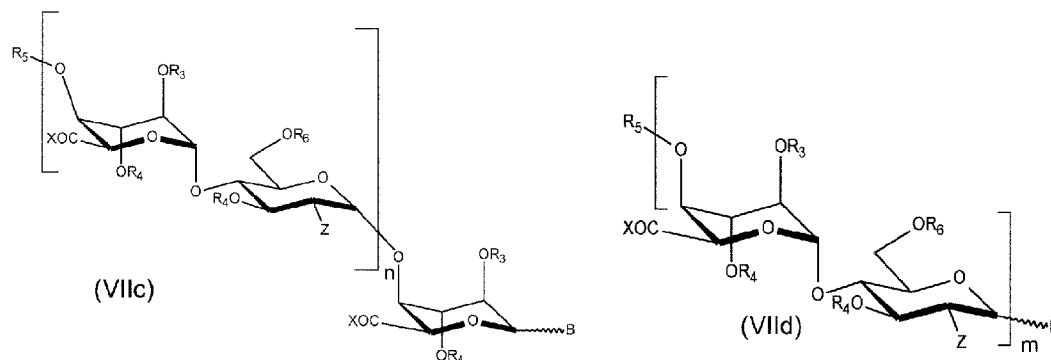
n is an integer of 8 or more, such as 8 to 19; and

m is an integer of 9 or more, such as 9 to 20.

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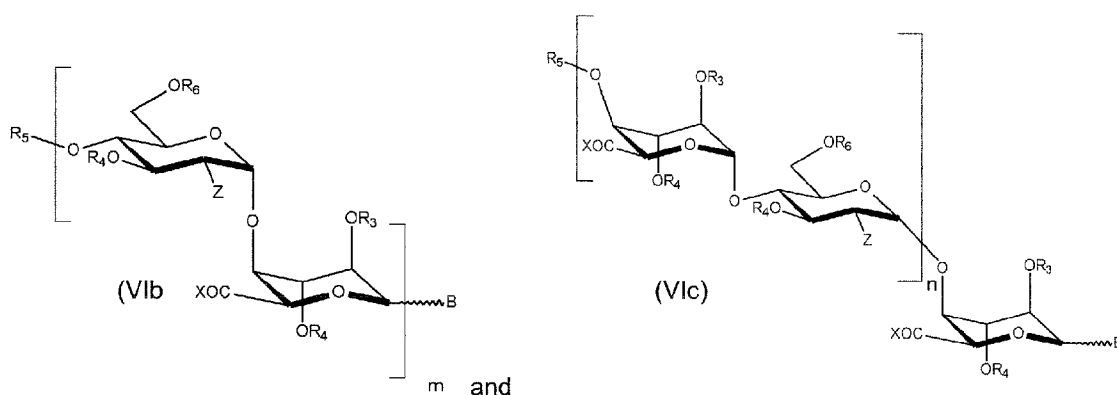
In embodiments, the polysaccharide is selected from formulae (VIIa-d):





wherein B, R₃ to R₆, X, Z, n and m are as defined above.

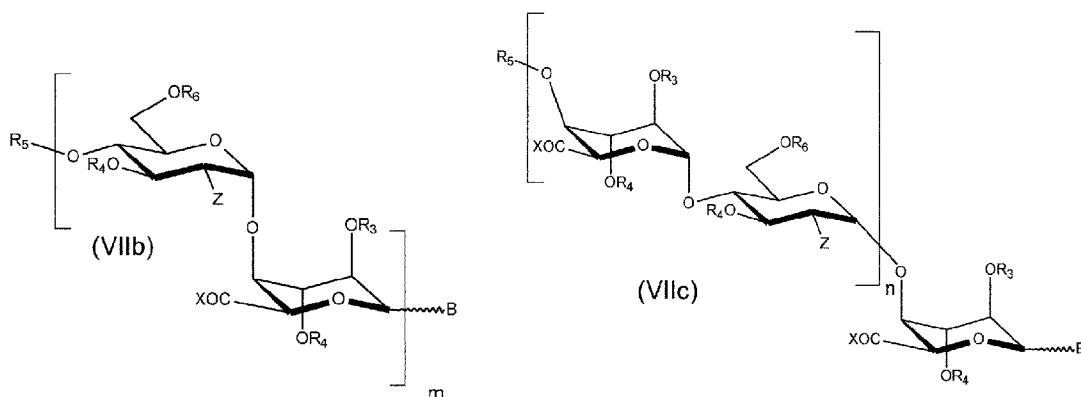
Preferably, the polysaccharide is selected from formulae (VIb) and (VIc):



5

wherein B, R₃ to R₆, X, Z, n and m are as defined above.

More preferably, the polysaccharide may be a compound selected from formulae (VIIb) and (VIIc):

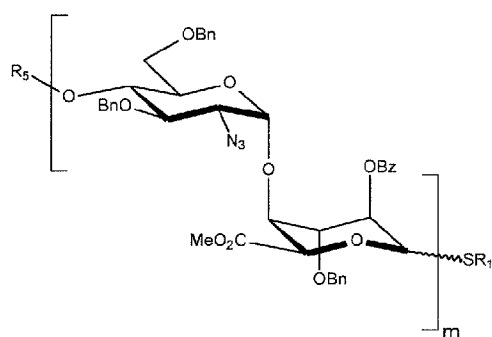
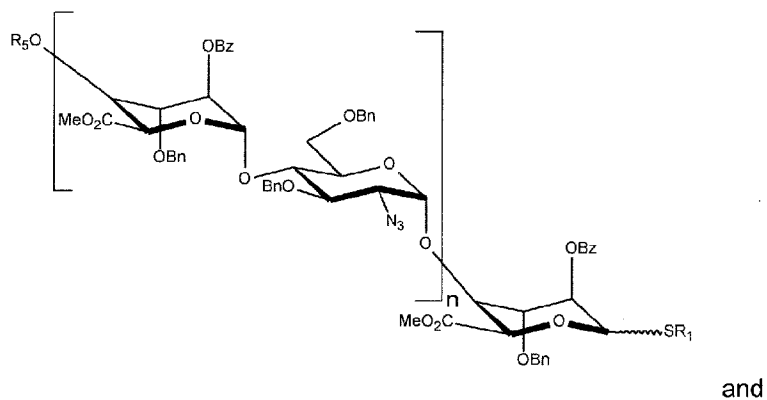


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wherein B, R₃ to R₆, X, Z, n and m are as defined in the embodiment above.

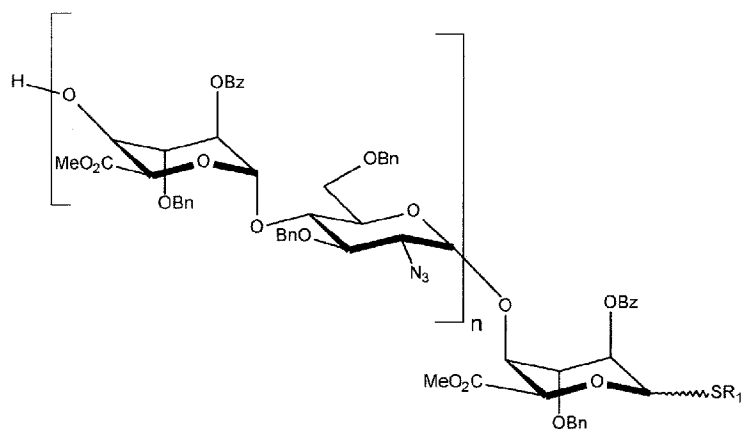
Suitably, the polysaccharide may be selected from formulae (VIa-d), preferably (VIIa-d) wherein B is a thio leaving group as defined above. In some embodiments, the polysaccharide is selected from formulae (VIb) and (VIc) (preferably formulae (VIIb) and (VIIc)) and B is a thio leaving group as defined above. In embodiments, the polysaccharide is selected from the following formulae:

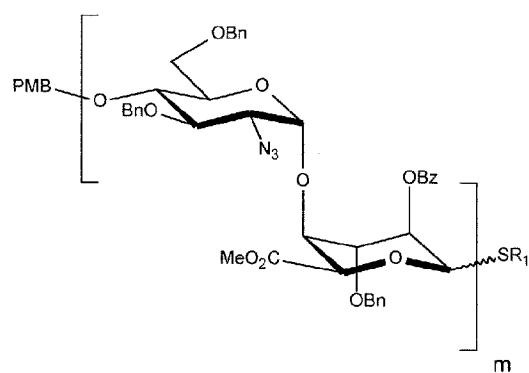
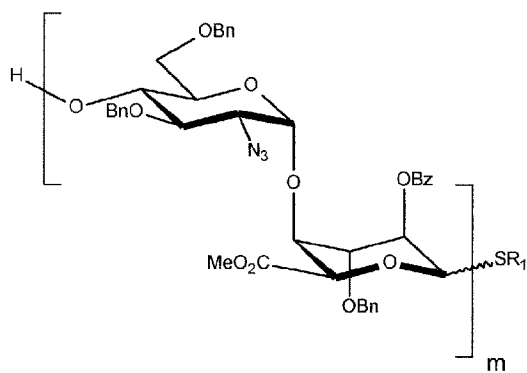
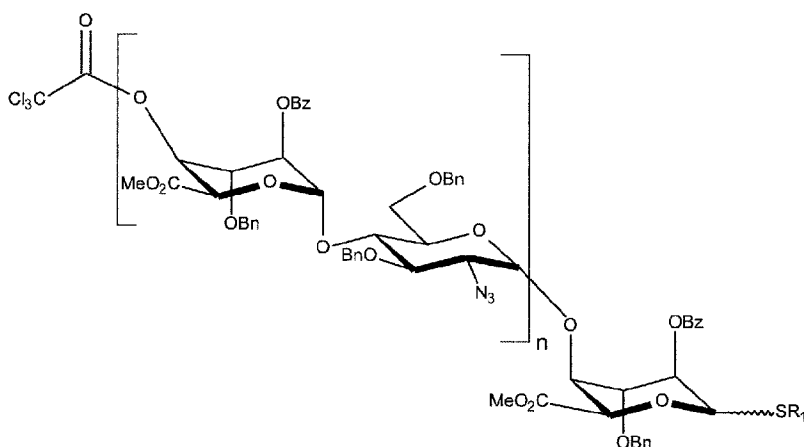
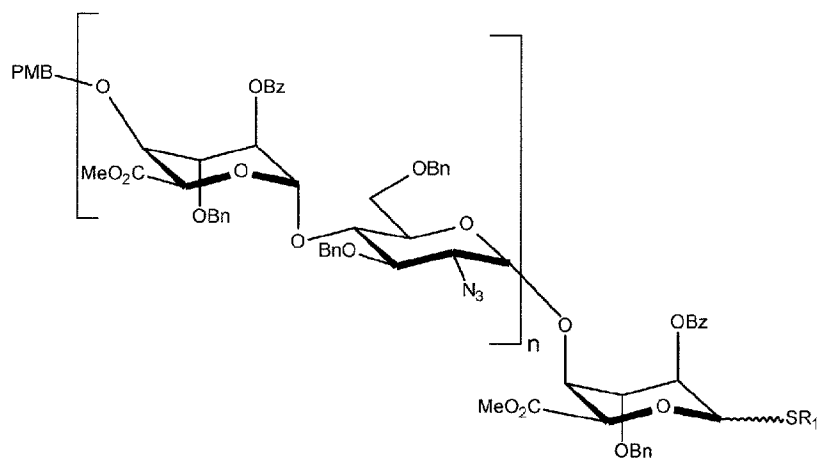
15



wherein R₁, R₅, n and m are as defined in the embodiment above.

- 5 In embodiments, the polysaccharide is selected from the following formulae:







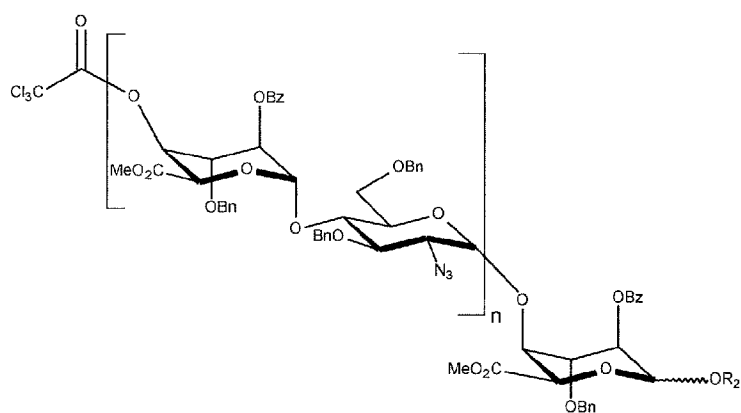
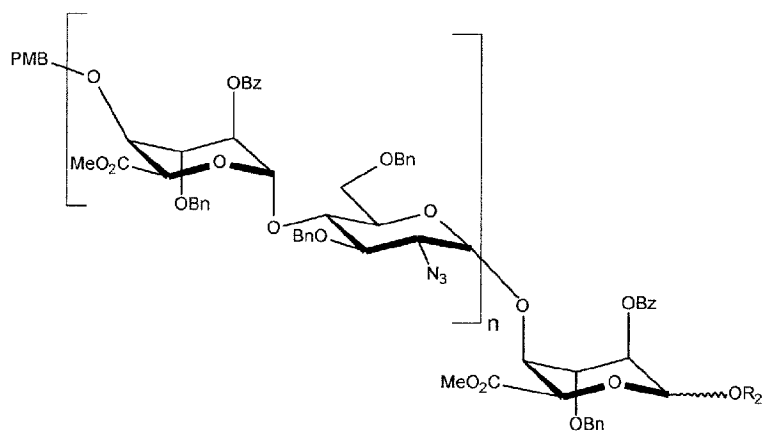
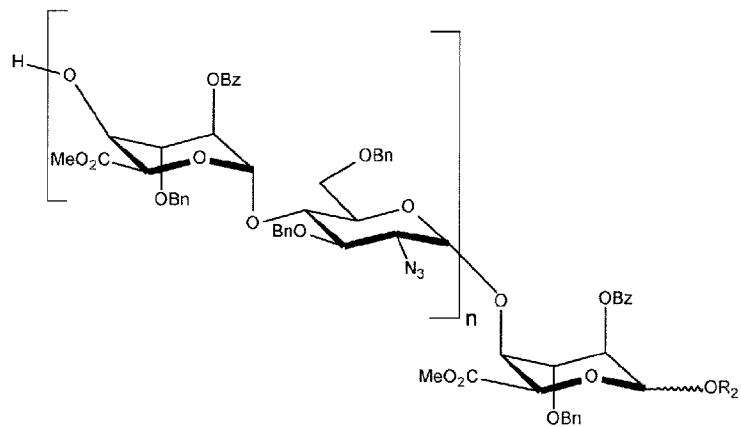
and

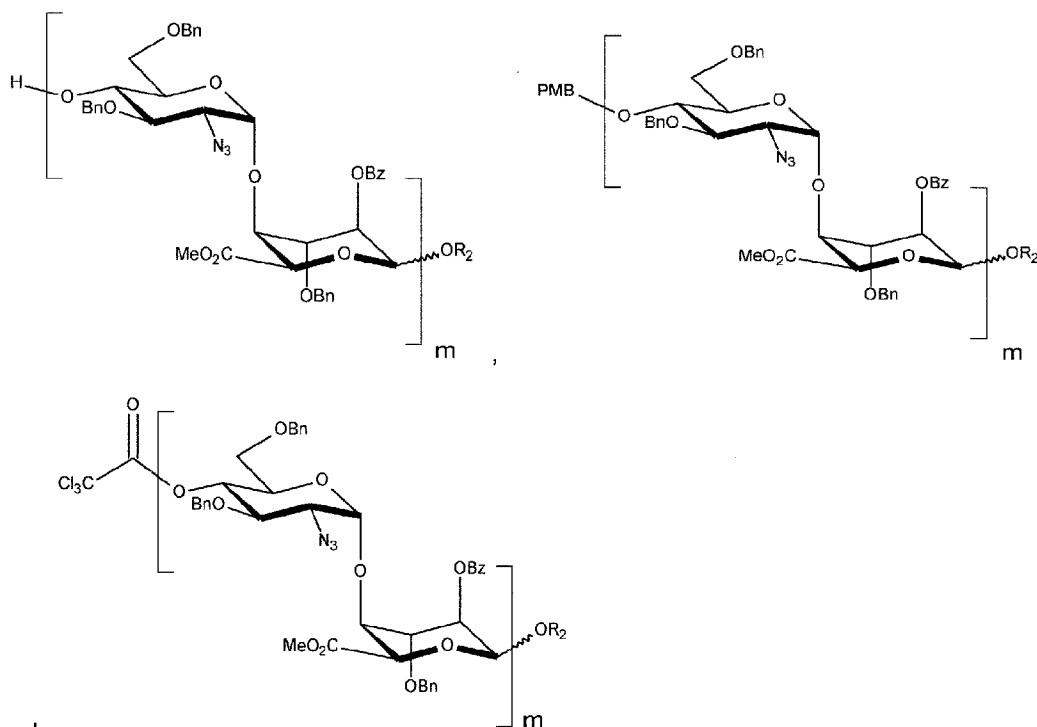
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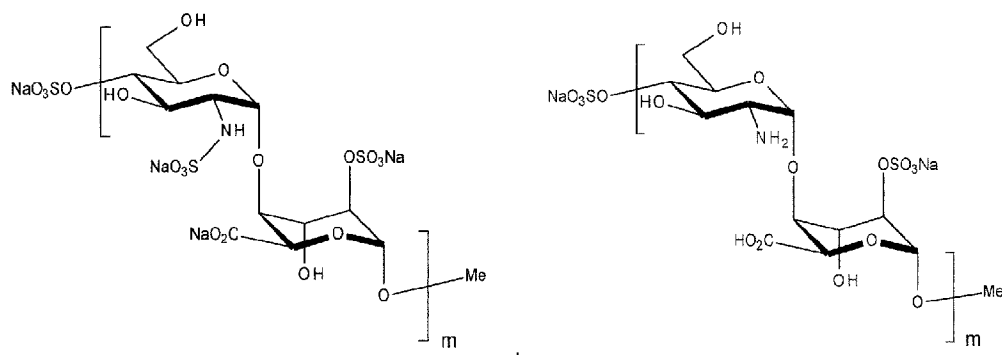


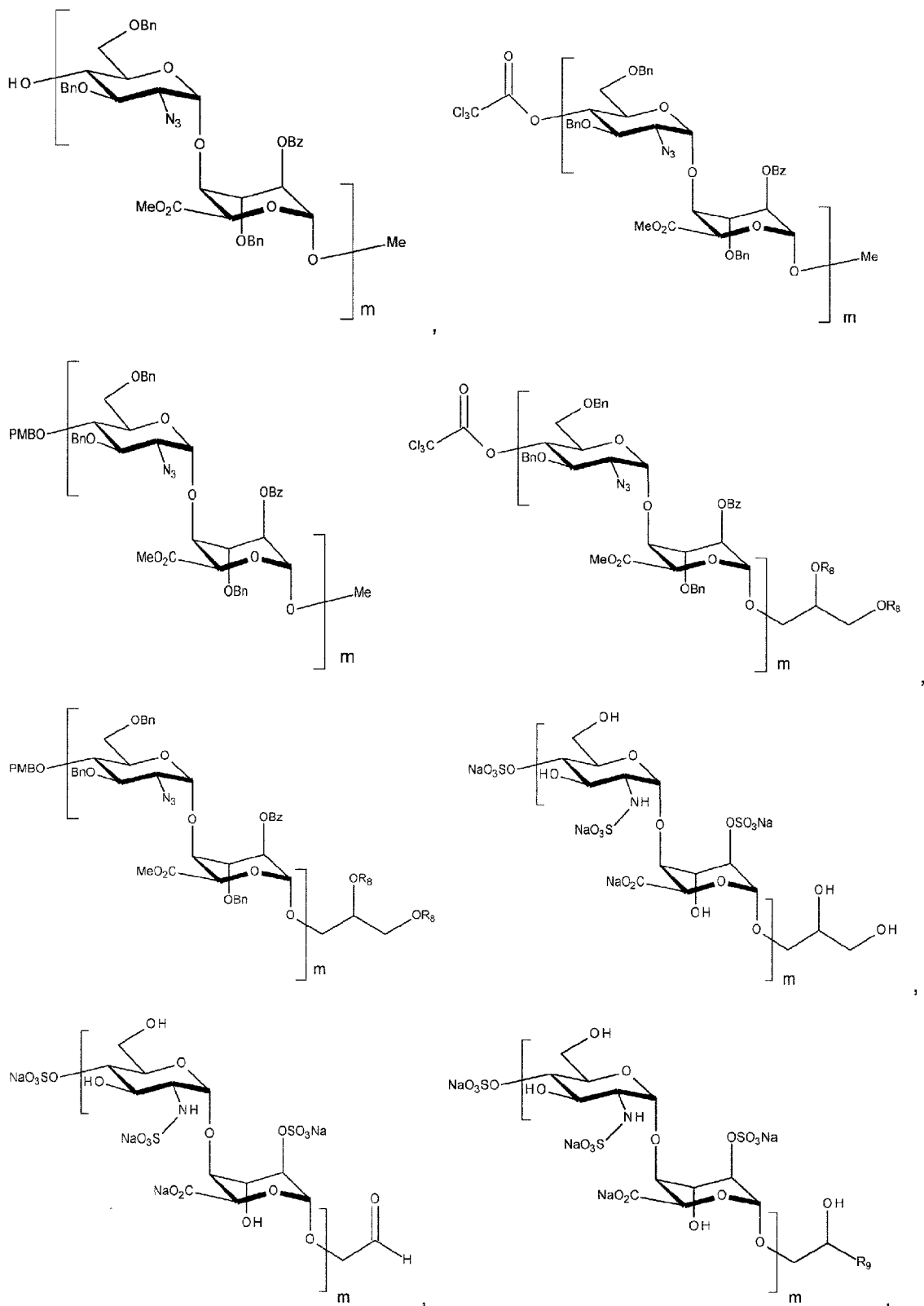
and

wherein R_2 , m and n are as defined above.

- 5 Typically, the OR_2 group is in an axial position.

Suitably, the polysaccharide is a compound selected from the following formulae:





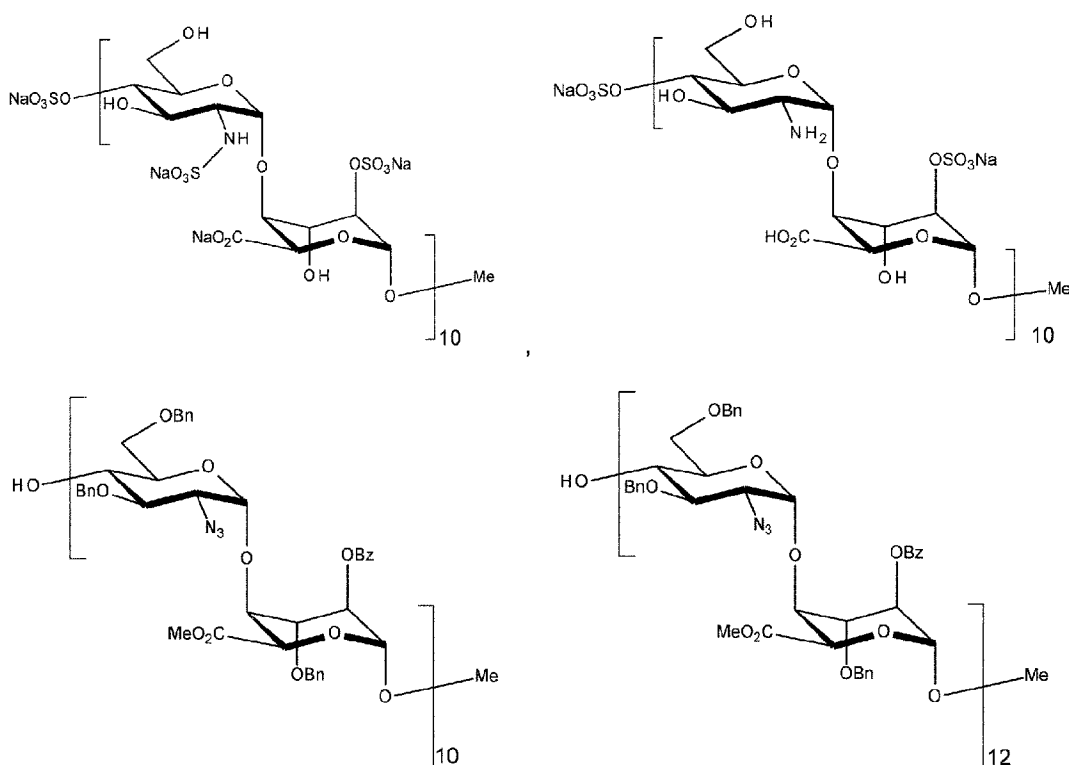
5 wherein R₈ is H or a hydroxyl protecting group;

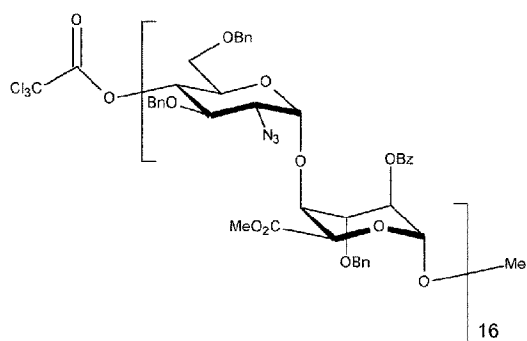
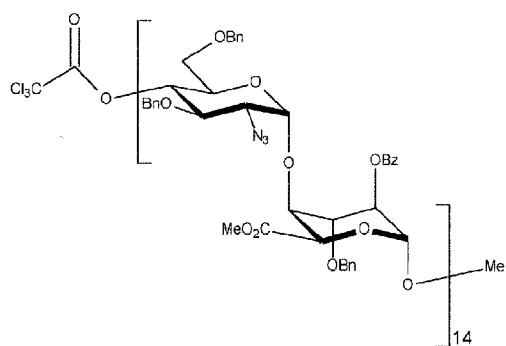
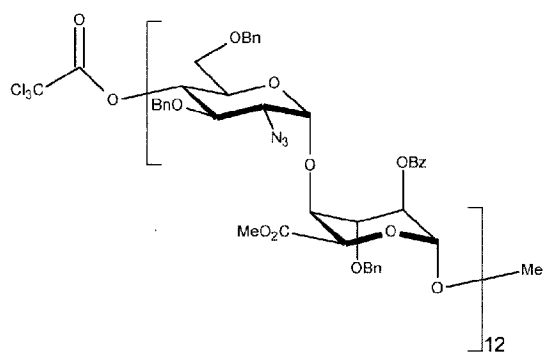
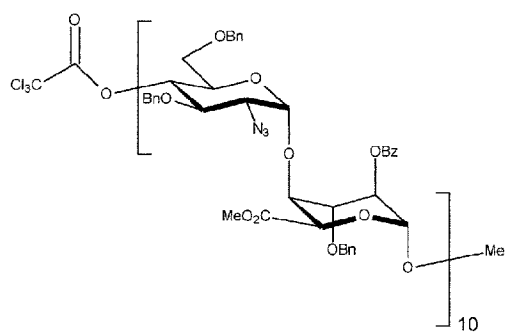
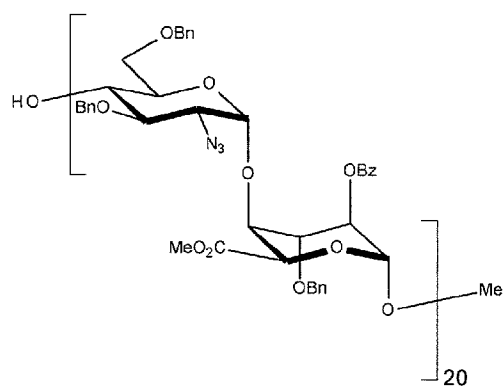
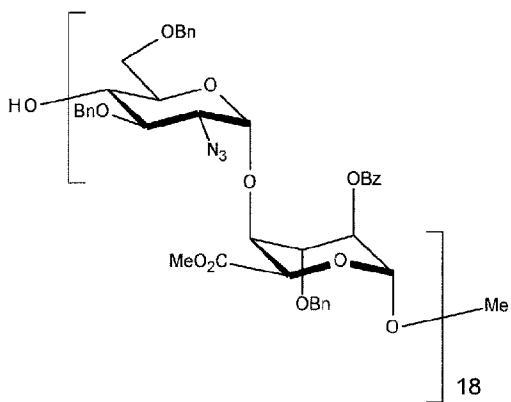
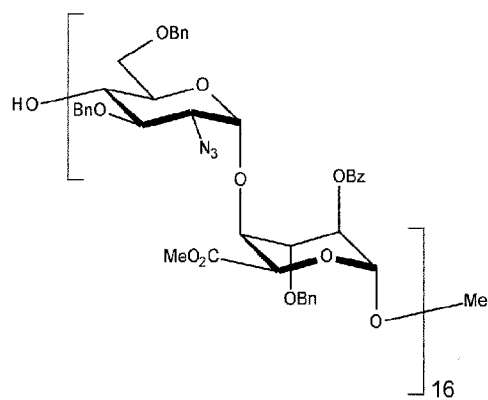
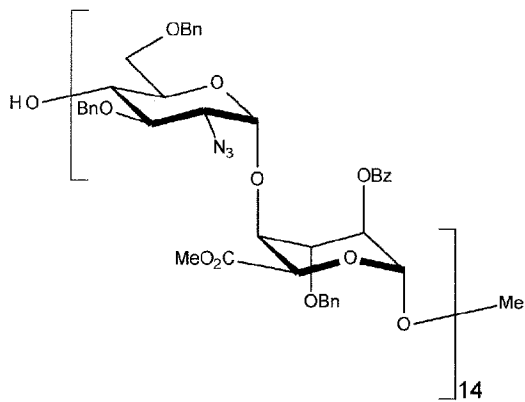
R₉ is H, optionally substituted C₁₋₁₀alkyl, optionally substituted C₂₋₁₀heteroalkyl, a radiolabel, a fluorogenic tag, a fluorescent label, a tether for attachment to a carrier molecule or solid support or a

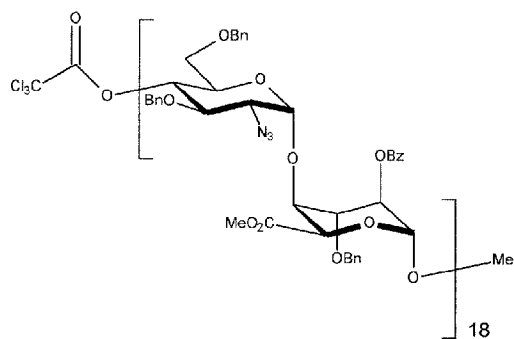
tether attached to a carrier molecule or solid support, preferably H, optionally substituted C₁₋₁₀alkyl or a radiolabel; and

m is an integer of 9 or more, such as 9 to 20.

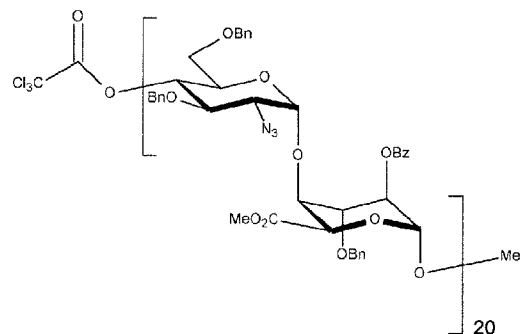
- 5 Suitably, m is an integer of 9 or more, preferably 10 or more, such as 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, or 16 or more, 17 or more, 18 or more, 19 or more, or 20 or more. In some embodiments, m is an integer of 9 to 20, preferably 10 to 20, 11 to 20, 12 to 20, 13 to 20, 14 to 20, 15 to 20, 16 to 20, or 17 to 20, 18 to 20 or 19 to 20, e.g. 20.
- 10 Suitably, n is an integer of 8 or more, such as 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, or 19 or more or 20 or more. In embodiments, n is an integer of 8 to 20, preferably 9 to 20, 10 to 20, 11 to 20, 12 to 20, 13 to 20, 14 to 20, 15 to 20, or 16 to 20, 17 to 20, 18 to 20, or 19 to 20.
- 15 In preferred embodiments, the polysaccharide is selected from the following formulae:



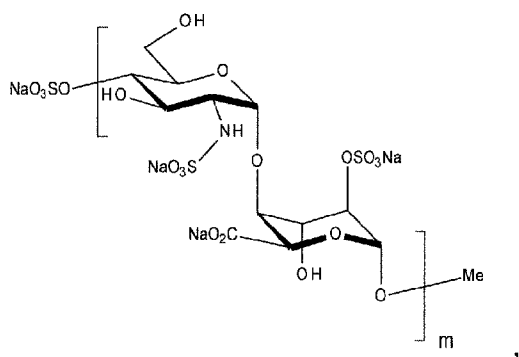




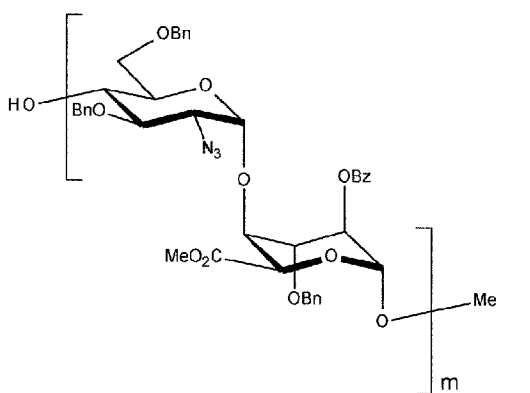
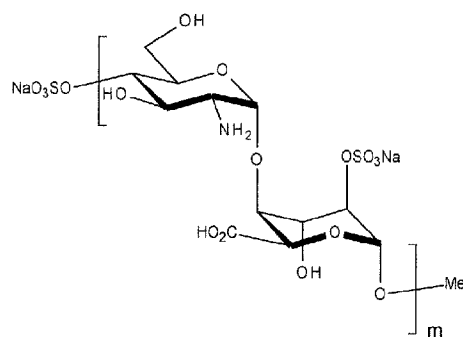
and

*Fourth aspect*

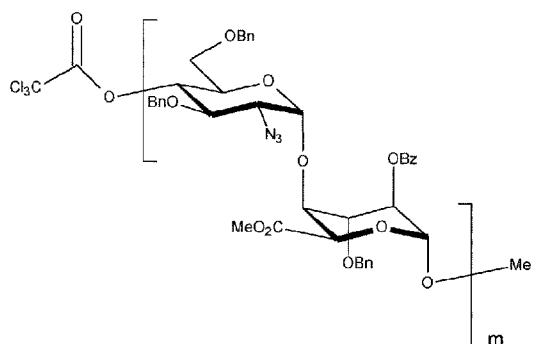
- 5 In a fourth aspect, the present invention provides a polysaccharide selected from the group consisting of:

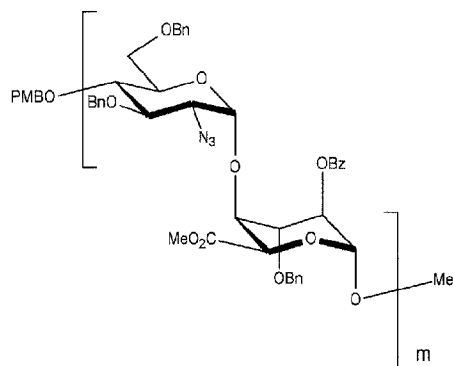


,



,



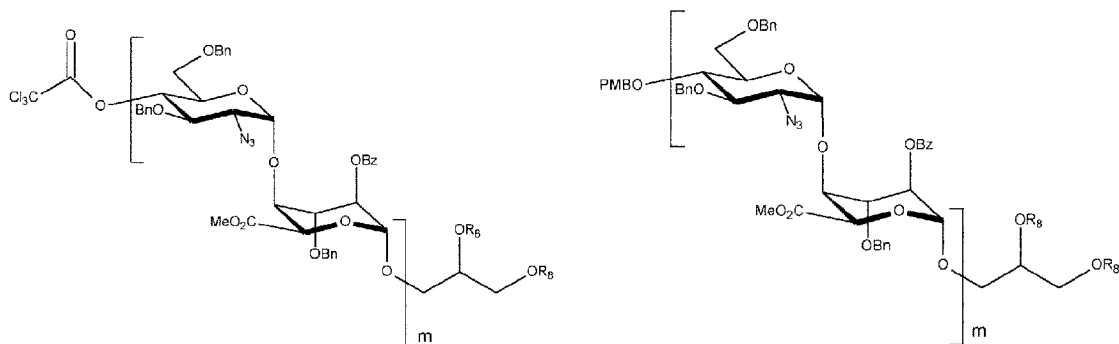


, wherein m is an integer of 7 or more, such as 7 to 20.

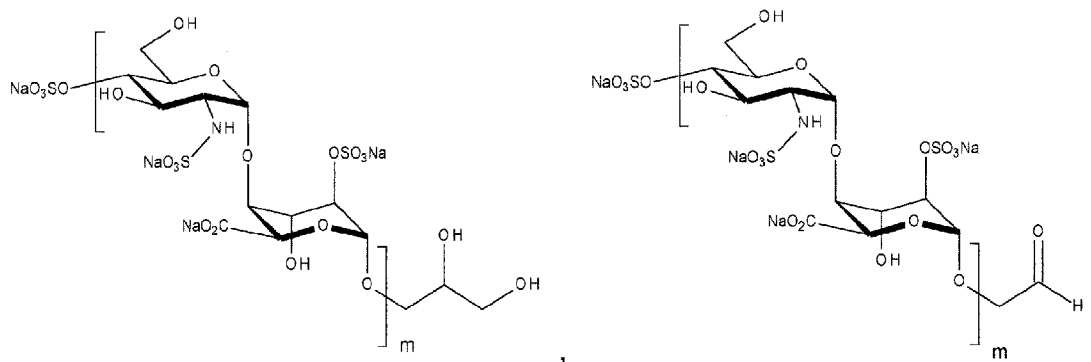
In embodiments, m is 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, or 16 or more, 17 or more, 18 or more, or 19 or more, such as 20. In embodiments, m is an integer of 8 to 20, 9 to 20, 10 to 20, 11 to 20, 12 to 20, 13 to 20, 14 to 20, 15 to 20, 16 to 20, or 17 to 20, 18 to 20 or 19 to 20 e.g. 20.

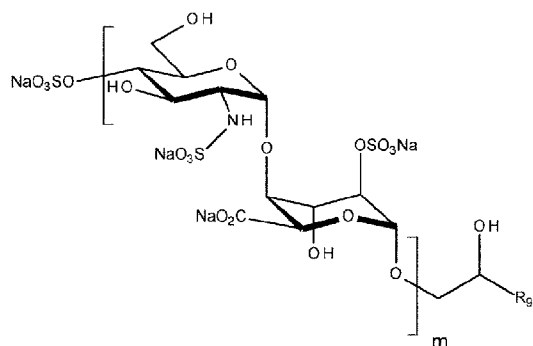
Fifth aspect

In a fifth aspect, the present invention provides a polysaccharide selected from the group consisting



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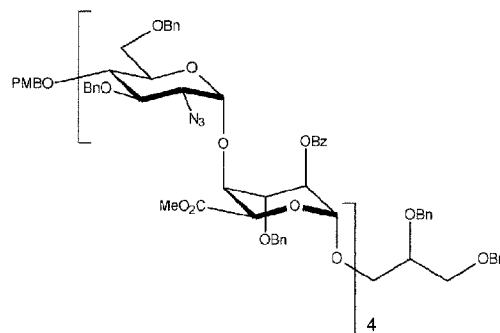
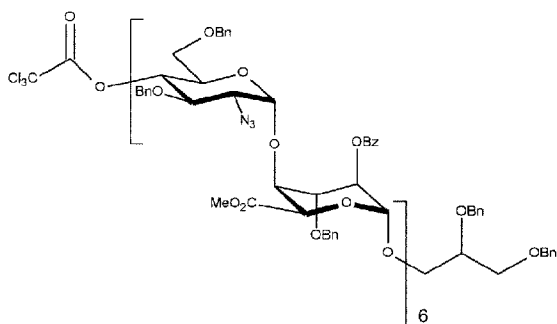
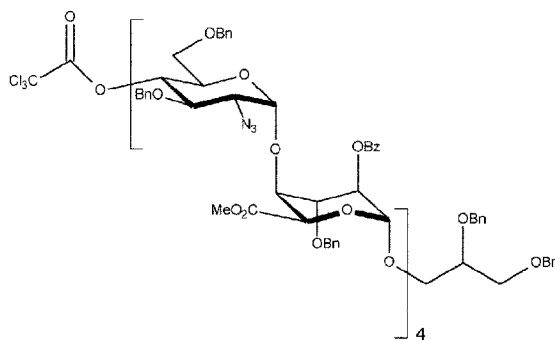
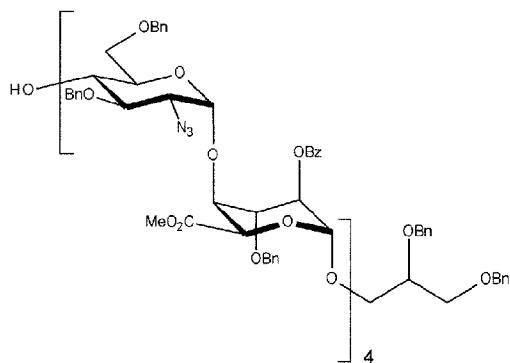


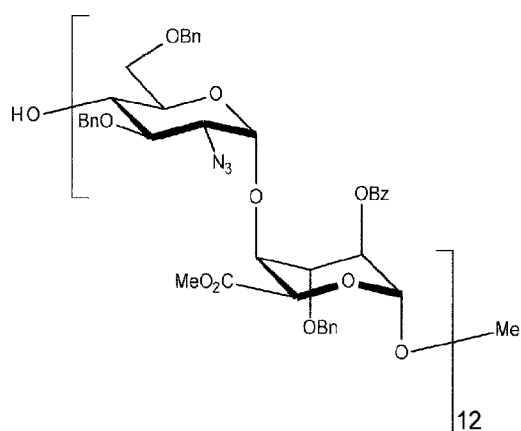
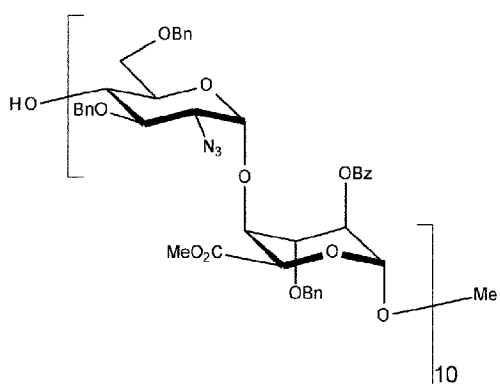
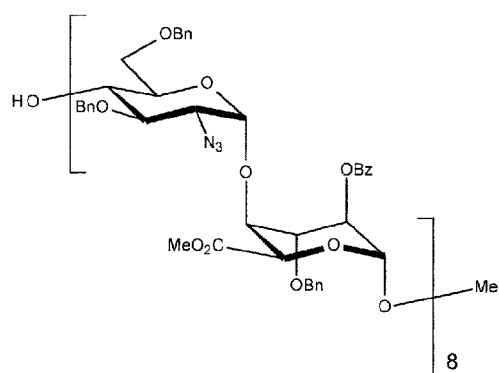
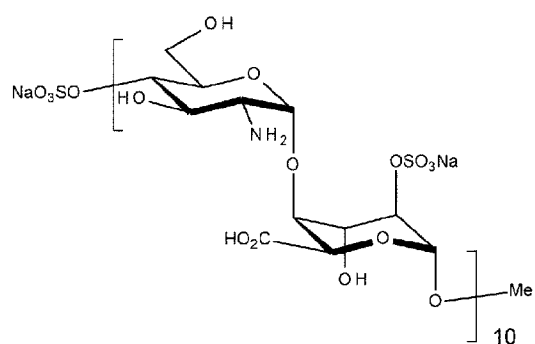
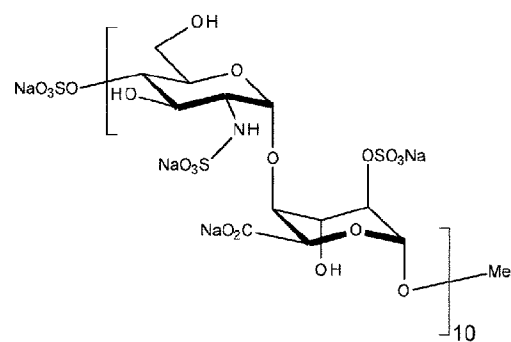
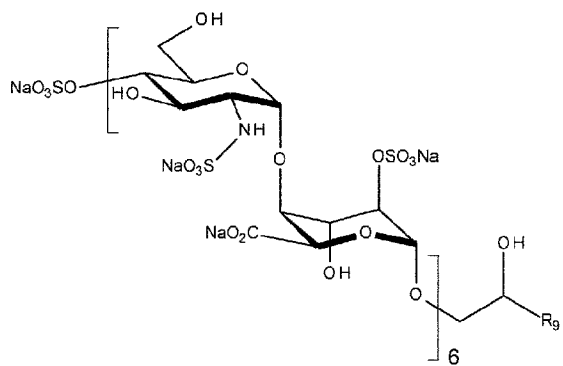
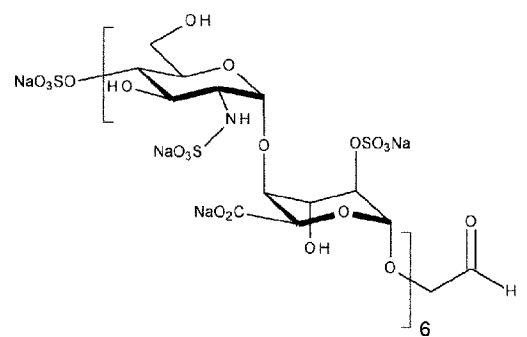
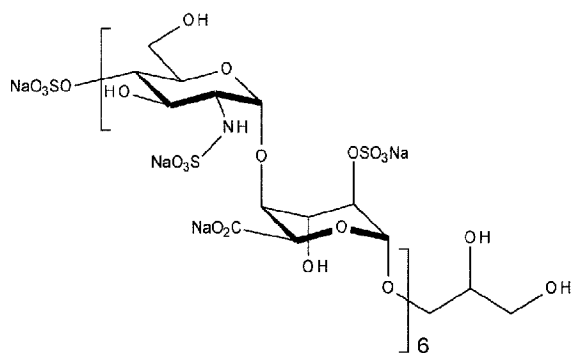


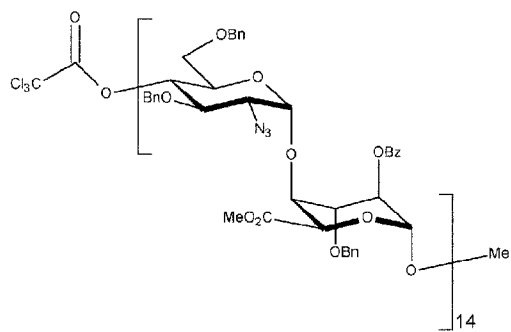
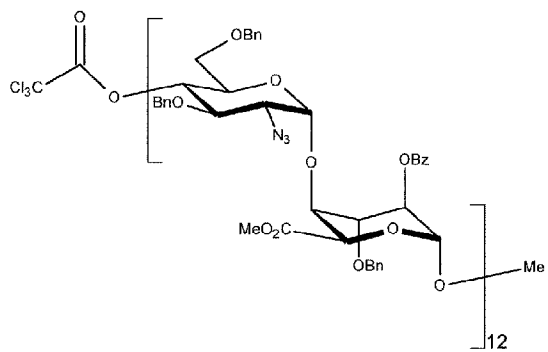
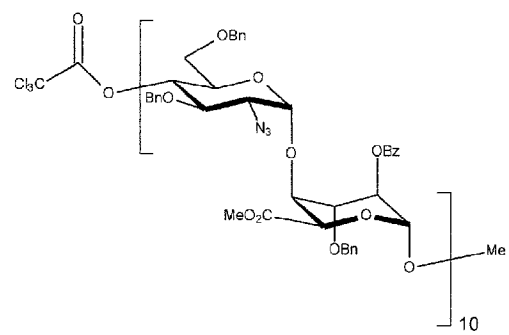
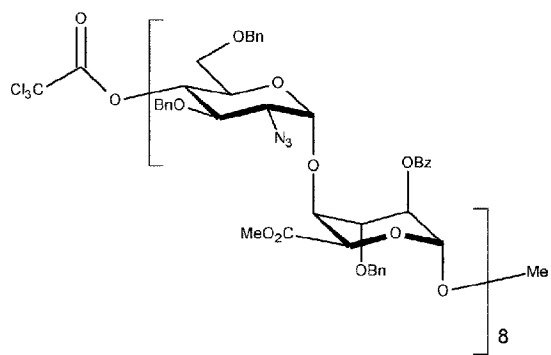
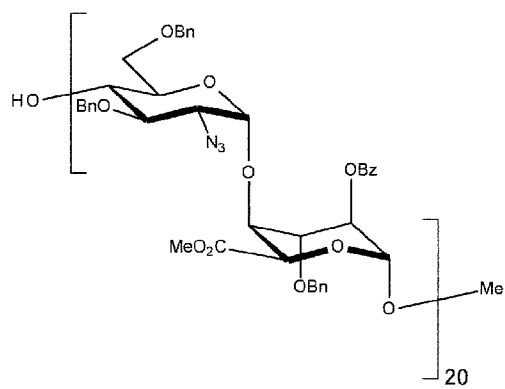
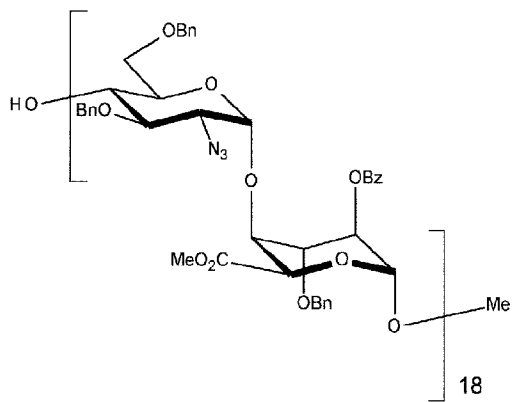
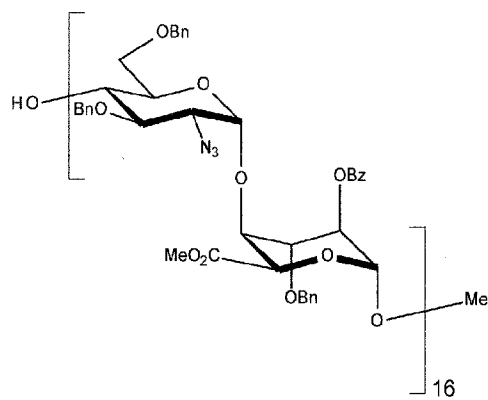
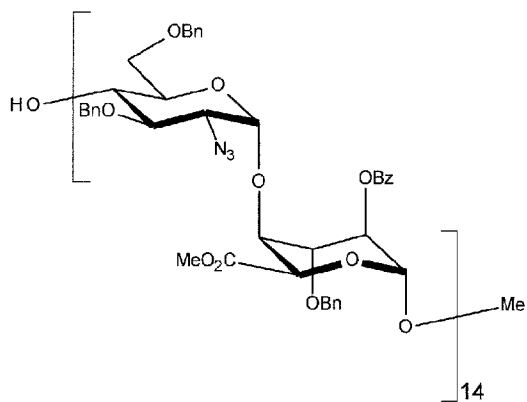
and , wherein R_9 is as defined above; and m is an integer of 4 to 20. In embodiments, m is 5 or more, preferably 6 or more, more preferably 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, or 16 or more, 17 or more, 18 or more, or 19 or more, such as 20. In embodiments, m is an integer of 4 to 20, preferably 5 to 20, 6 to 20, 7 to 20, 8 to 20, 9 to 20, 10 to 20, 11 to 20, 12 to 20, 13 to 20, 14 to 20, 15 to 20, 16 to 20, or 17 to 20, 18 to 20 or 19 to 20 e.g. 20.

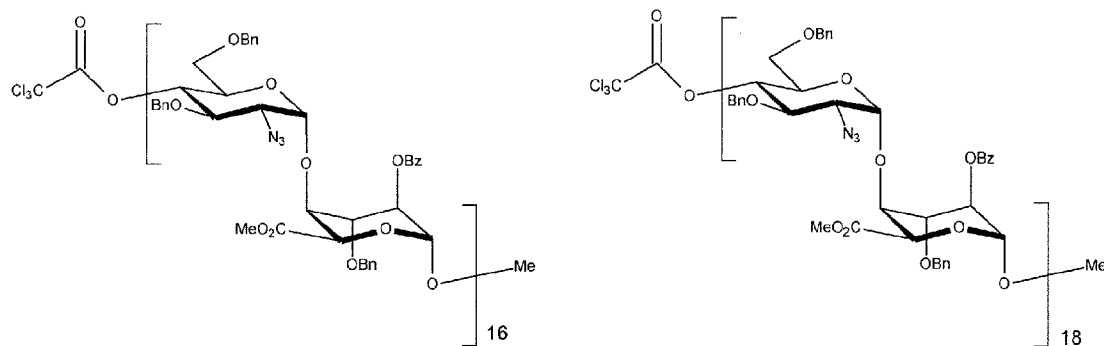
Sixth aspect

In a sixth aspect is provided a polysaccharide selected from the following formulae:

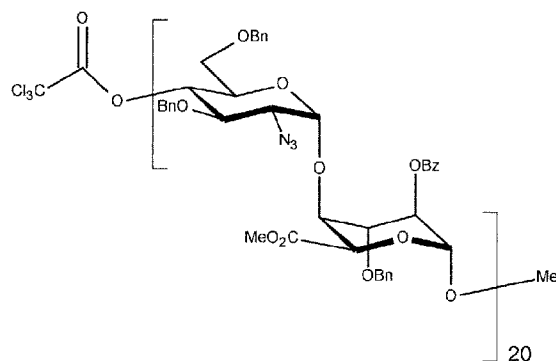








and



wherein R_9 is as defined above.

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Preferably the polysaccharide of any of the fourth to sixth aspects is isolated. Suitably, the polysaccharide may be substantially pure. In embodiments, "substantially pure" means that at least 80mol% of the total polysaccharide content of a sample of the polysaccharide consists of a polysaccharide of any of the fourth to sixth aspects and embodiments of the invention, such as at least 85 mol%, 90 mol%, 91 mol%, 92 mol%, 93 mol%, 94 mol%, 95 mol%, 96 mol%, 97 mol%, 98 mol% or more preferably at least 99mol%. In preferred embodiments, "substantially pure" means that at least 80mol% of the total content of a sample of the polysaccharide consists of a polysaccharide of any of the fourth to sixth aspects and embodiments of the invention, such as at least 85 mol%, 90 mol%, 91 mol%, 92 mol%, 93 mol%, 94 mol%, 95 mol%, 96 mol%, 97 mol%, 98 mol% or more preferably at least 99mol%.

In embodiments, the isolated polysaccharide is in solid form. Preferably, the isolated solid form of the polysaccharide comprises at least 80mol% of a polysaccharide of any of the fourth to sixth aspects and embodiments of the invention; more preferably 85mol%, 90mol%, 91mol%, 92mol%, 93mol%, 94mol%, 95mol%, 96mol%, 97mol%, 98mol% or most preferably at least 99mol%.

In embodiments, the polysaccharide is a synthetic polysaccharide.

In a further aspect, the invention provides a composition comprising the polysaccharide of the fourth aspect of the invention, for instance a solid composition comprising an isolated solid form as defined above.

5 *Seventh aspect*

As outlined above, the provision of structurally defined polysaccharides is of great interest due to their potential therapeutic applications. This is particularly the case for polysaccharides of the glycosaminoglycan family, such as heparin- or heparin sulphate-type compounds, which as explained above, are expected to have therapeutic potential in a number of areas due to their importance in the regulation of a variety of cell signalling pathways through modulation of interactions between cytokines and their receptors.

The inventors have now developed a new radiolabelling strategy that suitably allows for the quantification of tissue distribution and metabolic stability *in vivo* which should greatly assist the pre-clinical and clinical development of potential polysaccharide therapeutics.

Accordingly, the compounds described above in respect of the first to sixth aspects and embodiments may be provided with a radiolabel. Thus, in a seventh aspect of the invention is provided a polysaccharide of the formula (Ig)



wherein P is a polysaccharide residue;
L is a linker; and
R is a radiolabel.

Thus, the invention provides a polysaccharide that that is covalently bonded to a radiolabel via a linker.

As described in the Examples, an exemplary radiolabelled heparin-mimetic of formula (Ig) was employed to determine its *in vivo* clearance and tissue distribution in mice. The results show that the radiolabel provided an excellent means to probe the cellular distribution and dosage of the compound without showing any appreciable interference with ligand-binding potential.

P

In the compounds of formula (Ig), P is a polysaccharide residue. The polysaccharide may be any suitable polysaccharide as defined herein in respect of any of the first to sixth aspects and embodiments and thus may comprise any monosaccharide residues as defined above for the first to sixth aspects and embodiments and any substituent as also defined above for the first to sixth aspects and embodiments. Thus, any of the definitions of polysaccharide provided above for the first to sixth aspects and embodiments of the invention may be imported here. Preferably, the polysaccharide is a glycosaminoglycan.

In embodiments, P is a polysaccharide residue of 12 or more monosaccharide residues in length, suitably 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, or 39 or more, e.g. 40. Suitably, the polysaccharide may be up to 70 monosaccharide residues in length, such as up to 60 residues, 50 residues, 40 residues, 30 residues, 25 residues, 20 residues, 18 residues, or 16 residues.

In embodiments, the first polysaccharide is of 12 to 40 monosaccharide residues in length, preferably 6 to 40 monosaccharide residues in length, more preferably 7 to 40, 8 to 40, 9 to 40, 10 to 40, 11 to 40, 12 to 40, 13 to 40, 14 to 40, 15 to 40, 16 to 40, 17 to 40, 18 to 40, 19 to 40, 20 to 40, 21 to 40, 22 to 40, 23 to 40, 24 to 40, 25 to 40, 26 to 40, 27 to 40, 28 to 40, 29 to 40, 30 to 40, 31 to 40, 32 to 40, 33 to 40, 34 to 40, or 35 to 40 monosaccharide residues in length.

L

In the compounds of formula (Ig), L is a linker which bonds the polysaccharide residue to the radiolabel. The linker may be a covalent bond (in the case of direct attachment of the radiolabel to the polysaccharide) or a bridge of one or more atoms in length. In embodiments, the linker is bonded to an O, N, S or Se atom in the polysaccharide residue (e.g. via substitution of a pendant hydroxy group, amino group or thio or Se group), preferably O, N or S, more preferably O or N, most preferably an O atom.

In embodiments, the linker is a bridge of up to 15 atoms in length, more preferably up to 10 atoms, up to 9 atoms, up to 8 atoms, up to 7 atoms, up to 6 atoms, up to 5 atoms, preferably up to 4 atoms in length, for instance 3, 2, or 1 atom in length. In other embodiments, the linker is a covalent bond.

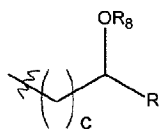
Where the linker is a bridge of one or more atoms in length bonded to the polysaccharide, it may suitably be a protecting group as defined herein, for instance, where the linker is a bridge of one or more atoms in length bonded to the polysaccharide via an oxygen atom, it may suitably be selected from hydroxyl protecting groups as defined herein. Where the linker is a bridge of one or more atoms in length bonded to the polysaccharide via a nitrogen atom, it may suitably be selected from amino protecting groups as defined herein. In embodiments, the linker is selected from the group consisting of optionally substituted C₁₋₁₀alkylene, optionally substituted C₃₋₁₀cycloalkylene, optionally substituted C₂₋₁₀alkenylene, optionally substituted C₂₋₁₀alkynylene, optionally substituted C₃₋₁₀cycloalkenylene, optionally substituted C₂₋₁₀heteroalkylene, optionally substituted C₃₋₁₀heterocycloalkylene, optionally substituted C₂₋₁₀heteroalkenylene, optionally substituted C₃₋₁₀heterocycloalkenylene, optionally substituted C₆₋₁₄arylene optionally substituted C₅₋₁₄heteroarylene, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support.

Suitably, the linker may be independently selected from the group consisting of optionally substituted C₁₋₁₀alkylene, optionally substituted C₂₋₁₀alkenylene, optionally substituted C₂₋₁₀heteroalkylene, optionally substituted C₃₋₁₀heterocycloalkylene, optionally substituted C₂₋₁₀heteroalkenylene and optionally substituted C₃₋₁₀heterocycloalkenylene, more preferably optionally substituted C₁₋₁₀alkylene and optionally substituted C₂₋₁₀alkenylene, and even more preferably optionally substituted C₁₋₁₀alkylene. Suitably, the optionally substituted C₁₋₁₀alkylene may be optionally substituted C₁₋₄alkylene, such as optionally substituted butylene, optionally substituted propylene, optionally substituted ethylene or optionally substituted methylene, preferably optionally substituted ethylene.

In embodiments where the linker is substituted as defined above, the respective substituents may be selected from OR₈, N(R^a)₂, =O, -C(=O)H, C₆₋₁₄aryl, halo, haloC₁₋₄alkyl, =N(R^b), a fluorogenic tag, a fluorescent label and a radiolabel, wherein C₆₋₁₄aryl may be further substituted with one to three substituents independently selected from halo, C₁₋₄alkoxy and C₁₋₄alkyl, wherein each R^a and R^b is independently as defined below and each R^b is independently H, C₁₋₄alkyl, or -C(=O)C₁₋₄alkyl. More preferably, when the linker is substituted as defined above, the respective substituents may be selected from OR₈, =O, halo, and a radiolabel, preferably OR₈, wherein R^b is as defined below.

In preferred embodiments, the linker is optionally substituted C₁₋₁₀alkylene wherein the optional substituents are selected from OR₈, N(R^a)₂, =O, halo, haloC₁₋₄alkyl and =N(R^b), preferably an OR₈ group wherein R^b is as defined below, wherein each R^b is independently H, C₁₋₄alkyl, or -C(=O)C₁₋₄alkyl.

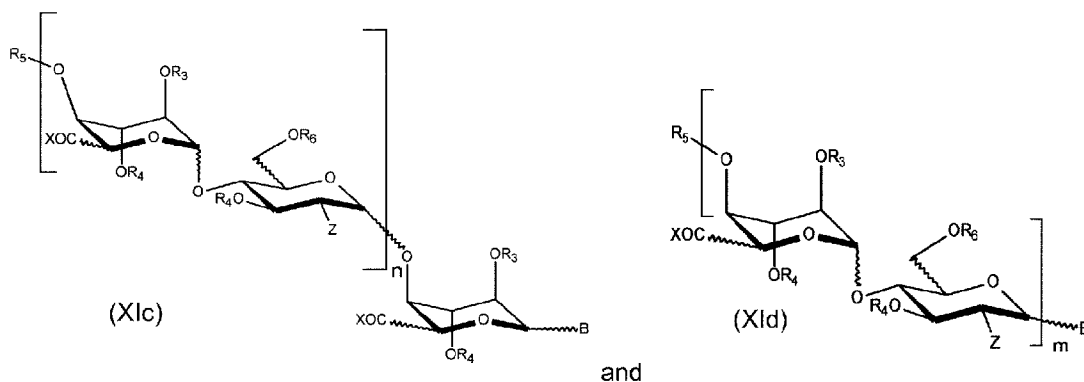
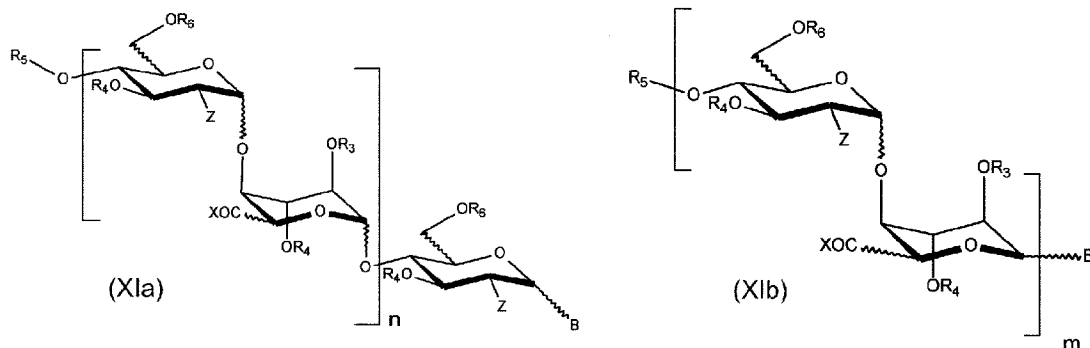
In embodiments, the -L-R group appended to the polysaccharide P in formula (I_g) is:



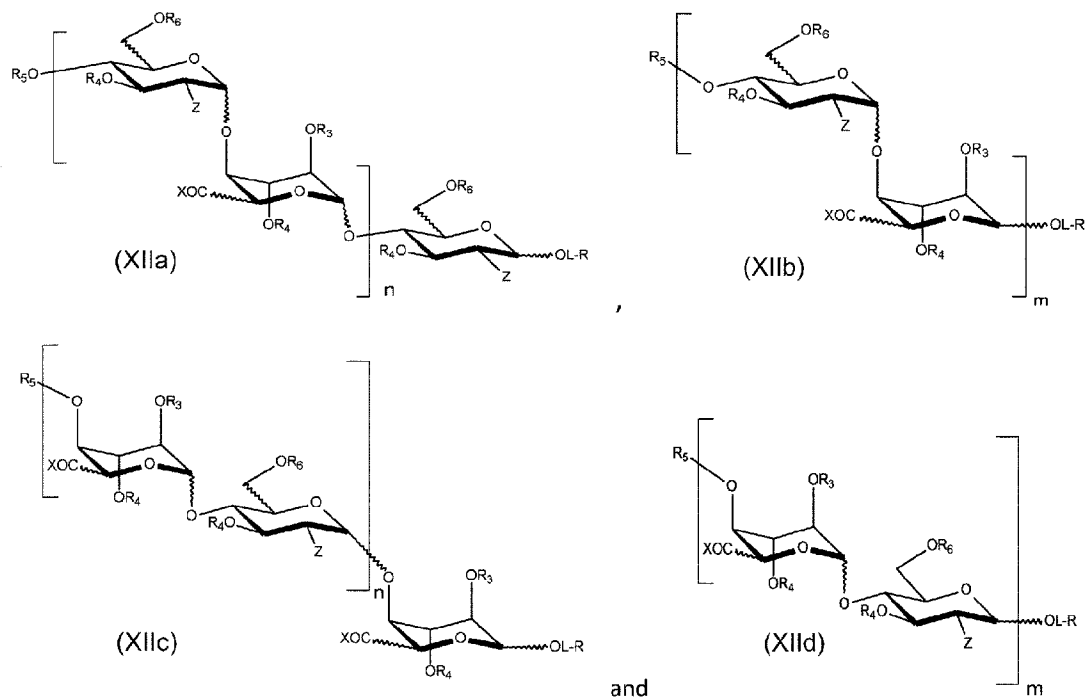
, wherein c is an integer of 1 to 9, suitably 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, preferably 1, more preferably wherein said group is bonded to the polysaccharide by an oxygen atom.

Suitably, the linker may be attached to any monosaccharide residue within the polysaccharide. In preferred embodiments, the linker is attached to a monosaccharide residue at a terminus of the polysaccharide chain (i.e. a monosaccharide residue that is not bonded to more than one other monosaccharide residue). In other words, in embodiments the label is an end-label. More preferably, the linker is attached to the anomeric position of a monosaccharide at a terminus of the polysaccharide residue. By attaching the linker (and thus radiolabel) to a monosaccharide located at the terminus of the polysaccharide chain, especially the anomeric position, disruption of the natural biological interactions of the polysaccharide are minimised. Thus, a suitably end-labelled compound advantageously provides a more reliable probe for investigating the natural pharmacokinetic behaviour of the corresponding unlabelled compound.

For instance, in compounds of the following general formulae (XIa-d), the group -L-R may be bonded to any of the monosaccharide residues in the chain and may be for instance bonded at any suitable position, i.e. any of R₃-R₆, Z, X or B. Typically, the -L-R group will be present at position B.

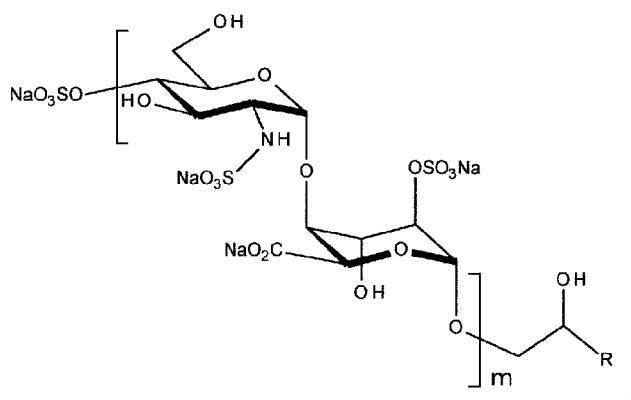
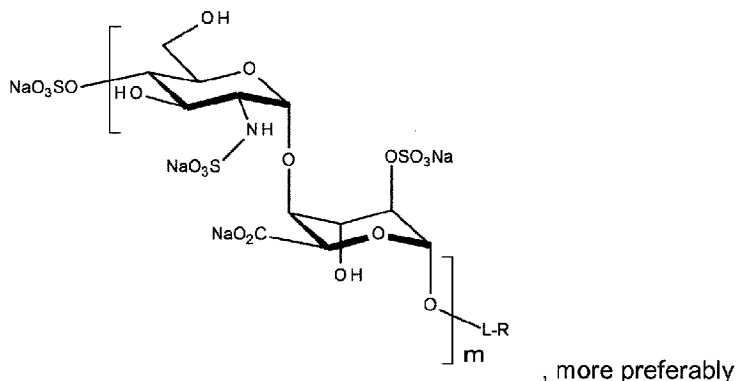


Thus, in embodiments, the compound of formula (Ig) is a compound of the following formulae:



wherein R_3 - R_6 , Z, X, B, m and n are as defined in any aspect or embodiment above and L-R is as defined in formula (Ig).

In preferred embodiments, the compound of formula (Ig) is a compound of the following formula:



wherein R_3 - R_6 , Z, X, B and m are as defined in any aspect or embodiment above and L and R is as defined in formula (Ig), most preferably wherein m is 6.

10 *R*

In the compounds of formula (Ig), R is a radiolabel. Suitably, the radiolabel comprises a radioactive isotope as defined herein. In embodiments, the radiolabel may comprise one or more radioactive isotopes as defined herein. In embodiments, the radiolabel comprises a radioactive isotope selected from ^3H , ^{14}C , ^{11}C , ^{18}F , ^{15}O and ^{13}N , such as ^{11}C , ^{18}F , ^{15}O and ^{13}N , preferably ^3H and ^{14}C , more preferably ^3H . In embodiments, the radiolabel consists of a radioactive isotope as defined herein, preferably ^3H .

^3H is particularly preferred for the present biological applications because of its ease of incorporation and ready means of detection. Positron-emitting isotopes, such as ^{11}C , ^{18}F , ^{15}O and ^{13}N , can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy.

In a further aspect, the invention provides a method of radiolabelling a polysaccharide comprising providing a group -L-R appended to the polysaccharide, wherein the definition of polysaccharide, L and R are each as defined above.

5 In a further aspect, the invention provides the use of a compound of formula (Ig) in a method of pharmacokinetic analysis, wherein the compound of formula (Ig) is as described above for the seventh aspect and embodiments. In embodiments, the method comprises administering a compound of formula (Ig) to a mammal, taking a blood or tissue sample from the mammal at a later point in time and determining the concentration of the compound of formula (Ig) in the sample.

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In a further aspect, the invention provides a polysaccharide as described in any of the second to seventh aspects and embodiments thereof *obtainable* by the process as described in the first aspect and embodiments thereof. In an embodiment, the invention provides a polysaccharide or polysaccharide as described any of the second to seventh aspects and embodiments thereof

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obtained by the process as described in the first aspect and embodiments thereof.

General definitions

Polysaccharide

Unless otherwise specified herein, the term "polysaccharide" refers to linear or branched polymer chain of two or more monosaccharide residues in length and suitable salts and derivatives thereof.

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Such polysaccharides include but are not limited to polysaccharides that contain only carbon, hydrogen and oxygen (i.e. carbohydrates) and thus the term includes polysaccharides containing one or more further heteroatoms in addition to carbon, hydrogen and oxygen, such as nitrogen (e.g. present in glucosamines), sulphur (e.g. present in sulphated polysaccharides such as heparin and heparin sulfate), phosphorous and halogens, etc.

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The term also includes substituted polysaccharides, which refers to compounds wherein one or more functional groups bonded to the monosaccharide backbone is notionally replaced by another functional group. Exemplary substituents include amino- and azido groups (wherein a hydroxy group of the corresponding carbohydrate monosaccharide is replaced by an amino or azido group); oxidised polysaccharides (e.g. wherein one or more hydroxyl groups in the polysaccharide chain is oxidised to a carboxylic acid or ketone moiety); deoxymonosaccharides (i.e. wherein one or more hydroxyl groups is replaced by hydrogen); carboxyl-, hydroxyl- and amino-protecting groups (i.e. in place of hydrogen atoms in the respective groups), and tethers suitable for bonding to (or actually bonded to) other non-saccharide moieties, such as peptides, lipids, fluorogenic tags, fluorescent labels, biotin, polyethers (e.g. PEGs) or a solid support. Other exemplary substituents include isotopic labels wherein a natural isotope of an atom in the polysaccharide is replaced by an atom of the same atomic number but different atomic mass (e.g. radiolabels).

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Synthetic polysaccharide

The term "synthetic polysaccharides" as use herein refers to polysaccharides of non-natural origin which have been prepared synthetically outside of an organism. Exemplary synthetic polysaccharides have been prepared synthetically outside of an organism comprising at least one polysaccharide chain extending step. Thus, polysaccharides of natural origin and polysaccharides that are the product of degradation of natural polysaccharides into smaller fragments are not encompassed within the meaning of this term.

Monosaccharide

The term "monosaccharide" means a compound containing a single saccharide residue that cannot be hydrolysed into a plurality of saccharide residues.

The term monosaccharide includes but is not limited to compounds that contain only carbon, hydrogen and oxygen (i.e. carbohydrates) and thus the term includes saccharide residues containing one or more further heteroatoms in addition to carbon, hydrogen and oxygen, such as nitrogen (e.g. in glucosamines and their azide analogues), sulphur (e.g. in thioglycosides and sulphated saccharide analogues) phosphorous and halogen atoms, etc.

The term also includes substituted monosaccharides, i.e. wherein one or more groups, such as one to three groups, for example two, e.g. one group bonded to the monosaccharide backbone, is notionally replaced by another functional group. Exemplary substituents include amino- and azido groups (wherein a hydroxy group of the corresponding carbohydrate monosaccharide is replaced by an amino or azido group); oxidised polysaccharides (e.g. wherein one or more hydroxyl groups in the monosaccharide chain is oxidised to a carboxylic acid or ketone moiety); deoxymonosaccharides (i.e. wherein one or more hydroxyl groups is replaced by hydrogen); carboxyl-, hydroxyl- and amino-protecting groups (i.e. in place of the hydrogen atom in the respective groups), and tethers suitable for bonding to (or actually bonded to) other non-saccharide moieties, such as peptides, lipids, fluorogenic tags, fluorescent labels, biotin residues, polyethers (e.g. PEGs) or a solid support. Other exemplary substituents include isotopic labels wherein a natural isotope of an atom in the monosaccharide is replaced by an atom of the same atomic number but different atomic mass (e.g. radiolabels).

Protecting groups

The term "protecting group" used herein refers to any moiety that may mask a chemical group such as by replacing a labile hydrogen atom in a nucleophilic group, such as a hydroxyl or amino group.

Hydroxyl protecting groups

The term "hydroxyl protecting group" used herein refers to any moiety that may replace hydrogen in a hydroxyl group. Any suitable protecting group known to the skilled person to be suitable for use with saccharides is intended to be included within the meaning of "hydroxyl protecting group" in the

present invention. The term is intended to encompass permanent, semi-permanent and temporary protecting groups. In other words, it is not a requirement that a protecting group is readily removable, although in the general context of this invention the skilled person will appreciate that protecting groups which can be easily removed, such as by hydrolysis or hydrogenation, are preferred.

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Examples of hydroxy protecting groups include optionally substituted C₁₋₁₀alkyl, optionally substituted C₂₋₁₀alkenyl, optionally substituted C₁₋₁₀heteroalkyl, optionally substituted C₁₋₁₀heterocycloalkyl, optionally substituted C₁₋₁₀heteroalkenyl, optionally substituted C₁₋₁₀heterocycloalkenyl, optionally substituted silyl, -PO(R^x)₂, -SO₂R^x, a tether for attachment to a carrier molecule or solid support and a
 10 tether attached to a carrier molecule or solid support, wherein each R^x is independently selected from OR^y, C₁₋₄alkyl, C₆₋₁₄aryl, C₁₋₄alkylC₆₋₁₄aryl, haloC₁₋₄alkyl and N(R^y)₂, wherein each R^y is independently selected from H, a cation and C₁₋₄alkyl. The optional substituents may be as defined herein. For instance, exemplary optional substituents are =O, C₆₋₁₄aryl, halo, haloC₁₋₄alkyl, and =N(R^b) wherein C₆₋₁₄aryl may be further substituted with one to three substituents independently selected from halo,
 15 C₁₋₄alkoxy and C₁₋₄alkyl and each R^b is independently H, C₁₋₄alkyl, or -C(=O)C₁₋₄alkyl.

Conventional hydroxyl protecting groups include groups which form:

- a) esters, such as acetyl (Ac), benzoyl (Bz), chloroacetyl (ClAc), trichloroacetyl (TCA), bromoacetyl (BrAc), pivaloyl (Piv), levulinoyl (Lev), difluorobenzoyl (dfBz);
- 20 b) alkoxyalkyl ethers, such as methoxymethyl (MOM), (2-Methoxyethoxy) methyl (MEM) benzyloxymethyl (BOM), p-methyl benzyloxymethyl (pMBOM), Trimethylsilylethoxymethyl (SEM);
- c) carbonates such as 9-Fluorenylmethyl carbonyl (Fmoc), [2,2,2-Trichloroethoxycarbonyl] (Troc), allyloxycarbonyl (Alloc);
- d) ethers, such as naphthylmethyl (Nap) (including both 2-naphthylmethyl, NAP, and
 25 1-naphthylmethyl, 1-NAP), benzyl (Bn), p-methoxybenzyl (PMB), trityl (Tr), tetrahydro-2-pyranyl (THP), methoxytrityl (MTr), dimethoxytrityl (DMTr), allyl (All);
- e) tosylates, such as p-toluene sulfonyl (Ts), methanesulfonyl (Ms) and trifluoromethane sulfonyl (Tf); and
- f) silyl ethers such as t-butyldimethylsilyl (TBS), t-exyldimethylsilyl (TDS), t-butyldiphenyl silyl
 30 (TBDPS), triisopropylsilyl (TIPS), trimethylsilyl (TMS), triethylsilyl (TES), triphenylsilyl (TPS), di-tert-butylmethylsilyl (DTBMS), diethylisopropylsilyl (DEIPS), dimethylisopropylsilyl (DMIPS),

The cation mentioned above may be a counter-ion of any base addition salt. Suitable base addition salts are defined herein. Typically, the cation is a sodium cation.

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Amino protecting groups

The term "amino protecting group" used herein refers to any moiety that may replace a hydrogen atom in an amino group (i.e. an NH₂ group). Any suitable protecting group known to the skilled person to be suitable for use with saccharides is intended to be included within the meaning of "amino
 40 protecting group" in the present invention. The term is intended to encompass permanent, semi-

permanent and temporary protecting groups. In other words, it is not a requirement that a protecting group is readily removable, although in the general context of this invention the skilled person will appreciate that protecting groups which can be easily removed, such as by hydrolysis or hydrogenation, are preferred.

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Examples of amino protecting groups are optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} heteroalkyl, optionally substituted C_{3-10} heterocycloalkyl, optionally substituted C_{2-10} heteroalkenyl, optionally substituted C_{3-10} heterocycloalkenyl, $-PO(R^x)_2$, $-SO_2R^x$, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support, wherein each R^x is independently selected from OR^y , C_{1-4} alkyl, C_{6-14} aryl, C_{1-4} alkyl C_{6-14} aryl, halo C_{1-4} alkyl and $N(R^y)_2$, wherein each R^y is independently selected from H, a cation and C_{1-4} alkyl.

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For instance the protecting group may be H, optionally substituted C_{1-10} alkyl and $-S(O)_2OR^y$, preferably H, Ac, Boc, Cbz, Bz, or $-S(O)_2OR^y$, such as H or $S(O)_2OR^y$, wherein R^y is selected from H, C_{1-4} alkyl and a cation, optionally wherein the cation is sodium.

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Suitable amino protecting groups are selected from acetyl (Ac), chloroacetyl, trichloroacetyl (TCA), tert-butyloxycarbonyl (Boc), benzyloxy carbonyl (Cbz), phthalimido (Phthal), tetrachlorophthaloyl (TCP), N-dithiasuccinyl (Dts) (cleavable orthogonally in the presence of an azide using propane-1,3-dithiol (PDT) and DIPEA), [2,2,2-Trichloroethoxycarbonyl] (Troc), levulinoyl (Lev), tosyl (Tos), nosyl (Nos), allyloxycarbonyl (Alloc), trifluoroacetyl (TFA), trityl (Tr), benzylideneamine, oxazolidine, diglycolyl (DG) and dimethylglutamyl (DMG).

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The cation may be a counter-ion of any base addition salt as defined herein, preferably a sodium cation.

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Preferred amine protecting groups as used in the invention are orthogonal with azido moieties and stable under conditions for the removal of the protecting groups mentioned above. Accordingly, such orthogonal amino and hydroxy protecting groups allow for a chemical flexibility that will allow a synthetic chemist to differentiate between different amine and hydroxyl groups in the polysaccharides of the present invention. This orthogonal protection is advantageous where selective functionalization / deprotection of the product polysaccharide is desired.

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Tether for attachment of polysaccharides to carrier molecules

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The processes of the present invention are particularly amenable to chain extension of a polysaccharide bound to a solid support and any of the chain extending, first, chain extended or other polysaccharides as described herein may be bonded via a tether to a carrier molecule (such as a peptide, nucleic acid, lipid, fluorogenic tag, a fluorescent label, biotin, polyether (e.g. PEGs) or radiolabel).

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In particular, as the first polysaccharide and chain extending polysaccharides of the invention are at least four monosaccharide residues in length, the coupling reaction of the present invention may be suitably removed from any attachment to a carrier molecule or solid phase support such that the presence of a tether is not expected to have an appreciable effect on the reaction progress.

Thus, the polysaccharides of the present invention may suitably comprise a tether for attachment of the respective polysaccharide to a carrier molecule or to a solid support (such as immobilising beads or a microarray). Suitably, the polysaccharides of the present invention may be bonded to the carrier molecule or solid support via the tether.

The skilled person has many ways of tethering polysaccharides to carrier substrates at his disposal and the present invention is intended to cover any suitable tether. The tether may be selected depending on the functionalities present in the polysaccharide and carrier substrate. For example, a tether for attachment to a carrier substrate may be an aliphatic or aromatic residue (e.g. optionally substituted C_{1-10} alkyl or optionally substituted C_{6-14} aryl group) comprising a reactive functional group, such as an amino group, carboxyl group, aldehyde, azide, alkenyl or alkynyl group. In some embodiments the tether may comprise a polyether or polyester chain. In some embodiments, the tether is a bond, i.e. in embodiments wherein the polysaccharides of the present invention are bonded to the respective carrier substrate directly.

The skilled person will appreciate that compounds of the present invention as defined herein may be tethered to the carrier substrate via any suitable group in the polysaccharide chain. In the present invention, a plurality of tethers to solid support may be theoretically possible in some embodiments. Preferably only one tether is present.

Chemical groups Unless indicated explicitly otherwise, where combinations of groups are referred to herein as one moiety, e.g. arylalkyl, the last mentioned group contains the atom by which the moiety is attached to the rest of the molecule.

Halo

The term "halogen" (or "halo") refers to fluorine, chlorine, bromine and iodine radicals.

Alkyl, alkenyl, alkynyl, cycloalkyl etc.

The term "alkyl" includes monovalent, straight or branched, saturated, acyclic hydrocarbonyl groups. Typically, the alkyl groups of the present invention are C_{1-10} alkyl groups, such as C_{1-6} alkyl, e.g. such as methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl or t-butyl groups.

The term "alkylene" includes divalent, straight or branched, saturated, acyclic hydrocarbyl groups. Typically the alkylene groups of the present invention are C₁₋₁₀alkylene, such as C₁₋₆alkylene, e.g. C₁₋₄alkylene, such as methylene, ethylene, n-propylene, i-propylene, n-butylene, i-butylene or t-butylene groups.

- 5 The term "cycloalkyl" includes monovalent, saturated, cyclic hydrocarbyl groups. Exemplary cycloalkyl groups of the present invention are C₃₋₁₀cycloalkyl, which includes C₃₋₆cycloalkyl such as cyclopentyl and cyclohexyl.

- The term "cycloalkylene" includes divalent, saturated, cyclic hydrocarbyl groups. Exemplary cycloalkylene groups of the present invention are C₃₋₁₀cycloalkylene, which includes C₃₋₆cycloalkylene
10 such as cyclopentylene and cyclohexylene.

The term "alkenyl" includes monovalent, straight or branched, unsaturated, acyclic hydrocarbyl groups having at least one carbon-carbon double bond and, in one embodiment, no carbon-carbon triple bonds. Typically, alkenyl groups are C₂₋₁₀alkenyl, such as C₂₋₆alkenyl, e.g. C₂₋₄alkenyl, such as allyl.

- The term "alkenylene" includes divalent, straight or branched, unsaturated, acyclic hydrocarbyl groups
15 having at least one carbon-carbon double bond and, in one embodiment, no carbon-carbon triple bonds. Typically, alkenylene groups are C₂₋₁₀alkenylene, such as C₂₋₆alkenylene, e.g. C₂₋₄alkenylene.

The term "cycloalkenyl" includes monovalent, partially unsaturated, cyclic hydrocarbyl groups having at least one carbon-carbon double bond and, in one embodiment, no carbon-carbon triple bonds. Typically cycloalkenyl refers to C₃₋₁₀cycloalkenyl, such as C₅₋₁₀cycloalkenyl, e.g. cyclohexenyl.

- 20 The term "cycloalkenylene" includes divalent, partially unsaturated, cyclic hydrocarbyl groups having at least one carbon-carbon double bond and, in one embodiment, no carbon-carbon triple bonds. Typically cycloalkenylene refers to C₃₋₁₀cycloalkenylene, such as C₅₋₁₀cycloalkenylene, e.g. cyclohexenylene.

- The term "alkynyl" includes monovalent, straight or branched, unsaturated, acyclic hydrocarbyl groups
25 having at least one carbon-carbon triple bond and, in one embodiment, no carbon-carbon double bonds. Suitably, the alkynyl group may be C₂₋₁₀alkynyl, such as C₂₋₆alkynyl, e.g. C₂₋₄alkynyl.

The term "alkynylene" includes divalent, straight or branched, unsaturated, acyclic hydrocarbyl groups having at least one carbon-carbon triple bond and, in one embodiment, no carbon-carbon double bonds. Suitably, the alkynylene group may be C₂₋₁₀alkynyl, such as C₂₋₆alkynyl, e.g. C₂₋₄alkynyl.

- 30 *Heteroalkyl, etc.*

The term "heteroalkyl" includes alkyl groups in which up to three carbon atoms, such as up to two carbon atoms, e.g. one carbon atom, are each replaced independently by O, S(O)_k or N, provided at

least one of the alkyl carbon atoms remains. The heteroalkyl group may be C-linked or hetero-linked, i.e. it may be linked to the remainder of the molecule through a carbon atom or through O, S(O)_t or N, wherein t is independently 0, 1 or 2, for example 2. Typically, t is 0. An exemplary heteroalkyl group is alkoxy.

- 5 The term “heteroalkylene” includes alkylene groups in which up to three carbon atoms, such as up to two carbon atoms, e.g. one carbon atom, are each replaced independently by O, S(O)_t or N, provided at least one of the alkylene carbon atoms remains.

- The term “heterocycloalkyl” includes cycloalkyl groups in which up to three carbon atoms, such as two carbon atoms, e.g. one carbon atom, are each replaced independently by O, S(O)_t or N, provided at
 10 least one of the cycloalkyl carbon atoms remains. Examples of heterocycloalkyl groups include oxiranyl, thiaranyl, aziridinyl, oxetanyl, thiatanyl, azetidiny, tetrahydrofuranyl, tetrahydrothiophenyl, pyrrolidinyl, tetrahydropyranyl, tetrahydrothiopyranyl, piperidinyl, 1,4-dioxanyl, 1,4-oxathianyl, morpholinyl, 1,4-dithianyl, piperazinyl, 1,4-azathianyl, oxepanyl, thiepanyl, azepanyl, 1,4-dioxepanyl, 1,4-oxathiepanyl, 1,4-oxaazepanyl, 1,4-dithiepanyl, 1,4-thieazepanyl and 1,4-diazepanyl. The
 15 heterocycloalkyl group may be C-linked or N-linked, i.e. it may be linked to the remainder of the molecule through a carbon atom or through a nitrogen atom.

- The term “heterocycloalkylene” includes cycloalkylene groups in which up to three carbon atoms, such as two carbon atoms, e.g. one carbon atom, are each replaced independently by O, S(O)_t or N, provided at least one of the cycloalkyl carbon atoms remains. The heterocycloalkylene group may be
 20 C-linked or N-linked, i.e. it may be linked to the remainder of the molecule through a carbon atom or through a nitrogen atom.

- The term “heteroalkenyl” includes alkenyl groups in which up to three carbon atoms, such as two carbon atoms, e.g. one carbon atom, are each replaced independently by O, S(O)_t or N, provided at least one of the alkenyl carbon atoms remains. The heteroalkenyl group may be C-linked or hetero-
 25 linked, i.e. it may be linked to the remainder of the molecule through a carbon atom or through O, S(O)_t or N.

The term “heteroalkenylene” includes alkenylene groups in which up to three carbon atoms, such as up to two carbon atoms, e.g. one carbon atom, are each replaced independently by O, S(O)_t or N, provided at least one of the alkenylene carbon atoms remains.

- 30 The term “heterocycloalkenyl” includes cycloalkenyl groups in which up to three carbon atoms, such as to two carbon atoms, e.g. one carbon atom, are each replaced independently by O, S(O)_t or N, provided at least one of the cycloalkenyl carbon atoms remains. Examples of heterocycloalkenyl groups include 3,4-dihydro-2H-pyranyl, 5-6-dihydro-2H-pyranyl, 2H-pyranyl, 1,2,3,4-tetrahydropyridinyl and 1,2,5,6-tetrahydropyridinyl. The heterocycloalkenyl group may be C-linked or

N-linked, i.e. it may be linked to the remainder of the molecule through a carbon atom or through a nitrogen atom.

5 The term "heterocycloalkenylene" includes cycloalkenylene groups in which up to three carbon atoms, such as up to two carbon atoms, e.g. one carbon atom, are each replaced independently by O, S(O)_t or N, provided at least one of the cycloalkenylene carbon atoms remains. The heterocycloalkenylene group may be C-linked or N-linked, i.e. it may be linked to the remainder of the molecule through a carbon atom or through a nitrogen atom.

10 The term "heteroalkynyl" includes alkynyl groups in which up to three carbon atoms, such as up to two carbon atoms, e.g. one carbon atom, are each replaced independently by O, S(O)_t or N, provided at least one of the alkynyl carbon atoms remains. The heteroalkynyl group may be C-linked or hetero-linked, i.e. it may be linked to the remainder of the molecule through a carbon atom or through O, S(O)_t or N.

15 The term "heteroalkynylene" includes alkynylene groups in which up to three carbon atoms, such as up to two carbon atoms, e.g. one carbon atom, are each replaced independently by O, S(O)_t or N, provided at least one of the alkynylene carbon atoms remains. The heteroalkynylene group may be C-linked or hetero-linked, i.e. it may be linked to the remainder of the molecule through a carbon atom or through O, S(O)_t or N.

Aryl

20 The term "aryl" includes monovalent, aromatic, cyclic hydrocarbyl groups, such as phenyl or naphthyl (e.g. 1-naphthyl or 2-naphthyl). In general, the aryl group may be a monocyclic or polycyclic fused ring aromatic group. Preferred aryl groups are C₆-C₁₄aryl, such as phenyl

25 Other examples of aryl groups are monovalent derivatives of aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, chrysene, coronene, fluoranthene, fluorene, as-indacene, s-indacene, indene, naphthalene, ovalene, perylene, phenalene, phenanthrene, picene, pleiadene, pyrene, pyranthrene and rubicene.

The term "arylene" includes divalent, aromatic, cyclic hydrocarbyl groups, such as phenylene or naphthylene. In general, the arylene group may be a monocyclic or polycyclic fused ring aromatic group.

The term "arylalkyl" means alkyl substituted with an aryl group, e.g. benzyl.

30 *Heteroaryl*

The term "heteroaryl" includes aromatic groups which contain a ring heteroatom, such as aryl groups in which one or more carbon atoms are each replaced by heteroatoms independently selected from O,

S, N and NR^{N} , where R^{N} is H, C_{1-10} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{5-14} heteroaryl, $-\text{C}(\text{O})-\text{C}_{1-10}$ alkyl, $-\text{C}(\text{O})-\text{C}_{6-14}$ aryl, $-\text{C}(\text{O})-\text{C}_{5-14}$ heteroaryl, $-\text{S}(\text{O})_t-\text{C}_{1-10}$ alkyl, $-\text{S}(\text{O})_t-\text{C}_{6-14}$ aryl or $-\text{S}(\text{O})_t-\text{C}_{5-10}$ heteroaryl, wherein t is as defined above.

R^{N} may, in particular, be H, alkyl (e.g. C_{1-6} alkyl) or cycloalkyl (e.g. C_{3-6} cycloalkyl).

- 5 In general, the heteroaryl groups may be monocyclic or polycyclic (e.g. bicyclic) fused ring heteroaromatic groups. Typically, heteroaryl groups contain 5-14 ring members (preferably 5-10 members) wherein 1, 2, 3 or 4 ring members are independently selected from O, S, N and NR^{N} . Typically, a heteroaryl group may be 5, 6, 9 or 10 membered, e.g. 5-membered monocyclic, 6-membered monocyclic, 9-membered fused-ring bicyclic or 10-membered fused-ring bicyclic.

- 10 Monocyclic heteroaromatic groups include heteroaromatic groups containing 5-6 ring members wherein 1, 2, 3 or 4 ring members are independently selected from O, S, N and NR^{N} .

Typically, 5-membered monocyclic heteroaryl groups may contain 1 ring member which is an $-\text{NR}^{\text{N}}$ group, an $-\text{O}-$ atom or an $-\text{S}-$ atom and, optionally, 1-3 ring members (e.g. 1 or 2 ring members) which are $=\text{N}-$ atoms (where the remainder of the 5 ring members are carbon atoms).

- 15 Examples of 5-membered monocyclic heteroaryl groups are pyrrolyl, furanyl, thiophenyl, pyrazolyl, imidazolyl, isoxazolyl, oxazolyl, isothiazolyl, thiazolyl, 1,2,3 triazolyl, 1,2,4 triazolyl, 1,2,3 oxadiazolyl, 1,2,4 oxadiazolyl, 1,2,5 oxadiazolyl, 1,3,4 oxadiazolyl, 1,3,4 thiadiazolyl, pyridyl, pyrimidinyl, pyridazinyl, pyrazinyl, 1,3,5 triazinyl, 1,2,4 triazinyl, 1,2,3 triazinyl and tetrazolyl.

- Examples of 6-membered monocyclic heteroaryl groups are pyridinyl, pyridazinyl, pyrimidinyl and
20 pyrazinyl.

In some examples, 6-membered monocyclic heteroaryl groups may contain 1 or 2 ring members which are $=\text{N}-$ atoms (where the remainder of the 6 ring members are carbon atoms).

Bicyclic heteroaromatic groups include fused-ring heteroaromatic groups containing 9-14 ring members wherein 1, 2, 3, 4 or more ring members are independently selected from O, S, N and NR^{N} .

- 25 In some examples, 9-membered bicyclic heteroaryl groups contain 1 ring member which is an $-\text{NR}^{\text{N}}$ group, an $-\text{O}-$ atom or an $-\text{S}-$ atom and, optionally, 1-3 ring members (e.g. 1 or 2 ring members) which are $=\text{N}-$ atoms (where the remainder of the 9 ring members are carbon atoms).

- Examples of 9-membered fused-ring bicyclic heteroaryl groups are benzofuranyl, benzothiophenyl, indolyl, benzimidazolyl, indazolyl, benzotriazolyl, pyrrolo[2,3-b]pyridinyl, pyrrolo[2,3-c]pyridinyl,
30 pyrrolo[3,2-c]pyridinyl, pyrrolo[3,2-b]pyridinyl, imidazo[4,5-b]pyridinyl, imidazo[4,5-c]pyridinyl, pyrazolo[4,3-d]pyridinyl, pyrazolo[4,3-c]pyridinyl, pyrazolo[3,4-c]pyridinyl, pyrazolo[3,4-b]pyridinyl,

isoindolyl, indazolyl, purinyl, indolinyl, imidazo[1,2-a]pyridinyl, imidazo[1,5-a]pyridinyl, pyrazolo[1,2-a]pyridinyl, pyrrolo[1,2-b]pyridazinyl and imidazo[1,2-c]pyrimidinyl.

In exemplary heteroaryl groups, 10-membered bicyclic heteroaryl groups contain 1-3 ring members which are =N- atoms (where the remainder of the 10 ring members are carbon atoms).

- 5 Examples of 10-membered fused-ring bicyclic heteroaryl groups are quinoliny, isoquinoliny, cinnoliny, quinazolinyl, quinoxaliny, phthalazinyl, 1,6-naphthyridinyl, 1,7-naphthyridinyl, 1,8-naphthyridinyl, 1,5-naphthyridinyl, 2,6-naphthyridinyl, 2,7-naphthyridinyl, pyrido[3,2-d]pyrimidinyl, pyrido[4,3-d]pyrimidinyl, pyrido[3,4-d]pyrimidinyl, pyrido[2,3-d]pyrimidinyl, pyrido[2,3-b]pyrazinyl, pyrido[3,4-b]pyrazinyl, pyrimido[5,4-d]pyrimidinyl, pyrazino[2,3-b]pyrazinyl and pyrimido[4,5-
- 10 d]pyrimidinyl.

The term "heteroarylene" includes arylene groups in which one or more carbon atoms are each replaced by heteroatoms independently selected from O, S, N and NR^{N} , where R^{N} is H, C_{1-10} alkyl, C_{3-10} cycloalkyl, C_{6-14} aryl, C_{5-14} heteroaryl, $-\text{C}(\text{O})-\text{C}_{1-10}$ alkyl, $-\text{C}(\text{O})-\text{C}_{6-14}$ aryl, $-\text{C}(\text{O})-\text{C}_{5-14}$ heteroaryl, $-\text{S}(\text{O})_t-\text{C}_{1-10}$ alkyl, $-\text{S}(\text{O})_t-\text{C}_{6-14}$ aryl or $-\text{S}(\text{O})_t-\text{C}_{5-10}$ heteroaryl, wherein t is as defined above.

15

Haloalkyl

The term "haloalkyl" refers to an alkyl group substituted with 1-5, such as 1-3, in some instances 1 or 2, such as 1 halo radical and includes groups such as $-\text{CF}_3$ and $-\text{CCl}_3$.

20

Silyl

The term silyl refers to $-\text{SiR}^{\text{c}}_3$, wherein each R^{c} is independently selected from C_{1-10} alkyl and C_{6-14} aryl.

By way of clarification, in relation to the above heteroatom containing groups (such as heteroalkyl *etc.*), where a numerical of carbon atoms is given, for instance C_{3-6} heteroalkyl, what is intended is a group based on C_{3-6} alkyl in which one of more of the 3-6 chain carbon atoms is replaced by O, $\text{S}(\text{O})_t$ or N. Accordingly, a C_{3-6} heteroalkyl group, for example, will contain less than 3-6 chain carbon atoms (but will contain at least one carbon atom).

25

Where mentioned above, t is independently 0, 1 or 2, for example 2. Typically, t is 0.

30

Optional substituents

Optionally substituted groups (e.g. alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, alkylene, heteroalkyl, heterocycloalkyl, heteroalkenyl, heterocycloalkenyl, aryl, arylalkyl, heteroaryl, *etc.*) may be substituted or unsubstituted, such as unsubstituted. Typically, substitution refers to the notional replacement of a hydrogen atom with a substituent group, or two hydrogen atoms in the case of substitution by $=\text{O}$ or $=\text{NR}$, *etc.* When a particular group is substituted, there will generally be substitution by 1 to 5 substituents, such as 1 to 3 substituents, for instance 1 or 2 substituents, e.g. 1 substituent.

35

Unless indicated otherwise herein, the optional substituent(s) may be independently OH, NH₂, halogen, trihalomethyl, trihaloethyl, -NO₂, -CN, -N⁺(C₁₋₆alkyl)₂O⁻, -CO₂H, -CO₂C₁₋₆alkyl, -SO₃H, -SOC₁₋₆alkyl, -SO₂C₁₋₆alkyl, -SO₃C₁₋₆alkyl, -OC(=O)OC₁₋₆alkyl, -C(=O)H, -C(=O)C₁₋₆alkyl, -OC(=O)C₁₋₆alkyl, =O, -N(C₁₋₆alkyl)₂, -C(=O)NH₂, -C(=O)N(C₁₋₆alkyl)₂, -N(C₁₋₆alkyl)C(=O)O(C₁₋₆alkyl), -N(C₁₋₆alkyl)C(=O)N(C₁₋₆alkyl)₂, -OC(=O)N(C₁₋₆alkyl)₂, -N(C₁₋₆alkyl)C(=O)C₁₋₆alkyl, -C(=S)N(C₁₋₆alkyl)₂, -N(C₁₋₆alkyl)C(=S)C₁₋₆alkyl, -SO₂N(C₁₋₆alkyl)₂, -N(C₁₋₆alkyl)SO₂C₁₋₆alkyl, -N(C₁₋₆alkyl)C(=S)N(C₁₋₆alkyl)₂, -N(C₁₋₆alkyl)SO₂N(C₁₋₆alkyl)₂, -C₁₋₆alkyl, -C₁₋₆heteroalkyl, -C₃₋₆cycloalkyl, -C₃₋₆heterocycloalkyl, -C₂₋₆alkenyl, -C₆₋₁₄aryl, -C₆₋₁₄heteroaryl, -C₂₋₆heteroalkenyl, -C₃₋₆cycloalkenyl, -C₃₋₆heterocycloalkenyl, -C₂₋₆alkynyl, -C₂₋₆heteroalkynyl, a biotin residue, a polyethylene glycol residue, a fluorogenic tag, a fluorescent label, an isotopic label, -Z^u-C₁₋₆alkyl, -Z^u-C₃₋₆cycloalkyl, -Z^u-C₂₋₆alkenyl, -Z^u-C₃₋₆cycloalkenyl, -Z^u-C₂₋₆alkynyl, or -Z^u-C₁₋₆alkylaryl wherein Z^u is independently O, S, NH or N(C₁₋₆alkyl), wherein -C₆₋₁₄aryl may be further substituted with one or more of OH, NH₂, halo, -CO₂H, and Z^u-C₁₋₆alkyl.

Exemplary optional substituent(s) is/are independently halogen, trihalomethyl, trihaloethyl, -NO₂, -CN, -N⁺(C₁₋₆alkyl)₂O⁻, -CO₂H, -SO₃H, -SOC₁₋₆alkyl, -SO₂C₁₋₆alkyl, -C(=O)H, -C(=O)C₁₋₆alkyl, =O, -N(C₁₋₆alkyl)₂, -C(=O)NH₂, -C₁₋₆alkyl, -C₃₋₆cycloalkyl, -C₃₋₆heterocycloalkyl, -Z^u-C₁₋₆alkyl or -Z^u-C₃₋₆cycloalkyl, wherein Z^u is defined above.

Isotopic labelling and radiolabels

The terms "Isotopic label" or "isotopically labelling" as recited herein refers to the replacement of one or more atoms in any monosaccharide by an atomic isotope that is not abundant in nature. For instance, a given atom may be replaced by an atom having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature.

Examples of isotopes suitable for inclusion in the monosaccharides of the invention include isotopes of hydrogen, such as ²H and ³H, carbon, such as ¹¹C, ¹³C and ¹⁴C, chlorine, such as ³⁶Cl, fluorine, such as ¹⁸F, iodine, such as ¹²³I and ¹²⁵I, nitrogen, such as ¹³N and ¹⁵N, oxygen, such as ¹⁵O, ¹⁷O and ¹⁸O, phosphorus, such as ³²P, and sulphur, such as ³⁵S.

The term "radiolabel" refers to a group comprising or consisting of a radioactive isotope. Exemplary radioactive isotopes include ³H, ¹⁴C, ¹¹C, ¹⁸F, ¹⁵O and ¹³N, such as ³H and ¹⁴C. Substitution with positron emitting isotopes, such as ¹¹C, ¹⁸F, ¹⁵O and ¹³N, can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. Isotopically-labelled compounds can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described herein using an appropriate isotopically-labelled reagent in place of the non-labelled reagent previously employed. The installation of a tritium atom into a polysaccharide of the invention is described in the examples.

Fluorogenic tags

Fluorogenic tags are substituents containing fluorescent groups that may be affixed to a polysaccharide of the invention and which fluoresce in response to an addition of another moiety. A variety of tags are available to the skilled person and suitable tags may be used depending on the respective functional groups present in the polysaccharide. Fluorogenic tags provide means for

5 probing biological mechanisms with high sensitivity and may be suitably used in bioimaging, immunochemistry, fluorescence in situ hybridization (FISH), cell tracing, receptor labeling, and cytochemistry applications as well as probing biological structure, function and interactions.

Carrier molecule

10 Carrier molecules include lipids, peptides, biotin residues, polyethyleneglycol residues, amino acids, radiolabels, a fluorogenic tags, fluorescent labels, etc.

Acid and base addition salts

Monosaccharides or polysaccharides which contain basic groups, *e.g.* amines etc. as in glucosamine) are capable of forming salts with acids. Acid addition salts of the monosaccharides and polysaccharides described herein include but are not limited to, those of inorganic acids such as hydrohalic acids (*e.g.* hydrochloric, hydrobromic and hydroiodic acid), sulfuric acid, nitric acid and phosphoric acids. In one embodiment, acid addition salts include, but are not limited to, those of organic acids such as aliphatic, aromatic, carboxylic and sulfonic classes of organic acids, examples

15 of which include: aliphatic monocarboxylic acids such as formic acid, acetic acid, propionic acid or butyric acid; aliphatic hydroxy acids such as lactic acid, citric acid, tartaric acid or malic acid; dicarboxylic acids such as maleic acid or succinic acid; aromatic carboxylic acids such as benzoic acid, *p*-chlorobenzoic acid, phenylacetic acid, diphenylacetic acid or triphenylacetic acid; aromatic hydroxyl acids such as *o*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, 1-hydroxynaphthalene-2-

20 carboxylic acid or 3-hydroxynaphthalene-2-carboxylic acid; and sulfonic acids such as methanesulfonic acid, ethanesulfonic acid or benzenesulfonic acid. Other acceptable acid addition salts include, but are not limited to, those of glycolic acid, glucuronic acid, furoic acid, glutamic acid, anthranilic acid, salicylic acid, mandelic acid, embonic (pamoic) acid, pantothenic acid, stearic acid, sulfanilic acid, algenic acid and galacturonic acid.

30 Where the monosaccharide or polysaccharide comprises a plurality of basic groups, multiple centres may be protonated to provide multiple salts, *e.g.* di- or tri-salts. For example, a hydrohalic acid salt as described herein may be a monohydrohalide, dihydrohalide or trihydrohalide, *etc.* In one embodiment, the salts include, but are not limited to those resulting from addition of any of the acids disclosed

35 above. In one embodiment, two basic groups form acid addition salts. In a further embodiment, the two addition salt counterions are the same species, *e.g.* dihydrochloride, dihydrosulphide *etc.*

Monosaccharides or polysaccharides which contain acidic, *e.g.* carboxyl, groups (*e.g.* hexuronic acids) are capable of forming salts with bases. Base addition salts of the monosaccharides or

40 polysaccharides include, but are not limited to, metal salts such as alkali metal or alkaline earth metal

salts (e.g. sodium, potassium, magnesium or calcium salts), zinc or aluminium salts, and salts formed with ammonia, organic amines (e.g. ammonium, mono-, di-, tri- and tetraalkylammonium salts), or heterocyclic bases such as ethanolamines (e.g. diethanolamine), benzylamines, N-methyl-glucamine, amino acids (e.g. lysine) or pyridine. In typical embodiments, the base addition salt is selected from sodium, potassium and ammonium, mono-, di-, tri- and tetraalkylammonium salts.

Hemisalts of acids and bases may also be formed, e.g. hemisulphate salts.

Salts of monosaccharides and polysaccharides or the invention may be prepared by methods well-known in the art. For a review of pharmaceutically acceptable salts, see Stahl and Wermuth, Handbook of Pharmaceutical Salts: Properties, Selection and Use (Wiley-VCH, Weinheim, Germany, 2002). As noted above, the term "polysaccharide" includes such salts.

Brief Description of the Drawings

Figure 1 provides a figurative illustration of a chain extending process according to the invention wherein both coupling partners possess an SR_1 leaving group. The circle represents a chain extending polysaccharide and the square represents the first polysaccharide.

Figure 2 provides a figurative illustration of a chain extending process according to the invention wherein only one coupling partner possesses the SR_1 leaving group. The circle represents a chain extending polysaccharide and the square represents the first polysaccharide. In this case, each coupling step is performed by substitution of the SR_1 group bonded to the anomeric carbon of the chain extending polysaccharide.

Figure 3 provides a figurative illustration of a chain extending process according to the invention wherein different chain extending polysaccharides are used in each chain extending step, i.e. wherein the chain extending polysaccharide of the first chain extending step differs from the second chain extending polysaccharides used in second and further chain extending steps, etc. The circle, triangle and pentagon represent respective first, second and third chain extending polysaccharides and the square represents the first polysaccharide.

Figure 4 describes the preparation of thioglycoside building blocks (both donors and acceptors) of four monosaccharide residues in length according to the present invention, i.e. synthesis of tetrasaccharide donor/acceptor building blocks.

Figure 5 provides an example of protecting group manipulation in the polysaccharide (in this example, tetrasaccharide) building blocks of the present invention.

Figure 6 shows alternative approaches to glycoside acceptors for coupling with thioglycoside donors. In particular, alternative exemplary syntheses a) and b) for the preparation of glycoside acceptors

having an OR₂ group bonded to the anomeric carbon of a monosaccharide residue in the polysaccharide. Synthesis b) is further described in (Gardiner, J.M. *et al. Org. Lett.* **2013**, 15,(1), 88-91).

- 5 Figure 7 shows an exemplary iterative chain extension process according to the present invention wherein acceptor 15 is coupled with chain extending thioglycoside **14** (see Figure 5) to form chain extended polysaccharide **22**, which is deprotected and coupled with a further chain extending thioglycoside **14** to provide further chain extended polysaccharide **24**.
- 10 Figure 8 shows the further manipulation of dodecassachride **24** prepared according to the process outlined in Figure 7. In particular, dodecasaccharide deprotection and functionalization. In this example, the further manipulation includes sequential deprotection and sulfation steps. A process for the direct introduction of a tritium radiolabel is also exemplified.
- 15 Figure 9 provides data for the tissue localisation of **27b** following subcutaneous injection of **27b** into : mice at doses of 20 mg/kg (A), 40 mg/kg (B) and 80 mg/kg (C). Tissue quantities are pre-solubilization. Error bars represent the standard error of the mean of cpm converted to oligo weight using specific activity.
- 20 Figure 10 shows a HPLC SEC of radiolabelled polysaccharide **27b** (see Figure 8) injected subcutaneously into mice and then extracted and purified from kidneys 4 h after injection.
- Figure 11 shows an exemplary synthesis of O4-end (C-4 oxygen) tagged oligosaccharides to 10-mer and 12-mer. Modification of the O4 end of the core heparin-type disaccharide provides an
25 exemplification of access to O4 end tags that are analogous to the O1 end tag oligosaccharides discussed herein.
- Figure 12 shows an exemplary modification of an O4 end tag (aldehyde) to provide an amino group, which in turn can be used, for example, for conjugation. The same modification chemistry can be
30 used to access amino tag functionality at other tag locations, including O1.

Figure 13 shows an exemplary preparation of synthetic sulphonated heparin.

General methods

35 Preparation of building blocks of four monosaccharide residues in length

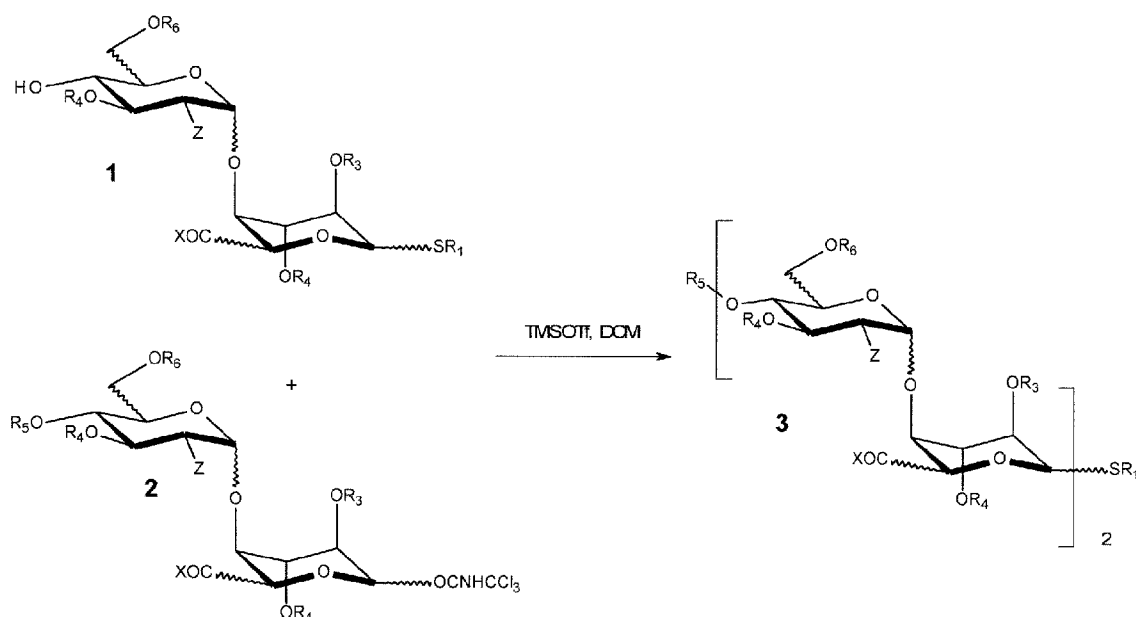
The chain extending and first oligosaccharide building blocks of four monosaccharide residues in length used in the present process may be accessed by conventional iterative glycosidic coupling of monosaccharides (i.e. 1+1+1), or alternatively by glycosidic bonding of two disaccharide residues.

A variety of methods are available to the skilled person for accessing suitably functionalised monosaccharides and disaccharides, including saccharides containing iduronic acid residues. Exemplary syntheses of such mono and disaccharides are disclosed in WO2006/129075A1, WO2009/098449A1 and Gardiner, J. M. *J. Org. Chem.* **2012**, 77, 7823-7843.

5

In a typical synthesis of a thioglycoside building block of four monosaccharide residues in length, a disaccharide **1** having a thio leaving group at its anomeric terminus is reacted with a further disaccharide **2** having a more reactive group at its anomeric terminus (e.g. trichloroacetimidate) to provide a tetrasaccharide building block **3** according to the invention having a thio leaving group at its anomeric terminus:

10



Preferably, a single nucleophilic group is provided in the acceptor disaccharide (e.g. the free OH in **1** above) to provide complete regiochemical control in the coupling. It is also preferred that each group in the donor glycoside (i.e. **2**, above) is protected to avoid self-condensation. An exemplary synthesis of a compound of formula **3** is provided in Figure 4.

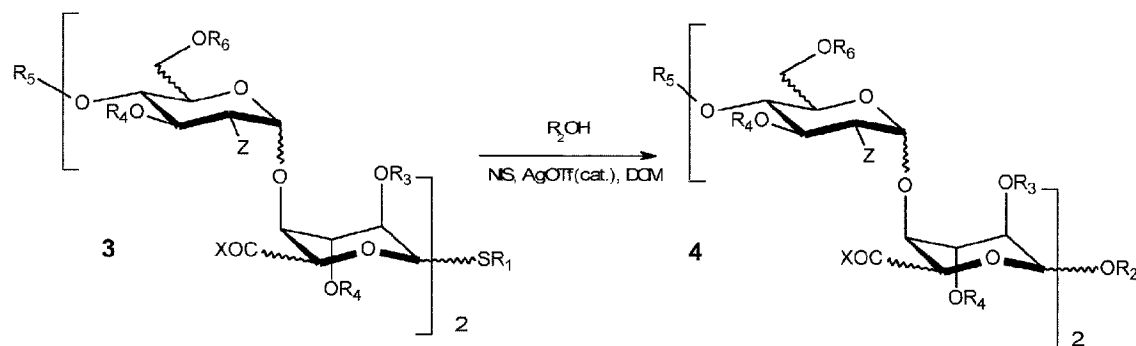
15

An analogous process can be also used to access building blocks of four or more monosaccharide units in length having a group other than a thio leaving group at its anomeric terminus by substituting thioglycoside **1** for a compound having a different anomeric group at its terminus, or by using a disaccharide donor thioglycoside with an acceptor disaccharide having a different anomeric group at its terminus, e.g. $-OR_2$ (see, e.g. Figure 6b).

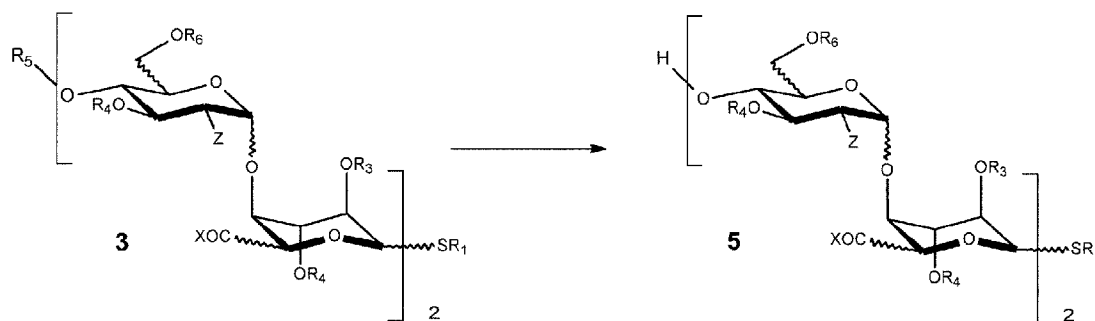
20

Alternatively, polysaccharides of four monosaccharide residues in length having an group other than a thio leaving group at the anomeric terminus (e.g. $-OR_2$), can be prepared using a tetrasaccharide **3** as an intermediate as depicted below (see, e.g. Figure 6a):

25

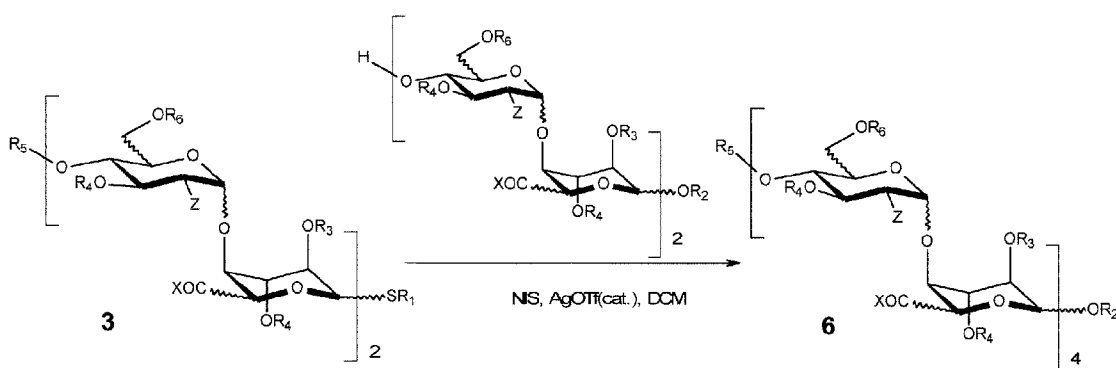


Furthermore, one or more nucleophilic groups in the tetrasaccharide **3** may be deprotected as exemplified below to enable the tetrasaccharide building block **3** to act as a glycoside acceptor (nucleophile) in further glycosidic couplings (e.g. with a further thioglycoside donor according to the invention). The skilled person can control which group or groups may be deprotected using a suitable orthogonal protecting group strategy in the preparation of **3** (see, e.g. the conversion of **12** to **13** in Figure 4). In the example below, R_5 is deprotected selectively.



Formation of polysaccharides of eight or more residues in length according to the invention

- 10 The tetrasaccharide **3** may be used as a glycoside donor in a glycosidic coupling to provide a chain extended polysaccharide of 8 monosaccharide residues in length according to the present invention, as depicted below:

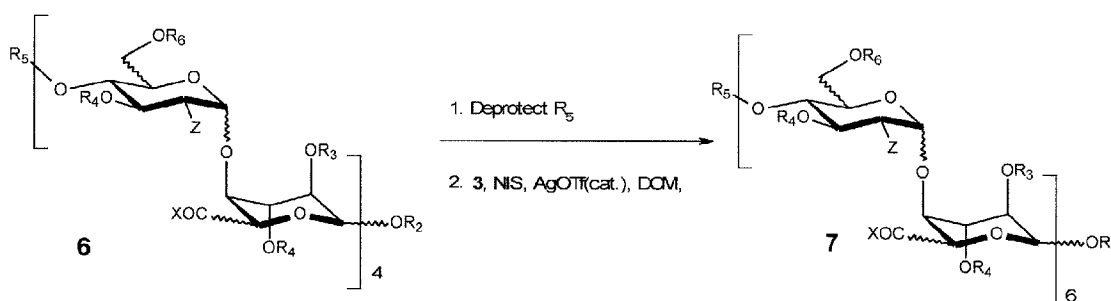


- 15 An exemplary process providing a chain extended polysaccharide of 8 monosaccharide residues in length is illustrated in Figure 7 (which shows the chain extension of a first polysaccharide **15** to provide a chain extended polysaccharide **21** or **22**).

Typically, a suitable thioglycoside activating system is used to promote the substitution of the thio leaving group in the glycosidic bond forming reaction. Examples of activating systems suitable for use with thio leaving groups include AgOTf, N-iodosuccinimide (NIS) / silver triflate (AgOTf), benzenesulfinyl piperidine (BSP) / trifluoromethanesulfonic anhydride (Tf₂O), and diphenylsulfoxide (DPS) / Tf₂O. Preferably, the activating system is NIS / AgOTf as depicted above.

Further chain extension

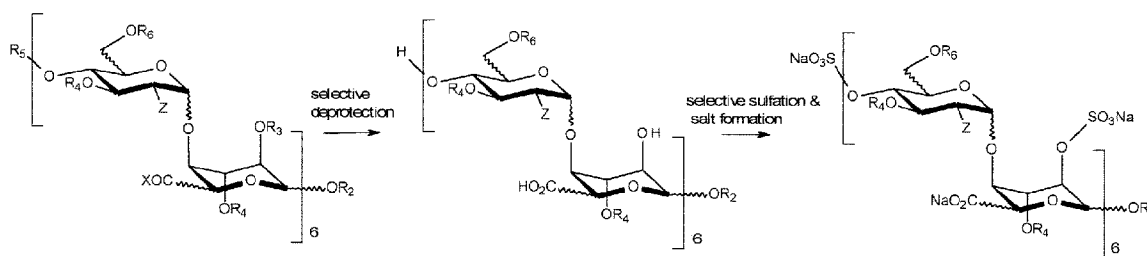
The chain extended polysaccharide **6** may be used in a further chain extending step with a further (i.e. second) chain extending thioglycoside donor **3**. If the chain extended polysaccharide is fully protected, it is necessary to first deprotect one or more nucleophilic groups in the acceptor. Typically only one nucleophilic group is deprotected to provide complete regiochemical control in the reaction (R⁵ in the reaction below). Orthogonal protecting group strategies known to the skilled person can be used to ensure a single group is deprotected (see, e.g. Figure 7):



An exemplary further (i.e. second) chain extending step to provide a polysaccharide of twelve monosaccharide residues in length is illustrated in Figure 7 (wherein chain extended polysaccharide **22** is extended using **14** to provide further chain extended polysaccharide **24**). This process can be reiterated analogously to provide the desired polysaccharide chain length and the Examples below show that this process can provide up to 40 monosaccharide residues in length cleanly and efficiently.

Further manipulation

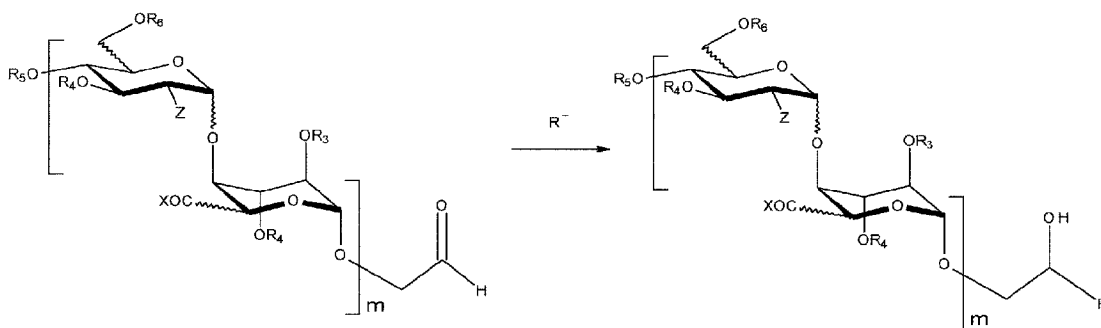
Once the skilled person has accessed a polysaccharide of a desired chain length using the iterative process of the present invention, the resulting polysaccharide can then be manipulated using conventional synthetic techniques if desired. For example, suitably labile protecting groups used during preparation of the polysaccharide chain may be removed. Orthogonal protecting group strategies will allow for the selective deprotection of nucleophilic groups which may then be functionalised independently if necessary, as exemplified below.



Radiolabelling

As described in more detail above, the addition of a radiolabel via a suitable linker suitably allows for analysis of the pharmacokinetic properties of the polysaccharide, providing an extremely useful tool for research chemists seeking to discover new drugs. Suitably, the radiolabel may be introduced into the polysaccharide as a constituent part of another functional group (for instance a modified protecting group / linker) or it may be introduced directly to the polysaccharide at any stage, for instance, by using an appropriate isotopically-labelled reagent in place of the non-labelled reagent usually employed.

- 10 The use of conventional carbonyl chemistry may for example be exploited for addition of a nucleophilic radionuclide species to form a corresponding radiolabelled polysaccharide. In the example below, a radiolabel is introduced directly to the polysaccharide by nucleophilic addition to an aldehyde precursor using a nucleophilic radiolabel source R^- (for instance ^3H may be introduced using NaB^3H_4):



- 15 wherein m is as defined in any embodiment above.

- An example of this methodology is provided in Figure 8. Whilst this methodology introduces a radiolabel appended to the end-capping group at the anomeric position, the skilled person will appreciate that the radiolabel may be introduced to groups appended to any other position in the polysaccharide chain using analogous methodology without detriment to the polysaccharide. Indeed, as shown in Figure 11, an aldehyde tag/linker can be provided at the C-4 oxygen (O4 position). This permits subsequent introduction of a radiolabel analogous to the scheme above. Furthermore, whilst this methodology is demonstrated herein using a tritium label, the skilled person will appreciate that this methodology is extremely flexible and provides innumerable avenues by which a given radiolabel may be introduced.

The present invention is described in more detail by way of example only with reference to the following Examples.

Examples

1. Synthesis of compounds

Preparation of thioglycoside donors

5

Preparation of Methyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl-L-idopyranoseuronate) (9)

To disaccharide **8** (1.85 g, 1.88 mmol, prepared in accordance with the methods disclosed in Gardiner, J. M. J. *Org. Chem.* **2012**, 77, 7823-7843) was added acetone 50 mL and cooled to 0 ° C in an ice bath. N-Bromosuccinimide (335 mg, 1.88 mmol) was then added and the mixture stirred for 45 min. The reaction was quenched by addition of saturated NaHCO₃ solution (5 mL), the acetone evaporated and DCM (200 mL) and water (200 mL) added. The organic phase was separated, dried (MgSO₄), filtered and evaporated. The crude product was purified using flash column chromatography (EtOAc/hexane 1:2) to yield 1.45 g (87%) of **3** as a white foam ($\alpha/\beta \sim 1:1$). *Rf* 0.17 (EtOAc/ Hexane 1:2). ¹H NMR (400 MHz; CDCl₃) δ 8.11-8.07 (m, 2H, Bz), 7.40-7.17 (m, 16H, Ph), 7.11-7.07 (m, 2H, Bz), 7.02 (d, *J* = 8.8 Hz, 2H, PMB), 6.84 (d, *J* = 8.8 Hz, 2H, PMB), 5.46-5.43 (m, 1H, H-1 α), 5.22 (dd, *J* = 11.6 Hz, 2 Hz, 1H, H-1 β), 5.08-5.06 (m, 2H, H-2 α , H-2 β), 4.91-4.90 (m, 1H, H-5 α), 4.90-4.75 (m, 2H, CH₂Ph), 4.69 (d, *J* = 3.6 Hz, 1H, H'-1), 4.66 (d, *J* = 3.6 Hz, 1H, H'-1), 4.62-4.35 (m, 4H, CH₂Ph, CH₂PMP), 4.60-4.59 (m, 1H, H-5 β), 4.33 (t, *J* = 2.8 Hz, 1H, H-3 β), 4.30 (dt, *J* = 2.8 Hz, 1.2 Hz, 1H, H-3 α), 4.20 (d, *J* = 9.2 Hz, 1H, OH α), 4.06-3.59 (m, 14H, H-4, H'-4, H'-5, H'-6, CH₂Ph), 3.82 (s, 3H, PhOCH₃), 3.81 (s, 3H, PhOCH₃), 3.74 (s, 3H, COOCH₃), 3.73 (s, 3H, COOCH₃), 3.41-3.36 (m, 2H, H'-3), 3.25-3.21 (m, 2H, H'-2). ¹³C NMR (100 MHz; CDCl₃) δ 169.5, 168.7, 165.9, 165.7, 159.2, 137.9, 137.9, 137.8, 137.0, 136.5, 133.4, 133.3, 130.4, 130.0, 129.5, 129.4, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 127.8, 127.7, 127.7, 127.6, 113.6, 100.4, 93.8, 92.6, 80.2, 80.0, 77.4, 77.3, 77.3, 76.2, 75.9, 74.5, 74.5, 74.0, 73.7, 73.6, 73.4, 73.0, 71.8, 71.7, 68.6, 67.6, 67.5, 67.0, 63.7, 63.7, 56.3, 55.3, 52.5, 52.4. HRMS (FT MS): *m/z*: calcd for C₄₉H₅₅N₄O₁₃ [*M*+NH₄]⁺: 907.3760; found: 907.3766.

Preparation of Methyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-p-methoxybenzyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl-1-trichloroacetimidate- α/β -L-idopyranuronate) (11)

To **9** (505 mg, 0.57 mmol) was added dry DCM (10 mL), CCl₃CN (0.40 mL, 3.99 mmol), 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (10 μ L, 0.07 mmol) and the mixture stirred under N₂ for 1 h. The solution was evaporated and the crude product was purified using flash column chromatography (EtOAc/hexane 1:3 with 1% NEt₃), yielding 535 mg (91%) of **4** as an oil ($\alpha/\beta \sim 3:1$). α -4: *Rf* 0.30 (EtOAc/Hexane 1:3). ¹H NMR (400 MHz; CDCl₃) δ 8.70 (s, 1H, C=NH), 8.15-8.12 (m, 2H, Bz), 7.44-7.11 (m, 18H, Ph), 7.07 (d, *J* = 8.6 Hz, 2H, PMB), 6.87 (d, *J* = 8.6 Hz, 2H, PMB), 6.57-6.56 (m, 1H, H-1), 5.36-5.35 (m, 1H, H-2), 5.03 (d, *J* = 2.6 Hz, 1H, H-5), 4.96-4.77 (m, 2H, CH₂Ph), 4.81 (d, *J* = 3.5 Hz, 1H, H'-1), 4.63-4.40 (m, 4H, CH₂Ph, CH₂PMP), 4.27-4.26 (m, 1H, H-3), 4.20-4.19 (m, 1H, H-4), 4.13-4.11 (m, 1H, CH₂Ph), 3.93-3.81 (m, 4H, H'-5, H'-6a, H'-6b, CH₂Ph), 3.83 (s, 3H, PhOCH₃),

3.75 (s, 3H, COOCH₃), 3.71-3.66 (m, 1H, H'-4), 3.50 (t, *J* = 10.0 Hz, 1H, H'-3), 3.25 (dd, *J* = 10.3 Hz, *J* = 3.5 Hz, 1H, H'-2). ¹³C NMR (100 MHz; CDCl₃) δ 168.7, 165.5, 160.2, 159.3, 137.9, 137.8, 137.3, 133.5, 130.5, 130.1, 129.5, 129.3, 128.9, 128.4, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.7, 113.7, 100.3, 96.1, 90.8, 80.1, 77.4, 77.1, 76.7, 75.8, 74.6, 74.5, 73.6, 72.5, 72.2, 71.8, 69.0, 67.7, 65.7, 63.7, 55.4, 52.5. β-4: *R*_f 0.16 (EtOAc/Hexane 1:3). ¹H NMR (400 MHz; CDCl₃) δ 8.68 (s, 1H, C=NH), 8.14-8.12 (m, 2H, Bz), 7.43-7.12 (m, 18H, Ph), 7.04 (d, *J* = 8.6 Hz, 2H, PMB), 6.85 (d, *J* = 8.6 Hz, 2H, PMB), 6.31 (d, *J* = 1.9 Hz, 1H, H-1), 5.48 (dd, *J* = 3.0 Hz, *J* = 1.9 Hz, 1H, H-2), 4.92-4.79 (m, 2H, CH₂Ph), 4.62-4.37 (m, 5H, H-3, CH₂Ph, CH₂PMP), 4.13-4.11 (m, 1H, CH₂Ph), 4.04-4.03 (m, 1H, H-4), 3.88-3.61 (m, 5H, H'-4 H'-5, H'-6a, H'-6b, CH₂Ph), 3.82 (s, 3H, PhOCH₃), 3.75 (s, 3H, COOCH₃), 3.48 (t, *J* = 10.2 Hz, 1H, H'-3), 3.28 (dd, *J* = 10.2 Hz, *J* = 3.5 Hz, 1H, H'-2). ¹³C NMR (100 MHz; CDCl₃) δ 167.9, 166.3, 163.6, 160.4, 159.3, 137.9, 137.8, 136.9, 133.3, 130.4, 130.1, 129.6, 128.8, 128.7, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 127.9, 127.8, 127.7, 113.7, 100.5, 95.2, 90.5, 80.2, 77.4, 77.1, 76.8, 76.7, 76.2, 74.7, 74.6, 74.5, 73.8, 73.6, 73.3, 71.8, 67.7, 66.5, 63.8, 55.4, 52.5. HRMS (FT MS): *m/z*: calcd for C₅₁H₅₁Cl₃N₄O₁₃ [*M*+NH₄]⁺: 1052.2844; found: 1052.2842.

Preparation of Methyl [(methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyl)uronate)-(1→4)-(phenyl(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-1-thio-β-L-idopyranoside)uronate (β-12)]

To **10** (1.05 g, 1.21 mmol) and **11** (1.43 g, 1.38 mmol) was added dry DCM (20 mL), the mixture cooled to -25 ° C and TMSOTf (2 μL, 0.01 mmol) added and the mixture stirred under a N₂ atmosphere for 30 min. More TMSOTf (2 μL, 0.01 mmol) was then added, the mixture left another 30 min. and then quenched by addition of a few drops of NEt₃. The solution was evaporated and the crude product was purified using flash column chromatography (EtOAc/hexane 1:3). A second column (DCM/EtOAc 20:1) removed remaining Cl₃CONH₂. This yielded 1.80 g (85%) of the product β-**6** as a foam. *R*_f 0.16 (EtOAc/Hexane 1:3). [α]_D²⁰ = +21.1 (*c* = 0.85, CH₂Cl₂). ¹H NMR (400 MHz; CDCl₃) δ 8.23-8.21 (m, 2H, Bz), 7.96-7.94 (m, 2H, Bz), 7.56-7.53 (m, 2H, Bz), 7.41-7.21 (m, 35H, Ph), 7.09-7.05 (m, 4H, Bz, PMB), 6.84 (d, *J* = 8.4 Hz, 2H, PMB), 5.50 (d, *J* = 4.0 Hz, 1H, H''-1), 5.26 (d, *J* = 2.0 Hz, 1H, H-1), 5.23-5.22 (m, 1H, H-2), 5.19 (t, *J* = 4.4 Hz, 1H, H''-2), 4.96 (d, 1H, *J* = 3.6 Hz, H'-1), 4.88-4.74 (m, 4H, CH₂Ph, CH₂PMP), 4.65-4.42 (m, 12H, H'''-1, H-4, H-5, H''-5, 4xCH₂Ph), 4.62-4.35 (m, 4H, CH₂Ph, CH₂PMP), 4.60-4.59 (m, 1H, H-5β), 4.33 (t, *J* = 2.8 Hz, 1H, H-3β), 4.30-4.21 (m, 4H, H-3, H''-3, CH₂Ph), 4.04 (t, *J* = 4.4 Hz, 1H, H''-4), 3.93-3.37 (m, 10H, H'-3, H'-4, H'-5, H'-6, H'''-3, H'''-4, H'''-5, H'''-6), 3.81 (s, 3H, PhOCH₃), 3.48 (s, 3H, COOCH₃), 3.43 (s, 3H, COOCH₃), 3.27-3.21 (m, 2H, H'-2, H'''-2). ¹³C NMR (100 MHz; CDCl₃) δ 169.2, 168.6, 166.4, 165.2, 159.3, 138.0, 137.9, 137.9, 137.4, 137.0, 134.8, 133.4, 133.3, 131.5, 130.4, 130.1, 129.9, 129.7, 129.5, 129.4, 129.0, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.2, 113.7, 99.7, 99.0, 98.3, 86.0, 79.9, 78.7, 77.6, 77.5, 77.4, 77.2, 76.8, 75.7, 75.5, 75.4, 75.1, 74.9, 74.6, 74.4, 73.9, 73.6, 72.9, 72.4, 71.7, 71.4, 70.2, 70.0, 69.7, 67.7, 67.6, 63.8, 63.5, 55.4, 52.1, 51.9. HRMS (FT MS): *m/z*: calcd for C₉₆H₁₀₀N₇O₂₃S [*M*+NH₄]⁺: 1750.6586; found: 1750.6578.

Preparation of Methyl [(methyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-(phenyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl-1-thio- β -L-idopyranoside]uronate (β -13)

To β -12 (952 mg, 0.55 mmol) was added CH₃CN (33 mL) and water (3 mL) followed by ammonium cerium (IV) nitrate (99.99+%, 751 mg, 1.37 mmol). The orange solution was stirred for 90 min and was then extracted with DCM (200 mL, 50 mL) and water (200 mL), dried (MgSO₄), filtered and evaporated. The crude product was purified using flash column chromatography (EtOAc/hexane 1:3) yielding β -7 (596 mg, 67%) as a white foam and recovered starting material (259 mg, 27%). *R_f* 0.20 (EtOAc/Hexane 1:2). $[\alpha]_{\text{D}20} = +10.4$ ($c = 0.12$, CH₂Cl₂). ¹H NMR (400 MHz; CDCl₃) δ (d, $J = 4.8$ Hz, 1H, H''-1), 5.23 (d, $J = 1.6$ Hz, 1H, H-1), 5.21-5.18 (m, 2H, H-2, H''-2), 4.95 (d, 1H, $J = 3.6$ Hz, H'-1), 4.86-4.72 (m, 4H, 2xCH₂Ph), 4.59-4.46 (m, 9H, H'''-1, H''-5, H-5, 3xCH₂Ph), 4.26-4.20 (m, 2H, H-3, H''-3), 4.26-3.34 (m, 13H, H-4, H''-4, H'-3, H'-4, H'-5, H'-6, H'''-3, H'''-5, H'''-6, CH₂Ph), 3.43 (s, 3H, COOCH₃), 3.41 (s, 3H, COOCH₃), 3.23-3.17 (m, 3H, H'-2, H''-2, H'''-4). ¹³C NMR (100 MHz; CDCl₃) δ 169.3, 168.6, 166.4, 165.2, 138.0, 138.0, 137.9, 137.6, 137.4, 136.9, 134.7, 133.4, 133.3, 131.4, 131.1, 130.1, 130.0, 129.9, 129.9, 129.6, 129.4, 129.0, 128.9, 128.8, 128.6, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.3, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.2, 99.3, 99.0, 98.1, 85.9, 79.2, 78.5, 77.4, 77.1, 77.1, 76.8, 75.8, 75.7, 75.6, 75.0, 75.0, 74.8, 74.5, 74.1, 73.8, 73.6, 72.8, 72.5, 72.3, 71.3, 70.8, 70.5, 70.2, 70.0, 69.4, 67.4, 63.6, 62.7, 52.1, 51.9. HRMS (FT MS): m/z : calcd for C₈₈H₉₂N₇O₂₂S [M+NH₄]⁺: 1630.6011; found: 1630.6009.

Preparation of Methyl [(methyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-trichloroacetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-(phenyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl-1-thio-Lidopyranoside] uronate (α -14 and β -14)

The tetrasaccharide α -13 (596 mg, 0.37 mmol) was dissolved in dry DCM (5 mL), cooled to 0 ° C in an ice bath, then dry pyridine (0.15 mL, 1.85 mmol) and trichloroacetyl chloride (0.10 mL, 0.93 mmol) was added. The solution was stirred for 1 h. The solution was extracted with DCM (50 mL) and water (50 mL), dried (MgSO₄), filtered and evaporated. The crude product was purified using flash column chromatography (EtOAc/hexane 1:3). This yielded α -14 (610 mg, 94%) as a white foam. The same procedure was used for converting β -13 into β -14. α -14: *R_f* 0.23 (EtOAc/Hexane 1:3). $[\alpha]_{\text{D}20} = -18.3$ ($c = 0.84$, CH₂Cl₂). ¹H NMR (500 MHz; CDCl₃) δ 8.13-8.11 (m, 2H, Bz), 8.02-8.00 (m, 2H, Bz), 7.53-7.12 (m, 35H, Ph), 5.78-5.77 (m, 1H, H-1), 5.55 (d, $J = 3.8$ Hz, 1H, H''-1), 5.37-5.38 (m, 1H, H-2), 5.31-5.27 (m, 2H, H'''-4, H-5), 5.17 (t, 1H, $J = 4.4$ Hz, H''-2), 4.97-4.75 (m, 4H, 2xCH₂Ph), 4.90 (d, $J = 3.6$ Hz, 1H, H'-1 or H'''-1), 4.69 (d, $J = 3.6$ Hz, 1H, H'-1 or H'''-1), 4.64 (d, $J = 4.1$ Hz, 1H, H''-5), 4.53-4.25 (m, 6H, 3xCH₂Ph), 4.21 (t, $J = 5.2$ Hz, 1H, H''-3), 4.19-4.18 (m, 1H, H-3), 4.02-4.01 (m, 1H, H-4), 3.99-3.41 (m, 13H, H''-4, H'-3, H'-4, H'-5, H'-6, H'''-3, H'''-4, H'''-5, H'''-6, CH₂Ph), 3.55 (s, 3H, COOCH₃), 3.45 (s, 3H, COOCH₃), 3.37 (dd, 1H, $J = 10.1$ Hz, 3.4 Hz H'-2 or H'''-2), 3.27 (dd, 1H, $J = 10.3$ Hz, 3.6 Hz H'-2 or H'''-2). ¹³C NMR (100 MHz; CDCl₃) δ 169.3, 169.2, 165.6, 165.3, 160.2,

138.0, 137.8, 137.3, 137.1, 136.8, 135.4, 133.6, 133.5, 131.3, 130.0, 129.9, 129.6, 129.5, 129.1, 128.8, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.7, 127.6, 127.4, 127.2, 99.8, 99.2, 98.5, 89.7, 86.8, 78.6, 77.6, 77.5, 77.2, 76.8, 76.5, 76.0, 75.9, 74.9, 74.8, 74.8, 74.1, 73.9, 73.8, 73.6, 72.8, 71.9, 71.4, 70.5, 69.6, 69.3, 69.2, 68.5, 67.7, 67.2, 63.8, 63.1, 60.5, 52.1, 51.9. ES MS: m/z : calcd for $C_{90}H_{87}Cl_3N_6O_{23}Na$ $[M+Na]^+$: 1779.5; found: 1779.5.

Elemental analysis calcd (%) for $C_{90}H_{87}Cl_3N_6O_{23}S$: C 61.45, H 4.98, N 4.78; found C 61.71, H 5.05, N 4.69. **β -14**: R_f 0.18 (EtOAc/Hexane 1:3). $[\alpha]_D^{20} = +32.9$ ($c = 0.61$, CH_2Cl_2). ^1H NMR (400 MHz; $CDCl_3$) δ 8.24-8.21 (m, 2H, Bz), 8.03-8.00 (m, 2H, Bz), 7.57-7.54 (m, 2H, Bz), 7.45-7.07 (m, 35H, Ph), 5.56 (d, $J = 3.8$ Hz, 1H, $H''-1$), 5.32 (t, $J = 9.4$ Hz, 1H, $H'''-4$), 5.27 (d, $J = 1.9$ Hz, 1H, $H-1$), 5.24-5.23 (m, 1H, $H-2$), 5.18 (t, $J = 4.4$ Hz, $H''-2$), 4.90-4.75 (m, 5H, $H'-1$, $2 \times CH_2Ph$), 4.64 (d, $J = 4.4$ Hz, $H-5$ or $H-5''$), 4.55-4.45 (m, 6H, $H'''-1$, $H''-5$ or $H-5$, $2 \times CH_2Ph$), 4.33-4.18 (m, 4H, $H-3$, $H''-3$, CH_2Ph), 4.01-3.90 (m, 4H, $H-4$, $H''-4$, CH_2Ph), 3.74-3.37 (m, 10H, $H'-2$, $H'-3$, $H'-4$, $H'-5$, $H'-6$, $H'''-3$, $H'''-5$, $H'''-6$), 3.50 (s, 3H, $COOCH_3$), 3.39 (s, 3H, $COOCH_3$), 3.25 (dd, $J = 10.3$ Hz, $J = 3.6$ Hz, 1H, $H'''-2$). ^{13}C NMR (100 MHz; $CDCl_3$) δ 169.2, 168.6, 166.4, 165.3, 160.2, 138.0, 137.8, 137.3, 137.3, 137.0, 136.8, 134.7, 133.4, 133.4, 131.5, 130.1, 129.9, 129.7, 129.5, 129.0, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.7, 127.7, 127.6, 127.5, 127.4, 127.1, 99.3, 99.2, 98.3, 89.7, 85.9, 78.5, 77.5, 77.4, 76.1, 75.7, 75.3, 74.9, 74.8, 74.6, 74.2, 73.8, 73.8, 73.6, 72.9, 72.5, 71.3, 70.2, 70.0, 69.4, 69.1, 67.8, 67.2, 63.9, 63.1, 52.1, 51.9.

Preparation of glycoside acceptors

Preparation of Methyl [(methyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl) uronate)-(1 $\rightarrow$ 4)-((S)-2,3-bis(benzyloxy)propyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranoside]uronate (15)

Tetrasaccharide **α -13** (145 mg, 0.082 mmol) was dissolved in dry DCM (2 mL) under N_2 . (S)-2,3-bis(benzyloxy)propanol (44 mg, 0.163 mmol) was added and the clear solution cooled to 0 ° C. Freshly activated 4Å powdered molecular sieves (137 mg) were added, after 10 min NIS (50 mg, 2.22 mmol) and after another 10 min AgOTf (catalytic amount). The suspension changed colour from pale yellow to deep red and was stirred for a further 20 min. The reaction was quenched into $NaHCO_3$ (250 mg) and $Na_2S_2O_3$ (250 mg) in water (5 mL). After stirring for 10 mins and the reaction colour changing to pale yellow the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (10 mL). The organics were combined, dried ($MgSO_4$) and solvent removed *in vacuo* to reveal the crude product as a pale yellow oil. This was purified by flash column chromatography (EtOAc/ Hexane 3:5) to give **15** (130 mg, 82%) as a white foam. R_f 0.22 (EtOAc/Hexane 1:2). $[\alpha]_D^{20} = +0.7$ ($c = 0.37$, CH_2Cl_2). ^1H NMR (500 MHz; $CDCl_3$) δ 8.11-8.09 (m, 2H, Bz), 8.00-7.98 (m, 2H, Bz), 7.54-7.14 (m, 40H, Ph), 5.55 (d, $J = 4.7$ Hz, $H''-1$), 5.21 (t, 1H, $J = 5.4$ Hz, $H''-2$), 5.14-5.13 (m, 1H, $H-1$), 5.12-5.11 (m, $H-2$), 4.94 (d, $J = 3.6$ Hz, 1H, $H'-1$), 4.87-4.41 (m, 19H, $H'''-1$, $H-5$, $H''-5$, $8 \times CH_2Ph$), 4.21 (t, $J = 6.0$ Hz, 1H, $H''-$

3), 4.10-4.09 (m, 1H, H-3), 4.04-4.01 (m, 1H, H''-4), 3.99-3.98 (m, 1H, H-4), 3.96-3.42 (m, 15H, H'-3, H'-4, H'-5, H'-6, H'''-3, H'''-4, H'''-5, H'''-6, CH₂CHOBnCH₂OBn), 3.46 (s, 3H, COOCH₃), 3.44 (s, 3H, COOCH₃), 3.23 (dd, 1H, *J* = 10.2 Hz, 3.6 Hz H'-2 or H'''-2), 3.19 (dd, 1H, *J* = 10.3 Hz, 3.6 Hz H'-2 or H'''-2). ¹³C NMR (100 MHz; CDCl₃) δ 169.6, 169.5, 169.4, 165.8, 165.4, 138.6, 138.3, 138.2, 138.1, 138.0, 137.7, 137.7, 137.5, 133.6, 130.1, 129.8, 129.6, 128.9, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.4, 99.4, 99.3, 98.4, 79.4, 78.6, 77.5, 76.8, 76.0, 75.9, 75.8, 75.1, 74.9, 74.5, 74.2, 73.9, 73.8, 73.5, 72.8, 72.5, 71.5, 71.2, 70.7, 70.5, 69.8, 69.6, 68.6, 67.8, 67.6, 67.5, 63.7, 62.9, 60.6, 52.1, 52.0. HRMS (FT MS): *m/z*: calcd for C₉₉H₁₀₆N₇O₂₅ [*M*+NH₄⁺]: 1792.7233; found: 1792.7212. Elemental analysis calcd (%) for C₉₉H₁₀₂N₆O₂₅: C 66.96, H 5.79, N 4.73; found C 66.71, H 5.69, N 4.68.

Chain extending steps

Preparation of Methyl [(methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-*p*-methoxybenzyl-α-Dglucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyl)uronate)-(1→4)-(methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-Lidopyranosyl)uronate)-(1→4)-(methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy-α-Dglucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyl)uronate)-(1→4)-((*S*)-2,3-bis(benzyloxy)propyl(2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside]uronate (21)

Tetrasaccharide **15** (55 mg, 0.031 mmol) and donor β-**12** (65 mg, 0.037 mmol) were dissolved in dry DCM (1 mL) under N₂. Freshly activated 4Å powdered molecular sieves (100 mg) were added and the solution cooled to 0 ° C in an ice bath. After 10 min NIS (13 mg, 0.046 mmol) was added, and after another 10 min AgOTf (catalytic amount) was added. The suspension changed colour from pale yellow to deep red and was stirred for a further 35 min. The reaction was quenched by pouring into a separating funnel containing a mixture of DCM (20 mL), saturated aqueous NaHCO₃ (20 mL) and Na₂S₂O₃ (2 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (10 mL). The organic layers were combined, dried (MgSO₄) and solvent removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (EtOAc/hexane 3:7) to give **21** (85 mg, 81%) as a white foam. *R*_f 0.24 (EtOAc/Hexane 1:2). [α]_D²⁰ = +9.1 (*c* = 0.78, CH₂Cl₂). ¹H NMR (400 MHz; CDCl₃) δ 8.10-8.08 (m, 2H, Bz), 7.95-7.91 (m, 6H, Bz), 7.55-7.00 (m, 84H, Ph, PMB), 6.83 (d, 1H, *J* = 8.8 Hz, PMB), 5.55-5.51 (m, 3H, H'-1, H'''-1, H''''-1), 5.19-5.15 (m, 3H, H'-2, H'''-2, H''''-2), 5.11-5.10 (m, 2H, H-1, H-2), 4.97 (d, 1H, *J* = 3.5 Hz, H'-1 or H'''-1 or H''''-1), 4.93 (d, 1H, *J* = 3.5 Hz, H'-1 or H'''-1 or H''''-1), 4.89 (d, 1H, *J* = 3.8 Hz, H'-1 or H'''-1 or H''''-1), 4.88-4.29 (m, 33H, H''''''-1, H-5, H'-5, H'''-5, H''''-5, 14xCH₂Ph), 4.23-4.07 (m, 4H, H-3, H'-3, H'''-3, H''''-3), 4.05-3.19 (m, 32H, H'-2, H'''-2, H''''-2, H''''''-2, H'-3, H'''-3, H-3'', H''''''-3, H-4, H'-4, H'''-4, H''''-4, H''''''-4, H'-5, H'''-5, H''''-5, H''''''-5, H'-6, H'''-6, H''''-6, H''''''-6, CH₂CHOBnCH₂OBn), 3.81 (s, 3H, PhOCH₃), 3.46 (s, 3H, COOCH₃), 3.41 (s, 3H, COOCH₃), 3.30 (s,

3H, COOCH₃), 3.23 (s, 3H, COOCH₃). HRMS (FTMS): m/z: calcd for C₁₈₉H₂₀₀N₁₄O₄₈ [M + 2NH₄]²⁺: 1717.6848; found: 1717.6827.

Preparation of Methyl [(methyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-trichloroacetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-(methyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-(methyl(2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-((S)-2,3-bis(benzyloxy)propyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranoside]uronate (22)

Tetrasaccharide **15** (68 mg, 0.038 mmol) and donor α -**14** (81 mg, 0.046 mmol) was dissolved in dry DCM (1.5 mL) under N₂. Freshly activated 4Å powdered molecular sieves (111 mg) were added and the solution cooled to 0 ° C in an ice bath. After 10 min. NIS (17 mg, 0.076 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The suspension changed colour from pale yellow to deep red and was stirred for a further 45 min. The reaction was quenched into a separating funnel containing a mixture of DCM (30 mL), saturated aqueous NaHCO₃ (25 mL) and Na₂S₂O₃ (2 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (10 mL). The organic layers were combined, dried (MgSO₄) and solvent removed *in vacuo*. The crude was purified by silica gel flash column chromatography (toluene/acetone 20:1) to give **22** (120 mg, 92%) of as a white foam. *R*_f 0.23 (EtOAc/Hexane 1:2). [α]_D²⁰ = +12.9 (c = 0.35, CH₂Cl₂). ¹H NMR (500 MHz; CDCl₃) δ 8.10-8.08 (m, 2H, Bz), 8.02-8.00 (m, 2H, Bz), 7.96-7.93 (m, 4H, Bz), 7.53-7.03 (m, 82H, Ph), 5.57-5.52 (m, 3H, H¹''', H¹''''-1, H¹'''''-1), 5.30 (t, 1H, *J* = 9.8 Hz, H¹''''''-4), 5.19-5.16 (m, 3H, H²''', H²''''-2, H²'''''-2), 5.12-5.11 (m, 2H, H-1, H-2), 4.94-4.90 (m, 3H, H¹''', H¹''''-1, H¹'''''-1), 4.87-4.24 (m, 33H, H¹''''''-1, H-5, H¹''-5, H¹''''-5, H¹'''''-5, 14xCH₂Ph), 4.24-4.09 (m, 4H, H-3, H¹''-3, H¹''''-3, H¹'''''-3), 4.04-3.20 (m, 32H, H¹''-2, H¹''''-2, H¹'''''-2, H¹''''''-2, H¹''-3, H¹''''-3, H¹'''''-3, H-4, H¹''-4, H¹''''-4, H¹'''''-4, H¹''''''-4, H¹''-5, H¹''''-5, H¹'''''-5, H¹''''''-5, H¹-6, H¹''-6, H¹''''-6, H¹'''''-6, CH₂CHOBnCH₂OBn), 3.47 (s, 3H, COOCH₃), 3.44 (s, 3H, COOCH₃), 3.33 (s, 3H, COOCH₃), 3.27 (s, 3H, COOCH₃). ¹³C NMR (100 MHz; CDCl₃) δ 169.4, 169.3, 169.2, 165.6, 165.2, 165.1, 160.2, 138.5, 138.1, 138.0, 137.9, 137.8, 137.7, 137.7, 137.5, 137.4, 137.4, 137.2, 137.2, 136.7, 133.6, 133.5, 129.9, 129.9, 129.8, 129.8, 129.6, 129.4, 129.3, 129.2, 128.8, 128.7, 128.7, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.3, 127.3, 127.1, 99.3, 99.3, 99.2, 99.1, 99.1, 98.3, 98.0, 98.0, 89.7, 78.3, 78.2, 78.1, 77.5, 77.4, 77.2, 77.0, 77.0, 76.8, 76.7, 76.7, 76.7, 76.6, 76.6, 76.5, 76.5, 75.9, 75.8, 75.7, 75.7, 75.6, 75.4, 75.4, 75.3, 74.9, 74.8, 74.8, 74.6, 74.4, 74.2, 74.1, 74.1, 73.8, 73.7, 73.6, 73.6, 73.5, 73.3, 73.2, 72.6, 72.4, 71.3, 71.2, 71.1, 70.5, 70.5, 70.4, 69.6, 69.2, 68.4, 68.3, 67.5, 67.3, 67.2, 67.2, 63.4, 63.4, 63.2, 63.2, 63.1, 60.4, 53.5, 51.9, 51.7, 51.6. MS (MALDITOF): m/z: calcd for C₁₈₃H₁₈₃Cl₃N₁₂NaO₄₈ [M+Na]⁺: 3444.1; found: 3444.1. Elemental analysis calcd (%) for C₁₈₃H₁₈₃Cl₃N₁₂O₄₈: C 64.18, H 5.39, N 4.91; found C 64.10, H 5.32, N 4.89.

Preparation of Methyl [(methyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-(methyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-(methyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-((S)-2,3-bis(benzyloxy)propyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranoside]uronate (23**)**

The octasaccharide **22** (346 mg, 0.10 mmol) was dissolved in a mixture of MeOH/pyridine (5 mL/2 mL) and heated to 50 ° C for 4 h. The solvents were evaporated and co-evaporated with toluene (2x20 mL). The crude product was purified using flash column chromatography (EtOAc/hexane 1:2 and 3:5). This yielded **23** (300 mg, 91%) as a white foam, along with recovered starting material (20 mg, 5%). *R_f* 0.10 (EtOAc/Hexane 1:2). $[\alpha]_{D20} = +9.8$ (*c* = 0.32, CH₂Cl₂). ¹H NMR (500 MHz; CDCl₃) δ 8.10-8.08 (m, 2H, Bz), 8.00-7.93 (m, 6H, Bz), 7.54-7.03 (m, 82H, Ph), 5.57-5.52 (m, 3H, H¹-1, H¹''-1, H¹'''-1), 5.22-5.15 (m, 3H, H¹-2, H¹''-2, H¹'''-2), 5.12-5.11 (m, 2H, H-1, H-2), 4.97 (d, 1H, *J* = 3.5 Hz, H¹-1 or H¹''-1 or H¹'''-1), 4.93 (d, 1H, *J* = 3.6 Hz, H¹-1 or H¹''-1 or H¹'''-1), 4.90 (d, 1H, *J* = 3.6 Hz, H¹-1 or H¹''-1 or H¹'''-1), 4.86-4.34 (m, 33H, H¹''''-1, H-5, H¹''-5, H¹'''-5, H¹''''-5, 14xCH₂Ph), 4.24-4.08 (m, 4H, H-3, H¹''-3, H¹'''-3, H¹''''-3), 4.05-3.20 (m, 32H, H¹-2, H¹''-2, H¹'''-2, H¹''''-2, H¹-3, H¹''-3, H¹'''-3, H¹''''-3, H-4, H¹''-4, H¹'''-4, H¹''''-4, H¹-4, H¹''-4, H¹'''-4, H¹''''-4, H¹-5, H¹''-5, H¹'''-5, H¹''''-5, H¹-6, H¹''-6, H¹'''-6, H¹''''-6, CH₂CHOBNCH₂OBn), 3.48 (s, 3H, COOCH₃), 3.44 (s, 3H, COOCH₃), 3.32 (s, 3H, COOCH₃), 3.27 (s, 3H, COOCH₃), 2.55 (d, 1H, *J* = 2 Hz, OH). ¹³C NMR (100 MHz; CDCl₃): δ 169.4, 169.3, 169.3, 169.2, 165.6, 165.2, 165.2, 138.5, 138.1, 138.0, 138.0, 137.9, 137.9, 137.8, 137.6, 137.5, 137.4, 137.4, 133.6, 133.5, 130.0, 130.0, 129.9, 129.8, 129.8, 129.6, 129.4, 129.3, 129.3, 129.0, 128.9, 128.9, 128.8, 128.7, 128.6, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 99.3, 99.2, 99.1, 99.1, 98.2, 98.1, 98.1, 98.0, 79.2, 78.3, 78.2, 78.1, 77.5, 77.4, 77.2, 77.1, 77.0, 76.8, 76.8, 76.7, 76.6, 75.9, 75.8, 75.5, 75.4, 75.3, 74.8, 74.8, 74.5, 74.4, 74.3, 74.2, 74.2, 74.1, 73.8, 73.7, 73.6, 73.6, 73.3, 72.7, 72.4, 71.4, 71.3, 71.3, 71.1, 70.6, 70.6, 70.4, 70.4, 69.6, 69.5, 68.4, 67.6, 67.4, 67.4, 67.3, 63.5, 63.1, 63.0, 63.0, 62.7, 53.5, 52.0, 51.9, 51.9, 51.7, 51.6. MS (MALDI-TOF): *m/z*: calcd for C₁₈₁H₁₈₄N₁₂NaO₄₇ [*M*+Na]⁺: 3300.2; found: 3300.2. Elemental analysis calcd (%) for C₁₈₁H₁₈₄N₁₂O₄₇: C 66.29, H 5.66, N 5.13; found C 66.57, H 5.98, N 4.96.

Further chain extending steps to provide polysaccharides of 12 or more residues in length

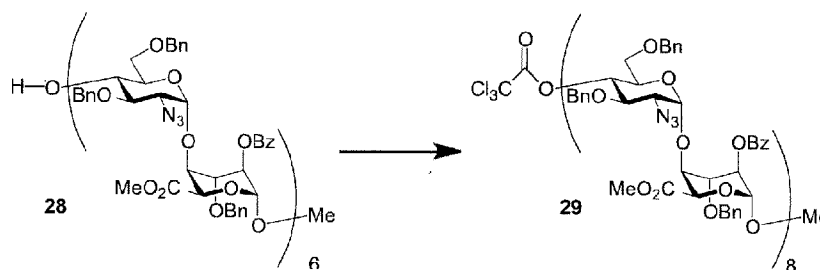
Preparation of Methyl [(methyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-trichloroacetyl- α -D-glucopyranosyl)- (1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-(methyl (2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-(methyl(2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-(methyl(2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-(methyl(2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl)uronate)-(1 $\rightarrow$ 4)-((S)-2,3-bis(benzyloxy)propyl

(2-azido-3,6-di-Obenzyl-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-O-benzoyl-3-O-benzyl- α -L-idopyranoside]uronate (24**)**

Acceptor **23** (252 mg, 0.077 mmol) and donor **14** (175 mg, 0.100 mmol) were dissolved in dry DCM (4 mL) under N₂. Freshly activated 4Å powdered molecular sieves (222 mg) were added and the solution cooled to 0 ° C in an icebath. After 10 min. NIS (47 mg, 0.21 mmol) and after another 10 min. AgOTf (catalytic amount) were added. The suspension changed colour from pale yellow to deep red and was stirred for a further 35 min. The reaction was quenched into a separating funnel containing a mixture of DCM (50 mL), saturated aqueous NaHCO₃ (50 mL) and Na₂S₂O₃ (5 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (10 mL). The organic layers were combined, dried (MgSO₄) and solvent removed *in vacuo*. The crude product was purified by silica gel flash column chromatography (EtOAc/hexane 7:13) to yield **24** (295 mg, 78%) as a white foam, along with recovered acceptor (37 mg, 15%). *R*_f 0.17 (EtOAc/Hexane 7:13). [α]_D20 = +18.1 (*c* = 0.68, CH₂Cl₂). ¹H NMR (500 MHz; CDCl₃) δ 8.10-8.08 (m, 2H, Bz), 8.02-8.00 (m, 2H, Bz), 7.96-7.91 (m, 8H, Bz), 7.53-7.02 (m, 118H, Ph), 5.57-5.52 (m, 5H, H¹-1, H¹'-1, H¹''-1, H¹'''-1, H¹''''-1), 5.29 (t, 1H, *J* = 9.7 Hz, H¹''''''-4), 5.19-5.14 (m, 5H, H²-2, H²'-2, H²''-2, H²'''-2, H²''''-2), 5.12-5.11 (m, 2H, H-1, H-2), 4.94-4.89 (m, 5H, H¹-1, H¹'-1, H¹''-1, H¹'''-1, H¹''''-1), 4.86-4.27 (m, 47H, H¹''''''-1, H-5, H¹'-5, H¹''-5, H¹'''-5, H¹''''-5, H¹''''''-5, 20xCH₂Ph), 4.24-4.08 (m, 6H, H-3, H³-3, H³'-3, H³''-3, H³'''-3, H³''''-3), 4.04-3.19 (m, 45H, H²-2, H²'-2, H²''-2, H²'''-2, H²''''-2, H²''''''-2, H³-3, H³'-3, H³''-3, H³'''-3, H³''''-3, H³''''''-3, H-4, H⁴-4, H⁴'-4, H⁴''-4, H⁴'''-4, H⁴''''-4, H⁴''''''-4, H⁵-5, H⁵'-5, H⁵''-5, H⁵'''-5, H⁵''''-5, H⁵''''''-5, H⁶-6, H⁶'-6, H⁶''-6, H⁶'''-6, H⁶''''-6, H⁶''''''-6, CH₂CHOBNCH₂OBn), 3.46 (s, 3H, COOCH₃), 3.44 (s, 3H, COOCH₃), 3.33 (s, 3H, COOCH₃), 3.30 (s, 3H, COOCH₃), 3.29 (s, 3H, COOCH₃), 3.26 (s, 3H, COOCH₃). ¹³C NMR (100 MHz; CDCl₃): δ 169.5, 169.4, 169.3, 165.7, 165.4, 165.3, 165.2, 160.3, 138.6, 138.2, 138.1, 138.0, 137.9, 137.8, 137.8, 137.6, 137.5, 137.5, 137.3, 137.3, 136.9, 133.7, 133.7, 133.6, 130.0, 130.0, 129.9, 129.7, 129.5, 129.5, 129.4, 129.3, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.4, 99.4, 99.4, 99.3, 99.2, 99.2, 99.1, 98.4, 98.2, 98.1, 98.1, 89.8, 78.4, 78.3, 76.8, 76.7, 76.7, 76.6, 76.6, 76.5, 76.1, 75.9, 75.8, 75.7, 75.7, 75.6, 75.5, 75.5, 75.4, 75.3, 75.0, 74.9, 74.8, 74.7, 74.6, 74.5, 74.3, 74.1, 74.1, 74.0, 74.0, 74.0, 73.9, 73.8, 73.7, 73.6, 73.6, 73.5, 73.5, 73.4, 73.3, 72.7, 72.7, 72.7, 72.5, 71.4, 71.4, 71.3, 71.3, 71.2, 71.2, 70.7, 70.7, 70.6, 70.6, 70.6, 70.5, 70.5, 70.4, 70.4, 69.7, 69.6, 69.3, 68.5, 68.4, 67.6, 67.6, 67.4, 67.3, 67.3, 67.2, 63.6, 63.3, 63.2, 63.1, 63.0, 53.6, 52.1, 51.8, 51.8, 51.7. MS (MALDI-TOF): *m/z*: calcd for C₂₆₅H₂₆₅Cl₃N₁₈NaO₇₀ [M+Na]⁺: 4946.7; found: 4946.6. Elemental analysis calcd (%) for C₂₆₅H₂₆₅Cl₃N₁₈O₇₀: C 64.58, H 5.42, N 5.12; found C 63.91, H 5.41, N 5.06.

16-mer protected polysaccharide

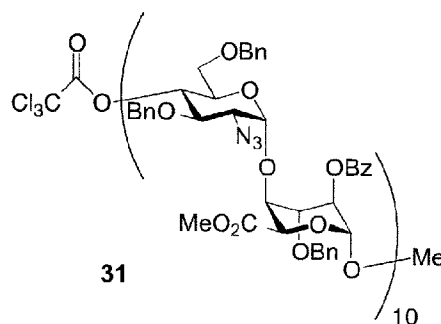
Methyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-trichloroacetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-
 (methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyluronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-
 deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyluronate)-
 (1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-
 5 benzyl- α -L-idopyranosyluronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl-
 (1 $\rightarrow$ 4)- (methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyluronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-
 benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl
 uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-
 benzoyl-3-O-benzyl- α -L-idopyranosyluronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-deoxy- α -D-
 10 glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyluronate)-(1 $\rightarrow$ 4)-2-
 azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-benzyl- α -L-
 idopyranoside) uronate (**29**)



The known dodecasaccharide **28** (114 mg, 0.025 mmol, accessible by iterative chain extension of
 15 tetrasaccharide **20** with thioglycoside building block **14** employing methods analogous to those
 disclosed above for the preparation of **22** to **24**) and thioglycoside tetrasaccharide donor **14** (53 mg,
 0.030 mmol) were dissolved in dry DCM (1 mL) under N₂. Freshly activated 4Å powdered molecular
 sieves (130 mg) was added and the solution cooled to 0°C in an ice bath. After 10 min. NIS (19 mg,
 0.084 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The
 20 suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was
 quenched into a separating funnel containing a mixture of DCM (30 mL), saturated aqueous NaHCO₃
 (20 mL) and Na₂S₂O₃ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the
 suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were
 separated and the aqueous extracted with DCM (10 mL). The organic layers were combined, dried
 25 (MgSO₄) and solvent removed *in vacuo*. The crude was purified by silica gel flash column
 chromatography (toluene/acetone 20:1) to give **29** (118 mg, 76%) as a white foam. *R*_f 0.45
 (toluene/acetone 10:1). ¹H NMR (400 MHz; CDCl₃): δ 8.15-8.13 (m, 2H, Bz), 8.07-8.05 (m, 2H, Bz),
 8.00-7.96 (m, 12H, Bz), 7.57-7.02 (m, 144H, Ph), 5.64-5.56 (m, 7H, H^{CEGIKMO}-1), 5.35 (t, *J* = 9.6 Hz,
 1H, H^P-4), 5.25-5.18 (m, 7H, H^{CEGIKMO}-2), 5.12-5.11 (m, 1H, H^A-2), 5.08-5.07 (m, 1H, H^A-1), 4.99-4.91
 30 (m, 8H, H^{BDFHJLNP}-1), 4.86-4.35 (m, 56H, H^{ACEGIKMO}-5, 24xCH₂Ph), 4.29-4.19 (m, 7H, H^{CEGIKMO}-3),
 4.12-3.20 (m, 80H, H^A-3, H^{ACEGIKMO}-4, H^{BDFHJLNP}-2, H^{BDFHJLNP}-3, H^{BDFHJLNP}-4, H^{BDFHJLNP}-5, H^{BDFHJLNP}-6_{ab},
 8xCOOCH₃), 3.27 (s, 3H, OCH₃). ¹³C NMR (101 MHz; CDCl₃): δ 169.5, 169.3, 169.2, 165.5, 165.2,
 165.1, 160.2, 138.0, 137.9, 137.8, 137.8, 137.7, 137.6, 137.4, 137.4, 137.4, 137.2, 137.2, 136.7,
 133.6, 133.6, 133.5, 130.0, 129.9, 129.9, 129.8, 129.7, 129.5, 129.4, 129.3, 129.2, 129.0, 129.0,

20-mer Protected Polysaccharide

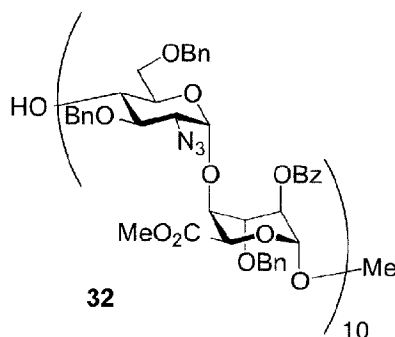
Methyl (2-azido-3,6-di-O-benzyl-2-deoxy-4-O-trichloroacetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-
 5 (methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-
 deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl uronate)-
 (1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-
 benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl-
 10 (1 $\rightarrow$ 4)- (methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-
 benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl
 uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-
 benzoyl-3-O-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-deoxy- α -D-
 glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-
 15 azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-benzyl- α -L-
 idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-
 (methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-O-benzyl-2-
 deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-O-benzoyl-3-O-benzyl- α -L-idopyranoside) uronate
 (31)



20 Acceptor hexadecasaccharide **30** (286 mg, 0.047 mmol) and thioglycoside tetrasaccharide donor **14**
 (108 mg, 0.061 mmol) was dissolved in dry DCM (2 mL) under N₂. Freshly activated 4Å powdered
 molecular sieves (171 mg) was added and the solution cooled to 0°C in an icebath. After 10 min. NIS
 (33 mg, 0.147 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The
 suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was
 25 quenched into a separating funnel containing a mixture of DCM (30 mL), saturated aqueous NaHCO₃
 (20 mL) and Na₂S₂O₃ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the
 suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were
 separated and the aqueous extracted with DCM (10 mL). The organic layers were combined, dried
 (MgSO₄) and solvent removed *in vacuo*. The crude was purified by silica gel flash column
 30 chromatography (toluene/acetone 20:1 to 15:1 to 10:1) followed by precipitation (dissolved in EtOAc
 (5 mL) and hexane (5 mL) added) and filtration to give **31** (295 mg, 81%) as a white solid. *R_f* 0.45
 (toluene/acetone 10:1). $[\alpha]_D^{20} = +19.1$ (*c* = 0.78, CH₂Cl₂). ¹H NMR (800 MHz; CDCl₃) δ 8.10-8.09 (m,

2H, Bz), 8.02-8.01 (m, 2H, Bz), 7.94-7.91 (m, 16H, Bz), 7.55-7.02 (m, 180H, Ph), 5.58-5.53 (m, 9H, H^{CEGIKMOQS}-1), 5.31 (t, *J* = 9.6 Hz, 1H, H^T-4), 5.19-5.14 (m, 9H, H^{CEGIKMOQS}-2), 5.09-5.08 (m, 1H, H^A-2), 5.05-5.04 (m, 1H, H^A-1), 4.95-4.88 (m, 10H, H^{BDFHJLNPR}-1), 4.83-4.34 (m, 70H, H^{ACEGIKMOQS}-5, 30xCH₂Ph), 4.26-4.16 (m, 9H, H^{CEGIKMOQS}-3), 4.06-3.18 (m, 100H, H^A-3, H^{ACEGIKMOQS}-4, H^{BDFHJLNPR}-2, H^{BDFHJLNPR}-3, H^{BDFHJLNPR}-4, H^{BDFHJLNPR}-5, H^{BDFHJLNPR}-6_{ab}, 10xCOOCH₃), 3.24 (s, 3H, OCH₃). ¹³C NMR (201 MHz; CDCl₃): δ 169.6, 169.4, 165.7, 165.2, 160.3, 138.0, 137.9, 137.5, 137.3, 133.7, 133.5, 130.0, 129.9, 129.4, 128.8, 128.5, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 127.2, 99.3, 98.4, 98.1, 78.1, 77.0, 76.1, 76.0, 75.6, 75.5, 75.0, 74.8, 74.5, 74.3, 73.9, 73.8, 72.7, 72.6, 71.4, 70.8, 70.7, 70.6, 69.8, 69.3, 67.4, 63.5, 63.1, 52.1, 52.0, 51.7. MALDI TOF MS: *m/z*: calcd for C₄₁₃H₄₁₃Cl₃N₃₀NaO₁₁₂ [*M*+Na]⁺: 7717.7; found: 7717.8.

20-mer Acceptor Polysaccharide

[illegible]

The icosasaccharide **31** (278 mg, 0.036 mmol) was dissolved in a mixture of MeOH/pyridine (5 mL/2.5 mL) and heated to 60°C for 5 h. The solvents were evaporated and co-evaporated with toluene (2x10 mL). The crude product was purified using flash column chromatography (toluene/acetone 15:1) followed by precipitation (dissolved in EtOAc (5 mL) and hexane (5 mL)).

5

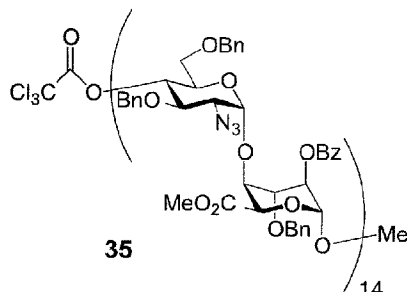
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30

(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranoside) uronate (35)



Acceptor tetracosasaccharide **34** (161 mg, 0.018 mmol) and thioglycoside tetrasaccharide donor **14** (41 mg, 0.023 mmol) was dissolved in dry DCM (1.5 mL) under N₂. Freshly activated 4Å powdered molecular sieves (174 mg) was added and the solution cooled to 0°C in an icebath. After 10 min. NIS (15 mg, 0.067 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was quenched into a separating funnel containing a mixture of DCM (20 mL), saturated aqueous NaHCO₃ (10 mL) and Na₂S₂O₃ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were separated and the aqueous extracted with DCM (10 mL). The organic layers were combined, dried (MgSO₄) and solvent removed *in vacuo*. The crude was purified by silica gel flash column chromatography (toluene/acetone 20:1 to 15:1 to 10:1) followed by precipitation (dissolved in warm EtOAc (5 mL) and hexane (5 mL) added) and filtration to give **35** (143 mg, 75%) as a white solid. Also acceptor starting material (35 mg, 21%) was recovered. *R_f* 0.45 (toluene/acetone 10:1). ¹H NMR (400 MHz; CDCl₃) δ 8.13-8.11 (m, 2H, Bz), 8.05-8.03 (m, 2H, Bz), 7.98-7.93 (m, 24H, Bz), 7.57-7.05 (m, 252H, Ph), 5.59-5.55 (m, 13H, H^{(BCDEFGHIJKLMN)ido}-1), 5.34 (t, *J* = 9.6 Hz, 1H, H^{(N)gicn}-4), 5.26-5.22 (m, 13H, H^{(BCDEFGHIJKLMN)ido}-2), 5.10-5.09 (m, 1H, H^{(A)ido}-2), 5.07-5.06 (m, 1H, H^{(A)ido}-1), 4.99-4.88 (m, 14H, H^{(ABCDEFGHIJKLMN)gicn}-1), 4.86-4.33 (m, 98H, H^{(ABCDEFGHIJKLMN)ido}-5, 42xCH₂Ph), 4.27-4.18 (m, 13H, H^{(BCDEFGHIJKLMN)ido}-3), 4.12-3.20 (m, 140H, H^{(A)ido}-3, H^{(ABCDEFGHIJKLMN)ido}-4, H^{(ABCDEFGHIJKLMN)gicn}-2, H^{(ABCDEFGHIJKLMN)gicn}-3, H^{(ABCDEFGHIJKLMN)gicn}-4, H^{(ABCDEFGHIJKLMN)gicn}-5, H^{(ABCDEFGHIJKLMN)gicn}-6_{ab}, 14xCOOCH₃), 3.25 (s, 3H, OCH₃). MALDI TOF MS: *m/z*: calcd for C₅₇₇H₅₇₇Cl₃N₄₂NaO₁₅₆ [*M*+Na]⁺: 10725; found: 10726.

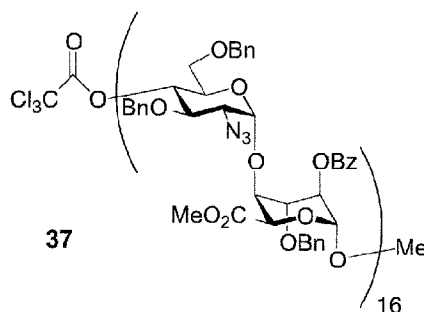
28-mer Acceptor Polysaccharide

14xCOOCH₃), 3.22 (s, 3H, OCH₃), 2.60 (d, *J* = 2.4 Hz, 1H, OH). MALDI TOF MS: *m/z*: calcd for C₅₇₅H₅₇₈N₄₂NaO₁₅₅ [*M*+Na]⁺: 10580; found: 10579.

32-mer Protected Polysaccharide

5

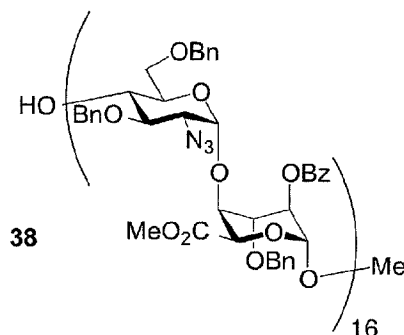
Methyl (2-azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-trichloroacetyl- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-
 (methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-
 deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-
 (1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-
 10 benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-
 (1 $\rightarrow$ 4)- (methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-
 benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl
 uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-
 15 benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-
 glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-
 azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-
 idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-
 (methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-
 20 deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-
 (1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-
 benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-
 (1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-
 25 benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl
 uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-
 benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-azido-3,6-di-*O*-benzyl-2-deoxy- α -D-
 glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-idopyranosyl uronate)-(1 $\rightarrow$ 4)-2-
 azido-3,6-di-*O*-benzyl-2-deoxy- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-(methyl 2-*O*-benzoyl-3-*O*-benzyl- α -L-
 idopyranoside) uronate (37)



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Acceptor octacosasaccharide **36** (147 mg, 0.014 mmol) and thioglycoside tetrasaccharide donor **14** (32 mg, 0.018 mmol) was dissolved in dry DCM (1.5 mL) under N₂. Freshly activated 4 Å powdered molecular sieves (125 mg) was added and the solution cooled to 0 °C in an icebath. After 10 min. NIS

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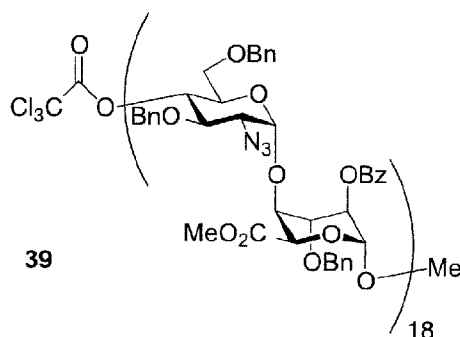
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20 36-mer Protected Polysaccharide

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idopyranosyl uronate)-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-
 (methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-O-benzyl-2-
 deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosyl uronate)-
 (1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-O-benzoyl-3-O-
 5 benzyl-α-L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-
 (1→4)-(methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-O-
 benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosyl
 uronate)-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-O-
 benzoyl-3-O-benzyl-α-L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-
 10 glucopyranosyl-(1→4)-(methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosyl uronate)-(1→4)-2-
 azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-O-benzoyl-3-O-benzyl-α-L-
 idopyranosyl uronate)-(1→4)-2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-
 (methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranosyl uronate)-(1→4)-2-azido-3,6-di-O-benzyl-2-
 deoxy-α-D-glucopyranosyl-(1→4)-(methyl 2-O-benzoyl-3-O-benzyl-α-L-idopyranoside) uronate
 15 (39)



Acceptor dotriacontasaccharide **38** (93 mg, 0.008 mmol) and thioglycoside tetrasaccharide donor **14**
 (20 mg, 0.011 mmol) was dissolved in dry DCM (1 mL) under N₂. Freshly activated 4Å powdered
 molecular sieves (74 mg) was added and the solution cooled to 0°C in an icebath. After 10 min. NIS
 20 (5 mg, 0.022 mmol) was added, and after another 10 min. AgOTf (catalytic amount) was added. The
 suspension changed colour from pale yellow to deep red, was stirred for 30 min. and the reaction was
 quenched into a separating funnel containing a mixture of DCM (20 mL), saturated aqueous NaHCO₃
 (10 mL) and Na₂S₂O₃ (1 mL, 10% aqueous). After shaking until the iodine colour was removed the
 suspension was filtered through a short pad of Celite® washing with water and DCM. The layers were
 25 separated and the aqueous extracted with DCM (5 mL). The organic layers were combined, dried
 (MgSO₄) and solvent removed *in vacuo*. The crude was purified by preparative TLC (toluene/acetone
 12:1) followed by precipitation (dissolved in warm EtOAc (4 mL) and hexane (4 mL) added) and
 filtration to give **39** (76 mg, 72%) as a white solid. Also acceptor starting material (22mg, 23%) was
 recovered. *R_f* 0.14 (toluene/acetone 12:1). ¹H NMR (800 MHz; CDCl₃) δ 8.09-8.08 (m, 2H, Bz), 8.01-
 30 8.00 (m, 2H, Bz), 7.95-7.91 (m, 32H, Bz), 7.54-7.02 (m, 324H, Ph), 5.57-5.52 (m, 17H,
 H^(BCDEFGHIJKLMNOPQR)ido-1), 5.30 (t, *J* = 9.6 Hz, 1H, H^{Rglcn}-4), 5.18-5.14 (m, 17H, H^(BCDEFGHIJKLMNOPQR)ido-2),
 5.08-5.07 (m, 1H, H^{Aido}-2), 5.04-5.03 (m, 1H, H^{Aido}-1), 4.94-4.87 (m, 18H, H^(ABCDEFGHIJKLMNPOQR)glcn-1),

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$\text{H}^{(\text{ABCDEFGHIJKLMNPOQRST})\text{glcn}}-2$, $\text{H}^{(\text{ABCDEFGHIJKLMNPOQRST})\text{glcn}}-3$, $\text{H}^{(\text{ABCDEFGHIJKLMOPQRS})\text{glcn}}-4$,
 $\text{H}^{(\text{ABCDEFGHIJKLMNPOQRST})\text{glcn}}-5$, $\text{H}^{(\text{ABCDEFGHIJKLMNPOQRST})\text{glcn}}-6_{\text{ab}}$, $20\times\text{COOCH}_3$, 3.23 (s, 3H, OCH_3). ^{13}C
 NMR (201 MHz; CDCl_3): δ 169.4, 165.2, 138.0, 137.9, 137.5, 133.7, 129.9, 129.4, 128.8, 128.5, 128.1,
 128.0, 127.9, 127.7, 127.6, 127.4, 99.3, 98.1, 78.1, 76.1, 75.6, 75.4, 74.5, 74.3, 73.8, 72.7, 72.6, 71.5,
 70.8, 70.7, 67.7, 67.4, 63.5, 63.3, 63.1, 52.1, 52.0, 51.8. MALDI TOF MS: m/z: calcd for
 $\text{C}_{823}\text{H}_{823}\text{Cl}_3\text{N}_{60}\text{NaO}_{222}$ [$M+\text{Na}$] $^{+}$: 15236; found: 15248.

Selective deprotection and selective sulfation of polysaccharides (see, e.g. Figure 8)

(S)-2,3-dihydroxypropyl-4-O-sulfonato-2-N-sulfonato-α-D-glucosaminopyranosyl-(1→4)-2-O-sulfonato-α-L-idopyranosyluronate-(1→4)-2-N-sulfonato-α-D-glucosaminopyranosyl-(1→4)-2-O-sulfonato-α-L-idopyranosyluronate-(1→4)-2-N-sulfonato-α-D-glucosaminopyranosyl-(1→4)-2-O-sulfonato-α-L-idopyranosyluronate-(1→4)-2-N-sulfonato-α-D-glucosaminopyranosyl-(1→4)-2-O-sulfonato-α-L-idopyranosyluronate-(1→4)-2-N-sulfonato-α-D-glucosaminopyranosyl-(1→4)-2-O-sulfonato-α-L-idopyranosyluronate nonadecasodium salt (25)

a) *Hydrolysis of esters*: The dodecasaccharide **24** (257 mg, 0.052 mmol) was dissolved in THF (5 mL) and MeOH (1.5 mL) and then cooled to 0 ° C in an icebath. Then LiOH.H₂O (55 mg, 1.30 mmol) dissolved in 1 mL water was added dropwise over 10 min. The solution was stirred for 5 h at 0 ° C and then at room temperature for another 19 h. The solution was then extracted with EtOAc (2x50 mL) and HCl (0.2 M, 40 mL), dried (MgSO₄), filtered and evaporated. The crude was purified using flash column chromatography (DCM/MeOH 30:1). This yielded the carboxylic acid dodecasaccharide **A** product (174 mg, 74%) as a white solid. *R*_f 0.18 (DCM/MeOH 20:1). The product was used directly in the next step without further characterisation.

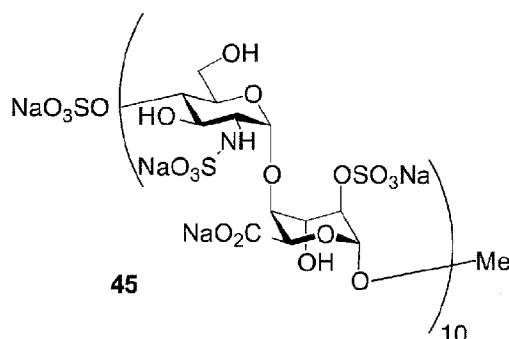
b) *Sulfation of hydroxyls*: The dodecasaccharide **A** (170 mg, 0.042 mmol) was dissolved in drypyridine (5 mL), pyridine sulfur trioxide complex (140 mg, 0.88 mmol) added and then heated to 50 ° C in an oil bath for 8 h. The solution was stirred at room temperature for another 12 h. The reaction was quenched with MeOH and evaporated. The crude was redissolved in MeOH/DCM (10 mL/5 mL), stirred with Amberlite IR-120 Na⁺ resin (1.3 g) for 8 h, filtered, resin washed with MeOH (2x5 mL) and filtrate evaporated. This residue was then purified using flash column chromatography (DCM/MeOH 20:1). This yielded 2-O-sulfated dodecasaccharide **B** (160 mg, 80%) as a white solid. *Rf* 0.29 (DCM/MeOH 5:1). ¹H NMR (400 MHz; CDCl₃/CD₃OD 10:1) δ 7.34–6.96 (m, 10H, Ph), 5.68–5.64 (m, 5H, H¹-1, H¹'-1, H¹''-1, H¹'''-1, H¹''''-1, H¹'''''-1), 5.36–5.34 (m, 1H, H-1), 5.06–3.36 (m, 104H, H¹-1, H¹'-1, H¹''-1, H¹'''-1, H¹''''-1, H¹'''''-1, H-5, H¹'-5, H¹''-5, H¹'''-5, H¹''''-5, H¹'''''-5, H-3, H¹'-3, H¹''-3, H¹'''-3, H¹''''-3, H¹'''''-3, H¹-2, H¹'-2, H¹''-2, H¹'''-2, H¹''''-2, H¹'''''-2, H¹''''''-2, H¹'''''''-2, H¹''''''''-2, H¹'''''''''-2, H¹-3, H¹'-3, H¹''-3, H¹'''-3, H¹''''-3, H¹'''''-3, H-4, H¹'-4, H¹''-4, H¹'''-4, H¹''''-4, H¹'''''-4, H¹''''''-4, H¹'''''''-4, H¹''''''''-4, H¹'''''''''-4, H¹-5, H¹'-5, H¹''-5, H¹'''-5, H¹''''-5, H¹'''''-5, H¹''''''-5, H¹'-6, H¹''-6, H¹'''-6, H¹''''-6, H¹'''''-6, H¹''''''-6, H¹'''''''-6, CH₂CHOBnCH₂OBN. 20xCH₂Ph).

c) *Hydrogenolysis of benzyls and azides*: The dodecasaccharide **B** (132 mg, 0.027 mmol) was dissolved in a mixture of MeOH/THF (4 mL/ 2 mL) and NaHCO₃ (14 mg, 0.165 mmol) in 2 mL of water was added. Then a three way tap was attached to the flask and fitted with a nitrogen balloon and the other tap was attached to water aspirator vacuum. Switching between vacuum and nitrogen balloon 5 times ensured removal of all oxygen from flask and solvent. Then was added Pd(OH)₂/C (120 mg, 10-20%) and again flushed with nitrogen. The nitrogen balloon was replaced with a hydrogen balloon and the flask again subjected to vacuum and hydrogen 5 times to ensure all the nitrogen was replaced with hydrogen. The reaction was heated to 40 ° C in an oil bath for 48 h with vigorous stirring. The product mixture was filtered through Celite®, washed with MeOH/water (3x3 mL) and water (3x3 mL). The combined filtrate was then evaporated to give dodecasaccharide amine **C** (see note 1) (78 mg, 96%) as a glassy solid. ¹H NMR (400 MHz; D₂O) δ 5.16-5.09 (m, 12H, H-1, H'-1, H''-1, H'''-1, H''''-1, H'''''-1, H''''''-1, H'''''''-1, H''''''''-1, H'''''''''-1, H''''''''''-1), 4.88-4.85 (m, 6H, H-2, H'-2, H''-2, H'''-2, H''''-2, H'''''-2, H''''''-2, H'''''''-2, H''''''''-2, H'''''''''-2, H''''''''''-2), 4.52-4.51 (m, 1H, H''''''''''''-4), 4.29-3.53 (m, 51H, H-5, H'-5, H''-5, H'''-5, H''''-5, H'''''-5, H''''''-5, H-3, H'-3, H''-3, H'''-3, H''''-3, H'''''-3, H''''''-3, H'-3, H''-3, H'''-3, H''''-3, H'''''-3, H''''''-3, H'''''''-3, H-4, H'-4, H''-4, H'''-4, H''''-4, H'''''-4, H''''''-4, H'-4, H''-4, H'''-4, H''''-4, H'''''-4, H''''''-4, H'''''''-4, H'-5, H''-5, H'''-5, H''''-5, H'''''-5, H''''''-5, H'''''''-5, H'-6, H''-6, H'''-6, H''''-6, H'''''-6, H''''''-6, H'''''''-6, H''''''''-6, CH₂CHOHCH₂OH), 2.92-2.89 (m, 6H, H'-2, H''-2, H'''-2, H''''-2, H'''''-2, H''''''-2).

d) *Sulfation of amines*: The dodecasaccharide amine **C** (65 mg, 0.022 mmol) was dissolved in water (4 mL), NaHCO₃ (108 mg, 1.29 mmol) and pyridine sulfur trioxide complex (97 mg, 0.61 mmol) was added with vigorous stirring. This procedure was repeated after 1.30 h, 3.30 h, 5.30 h, 17.30 h and 19.30 h (NaHCO₃: 109 mg, 121 mg, 113 mg, 110 mg, 110 mg. Py.SO₃: 92 mg, 88 mg, 111 mg, 100 mg, 70 mg.). After 24 h the mixture was evaporated. The crude containing Na₂SO₄ salts was redissolved in minimum amount of water and purified by passage through a Sephadex G-25 column (40 mL) by eluting with water. The fractions containing polysaccharide were pooled and evaporated to yield **25** (78 mg, 100%) of as a glassy solid. ¹H NMR (400 MHz; D₂O) δ 5.32-5.14 (m, 12H, H-1, H'-1, H''-1, H'''-1, H''''-1, H'''''-1, H''''''-1, H'''''''-1, H''''''''-1, H'''''''''-1, H''''''''''-1), 4.88-4.84 (m, 6H, H-2, H'-2, H''-2, H'''-2, H''''-2, H'''''-2, H''''''-2, H'''''''-2, H''''''''-2, H'''''''''-2, H''''''''''-2), 4.51-4.49 (m, 1H, H''''''''''''-4), 4.32-3.55 (m, 51H, H-5, H'-5, H''-5, H'''-5, H''''-5, H'''''-5, H''''''-5, H-3, H'-3, H''-3, H'''-3, H''''-3, H'''''-3, H''''''-3, H'-3, H''-3, H'''-3, H''''-3, H'''''-3, H''''''-3, H'''''''-3, H-4, H'-4, H''-4, H'''-4, H''''-4, H'''''-4, H''''''-4, H'-4, H''-4, H'''-4, H''''-4, H'''''-4, H''''''-4, H'''''''-4, H'-5, H''-5, H'''-5, H''''-5, H'''''-5, H''''''-5, H'''''''-5, H'-6, H''-6, H'''-6, H''''-6, H'''''-6, H''''''-6, H'''''''-6, H''''''''-6, CH₂CHOHCH₂OH), 3.32-3.19 (m, 6H, H'-2, H''-2, H'''-2, H''''-2, H'''''-2, H''''''-2). ¹³C NMR (100 MHz; D₂O): δ 178.5, 178.4, 178.4, 178.3, 178.2, 177.7, 102.2, 102.1, 102.1, 102.01, 102.0, 101.9, 101.6, 100.3, 100.2, 100.1, 100.1, 99.8, 80.3, 80.2, 79.9, 78.8, 78.7, 78.7, 78.5, 78.5, 78.1, 77.2, 77.2, 77.1, 77.1, 74.1, 73.9, 73.2, 73.1, 73.0, 73.0, 72.5, 72.5, 72.3, 72.2, 72.1, 71.2, 71.1, 70.9, 70.9, 70.9, 70.8, 70.8, 70.3, 70.3, 70.2, 65.4, 62.9, 62.5, 61.1, 60.7, 57.2. HRMS (FT MS): m/z: calcd for C₇₅H₁₁₁N₆Na₄O₁₀₂S₁₃ [M-15Na+8H]⁻⁷: 462.4234; found: 462.4244 (See note 2).

vacuum and nitrogen balloon 5 times ensured removal of all oxygen from flask and solvent. Then was added Pd(OH)₂/C (32 mg, 10-20%) and again flushed with nitrogen. The nitrogen balloon was replaced with a hydrogen balloon and the flask again subjected to vacuum and hydrogen 5 times to ensure all the nitrogen was replaced with hydrogen. The reaction was heated to 50°C for 36 h with vigorous stirring. The product mixture was filtered through Celite® and washed with EtOH/water 1:2 (3x2 mL). The combined filtrate was then evaporated and purified by a short Sephadex® G-25 column to give icosasaccharide amine **44** (4.1 mg, 22% 3 steps) as a glassy solid. $[\alpha]_D^{20} = +57.3$ ($c = 0.18$, H₂O). ¹H NMR (400 MHz; D₂O) δ 5.35-5.29 (m, 9H, H^{CEGIKMOQS}-1), 5.22-5.16 (m, 10H, H^{BDFHJLNPR}-1), 5.01-4.99 (m, 1H, H^A-1), 4.89-4.85 (m, 9H, H^{CEGIKMOQS}-5), 4.50-4.49 (m, 1H, H^A-5), 4.35-4.17 (m, 21H, H^{ACEGIKMOQS}-2, H^{ACEGIKMOQS}-3, H^T-4), 4.13-4.07 (m, 10H, H^{ACEGIKMOQS}-4), 3.90-3.66 (m, 49H, H^{BDFHJLNPR}-3, H^{BDFHJLNPR}-4, H^{BDFHJLNPR}-5, H^{BDFHJLNPR}-6_{ab}), 3.39 (s, 3H, OMe), 3.26-3.18 (m, 10H, H^{BDFHJLNPR}-2). ¹³C NMR (201 MHz; D₂O): δ 175.3, 99.6, 99.0, 98.8, 91.7, 76.2, 76.0, 72.5, 71.4, 70.3, 70.1, 68.6, 66.9, 66.8, 66.2, 62.8, 62.6, 59.7, 59.3, 54.6, 54.1. FT MS: m/z : calcd for C₁₂₁H₁₈₂N₁₀O₁₃₄S₁₁ [M-11Na-1H]¹²⁻: 355.9563; found: 356.8792 (Na⁺ ion exchanged with NH₄⁺ before submitting for MS).

Methyl 4-O-sulfonato-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranoside uronate hentriaconta sodium salt (45**)**



The icosasaccharide **44** (2.3 mg, 0.51 μ mol) was dissolved in water (0.5 mL), NaHCO₃ (2.8 mg, 0.0335 mmol) and pyridine sulfur trioxide complex (2.4 mg, 0.0152 mmol) was added with stirring. This procedure was repeated after 1 h, 2 h, 4 h, 5 h, 7 h, 10 h, 12 h and 16 h (NaHCO₃: 6.7 mg, 9 mg,

5 93%) as a glassy solid. $[\alpha]_D^{20} = +64.8$ ($c = 0.13$, H_2O). ^1H NMR (800 MHz; D_2O) δ 5.36-5.25 (m, 19H, $H^{BCDEFGHIJKLMNPOQRST-1}$), 5.04-5.03 (m, 1H, H^A-1), 4.89-4.85 (m, 9H, $H^{CEGIKMOQS-5}$), 4.45 (d, $J = 2.4$ Hz, 1H, H^A-5), 4.37-4.29 (m, 9H, $H^{CEGIKMOQS-2}$), 4.27-4.21 (m, 11H, H^A-2 , $H^{ACEGIKMOQS-3}$, H^T-4), 4.06-4.02 (m, 10H, $H^{ACEGIKMOQS-4}$), 3.93-3.81 (m, 30H, $H^{BDFHJLNPR-5}$, $H^{BDFHJLNPR-6_{ab}}$), 3.75-3.68 (m, 20H, $H^{BDFHJLNPR-3}$, $H^{BDFHJLNPR-4}$), 3.43 (s, 3H, OMe), 3.33-3.21 (m, 10H, $H^{BDFHJLNPR-2}$). ^{13}C NMR (201 MHz; D_2O): δ 175.7, 99.8, 99.2, 97.3, 96.9, 77.3, 77.1, 75.8, 75.6, 75.0, 74.5, 71.8, 71.5, 71.3, 71.1, 69.5, 68.3, 68.2, 67.6, 59.7, 58.4, 58.3, 57.9, 55.3. FT MS: m/z : calcd for $C_{121}H_{175}N_{12}Na_4O_{164}S_{21}$ [$M-27Na+2NH_4+4H$] $^{21+}$: 246.8552; found: 246.8698 (Na^+ ion exchanged with NH_4^+ before submitting for MS).

Oxidative cleavage of diol 25

2-oxoethyl 4-O-sulfonato-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-lucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-lucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosideuronate nonadecasodium salt (26)

The dodecasaccharide diol **25** (61 mg, 0.017 mmol) was dissolved in water (1 mL). Sodium periodate (3.9 mg, 0.018 mmol) was added and stirred 6 h. The crude was purified by passage through a Sephadex G-25 column by eluting with water. The fractions containing oligosaccharide were pooled and evaporated to yield 58 mg (97%) of **15** as a glassy solid. ¹H NMR (400 MHz; D₂O) δ 5.32-5.14 (m, 13H, H-1, H'-1, H''-1, H'''-1, H''''-1, H'''''-1, H''''''-1, H'''''''-1, H''''''''-1, H'''''''''-1, H''''''''''-1, H'''''''''''-1, CH(OH)₂), 4.87-4.83 (m, 6H, H-2, H'-2, H''-2, H'''-2, H''''-2, H'''''-2), 4.51-4.49 (m, 1H, H''''''''''-4), 4.31-3.66 (m, 47H, H-5, H'-5, H''-5, H'''-5, H''''-5, H'''''-5, H''''''-5, H-3, H'-3, H''-3, H'''-3, H''''-3, H'''''-3, H''''''-3, H'-3, H''-3, H'''-3, H''''-3, H'''''-3, H''''''-3, H'''''''-3, H-4, H'-4, H''-4, H'''-4, H''''-4, H'''''-4, H''''''-4, H'-4, H''-4, H'''-4, H''''-4, H'''''-4, H''''''-4, H'''''''-4, H-5, H'-5, H''-5, H'''-5, H''''-5, H'''''-5, H''''''-5, H'''''''-5, H-6, H'-6, H''-6, H'''-6, H''''-6, H'''''-6, H''''''-6, H'''''''-6, H''''''''-6), 3.55 (dd, 2H, *J* = 10.9 Hz, *J* = 5.0 Hz, CH₂CH(OH)₂), 3.31-3.19 (m, 6H, H'-2, H''-2, H'''-2, H''''-2, H'''''-2, H''''''-2).

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2-hydroxyethyl 4-O-sulfonato-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyluronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyluronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosideuronate nonadecasodium salt (27a)

The dodecasaccharide **26** (41 mg, 0.012 mmol) was dissolved in water (1 mL), Sodium borohydride (0.44 mg, 0.012 mmol) was added and stirred 2 h. Then more NaBH₄ (1.0 mg, 0.026 mmol) was added and stirred another 3 h. The crude was purified by passage through a Sephadex G-25 column by eluting with water. The fractions containing polysaccharide were pooled and evaporated to yield 40 mg (98%) of **27** as a glassy solid. ¹H NMR (400 MHz; D₂O) δ 5.31-5.15 (m, 12H, H-1, H'-1, H''-1, H'''-1, H''''-1, H'''''-1, H''''''-1, H'''''''-1, H''''''''-1, H'''''''''-1), 4.87-4.83 (m, 6H, H-2, H''-2, H'''-2, H''''-2, H'''''-2, H''''''-2), 4.51-4.49 (m, 1H, H''''''''-4), 4.31-3.60 (m, 50H, H-5, H'-5, H''-5, H'''-5, H''''-5, H'''''-5, H''''''-5, H-3, H'-3, H''-3, H'''-3, H''''-3, H'''''-3, H''''''-3, H-4, H'-4, H''-4, H'''-4, H''''-4, H'''''-4, H''''''-4, H-6, H'-6, H''-6, H'''-6, H''''-6, H'''''-6, H''''''-6, CH₂CH₂OH), 3.30-3.18 (m, 6H, H'-2, H''-2, H'''-2, H''''-2, H'''''-2, H''''''-2). HRMS (FT MS): m/z: calcd for C₇₄H₁₀₇N₆Na₆O₁₀₁S₁₃ [M-13Na+6H]⁻: 464.4167; found: 464.4173.

Addition of tritium radiolabel to aldehyde **26 to form compound **27b****

2-tritium-2-hydroxyethyl 4-O-sulfonato-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyluronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyl uronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosyluronate-(1 $\rightarrow$ 4)-2-N-sulfonato- α -D-glucosaminopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfonato- α -L-idopyranosideuronate nonadecasodium salt (27a)

Three micrograms of ³H-labelled sodium borohydride (1 mCi) was reacted with 1.2 mg of oligosaccharide **26** in 20 μ L of 50 mM NaOH in a sealed reinforced glass Reacti-Vial at 45 ° C for 2h. To ensure that all aldehyde was reduced, an excess of unlabelled 1M sodium borohydride was then added and sample incubated for a further 2 h at 45 °C. Reaction was halted by the addition of 5 μ L of 1 M sulfuric acid. Tritium-labelled oligosaccharide was then desalted on PD-10 (G-25) column that was pre-equilibrated with water and 0.5 mL fractions were collected. Labelled oligosaccharide **27b**, which eluted in fractions 10-14, was collected and freeze-dried. To confirm the size of radiolabelled material the tritiated compound **27b** was subjected to HPLC size exclusion chromatography on an Agilent 1200 HPLC system. The sample was run on a Superdex 75 column (10 mm x 300 mm; GE Healthcare) in PBS at 0.5 mL/min. Aliquots from 0.5 mL fractions were taken, mixed with 2 mL of Hisafe scintillation fluid (Perkin-Elmer) and 3H level was counted on a Wallac 1400 scintillation counter. An unlabelled de-6-O-sulphated 12-mer of heparin (Iduron), which is approximately the same

size as **27b**, was used to calibrate the column and was monitored at 232 nm by an in-line UV detector. Fractions containing **27b** were collected, desalted, freeze-dried and weighed. Specific activity was determined as 5.5×10^6 cpm per mg of polysaccharide.

5 O4 reactive tag chemistry

Figure 11 illustrates a synthesis to achieve tag/linker functionality at the C-4 oxygen (O4 position). Modification of the O4 end of the core heparin-type disaccharide provides an example of access to the analogous oligosaccharides with a latent tag at the O4 terminus. Examples are provided below of synthesis of 10-mer and 12-mer targets, and the release of the terminal tag aldehyde on the final sulfated oligosaccharides (analogous deprotection and sulfation chemistry to O1 targets). The 8-mer acceptor employed to generate the 10-mer here is accessible using the 4+4 chemistry described herein.

15 Phenyl-2-azido-2-deoxy-3,6-di-O-benzyl-4-O-[(S)-2,3-bis(benzyloxy)propoxy]-1-thio- α -D-glucopyranoside (**102**)

To **101** [R=Bn] (611 mg, 1.28 mmol) was added dry THF (10 mL) under N₂ and the solution cooled to 0 °C. NaH (60% in mineral oil) (76.0 mg, 1.9 mmol) was added in two portions over 30 min. while being kept under N₂. Triflate reagent (600 mg, 1.41 mmol) in dry THF (5 mL) was then added dropwise and the suspension allowed to warm to RT and heated at 50 °C overnight. TLC analysis (4/1, hexane/EtOAc) showed the reaction to be complete and quenching was effected with aqueous NaHCO₃ (1 mL). The solution was partitioned between EtOAc and H₂O. The layers were separated and the organic phase washed with 1M HCl, H₂O, saturated aqueous NaCl, dried (MgSO₄), filtered and evaporated. The crude product was purified twice by flash column chromatography (EtOAc/hexane gradient 1:10, 1:8, then DCM/Ether gradient 99:1) yielding **102** (115 mg, 0.16 mmol, 12%) as a clear oil. *R*_f 0.40 (EtOAc/hexane, 1:4); [α]_D -19.4 (c = 0.7, DCM); ¹H NMR (400 MHz; CDCl₃) δ 7.44-7.39 (m, 2H, ArH), 7.30-7.25 (m, 2H, ArH), 7.25-7.11 (m, 21H, ArH), 5.48 (d, *J* = 5.3 Hz, 1H, H₁), 4.81-4.74 (m, 2H, CH₂Ar), 4.57-4.49 (m, 2H, CH₂Ar), 4.39 (s, 2H, CH₂Ar), 4.35 (d, *J* = 12.0 Hz, 1H, CH₂Ar), 4.28 (d, *J* = 12.0 Hz, 1H, CH₂Ar), 4.23 (ddd, *J* = 9.9, 3.8, 1.7 Hz, 1H, H₅), 3.94-3.91 (m, 1H, CH₂CH[OBn]CH₂OBn), 3.79 (dd, *J* = 10.3, 5.3 Hz, 1H, H₂), 3.69-3.63 (m, 2H, H₃, H₄), 3.63-3.52 (m, 3H, H_{6A}, CH₂CH[OBn]CH₂OBn), 3.49-3.41 (m, 3H, H_{6B}, CH₂CH[OBn]CH₂OBn); ¹³C NMR (100 MHz; CDCl₃) δ 138.6, 138.1, 138.0, 137.8, 133.7, 132.1, 129.1, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 87.3, 81.6, 78.9, 77.4, 75.6, 73.5, 73.4, 73.3, 72.5, 71.9, 69.8, 68.5, 64.0; MS ES [M+Na]⁺ *m/z* 754.0; HRMS (ES-TOF⁺) *m/z* calcd for C₄₃H₄₅N₃O₆SNa [M+Na]⁺ 754.2921, found 754.2918.

Phenyl-2-azido-2-deoxy-3-O-benzyl-4-O-[(S)-2,3-bis(benzyloxy)propoxy]-6-O-benzoyl-1-thio- β -D-glucopyranoside (**β -103**)

To **101** [R=Bz] (1.83 g, 3.72 mmol) was added dry THF (20 mL) under N₂ and the solution cooled to 0 °C. NaH (60% in mineral oil) (164.0 mg, 4.10 mmol) was added in two portions over 30 min. while

being kept under N₂. Triflate reagent (1.81 g, 4.46 mmol) in dry THF (10 mL) was then added dropwise and the suspension allowed to warm to RT and stirred for a further 2 h. TLC analysis (3/1, hexane/EtOAc) showed the reaction to be virtually complete (NOTE: allowing the reaction continue for longer resulted no further consumption of starting material and the formation of a transesterified product) and quenching was effected with glacial AcOH (4.1 mmol) and solvents removed *in vacuo*. The solution was partitioned between EtOAc and H₂O. The layers were separated and the organic phase washed with 1M HCl, H₂O, brine, dried (MgSO₄), filtered and evaporated. The crude product was purified by flash column chromatography (EtOAc/hexane gradient 1:9, 1:7) yielding **103** (1.26 g, 1.60 mmol, 69%) as a clear oil along with recovered starting material GM_222-2 (979 mg). R_f 0.59 (EtOAc/hexane 1:3); [α]_D (c = 3.8, DCM) -19.7; ¹H NMR (400 MHz; CDCl₃) δ 7.97-7.94 (m, 2H, ArH), 7.56-7.52 (m, 1H, ArH), 7.45-7.38 (m, 4H, ArH), 7.30-7.11 (m, 16H, ArH), 7.02-6.98 (m, 2H, ArH), 4.78-4.73 (m, 2H, CH₂Ar), 4.71 (dd, J = 12.0, 2.0 Hz, 1H, H_{6A} or H_{6B}), 4.57-4.49 (m, 2H, CH₂Ar), 4.42-4.37 (m, 2H, CH₂Ar), 4.35-4.31 (m, 2H, H₁, H_{6A} or H_{6B}), 3.99-3.98 (m, 1H, CH₂CH[OBn]CH₂OBn), 3.64-3.58 (m, 2H, CH₂CH[OBn]CH₂OBn, CH₂CH[OBn]CH₂OBn), 3.54-3.50 (m, 1H, H₅), 3.49-3.47 (m, 2H, CH₂CH[OBn]CH₂OBn), 3.40 (t, J = 9.6 Hz, 1H, H₃), 3.34 (d, J = 9.6 Hz, 1H, H₄), 3.20 (dd, J = 10.1, 9.6 Hz, 1H, H₂); ¹³C NMR (100 MHz; CDCl₃) δ 166.0, 138.4, 138.1, 137.5, 134.2, 133.4, 130.5, 130.0, 129.8, 129.0, 128.7, 128.6, 128.5, 128.4, 128.2, 127.8, 127.7, 85.6, 84.7, 78.4, 77.4, 77.2, 76.0, 73.5, 72.5, 69.5, 64.8, 63.1; MS ES [M+Na]⁺ m/z 768.0; HRMS (TOF⁺) m/z calcd for C₄₃H₄₉N₅O₆S [M+NH₄]⁺ 763.3399, found 763.3398.

Methyl (phenyl 4-O-(2-azido-3,6-di-O-benzyl-2-deoxy-4-O-[(S)-2,3-bis(benzylloxy)propoxy]-α-D-glucopyranosyl)-2-O-benzoyl-3-O-benzyl-1-thio-α-L-idopyranoside)-uronate (105)

To iduronic ester acceptor **104** (833 mg, 1.78 mmol) and **102** (1.70 g, 2.14 mmol) was twice added dry toluene and the solvent evaporated. The residue was dried under high vacuum for 2 h and, while kept under nitrogen, dry DCM (25 mL) was added. The solution was cooled to -30 °C using a 65:35 mixture of ⁱPrOH/H₂O and a dry ice bath and TMSOTf (16 μL, 0.09 mmol) was then added. After 2 h the reaction was quenched with two drops of NEt₃, solvents were removed *in vacuo* and flash column chromatography (EtOAc/hexane, 1:5, 1:2) as the eluent yielded **105** (1.10 g, 0.99 mmol, 55%) as a white foam, along with recovered acceptor **104** (300 mg); R_f 0.31 (EtOAc/hexane 1:3); [α]_D (c = 1.0, DCM) +327.1; ¹H NMR (400 MHz; CDCl₃) δ 8.07-8.04 (m, 2H, ArH), 7.49-7.44 (m, 2H, ArH), 7.43-7.38 (m, 2H, ArH), 7.35-7.29 (m, 4H, ArH), 7.27-7.09 (m, 23H, ArH), 7.04-7.00 (m, 2H, ArH), 5.72 (s, 1H, H₅), 5.35 (s, 1H, H₄), 5.29 (d, J = 2.0 Hz, 1H, H₁), 4.91 (d, J = 11.7 Hz, 1H, CH₂Ph), 4.69 (d, J = 11.7 Hz, 1H, CH₂Ph), 4.61 (d, J = 3.6 Hz, 1H, H₁), 4.55-4.48 (m, 2H, CH₂Ph), 4.43 (s, 2H, CH₂Ph), 4.38-4.31 (m, 2H, CH₂Ph), 4.18 (d, J = 10.6 Hz, 1H, CH₂Ph), 4.12 (t, J = 2.8 Hz, 1H, H₃), 3.97 (s, 1H, H₂), 3.81 (dd, J = 9.7, 3.3 Hz, 1H, H_{6A}), 3.71-3.63 (m, 3H, CH₂Ph, CH₂CH[OBn]CH₂OBn), 3.61 (s, 3H, OCH₃), 3.59-3.37 (m, 7H, H₅, H_{6B}, H₄, H₃, CH₂CH[OBn]CH₂OBn, CH₂CH[OBn]CH₂OBn), 3.14 (dd, J = 10.0, 3.7 Hz, 1H, H₂); ¹³C NMR (100 MHz; CDCl₃) δ 169.3, 165.6, 138.6, 138.2, 137.9, 137.8, 137.1, 135.4, 133.4, 131.5, 130.0, 129.5, 129.1, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 127.5, 100.4, 87.0, 80.0, 78.0, 77.6, 77.4, 74.6, 73.4, 73.3, 73.1, 72.8, 72.4, 72.2,

71.6, 70.1, 69.2, 68.4, 67.6, 63.6, 52.3; MS ES $[M+NH_4]^+$ m/z 1133.5; HRMS (FTMS- NSI^+) m/z calcd for $C_{64}H_{69}N_4O_{13}S$ $[M+NH_4]^+$ 1133.4576, found 1133.4578.

5 Decasaccharide 110

Octasaccharide acceptor **108** (161.0 mg, 52.0 μ mol) and disaccharide donor **106** (71 mg, 62.0 μ mol) were combined in dry toluene (10 mL). Solvent was removed *in vacuo* and the residue dried under high vacuum for 1 h. The resulting foam was then dissolved in dry DCM (5.0 mL) and cooled to 0 °C. 4Å molecular sieves (100 mg) were added followed by NIS (15.2 mg, 68.0 μ mol) and AgOTf (1.3 mg, 5.2 μ mol). The resultant suspension was stirred at this temperature for 0.5 h whereupon a deep red colour persisted. The reaction was quenched with $NaHCO_3$ (25 mg) and $Na_2S_2O_3$ (25 mg) in H_2O (1.0 mL) and the mixture filtered through a Celite™ plug, washing with DCM. The layers were separated and the organics dried ($MgSO_4$) and solvent removed *in vacuo* to reveal crude **110** as a yellow gum. The material was then purified by silica gel flash chromatography eluting with toluene/acetone, 30/25/1, 20/1 to separate the product and unreacted octasaccharide acceptor (52 mg recovered). The product was then purified again by silica gel flash chromatography eluting with hexane/EtOAc, 2/1, 1/1 to give **110** (82 mg, 20.0 μ mol, 57%) as a white foam. R_f 0.29 (Ether/DCM 5:95); $[\alpha]_D$ (c = 0.5, DCM) +63.4; ^1H NMR (400 MHz; $CDCl_3$) δ 8.01-7.95 (m, 24H, ArH), 7.17-7.11 (m, 86H, ArH), 5.50-5.48 (m, 4H, H_{1IdoA}), 5.13-5.08 (m, 4H, H_{2IdoA}), 4.99 (brs, 1H, H_{1IdoA}), 4.97 (brs, 1H, H_{2IdoA}), 4.83-4.16 (m, 43H, CH_2Ar x 23, H_{5IdoA} , H_{1GlcN} , $H_{6ABGlcN}$), 4.06-4.70 (m, 21H, CH_2Ar , x 4, H_{3IdoA} , H_{4IdoA} , H_{5GlcN} , H_{4GlcN} x 4, $CH_2CH[OBn]CH_2OBn$), 3.65-3.56 (m, 2H, $CH_2CH[OBn]CH_2OBn$), 3.53 (s, 3H, OCH_3), 3.47-3.42 (m, 14H, H_{3GlcN} , H_{4GlcN} x 1, $CH_2CH[OBn]CH_2OBn$ C(O) OCH_3 x 2), 3.25-3.04 (m, 14H, H_{2GlcN} , C(O) OCH_3 x 3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 169.6, 169.3, 169.2, 166.0, 165.9, 165.6, 165.2, 165.1, 138.3, 138.0, 137.7, 137.5, 137.4, 137.3, 137.2, 133.7, 133.5, 133.1, 133.0, 132.5, 129.9, 129.6, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 100.3, 99.1, 98.7, 98.4, 98.3, 98.3, 98.2, 98.1, 98.1, 98.0 anomeric carbons], 79.7, 78.6, 78.4, 78.3, 78.2, 77.8, 77.7, 77.6, 77.5, 77.3, 77.2, 77.0, 76.9, 76.7, 76.3, 75.9, 75.8, 75.7, 75.6, 75.4, 75.1, 75.0, 74.6, 74.5, 74.4, 74.3, 74.2, 73.9, 73.5, 72.4, 72.3, 71.7, 71.2, 70.2, 70.0, 69.9, 69.8, 69.6, 68.0, 67.1, 63.4, 63.3, 63.2, 63.0, 62.3, 61.9, 61.8, 56.2, 52.3, 51.9, 51.7, 51.6, 51.6; (FTMS NSI^+) m/z calcd for $C_{223}H_{225}N_{17}O_{63}$ $[M+2NH_4]^{2+}$ 2074.2457, found 2074.2422.

Dodecasaccharide 109

Decasaccharide acceptor **107** (391.0 mg, 0.10 mmol) and disaccharide donor **105** (138.0 mg, 0.12 mmol) were combined in dry toluene (10 mL). Solvent was removed *in vacuo* and the residue dried under high vacuum for 1 h. The resulting foam was then dissolved in dry DCM (5.0 mL) and cooled to 0 °C. 4Å molecular sieves (100 mg) were added followed by NIS (29.0 mg, 0.13 mmol) and AgOTf (2.6 mg, 10.0 μ mol). The resultant suspension was stirred at this temperature for 0.5 h whereupon a deep red colour persisted. The reaction was quenched with $NaHCO_3$ (25 mg) and $Na_2S_2O_3$ (25 mg) in H_2O (1.0 mL) and the mixture filtered through a Celite™ plug, washing with DCM. The layers were separated and the organics dried ($MgSO_4$) and solvent removed *in vacuo* to reveal crude **109** as a yellow gum. The material was then purified by silica gel flash chromatography eluting with

toluene/acetone, 20/1 to separate the product and unreacted deca-saccharide acceptor (131 mg recovered). The product **109** (367 mg, 76.0 μ mol, 77%) was isolated as a white foam. R_f 0.38 (toluene/acetone 10:1); $[\alpha]_D$ ($c = 1.1$, DCM) +42.9; ^1H NMR (400 MHz; $CDCl_3$) δ 8.13-8.10 (m, 3H, ArH), 8.01-7.92 (m, 11H, ArH), 7.54-7.07 (m, 116H, ArH), 5.57-5.56 (m, 5H, H_{1IdoA}), 5.21-5.18 (m, 5H, H_{2IdoA}), 5.10 (s, 1H, H_{1IdoA}), 5.06 (s, 1H, H_{2IdoA}), 4.97-4.90 (m, 6H, H_{1GlcN}), 4.82-4.38 (m, 43H), 4.24-3.25 (m, 78H, incl. $C(O)OCH_3 \times 6$, OCH_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 169.6, 169.4, 169.3, 167.7, 165.6, 165.2, 165.1, 138.6, 138.1, 138.0, 137.9, 137.8, 137.7, 137.5, 137.4, 133.6, 133.5, 132.3, 131.0, 130.0, 129.9, 129.8, 129.6, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 125.4, 100.3, 99.5, 99.3, 99.2, 98.2, 98.1, 98.0, 79.6, 78.3, 78.2, 78.1, 77.5, 77.4, 77.2, 76.9, 76.0, 75.6, 75.5, 75.4, 74.9, 74.4, 74.2, 74.0, 73.7, 73.6, 73.5, 73.4, 73.2, 72.5, 72.4, 71.6, 71.4, 70.7, 70.1, 68.0, 67.7, 67.4, 67.3, 67.2, 63.4, 63.3, 63.2, 63.1, 63.0, 61.7, 56.3, 52.1, 51.9, 51.8, 51.7, 51.6; (MS MALDI) m/z calcd for $C_{264}H_{268}N_{18}O_{69}Na$ $[M+Na]^+$ 4819.8, found 4819.8.

15 **Deprotection, sulfation and release of aldehyde for O1 end modified oligosaccharides**

Aldehyde release to form 112

Decasaccharide diol intermediate (4.6 mg, 1.4 μ mol) was dissolved in H_2O (200 μ L) at room temperature. $NaIO_4$ (0.4 mg, 1.5 μ mol) was added and the solution shaken in a 2.0 mL Eppendorf tube for 24 h. The reaction solution was diluted up to 1.0 mL with H_2O and duly passed through a Sephadex G-20 resin using water as eluent. The target material **120** (4.3 mg, 1.3 μ mol, 95%) was isolated as a white glass after freeze-drying. ^1H NMR (600 MHz; D_2O) δ 5.27-2.24 (m, 5H, H_{1GlcN}), 5.08 (brs, 4H, H_{1IdoA}), 4.88 (s, 1H, H_{1IdoA}), 4.78 (brs, 4H, H_{5IdoA}), 4.39 (brs, 1H, H_{5IdoA}), 4.21-4.00 (m, 23H), 3.89-3.77 (m, 11H), 3.67-3.65 (m, 6H), 3.26 (s, 3H, OCH_3), 3.24-3.21 (m, 5H, H_{2GlcN}); HRMS (FTMS NSI^-) m/z calcd for $C_{63}H_{95}N_5O_{82}S_{10}$ $[M-6H]^{6-}$ 425.5110, found 425.5115.

Chemical modification of O4 end aldehyde

The O4 terminus aldehyde, like the analogous O1 group, is an effective unit to provide extension linkers which deliver, for example, a terminal amino reactive group. Figure 12 shows an illustrative synthesis.

Pure synthetic heparin 20-mer (118)

Figure 13 shows an illustrative synthesis for preparation of an isolated sulphonated heparin-type polysaccharide, in this case a 20-mer.

a) The protected icosasaccharide **117** (40 mg, 0.0052 mmol) was dissolved in THF (2 mL) and MeOH (0.5 mL) and then cooled to 0 $^{\circ}C$ in an icebath. Then $LiOH \cdot H_2O$ (14 mg, 0.33 mmol) dissolved in 0.5 mL water was added in portions over 20 min. The solution was stirred 12 h slowly warming to room temperature. The solution was then extracted with EtOAc (30 mL) and HCl (0.1 M, 20 mL). The

organic phase was washed with water (2x10 mL), dried (MgSO₄), filtered and evaporated. The crude was purified using flash column chromatography (DCM/MeOH gradient 40:1 to 30:1). This yielded the carboxylic acid icosasaccharide intermediate (16.0 mg) as solid. *R_f* 0.21 (DCM/MeOH 15:1).

5 b) The icosasaccharide intermediate (7.0 mg, 1.1 μmol) was dissolved in dry DMF (0.5 mL) under N₂ in a microwave compatible tube. SO₃NMe₃ (8.5 mg, 60.5 μmol) was added and the solution heated in a microwave reactor for 1 h at 100 °C. Tlc analysis (5/1, DCM/MeOH) showed one product spot and the reaction was quenched with Et₃N and loaded onto a column of Sephadex LH20. The column was eluted with DCM/MeOH, 9/1 and the fractions containing the oligosaccharide pooled and then passed
10 though an additional column containing Amberlite® IRC 86 Na⁺ resin to convert the triethylammonium salts to sodium salt. This yielded 2-O-sulfated icosasaccharide (7.5 mg) as a glassy solid. *R_f* = 0.21 (DCM/MeOH 5:1).

c) This 2-OS icosasaccharide (8 mg, 0.0010 mmol) was dissolved in a mixture of EtOH/H₂O (1 mL / 1
15 mL). Then a three way tap was attached to the flask and fitted with a nitrogen balloon and the other tap was attached to a water aspirator vacuum. Switching between vacuum and nitrogen balloon 5 times ensured removal of all oxygen from flask and solvent. Then was added Pd(OH)₂/C (32 mg, 10-20%) and again flushed with nitrogen. The nitrogen balloon was replaced with a hydrogen balloon and the flask again subjected to vacuum and hydrogen 5 times to ensure all the nitrogen was
20 replaced with hydrogen. The reaction was heated to 50 °C for 36 h with vigorous stirring. The product mixture was filtered through Celite® and washed with EtOH/water 1:2 (3x2 mL). The combined filtrate was then evaporated and purified by a short Sephadex® G-25 column to give icosasaccharide amine (4.1 mg, 22% 3 steps) as a glassy solid. $[\alpha]_D^{20} = +57.3$ (*c* = 0.18, H₂O). ¹H NMR (400 MHz; D₂O) δ 5.35-5.29 (m, 9H, H^{CEGIKMOQS}-1), 5.22-5.16 (m, 10H, H^{BDFHJLNPR}-1), 5.01-4.99 (m, 1H, H^A-1), 4.89-4.85
25 (m, 9H, H^{CEGIKMOQS}-5), 4.50-4.49 (m, 1H, H^A-5), 4.35-4.17 (m, 21H, H^{ACEGIKMOQS}-2, H^{ACEGIKMOQS}-3, H^T-4), 4.13-4.07 (m, 10H, H^{ACEGIKMOQS}-4), 3.90-3.66 (m, 49H, H^{BDFHJLNPR}-3, H^{BDFHJLNPR}-4, H^{BDFHJLNPR}-5, H^{BDFHJLNPR}-6_{ab}), 3.39 (s, 3H, OMe), 3.26-3.18 (m, 10H, H^{BDFHJLNPR}-2). ¹³C NMR (201 MHz; D₂O): δ 175.3, 99.6, 99.0, 98.8, 91.7, 76.2, 76.0, 72.5, 71.4, 70.3, 70.1, 68.6, 66.9, 66.8, 66.2, 62.8, 62.6, 59.7, 59.3, 54.6, 54.1. NSI MS: *m/z*: calcd for C₁₂₁H₁₈₅N₁₀O₁₃₄S₁₁ [*M*-9H]⁹⁻: 474.8327; found: 474.8324 (Na⁺
30 ion exchanged with NH₄⁺ before submitting for MS).

d) The icosasaccharide amine intermediate (2.3 mg, 0.51 μmol) was dissolved in water (0.5 mL), NaHCO₃ (2.8 mg, 0.0335 mmol) and pyridine sulfur trioxide complex (2.4 mg, 0.0152 mmol) was added with stirring. This procedure was repeated after 1 h, 2 h, 4 h, 5 h, 7 h, 10 h, 12 h and 16 h
35 (NaHCO₃: 6.7 mg, 9 mg, 8.5 mg, 10 mg, 7.3 mg, 13 mg, 9 mg. Py.SO₃: 6.5 mg, 7 mg, 5.7 mg, 6 mg, 5 mg, 10 mg, 5 mg.). After 20 h the mixture was evaporated. The crude containing Na₂SO₄ salts was redissolved in minimum amount of water and purified by passage through a Sephadex® G-25 column (18x2.5 cm) by eluting with water. The fractions containing oligosaccharide were pooled and evaporated to yield **118** (2.7 mg, 93%) as a glassy solid. $[\alpha]_D^{20} = +64.8$ (*c* = 0.13, H₂O). ¹H NMR (800
40 MHz; D₂O) δ 5.36-5.25 (m, 19H, H^{BCDEFGHIJKLMNOPQRST}-1), 5.04-5.03 (m, 1H, H^A-1), 4.89-4.85 (m, 9H,

$H^{CEGIKMQS-5}$, 4.45 (d, $J = 2.4$ Hz, 1H, H^A-5), 4.37-4.29 (m, 9H, $H^{CEGIKMQS-2}$), 4.27-4.21 (m, 11H, H^A-2 , $H^{ACEGIKMQS-3}$, H^T-4), 4.06-4.02 (m, 10H, $H^{ACEGIKMQS-4}$), 3.93-3.81 (m, 30H, $H^{BDFHJLNPRT-5}$, $H^{BDFHJLNPRT-6_{ab}}$), 3.75-3.68 (m, 20H, $H^{BDFHJLNPRT-3}$, $H^{BDFHJLNPRT-4}$), 3.43 (s, 3H, OMe), 3.33-3.21 (m, 10H, $H^{BDFHJLNPRT-2}$). ^{13}C NMR (200 MHz; D_2O): δ 175.7, 99.8, 99.2, 97.3, 96.9, 77.3, 77.1, 75.8, 75.6, 75.0, 74.5, 71.8, 71.5, 71.3, 71.1, 69.5, 68.3, 68.2, 67.6, 59.7, 58.4, 58.3, 57.9, 55.3.

2. Application of radiolabelled polysaccharides

Radiolabelled heparin-mimetic **27b** was employed to determine its *in vivo* clearance and tissue distribution in mice (the biological efficacy of the analogous compound having an OMe group at the anomeric terminus has previously been established *in vitro* – see Gardner J.M. *et al. PLoS ONE* 5, e11644, 2010).

In vivo assay methods

Mice were dosed subcutaneously with 20, 40 and 80 mg/kg of oligosaccharide spiked with 140,000 cpm of **27b** as a radio-tracer. Tissue concentrations were determined from the level of radiolabelled oligosaccharide in tissue samples of known mass during a 16 hour period (Figure 9). A maximum plasma concentration of 18 μ g/mL was observed 15 minutes after dosing mice with 20 mg/kg of oligosaccharide (Figure 9A). When dosed with 40 or 80 mg/kg maximum plasma concentrations of 44.8 μ g/mL and 84.0 μ g/mL were observed after 60 minutes respectively (Figures 9B and 9C). Critically these data demonstrate that the plasma concentration of polysaccharide, *in vivo* was sufficient to inhibit the biological activity of FGF2 based on *in vitro* data (for *in vitro* data see Gardner J.M. *et al. PLoS ONE* 5, e11644, 2010).

All tissues, except lungs, showed a time-dependent accumulation of **27b** at all doses and the maximum concentrations increased with a higher initial dose (Figure 9). The highest concentration of **27b** was detected in tissues when mice were treated with 80 mg/kg. In addition, all tissues with, exception of the liver, attained maximal levels of 1 after 120 minutes. The tissue distribution data described here show that at 40 mg/kg and 80 mg/kg biologically active concentrations (based on *in vitro* data in data in Gardner J.M. *et al. PLoS ONE* 5, e11644, 2010) were achieved in liver, lungs and spleen within a two hour period. The half-life of polysaccharides in mice was estimated to be approximately 2 hours. As murine clearance is so rapid, this result is particularly encouraging for the development of oligosaccharide therapeutics. From the results shown in Figure 9 there is good evidence for sustained plasma concentrations of **27b** up to around 1 h at the higher two-dose levels and that the polysaccharide is well retained in the plasma at these concentrations. At lower doses there is a more even distribution among the examined tissues, suggesting that higher doses would be needed to sustain sufficient oligosaccharide concentration in plasma.

In order to assess the metabolic stability of **27b** *in vivo*, **27b** was extracted and purified from mouse kidney after a 4 hour treatment, to determine the extent of any degradation or metabolism. The

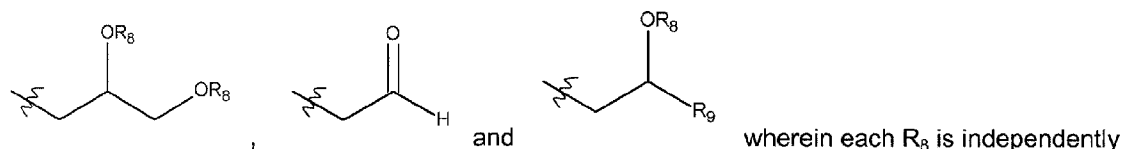
majority of the material eluted in a single peak of 3500 Da which corresponds to a mass of dodecasaccharide **27** (Figure 10), showing that the compound was stable.

Claims

1. A process for preparing a polysaccharide of 8 to 40 monosaccharide residues in length, the process comprising coupling a chain extending polysaccharide of four monosaccharide residues in length with a first polysaccharide of four or more monosaccharide residues in length by substitution of a thio leaving group bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue in either the chain extending polysaccharide or the first polysaccharide to form a chain extended polysaccharide of 8 or more residues in length.
2. A process of claim 1 wherein the thio leaving group is an $-SR^1$ group, wherein R^1 is selected from optionally substituted C_{1-10} alkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} alkynyl, optionally substituted C_{3-10} cycloalkyl, optionally substituted C_{3-10} cycloalkenyl, optionally substituted C_{3-10} heterocycloalkenyl, optionally substituted C_{6-14} aryl, optionally substituted C_{5-14} heteroaryl, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support.
3. A process according to any previous claim, wherein the first polysaccharide is a compound of eight or more monosaccharide residues in length.
4. A process according to any previous claim, wherein a thio leaving group is bonded by a sulphur atom to the anomeric carbon of a monosaccharide residue in the chain extending polysaccharide.
5. A process according to any previous claim, wherein an $-OR_2$ group is bonded to the anomeric carbon of a monosaccharide residue in the first polysaccharide, wherein R_2 is H or an end-capping group R_{ec} .
6. A process of claim 5 wherein R_{ec} is a hydroxyl protecting group, optionally wherein R_{ec} is selected from the group consisting of optionally substituted C_{1-10} alkyl, optionally substituted C_{3-10} cycloalkyl, optionally substituted C_{2-10} alkenyl, optionally substituted C_{2-10} alkynyl, optionally substituted C_{3-10} cycloalkenyl, optionally substituted C_{2-10} heteroalkyl, optionally substituted C_{3-10} heterocycloalkyl, optionally substituted C_{2-10} heteroalkenyl, optionally substituted C_{3-10} heterocycloalkenyl, optionally substituted C_{6-14} aryl, optionally substituted silyl, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support.
7. A process of claim 6 wherein the optional R_{ec} substituents are selected from OR_8 , $N(R^a)_2$, $=O$, $-C(=O)H$, C_{6-14} aryl, halo, halo C_{1-4} alkyl, $=N(R^b)$, a fluorescent label, a fluorescent label, a fluorescent label, a fluorogenic tag, a biotin residue, polyethyleneglycol and a radiolabel, wherein C_{6-14} aryl may be further substituted with one to three substituents independently selected from

halo, C₁₋₄alkoxy and C₁₋₄alkyl, each R^a is independently H or an amino protecting group, each R^b is independently H, C₁₋₄alkyl or -C(=O)C₁₋₄alkyl and R⁸ is H or a hydroxyl protecting group.

8. A process of claim 7 wherein R_{ec} is selected from methyl,



H or a hydroxyl protecting group, and R₉ is H, optionally substituted C₁₋₁₀alkyl, optionally substituted C₂₋₁₀heteroalkyl, a radiolabel, a fluorescent label, a fluorogenic tag, a tether for attachment to a carrier molecule or solid support or a tether attached to a carrier molecule or solid support.

9. A process of any previous claim wherein one or more monosaccharide residues in the chain extending polysaccharide and / or first polysaccharide is a pyranose, optionally a hexose.

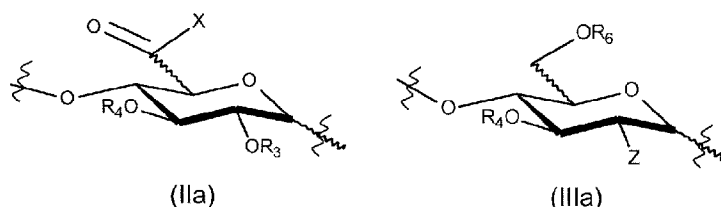
10. A process of claim 9 wherein one or more monosaccharide residues in the chain extending polysaccharide and / or first polysaccharide is selected from the group consisting of optionally substituted hexuronic acid residues and optionally substituted glucose residues.

11. A process of claim 10 wherein one or more monosaccharide residues in the chain extending polysaccharide and / or first polysaccharide is selected from optionally substituted iduronic acid residues and optionally substituted glucosamine residues.

12. A process of any of any previous claim wherein one or more hydroxyl and / or amino moieties in the chain extending polysaccharide and / or first polysaccharide are protected.

13. A process of any previous claim wherein the chain extending polysaccharide and / or first polysaccharide comprises monosaccharides coupled by a C1-C4 glycosidic bond, optionally wherein each monosaccharide residue in the chain extending polysaccharide and / or first polysaccharide is coupled by a C1-C4 glycosidic bond.

14. A process of any previous claim, wherein the first polysaccharide and / or chain extending polysaccharide comprises one or more monosaccharide residues independently selected from the group consisting of formulae (IIa) and (IIIa):



wherein

X is OR_7 or $\text{N}(\text{R}^a)_2$, wherein R_7 is H, a metal cation or optionally substituted C_{1-10} alkyl;

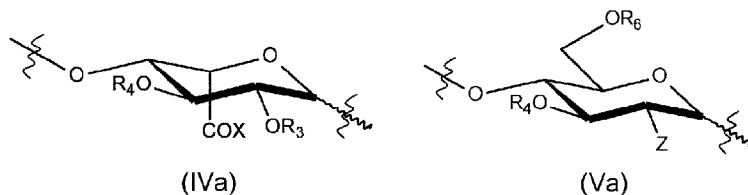
Z is OR_3 , N_3 or $\text{N}(\text{R}^a)_2$;

each R_3 , R_4 and R_6 is independently H, or a hydroxyl protecting group, optionally wherein R_3

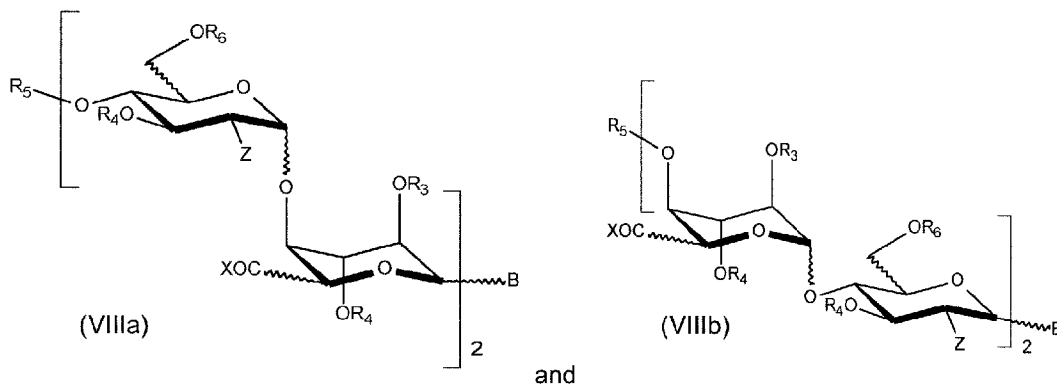
5 and R_4 are taken together to form a cyclic protecting group; and

each R^a is independently H or an amino protecting group.

15. A process of claim 14, wherein the first polysaccharide and / or chain extending polysaccharide
comprises one or more monosaccharide residues independently selected from the group
10 consisting of formulae (IVa) and (Va):



16. A process of any of claims 1 to 15 wherein the chain extending polysaccharide and / or first
polysaccharide is a compound of formula (VIIIa) or (VIIIb):

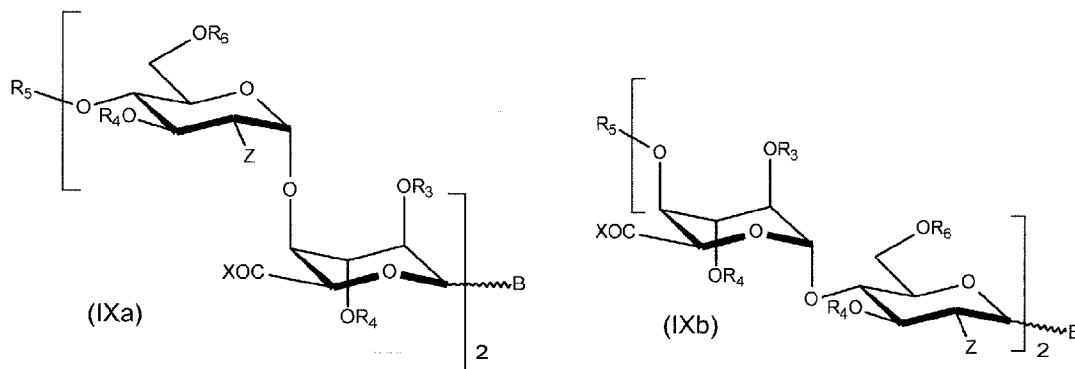


15 wherein R_5 is H, or a hydroxyl protecting group;

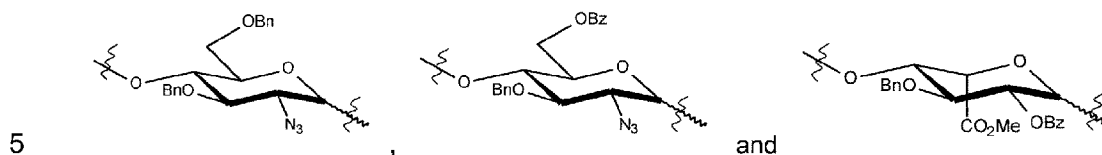
B is a thio leaving group as defined in any previous claim, or an $-\text{OR}_2$ group as
defined in any of claims 2-14; and

R_3 , R_4 , R_6 , X and Z are as defined in claim 14.

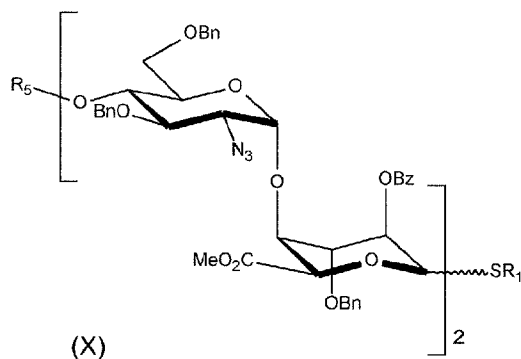
20 17. A process of claim 16 wherein the chain extending polysaccharide and / or first polysaccharide is
a compound of formula (IXa) or (IXb):



18. A process of any previous claim, wherein the first polysaccharide and / or chain extending polysaccharide comprises monosaccharide residues independently selected from the group consisting of:



19. A process of any previous claim wherein the chain extending polysaccharide and / or first polysaccharide is a compound of formula (X):

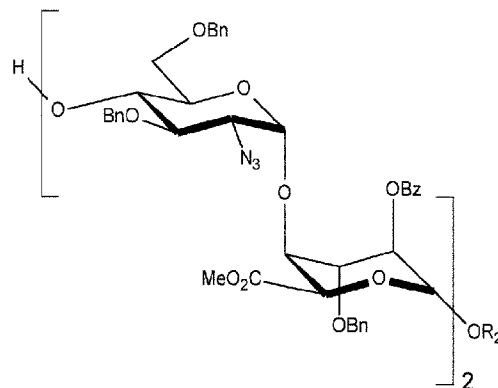


10 wherein R_5 is H or a hydroxyl protecting group.

20. A process of any of claims 16 to 19 wherein R_5 is H, $-C(O)CCl_3$, or PMB.

15 21. A process of any of claims 16 to 18, wherein the first polysaccharide or chain extending polysaccharide is a compound wherein R_5 is H and B is OR_2 , wherein R_2 is as defined in any of claims 5 to 16.

22. A process of claim 21 wherein the first polysaccharide or chain extending polysaccharide is selected from compounds of the following formula:



23. A process according to any previous claim comprising a further chain extending step, the further chain extending step comprising coupling a further chain extending polysaccharide of four monosaccharide residues in length with the chain extended polysaccharide by substitution of a thio leaving group bonded by a sulphur atom to the anomeric carbon of a monosaccharide in the chain extending polysaccharide or chain extended polysaccharide to form a further chain extended polysaccharide of 12 to 40 monosaccharide residues in length.

24. A process according to any previous claim comprising two to seven further chain extending steps, wherein each further chain extending step comprises coupling a further chain extending polysaccharide of four monosaccharide residues in length with a chain extended polysaccharide by substitution of a thio leaving group bonded by a sulphur atom to the anomeric carbon of a monosaccharide in the further chain extending polysaccharide or the chain extended polysaccharide to form a further chain extended polysaccharide of 16 to 40 monosaccharide residues in length.

25. A process according to any previous claim, comprising the further step of coupling a chain extended polysaccharide with a monosaccharide, or a polysaccharide of two or three monosaccharide residues in length to form a polysaccharide of 9 to 40 monosaccharide residues in length.

26. A process according to any previous claim, wherein the coupling forms an O-glycosidic bond.

27. A process according to any previous claim, wherein the coupling forms a C1-C4 O-glycosidic bond.

28. A process according to any previous claim further comprising one or more derivatization steps.

29. A process according to claim 28 wherein the one or more derivatization steps comprise:

- a) deprotection and / or protection of the polysaccharide at any stage in the process;
- b) conjugation of the polysaccharide to a non-saccharide moiety;

- c) sulfation, sulphonation, phosphation and / or phosphonation of one or more nucleophilic groups; and / or
- c) formation of the acid or base addition salts of any respective basic or acidic functionalities in the polysaccharide.

5

30. A process of any previous claim for preparing a polysaccharide of 12 to 40 monosaccharide residues in length, optionally 16 to 40, optionally 20 to 40, optionally 25 to 40.

31. A process of any previous claim wherein the polysaccharide is a glycosaminoglycan.

10

32. A polysaccharide of 8 to 40 monosaccharide residues in length wherein a thio leaving group is bonded to the anomeric carbon of a monosaccharide residue in the polysaccharide, wherein the thio leaving group is as defined in any previous claim.

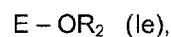
15

33. An isolated polysaccharide according to claim 32.

34. An isolated polysaccharide of 17 to 40 monosaccharide residues in length.

35. A polysaccharide according to claim 34, wherein the polysaccharide is a compound of formula (Ie):

20



wherein

E is a chain of 17 to 40 monosaccharide residues in length;

-OR₂ is bonded to the anomeric carbon of a monosaccharide residue in the polysaccharide,

25

wherein R₂ is H or an end-capping group R_{ec}.

36. A polysaccharide according to any of claims 32 to 35 comprising one or more glucosamine residues.

30

37. A polysaccharide of any of claims 32 to 36, wherein the polysaccharide is of 20 to 40 monosaccharide residues in length, optionally 24-40, optionally 28-40.

38. A polysaccharide of any previous claim wherein one or more monosaccharide residues in the polysaccharide is a pyranose, optionally a hexose.

35

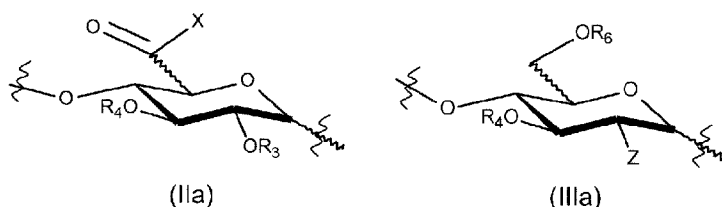
39. A polysaccharide of claim 38 wherein one or more monosaccharide residues in the polysaccharide is selected from the group consisting of optionally substituted hexuronic acid residues and optionally substituted glucose residues.

40. A polysaccharide of claim 39 wherein one or more monosaccharide residues in the polysaccharide is selected from optionally substituted iduronic acid residues and optionally substituted glucosamine residues.

5 41. A polysaccharide of any of claims 32 to 40 wherein one or more hydroxyl and / or amino moieties in the polysaccharide are protected.

42. A polysaccharide of any of claims 32 to 41 wherein the polysaccharide comprises monosaccharides coupled by a C1-C4 glycosidic bond, optionally wherein each monosaccharide
10 residue is coupled by a C1-C4 glycosidic bond, optionally α -glycosidic bonds.

43. A polysaccharide of any of claims 32-42 comprising one or more monosaccharide residues independently selected from the group consisting of formulae (IIa) and (IIIa):



15 wherein

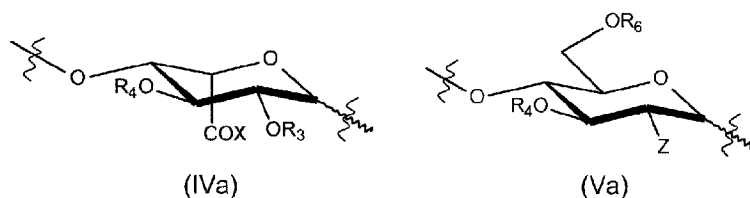
X is OR_7 or $N(R^a)_2$, wherein R_7 is H, a metal cation or optionally substituted C_{1-10} alkyl;

Z is OR_3 , N_3 or $N(R^a)_2$;

each R_3 , R_4 and R_6 is independently H, or a hydroxyl protecting group, optionally wherein R_3 and R_4 are taken together to form a cyclic protecting group; and

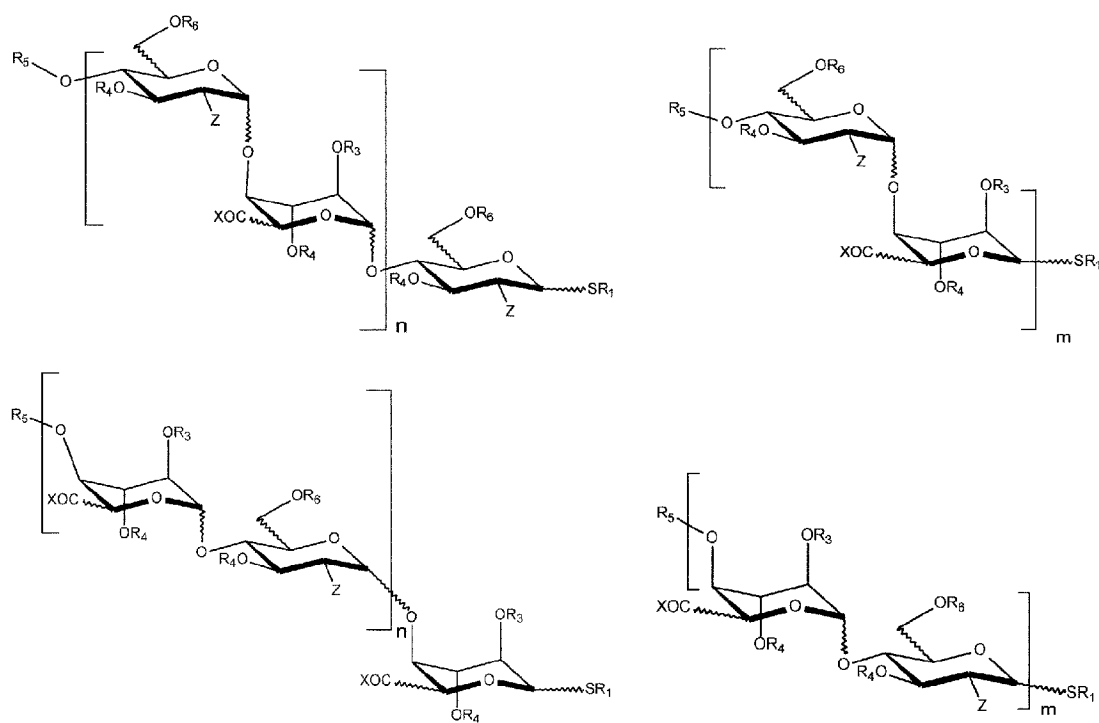
20 each R^a is independently H or an amino protecting group.

44. A polysaccharide of claim 43, wherein the polysaccharide comprises one or more monosaccharide residues independently selected from the group consisting of formulae (IVa) and (Va):



25

45. A polysaccharide of claim 32, 33 or any of claims 36 to 44 when dependent on claim 32 or 33, selected from the following formulae:



wherein R₁ is as defined in claim 2;

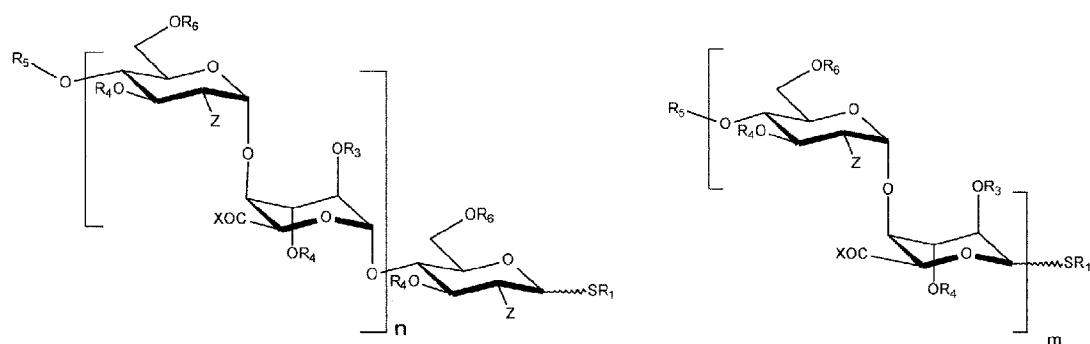
each R₃, R₄, R₆, X and Z is independently as defined in claim 43;

5 R₅ is H or a hydroxyl protecting group;

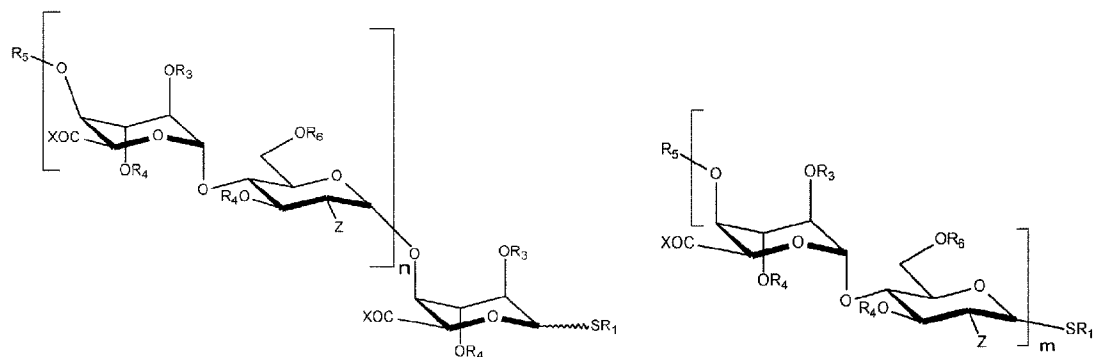
n is an integer from 4 to 19; and

m is an integer from 4 to 20.

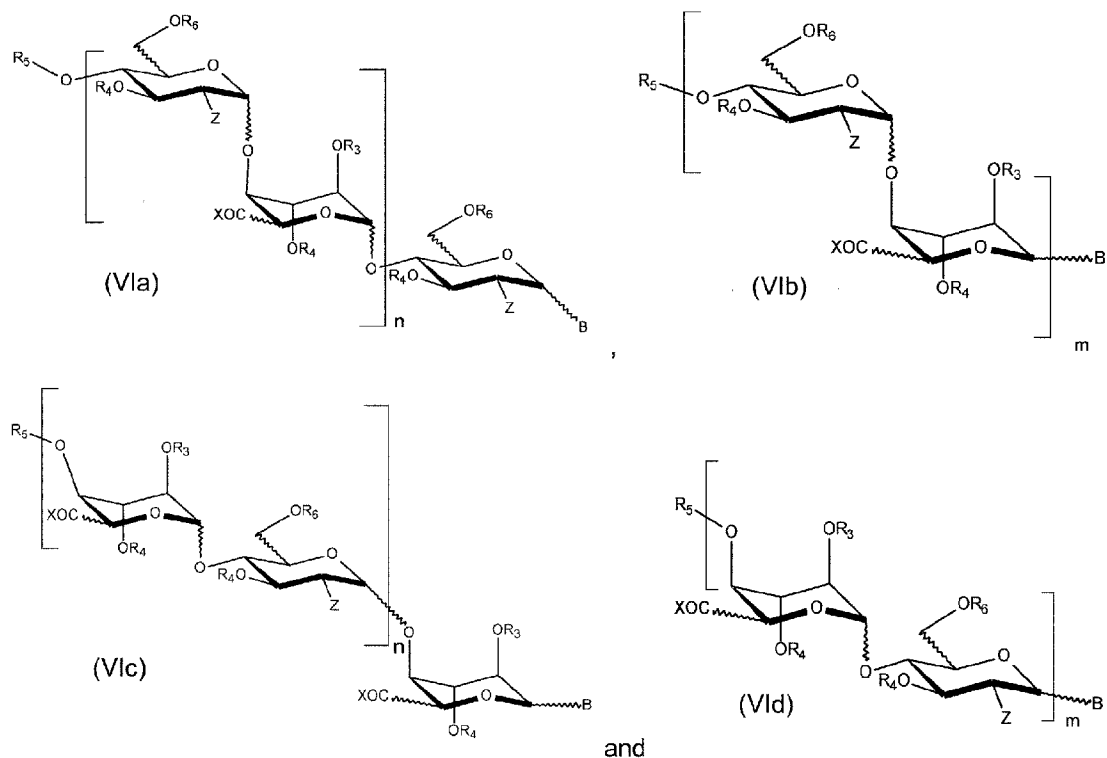
46. A polysaccharide of claim 45 selected from the group consisting of:



10



47. A polysaccharide of any of claims 34 to 44, selected from the group consisting of:

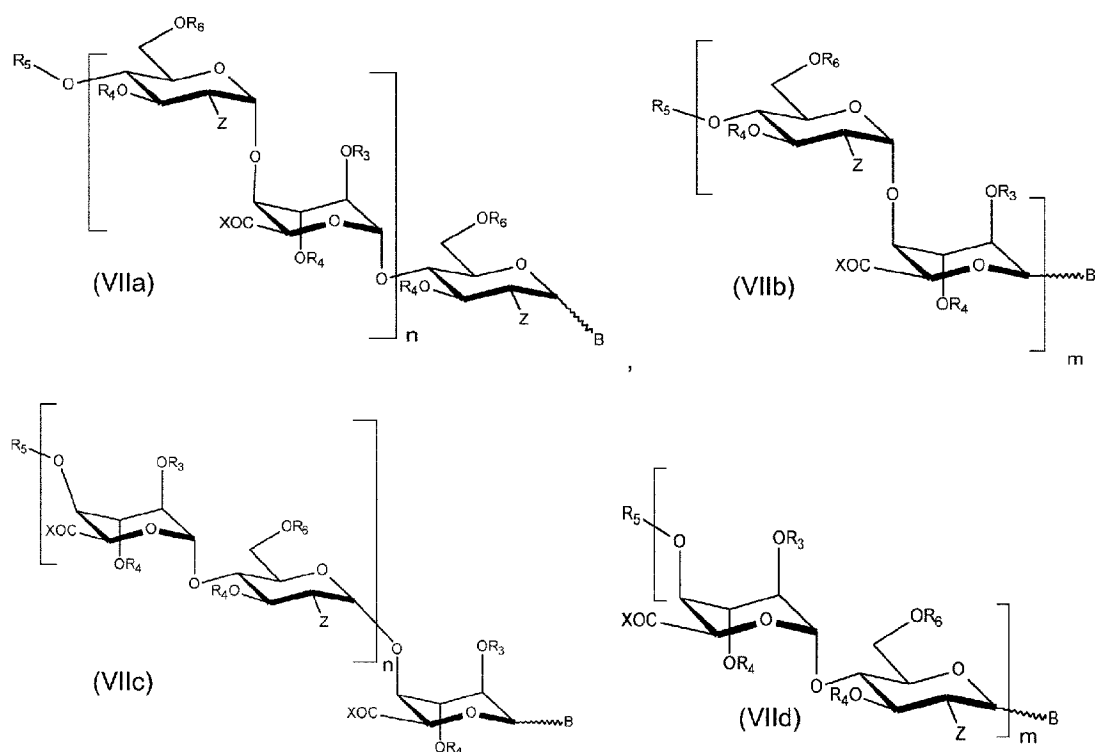


and

- 5 wherein B is as defined in claim 16;
 each of R_2 , R_3 , R_4 , R_6 , X and Z is independently as defined in claim 43.
 R_5 is H or a hydroxyl protecting group;
 n is an integer from 8 to 19; and
 m is an integer from 9 to 20.

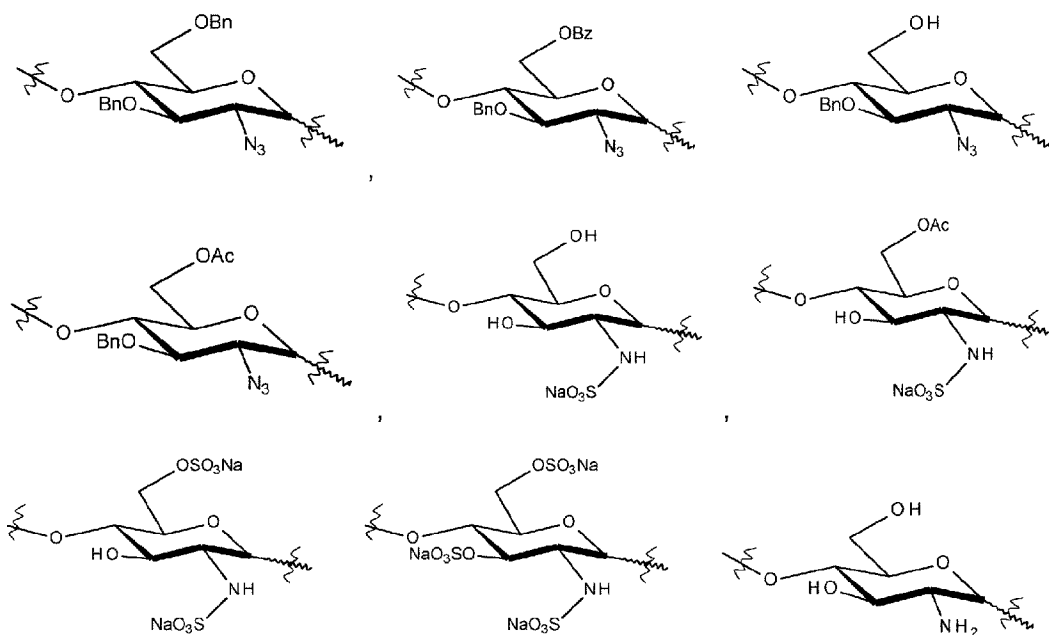
10

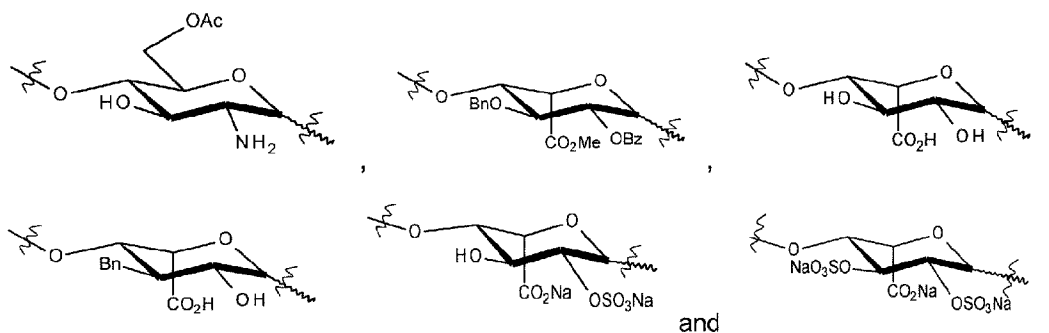
48. A polysaccharide of claim 47, selected from the group consisting of:



49. A polysaccharide of any of claims 32-48, wherein the monosaccharide residues are independently selected from the group consisting of:

50.



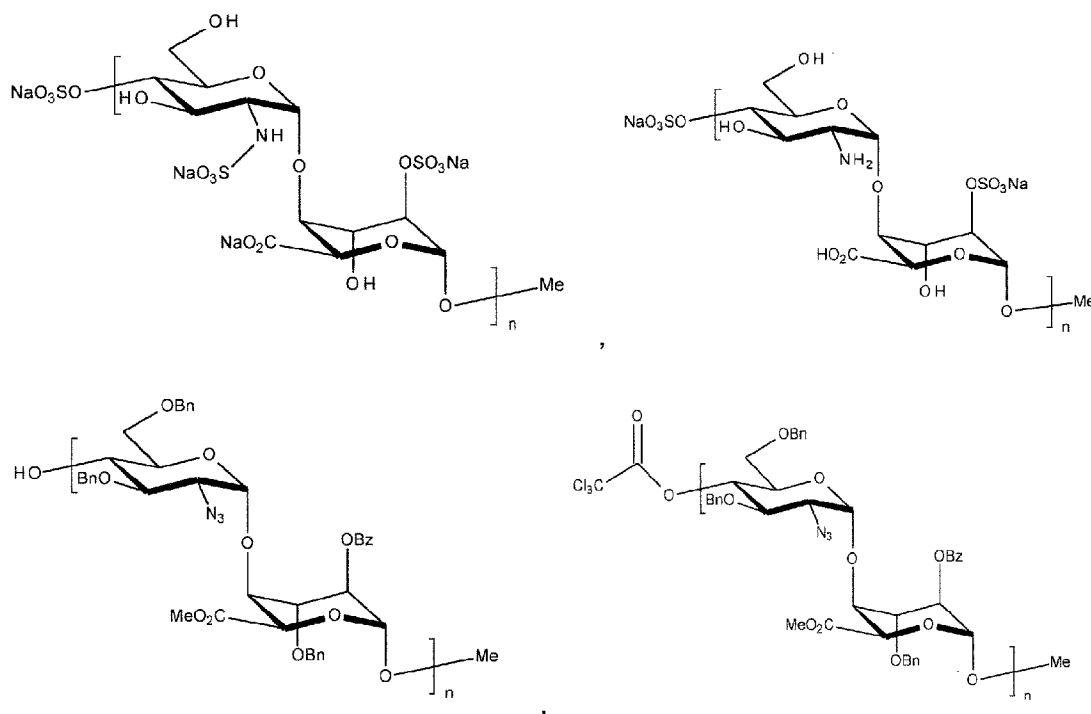


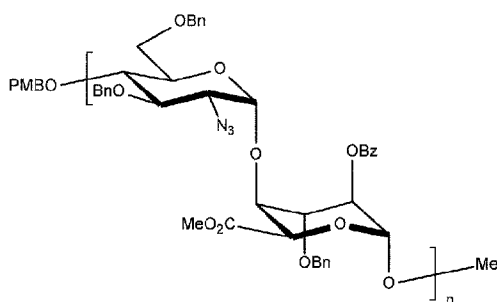
51. A polysaccharide of any of claims 34-49, wherein B is an -OR₂ group, wherein R₂ is H or an end-capping group R_{ec}, optionally wherein R₂ is as defined in any of claims 6 to 8.

5

52. A polysaccharide of any of claims 32 to 51, wherein R₃, R₄, R₅ and R₆, are independently selected from H, optionally substituted C₁₋₁₀alkyl and -S(O₂)OR^y, wherein R^y is selected from H, C₁₋₁₀alkyl and a metal cation, optionally wherein the metal cation is a sodium cation.

10 53. A polysaccharide selected from the group consisting of:

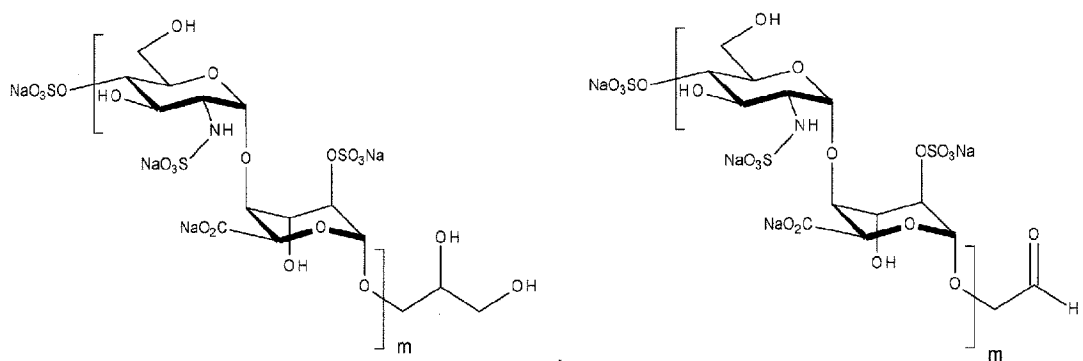
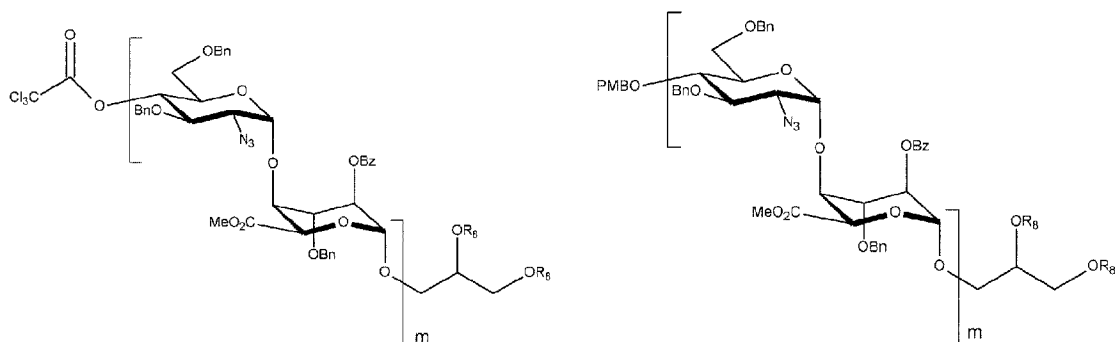




and

, wherein n is an integer from 7 to 20.

54. A polysaccharide selected from the group consisting of:



and

, wherein R₉ is H, optionally substituted C₁-

¹⁰alkyl, optionally substituted C₂₋₁₀heteroalkyl, a radiolabel, a fluorescent label, a fluorogenic tag, a tether for attachment to a carrier molecule or solid support and a tether attached to a carrier molecule or solid support; and m is an integer of 4 to 20.

55. An isolated polysaccharide of any of claims 33 to 54 in solid form.

56. A polysaccharide of any of claims 32 to 55 which is substantially pure.

5 57. An isolated polysaccharide in solid form according to claim 55 or 56 wherein the solid form comprises at least 80mol% of the polysaccharide.

58. A solid composition comprising an isolated polysaccharide as described in any of claims 55 to 57.

10 59. A process of any of claims 1-31 for producing a polysaccharide as defined in any of claims 32-57 or a composition according to claim 58.

60. A polysaccharide according to any of claims 32-57 or a composition according to claim 58 prepared according to a process of any of claims 1-31.

15 61. A polysaccharide of the formula (Ig)



wherein P is a polysaccharide residue;

L is a linker; and

20 R is a radiolabel.

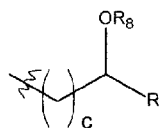
62. A polysaccharide of claim 61 wherein the polysaccharide is a polysaccharide as defined in any previous claim, optionally wherein the polysaccharide is a glycosaminoglycan.

25 63. A polysaccharide of any of claims 61 or 62 wherein L is bonded to an O, N, S or Se atom in the polysaccharide residue, optionally an O atom.

64. A polysaccharide of any one of claims 61 to 63 wherein L is a bridge of up to 15 atoms in length, optionally up to 4 atoms in length.

30 65. A polysaccharide as claimed in any one of claims 61 to 64 wherein L is selected from the group consisting of optionally substituted C₁₋₁₀alkylene, optionally substituted C₂₋₁₀alkenylene, optionally substituted C₂₋₁₀heteroalkylene, optionally substituted C₃₋₁₀heterocycloalkylene, optionally substituted C₂₋₁₀heteroalkenylene and optionally substituted C₃₋₁₀heterocycloalkenylene; optionally wherein L is
35 optionally substituted C₁₋₁₀alkylene; or optionally wherein the linker is optionally substituted C₁₋₄alkylene.

66. A polysaccharide as claimed in any one of claims 61 to 65, wherein the -L-R group appended to the polysaccharide P in formula (Ig) is:



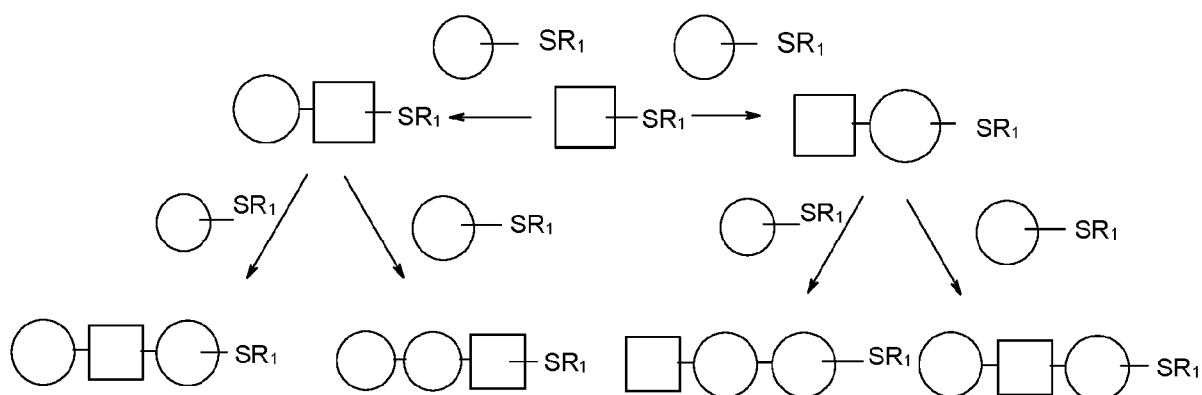
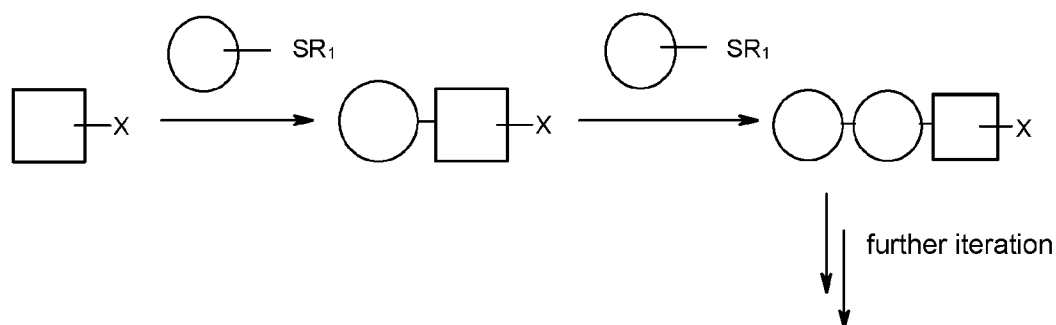
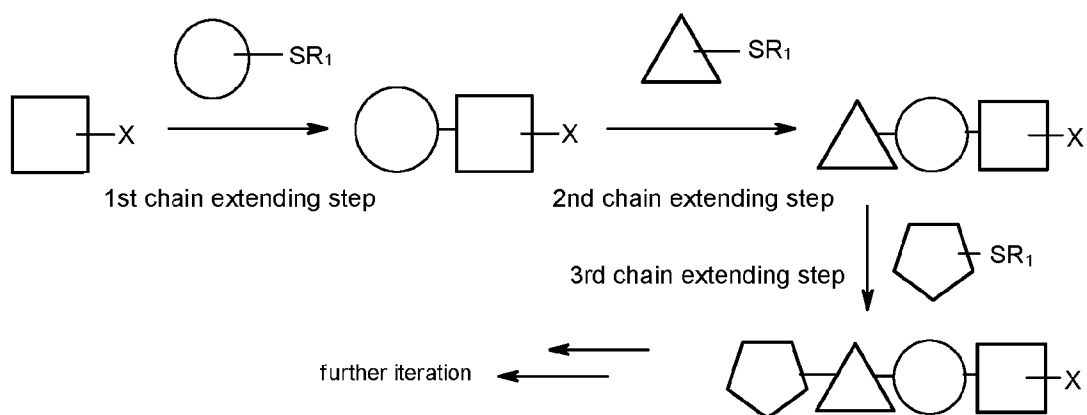
, wherein c is an integer of 1 to 9, optionally 1; and R^8 is H or a hydroxyl protecting group.

5 67. A polysaccharide of any one of claims 61 to 66 wherein the linker is attached to a monosaccharide residue at a terminus of the polysaccharide chain, optionally wherein the linker is attached to the anomeric position of the monosaccharide of the terminus of the polysaccharide chain.

68. A polysaccharide according to any one of claims 61 to 67 wherein R is a radiolabel comprising a radioactive isotope selected from ^3H , ^{14}C , ^{11}C , ^{18}F , ^{15}O and ^{13}N , optionally wherein the radiolabel is ^3H .

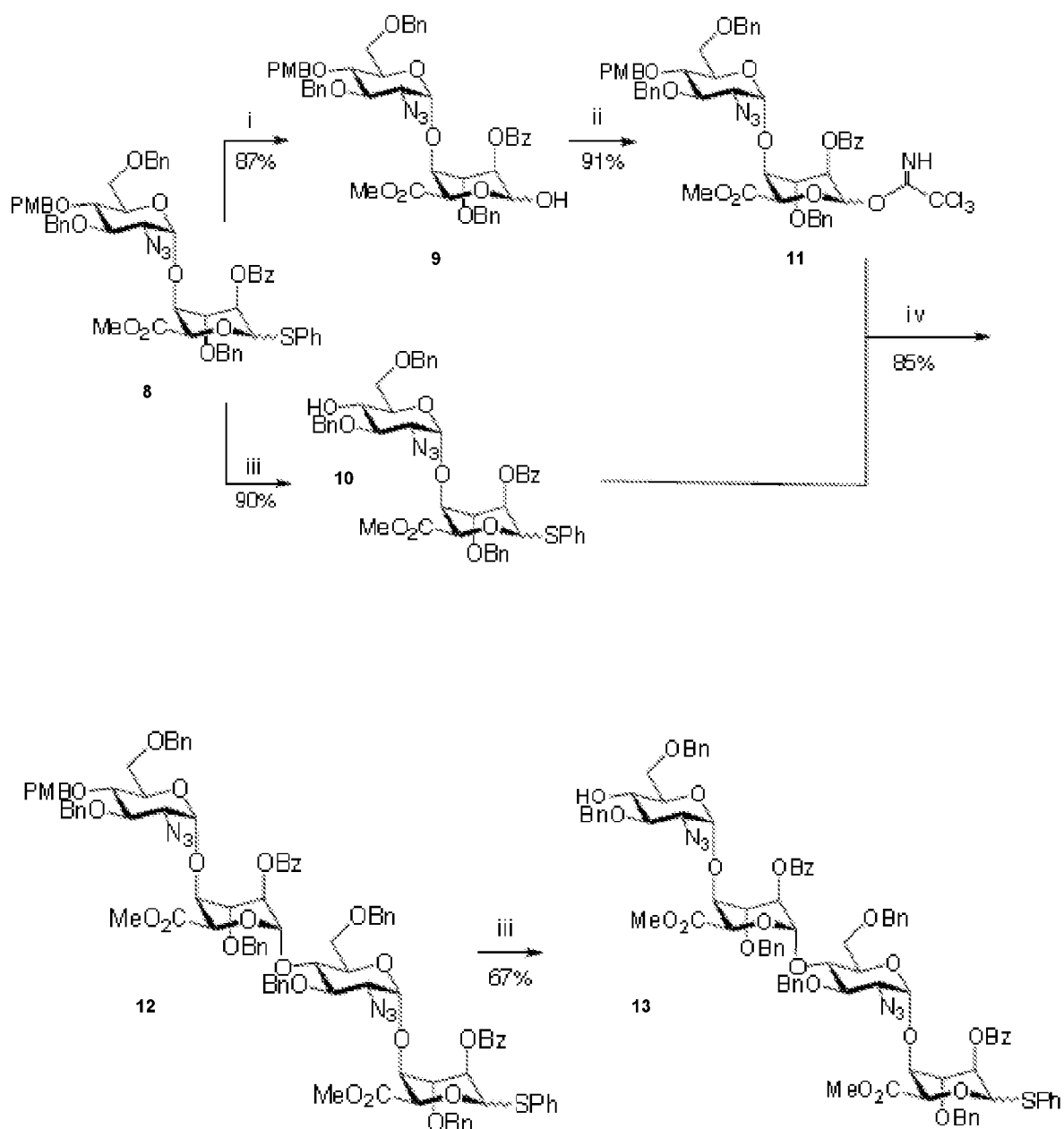
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Figure 1**Figure 2****Figure 3**

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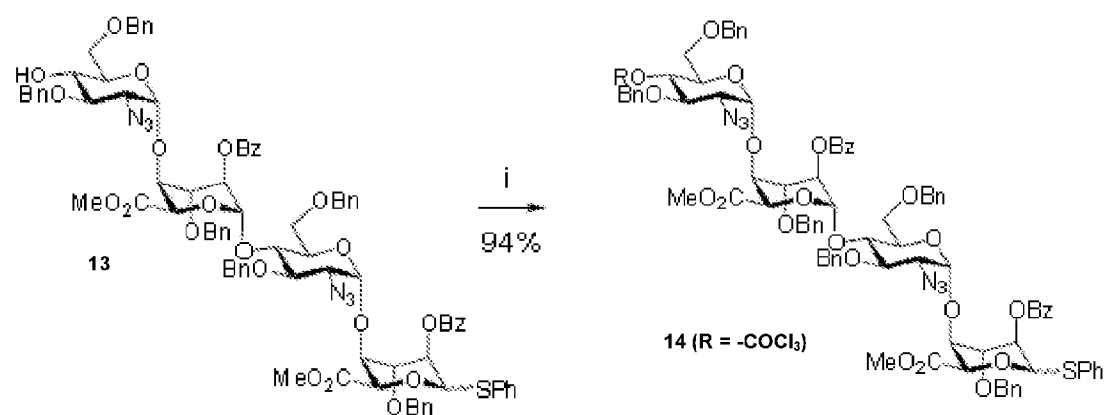
Figure 4



i) NBS, acetone; ii) CCl₃CN, DBU, DCM; iii) CAN, CH₃CN/H₂O; iv) TMSOTf, DCM.

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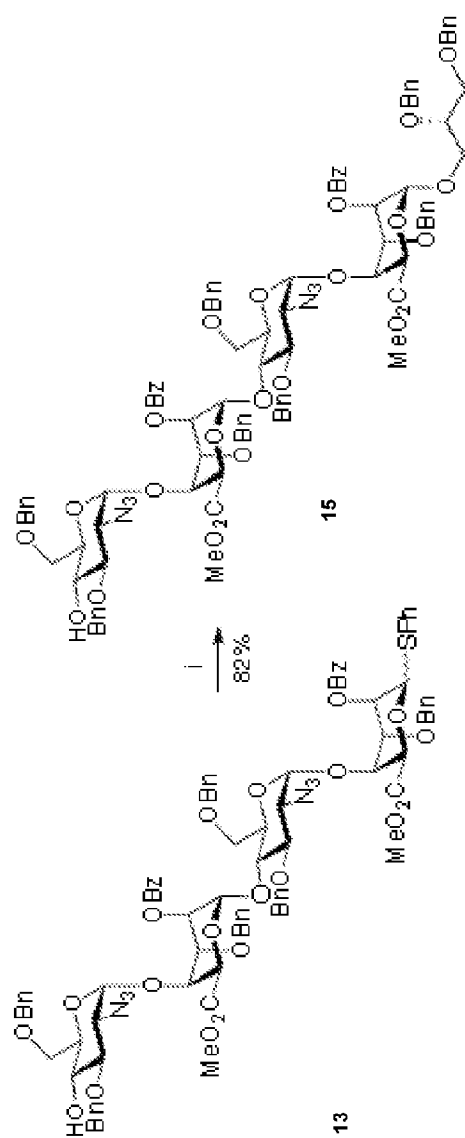
Figure 5

i) CCl₃COCl, Py, DCM.

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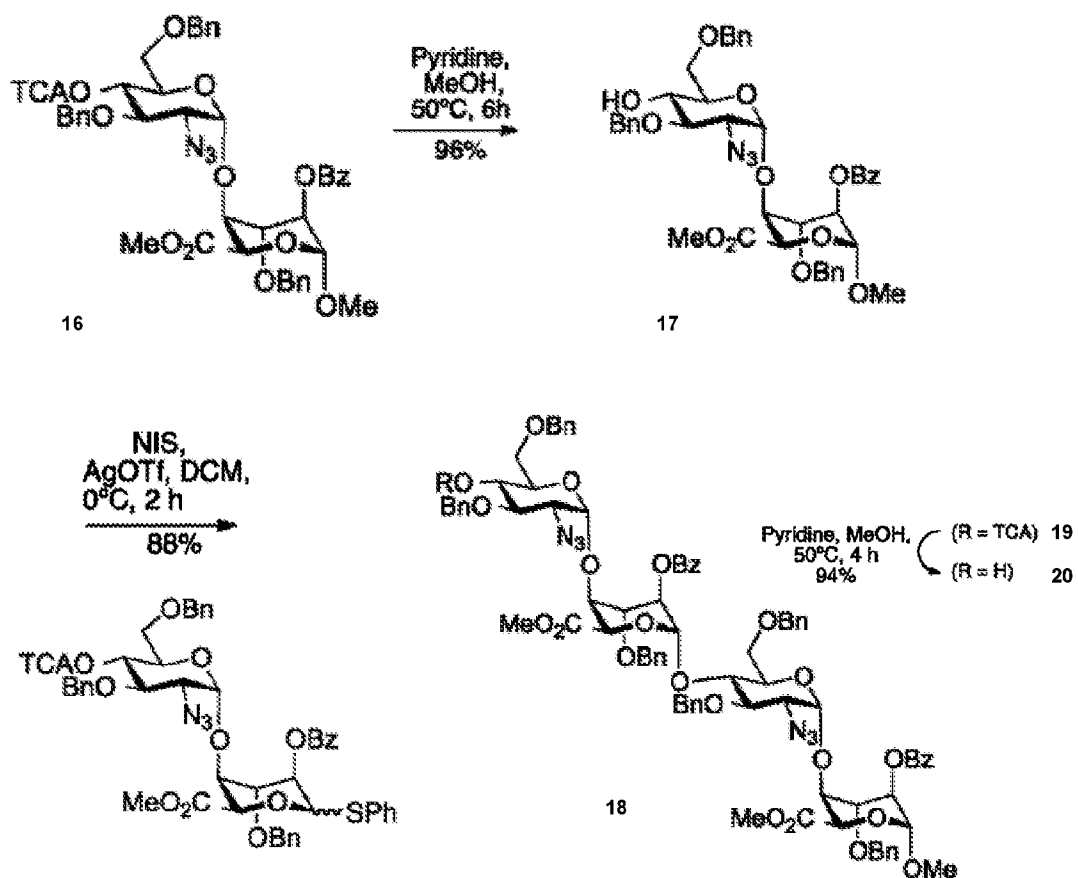
Figure 6

a)



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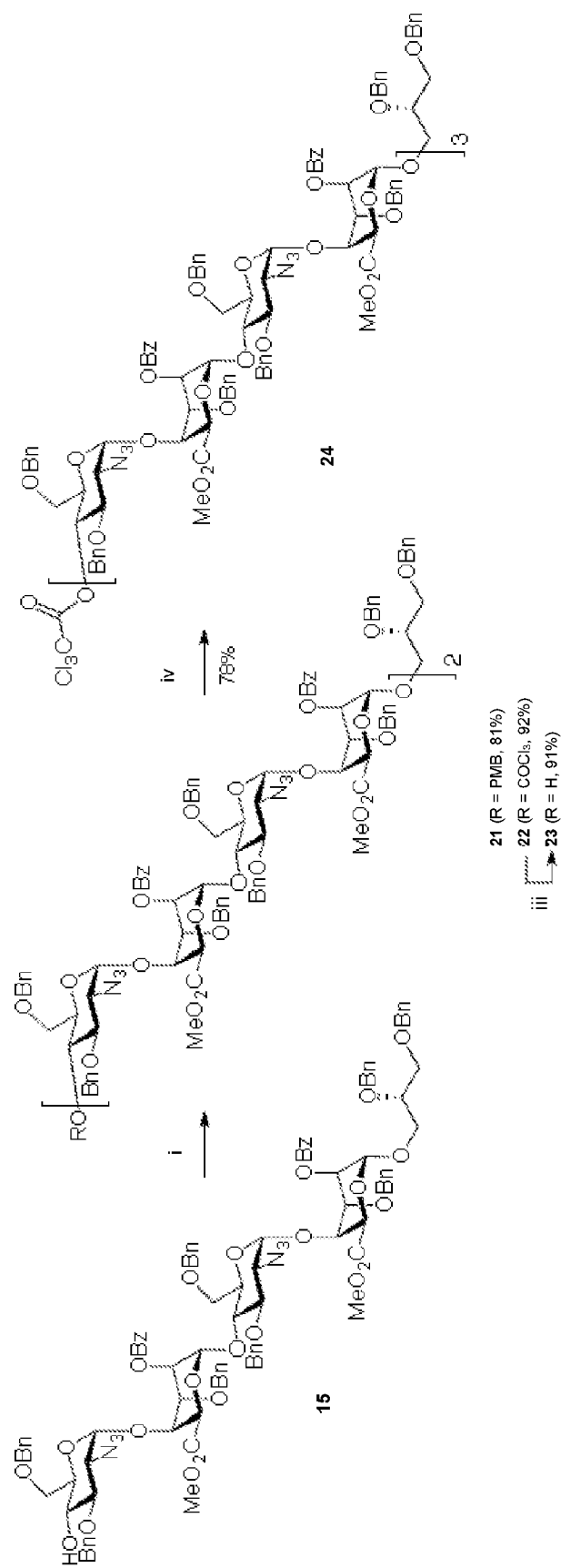
b)



i) (S)-2,3-bis(benzyloxy)propanol, NIS, AgOTf(cat.), DCM.

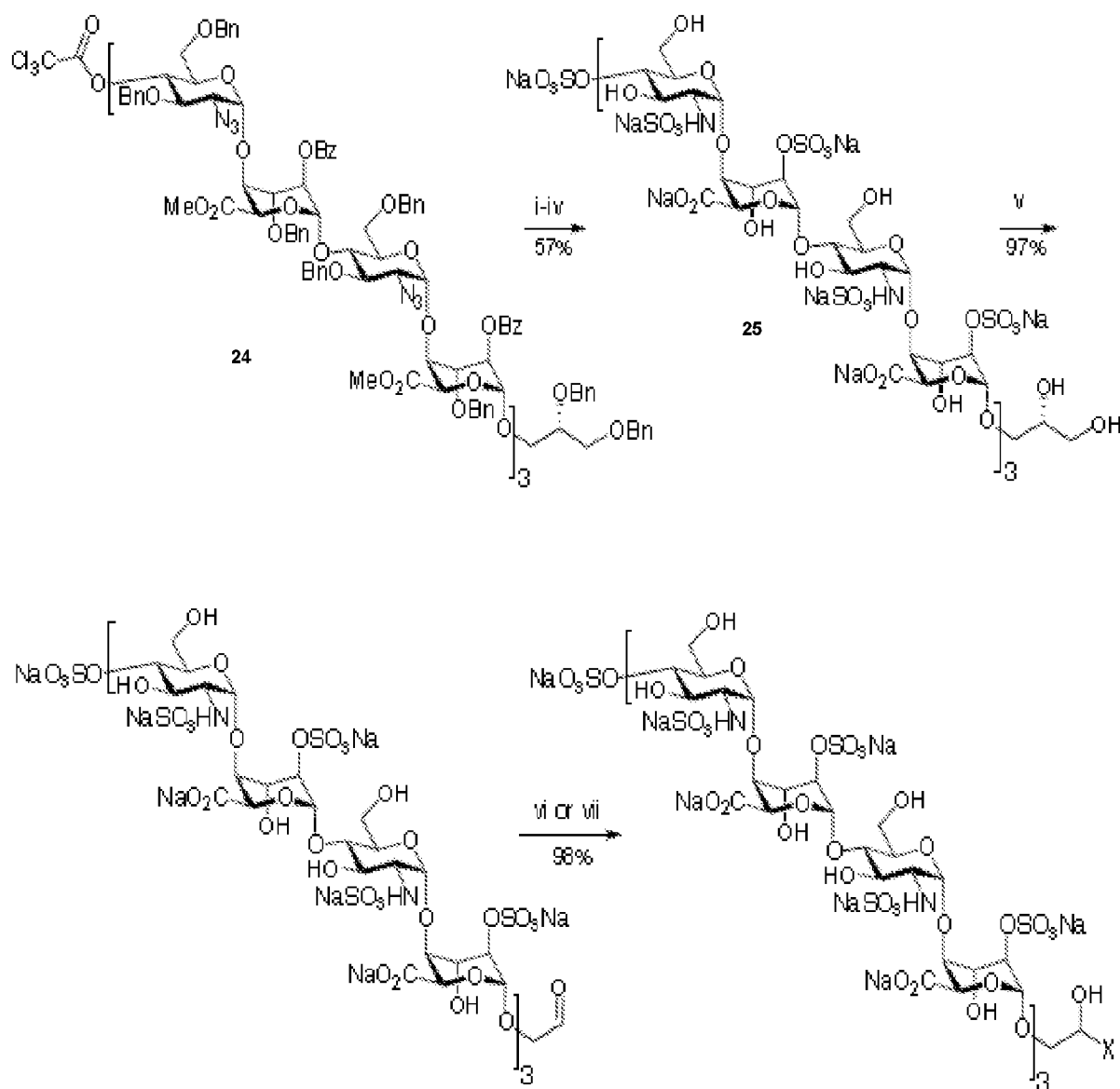
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Figure 7



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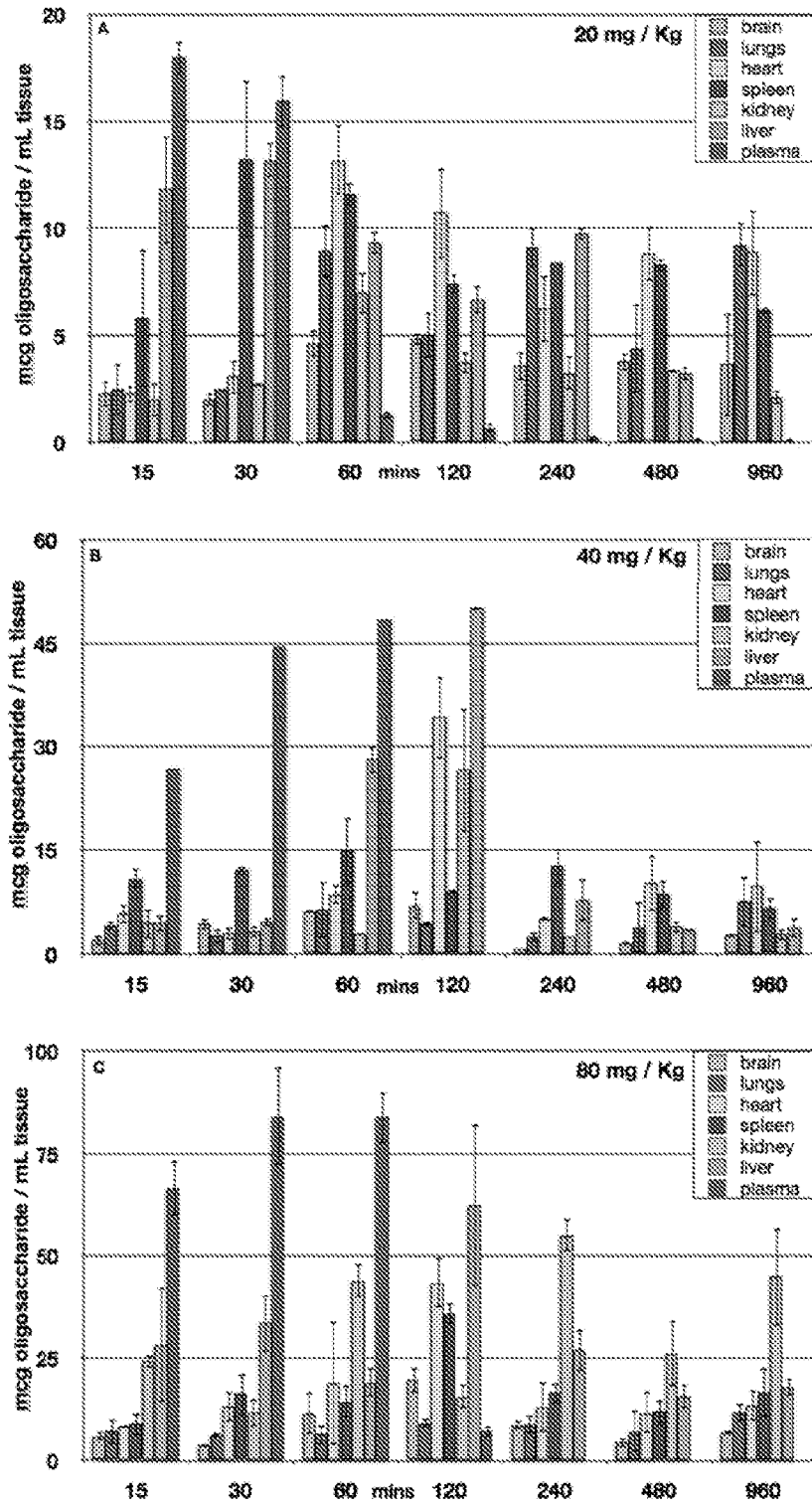
Figure 8



- i) LiOH, THF/MeOH/H₂O, 74%
 ii) Py.SO₃ complex, pyridine, 80%
 iii) H₂, Pd(OH)₂/C, MeOH/THF/H₂O; 2:1:1, 96%
 iv) Py.SO₃ complex, NaHCO₃, H₂O, 100%
 v) NaIO₄, H₂O
 vi) NaBH₄, H₂O (X = H)
 vii) NaB³H₄, H₂O (X = ³H).

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Figure 9



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Figure 10

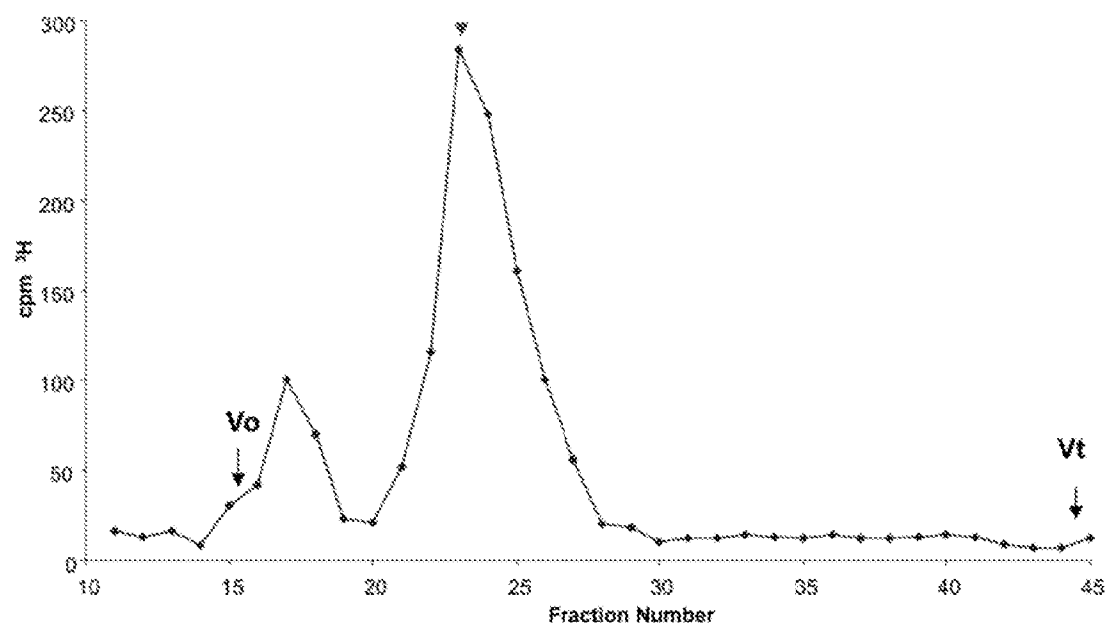
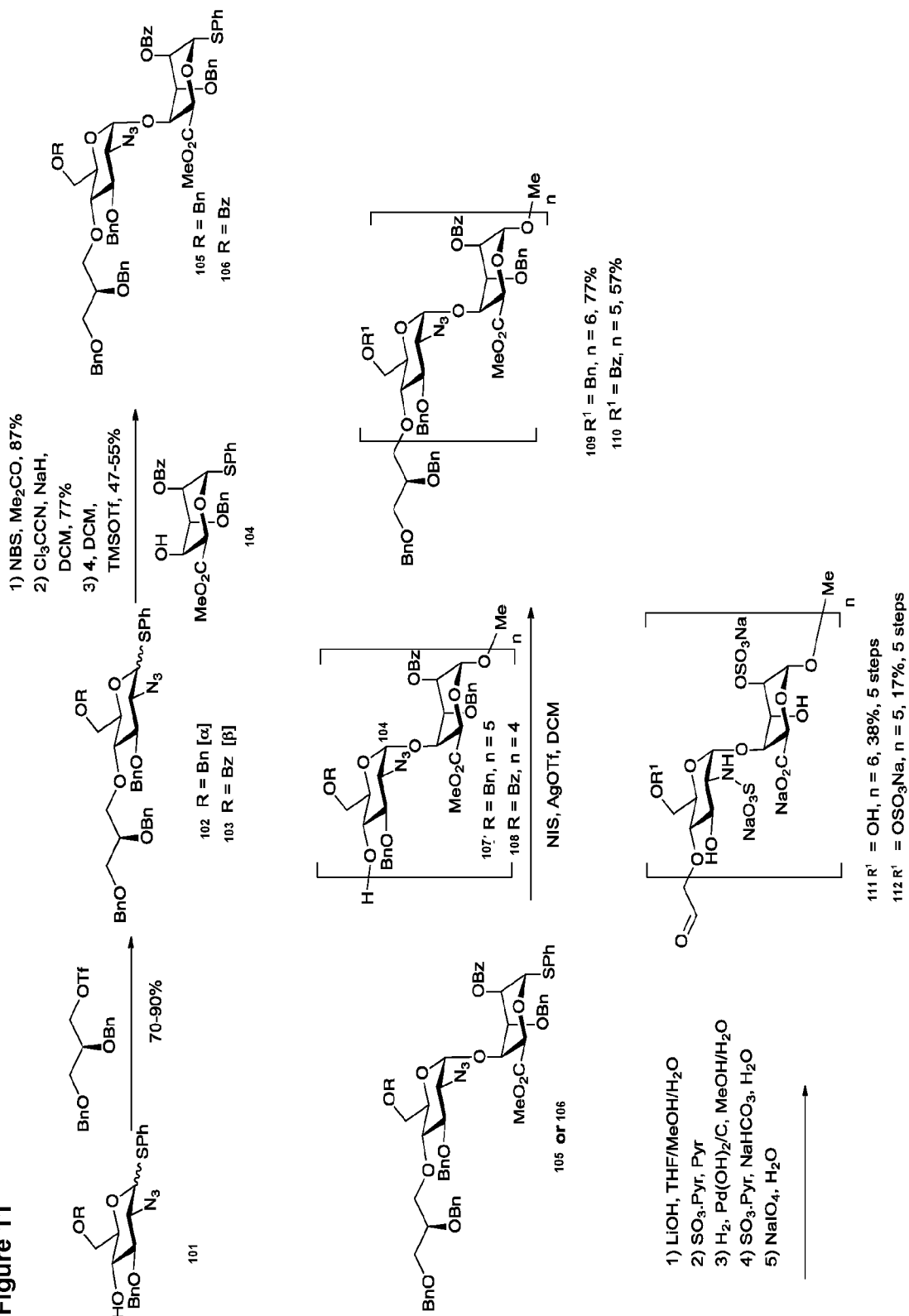


Figure 11

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Chemical reaction scheme showing the synthesis of model O4 terminal aldehyde 114 from compound 113.

Compound 113 (a disaccharide derivative with various protecting groups: OBn, MeOC, OBz, N₃) reacts with hydrazine (NH₂NH₂) and sodium borohydride (NaCNBH₃) to form intermediate 114 (a disaccharide derivative with a terminal aldehyde group and a hydrazide linkage).

Intermediate 114 then reacts with benzylamine (H₂NCH₂CH₂NHBz) to form the final product 116 (a disaccharide derivative with a terminal aldehyde group and a secondary amine linkage).

