[0007] There is a need to provide structurally well-defined, non-saccharide and multivalent glycosaminoglycan mimetics in which the spatial arrangement of pendant groups can be precisely controlled using a polyproline scaffold, for controlling and enhancing binding specificity to a target molecule. Each target molecule has its own unique structural confirmation, which in turn dictates the requirements of the spatial arrangement of the pendant groups of the glycosaminoglycan mimetic for achieving the optimal binding fit. Thus, there is a need to provide a method to tailor non-saccharide glycosaminoglycan mimetic molecules for binding to specific target molecules. There is also a need to provide a closer mimic to the longer natural glycosaminoglycan that also allows for a greater degree of control.

## SUMMARY

[0008] Disclosed is a non-saccharide glycosaminoglycan mimetic molecule comprising a polyproline backbone and one or more non-saccharide molecules. Advantageously, the non-saccharide glycosaminoglycan mimetic molecule is a well-defined, non-saccharide, multivalent glycosaminoglycan mimetic in which the spatial arrangement of the pendant groups has been precisely controlled using a polyproline scaffold, which allows for the use of this mimetic in controlling protein binding specificity and efficacy.

[0008a] According to a first aspect, there is provided a non-saccharide glycosaminoglycan mimetic molecule comprising a polyproline backbone and one or more non-saccharide molecules; wherein the non-saccharide molecules comprise primary, secondary or tertiary negatively charged groups, or a combination thereof; and wherein the negatively charged groups are selected from the group consisting of sulfates, and phosphates.

[0009] Non-saccharide molecules may be attached to one or more prolines and/or proline derivatives that make up the polyproline backbone. Advantageously, the non-saccharide pendant groups allow for controlling the specificity of these mimetics by precise positioning of non-saccharide bioactive moieties to direct multivalent interactions, allowing for a wide range of potential applications.

[0010] According to a second aspect, there is provided a method of synthesizing a non-saccharide glycosaminoglycan mimetic molecule as defined above, comprising attaching one or more non-saccharide molecules to a polyproline backbone. Advantageously, the non-carbohydrate alternative avoids the need for time consuming, atom inefficient, costly and laborious carbohydrate synthesis.

[0011] According to a third aspect, there is provided a non-saccharide glycosaminoglycan mimetic molecule as defined above for use in therapy. Advantageously, the use of the non-

saccharide glycosaminoglycan mimetic molecule enables specific binding to a target molecule (for example a protein such as selectin) that is implicated in disease conditions, thereby avoiding undesirable consequences (such as side effects that may arise from unspecific cellular binding).

[0012] Disclosed is a method for inhibiting cell adhesion molecules comprising administering a non-saccharide glycosaminoglycan mimetic molecule as defined above. Advantageously, the non-saccharide glycosaminoglycan mimetic molecule has non-anti-coagulant property compared to heparin (which is used as an anti-coagulant itself) when used as a cell adhesion molecule inhibitor.

[0013] Disclosed is a method of treating a patient in need of a target-specific therapy, comprising administering a non-saccharide glycosaminoglycan mimetic molecule as defined above.

[0013a] According to a fourth aspect, there is provided use of a non-saccharide glycosaminoglycan mimetic molecule as defined above in the manufacture of a medicament for:

(a) inhibiting cell adhesion molecules; or

(b) the treatment of a patient in need of a target-specific therapy; or

(c) promoting neuritogenesis in a patient; or

(d) inhibiting extravasation of circulating tumor cell into potential metastatic sites in a patient; or

(e) treating a viral infection.

[0014] Disclosed is a target-specific therapeutic agent comprising a non-saccharide glycosaminoglycan mimetic molecule as defined above.

[0015] Disclosed is a non-saccharide glycosaminoglycan mimetic molecule as defined above for use as target-specific biopolymers.

[0016] Disclosed is a non-saccharide glycosaminoglycan mimetic molecule as defined above for use in glycosaminoglycans (GAG)-based pharmaceutics. Disclosed is a non-saccharide glycosaminoglycan mimetic molecule as defined above for use as diagnostic tools.

[0017] Disclosed is a method of controlling the binding affinity of a non-saccharide glycosaminoglycan mimetic molecule to one or more binding molecules, comprising attaching one or more non-saccharide molecules at pre-determined positions along a polyproline backbone. Advantageously, the precise positioning of non-saccharide molecules on the polypeptide backbone of the non-saccharide glycosaminoglycan mimetic molecule may be used to direct multivalent interactions in a more controlled and specific manner, allowing for a wide range of potential applications.

[0017a] According to a fifth aspect, there is provided a method of controlling the binding affinity of a non-saccharide glycosaminoglycan mimetic molecule to one or more binding molecules, comprising attaching one or more non-saccharide molecules at pre-determined positions along a polyproline backbone; wherein the non-saccharide molecules comprise primary, secondary or tertiary negatively charged groups, or a combination thereof; and wherein the negatively charged groups are selected from the group consisting of sulfates, and phosphates.

[0018] Disclosed is a method of promoting neuritogenesis in a patient, comprising administering a non-saccharide glycosaminoglycan mimetic molecule as defined above.

[0019] Disclosed is a method of inhibiting extravasation of circulating tumor cell into potential metastatic sites in a patient, comprising administering a non-saccharide glycosaminoglycan mimetic molecule as defined above.

## DEFINITION OF TERMS

[0020] The following words and terms used herein shall have the meaning indicated:

[0021] The term "glycosaminoglycan" refers to any complex polysaccharides having repeating units of either the same saccharide subunit or two different saccharide subunits. Some examples of natural glycosaminoglycans include dermatan sulfates, hyaluronic acid, the chondroitin sulfates, chitin, heparin, keratan sulfates, keratosulfates, heparan sulfates, and derivatives thereof.

[0022] The term "protein", "polypeptide," and "peptide" used interchangeably herein refers to a polymer of at least two amino acids that are covalently linked. The amino acids may be D- or L-amino acids, or mixtures of D- and L-amino acids, as well as naturally occurring or synthetically produced amino acids.

[0023] The term “mimetic” refers to a molecule that has a structure, and typically biological properties, that are similar to the molecule it is imitating. For example, when used with reference to a non-saccharide glycosaminoglycan molecule as defined herein, the term refers to a molecule which because of its structural properties, is capable of mimicking the biological function of a glycosaminoglycan.

[0024] The term “derivative” refers to a chemically or biologically modified version of a compound or molecule that is structurally similar to a parent compound or molecule and is derived from that parent compound or molecule.

[0025] The term “pendant group” refers to any functional group that may be attached to, and forms a side-chain of a macromolecule. Typically, the pendant group is attached to the backbone of the macromolecule. For example, in the case of a non-saccharide glycosaminoglycan mimetic, the non-saccharide molecule may form the pendant group that is attached to the polyproline backbone of the glycosaminoglycan mimetic, via linkages such as a covalent bond. Exemplary pendant groups on the polyproline backbone of a non-saccharide glycosaminoglycan mimetic include, but are not limited to, hydroxyl, sulfate, phosphate or carboxylate group-containing non-saccharides. The non-saccharide glycosaminoglycan

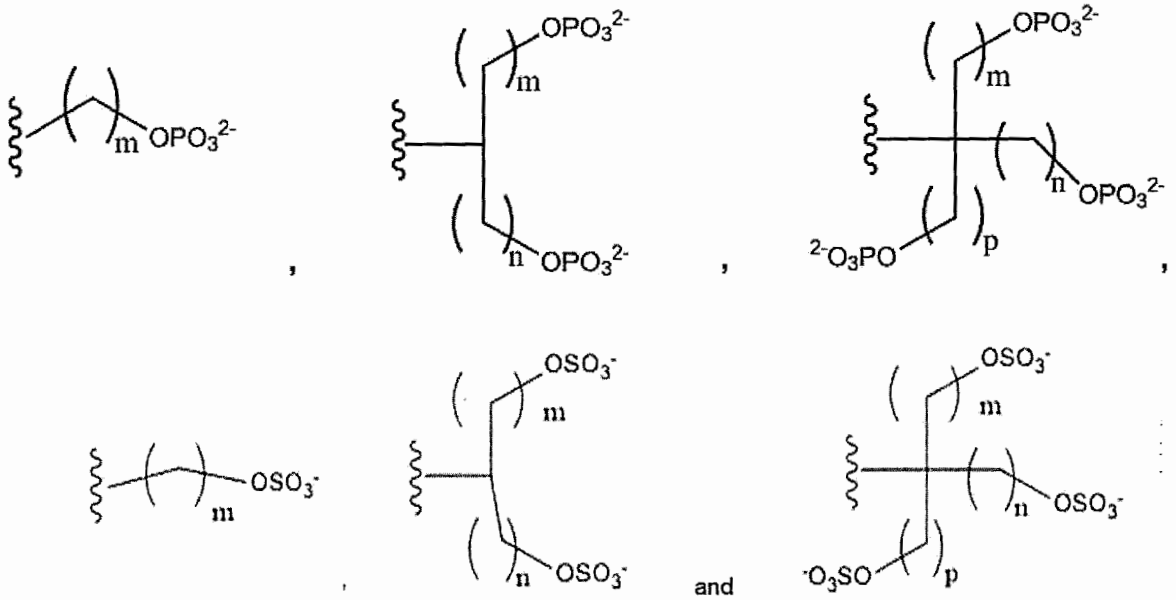
## CLAIMS

1. A non-saccharide glycosaminoglycan mimetic molecule comprising a polyproline backbone and one or more non-saccharide molecules; wherein the non-saccharide molecules comprise primary, secondary or tertiary negatively charged groups, or a combination thereof; and wherein the negatively charged groups are selected from the group consisting of sulfates, and phosphates.

2. The non-saccharide glycosaminoglycan mimetic molecule of claim 1, wherein each proline in the polyproline backbone is independently a proline or a proline derivative,

wherein optionally the proline derivative comprises a functional group for conjugation to the non-saccharide molecule, wherein the proline derivative is optionally selected from the group consisting of azidoproline, aminoproline, mercaptoproline, prolinecarboxylic acid, hydroxyproline and enantiomers thereof;

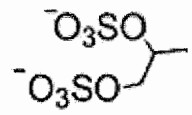
wherein optionally the non-saccharide molecules have a structure selected from the group consisting of:




and

wherein m, n, and p are 0 or a positive integer greater than 1,

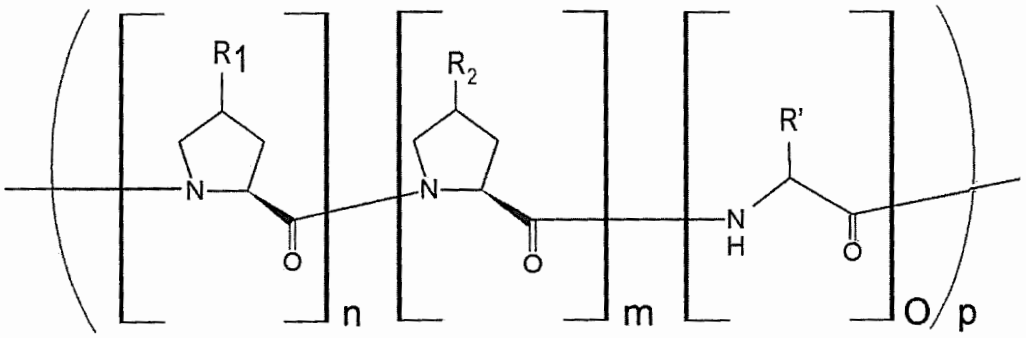
wherein optionally the non-saccharide molecules have the following structure:



; and

wherein optionally the non-saccharide molecules are bound to one or more prolines and/or proline derivatives on the polyproline backbone.

3. The non-saccharide glycosaminoglycan mimetic molecule of any one of the preceding claims, wherein the polyproline backbone has the following general formula (I):




wherein $R_1$ and $R_2$ is H or a functional group for conjugation to a non-saccharide molecule; R' is any amino acid side chain, n is a positive integer greater than 1; m is 0 or a positive integer, O is 0 or a positive integer, wherein at least one of $R_1$ or $R_2$ is a functional group for conjugation to a non-saccharide molecule, and p is a positive integer greater than 1.

4. The non-saccharide glycosaminoglycan mimetic molecule of claim 3, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12,

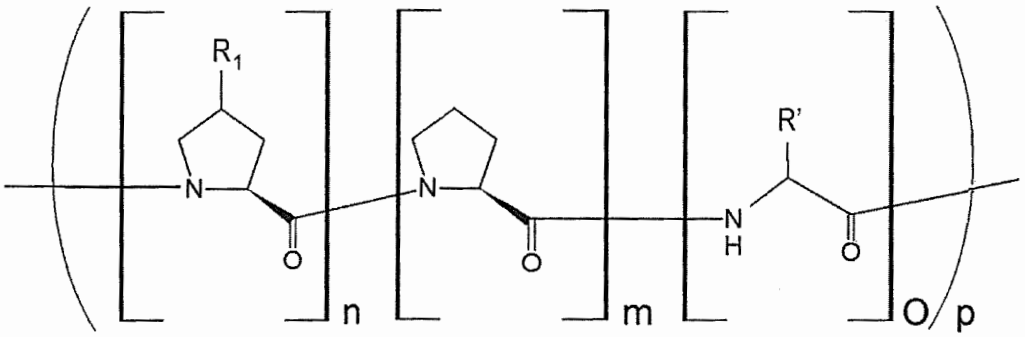
wherein optionally m is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12; or

wherein optionally n is 1, m is 0, O is 0, $R_1$ is an azido group, and p is 12; or

wherein optionally n is 2, m is 1, O is 0, $R_1$ is H, $R_2$ is azido, and p is 12; or

wherein optionally n is 4, m is 0, and O is 1, $R_1$ is azido, R' is H, and p is 3.

5. The non-saccharide glycosaminoglycan mimetic molecule of any one of the preceding claims, wherein the polyproline backbone has the following formula (II):




wherein $R_1$ is a functional group for conjugation to a non-saccharide molecule; R' is any amino acid side chain; n is a positive integer greater than 1; m is 0 or a positive integer; O is 0 or a positive integer, and p is a positive integer greater than 1.

6. The non-saccharide glycosaminoglycan mimetic molecule of any one of the preceding claims, wherein the functional group for conjugation to a non-saccharide molecule is $N_3$; wherein optionally the polyproline backbone is rigid or semi-flexible;

wherein optionally the polyproline backbone comprises one or more glycine;
wherein optionally the non-saccharide glycosaminoglycan mimetic molecule comprises more than one non-saccharide molecules; and

wherein optionally the non-saccharide glycosaminoglycan mimetic molecule is capable of binding cell adhesion molecules.

7. The non-saccharide glycosaminoglycan mimetic molecule of any one of the preceding claims, wherein the non-saccharide molecules are attached at pre-determined positions along the polyproline backbone;

wherein optionally the non-saccharide molecules are attached at equal distances along the polyproline backbone;

wherein optionally the non-saccharide molecules are attached along more than one different faces of the polyproline backbone,

wherein optionally the non-saccharide molecules are attached along three faces and project from the polyproline backbone; and

wherein optionally the non-saccharide molecules are attached along the same face of the polyproline backbone.

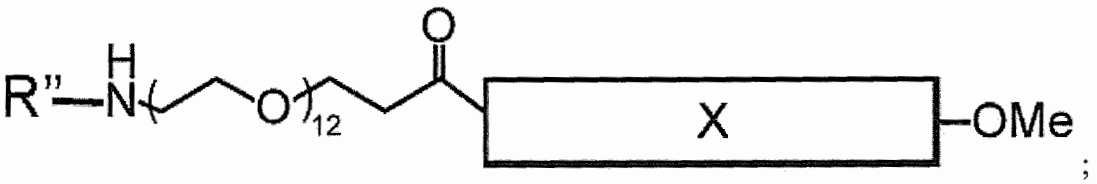
8. The non-saccharide glycosaminoglycan mimetic molecule of any one of the preceding claims, further comprising polyethylene glycol (PEG) at one end of the polyproline backbone,

wherein optionally the PEG is biotin conjugated; or

wherein optionally the PEG is 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(succinyl) conjugated; and

wherein optionally the non-saccharide glycosaminoglycan mimetic molecule further comprises a lipid.

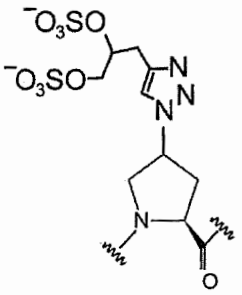
9. The non-saccharide glycosaminoglycan mimetic molecule of claim 8, wherein the non-saccharide glycosaminoglycan mimetic molecule has the following general formula (III):




;

wherein R'' is any functional moiety, and wherein X is a non-saccharide glycosaminoglycan mimetic molecule as defined in any one of claims 1-8.

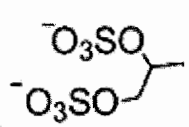
10. The non-saccharide glycosaminoglycan mimetic molecule of claim 9, wherein R'' is selected from the group consisting of H, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(succinyl)] and biotin; or

wherein optionally X is $(P_E)_{12}$, $(PPP_E)_{12}$, or $(P_E)_4G(P_E)_4G(P_E)_4G$ wherein P is proline; G is glycine; and $P_E$ is 

.

11. A method of synthesizing a non-saccharide glycosaminoglycan mimetic molecule according to any one of claims 1-10, comprising attaching one or more non-saccharide molecules to a polyproline backbone.

12. The method of claim 11, wherein the non-saccharide molecules comprise sulfated groups,




wherein optionally the non-saccharide molecules have the structure of ,

wherein optionally the non-saccharide molecules are alkyne-functionalized; and

wherein optionally the polyproline backbone is azido-functionalized.

13. The method of claim 11 or 12, wherein the one or more non-saccharide molecules are attached to the polyproline backbone via click reaction,

wherein optionally the method comprises conducting the click reaction in dimethyl sulfoxide (DMSO) at ambient temperature for 14 days in the presence of copper(I) idode, N,N-diisopropylethylamine (DIPEA) and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) under argon atmosphere,

wherein optionally the method further comprises the steps of:

(i) precipitating the reaction mixture resulting from the click reaction from a THF/methanol mixture and removing the solvent of the reaction mixture by vacuum and decanting the solid;

(ii) converting the reaction mixture into their sodium salt form; and

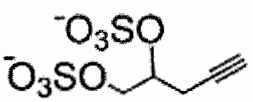
(iii) purifying the salt by size-exclusion chromatography;

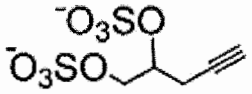
wherein optionally prior to the click reaction, the method comprises:

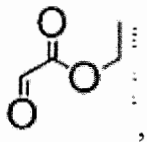
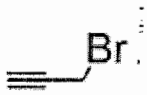
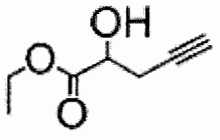
(a) conjugating $PEG_{12}$ to the polyproline backbone in the presence of N,N-diisopropylethylamine (DIPEA) base and Dimethylformamide (DMF) at room temperature,

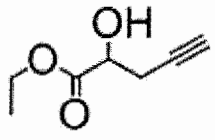
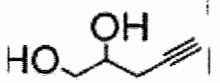
and optionally

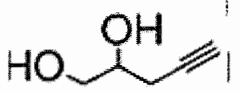
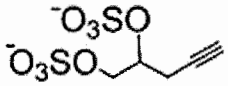
(b) coupling 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(succinyl) sodium salt or biotin to the $PEG_{12}$ via amide coupling in the presence of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate(PyBOB), hydroxybenzotriazole (HOBt), N,N-diisopropylethylamine (DIPEA) base and Dimethylformamide (DMF) at room temperature.

14. The method of any one of claims 11-13, wherein the non-saccharide molecules are alkyne-functionalized having the following structure:  ;

wherein optionally  is synthesised by:

(i) reacting ethyl glyoxylate, , and propargyl bromide, , to form Ethyl 2-hydroxypent-4-ynoate, , via a zinc Barbier reaction;

(ii) reducing the (Ethyl 2-hydroxypent-4-ynoate), , by lithium aluminum hydride to form Pent-4-yne-1,2-diol), , and

(iii) sulfating (Pent-4-yne-1,2-diol), , with an SO3.trimethyllamine complex (Sulfur trioxide trimethylamine complex) to form (Pent-4-yne-1,2-diyl bis(sulfate), ,

wherein optionally the Barbier reaction in step (i) is conducted in the presence of zinc (Zn), calcium chloride ($CaCl_2$), ammonium chloride ($NH_4Cl$) and tetrahydrofuran-water (THF-$H_2O$) at room temperature;

wherein optionally step (ii) is conducted in the presence of tetrahydrofuran (THF) at room temperature; and

wherein optionally step (iii) is conducted in the presence of dimethylformamide (DMF) at room temperature.

15. The non-saccharide glycosaminoglycan mimetic molecule of any one of claims 1-10 for use in therapy.

16. Use of a non-saccharide glycosaminoglycan mimetic molecule of any one of claims 1 to 10 in the manufacture of a medicament for:

(a) inhibiting cell adhesion molecules; or

(b) the treatment of a patient in need of a target-specific therapy; or

(c) promoting neuritogenesis in a patient; or

(d) inhibiting extravasation of circulating tumor cell into potential metastatic sites in a patient; or

(e) treating a viral infection.

17. The use of claim 16, wherein (i) the patient in the method of (b) is one suffering from a disease selected from the group consisting of Parkinson's disease, Alzheimer's disease and cancer;
(ii) the patient in the method of (c) is one suffering from a neurodegenerative disease selected from the group consisting of Alzheimer's disease, Parkinson's disease and Huntington's disease; and
(iii) the patient in the method of (d) is one suffering from a cancer selected from the group consisting of biliary tract cancer, brain cancer, breast cancer, cervical cancer, choriocarcinoma, colon cancer, endometrial cancer, esophageal cancer, gastric cancer, intraepithelial neoplasms, lymphomas, liver cancer, lung cancer (e.g. small cell and non-small cell), melanoma, neuroblastomas, oral cancer, ovarian cancer, pancreas cancer, prostate cancer, rectal cancer, sarcomas, skin cancer, testicular cancer, thyroid cancer, and renal cancer.

18. A method of controlling the binding affinity of a non-saccharide glycosaminoglycan mimetic molecule to one or more binding molecules, comprising attaching one or more non-saccharide molecules at pre-determined positions along a polyproline backbone; wherein the non-saccharide molecules comprise primary, secondary or tertiary negatively charged groups, or a combination thereof; and wherein the negatively charged groups are selected from the group consisting of sulfates, and phosphates.

19. The method of claim 18, wherein each proline in the polyproline backbone is independently a proline or a proline derivative,

wherein optionally the proline derivative comprises a functional group for conjugation to a non-saccharide molecule, wherein the proline derivative is optionally selected from the group consisting of azidoproline, aminoproline, mercaptoproline, prolinecarboxylic acid, hydroxyproline and enantiomers thereof;

wherein optionally the non-saccharide molecules are as defined in claim 2;

wherein optionally the polyproline backbone has a formula as defined in any one of claims 3 to 6;

wherein optionally the method comprises attaching the non-saccharide molecules at pre-determined positions along the polyproline backbone;

wherein optionally the method comprises attaching the non-saccharide molecules at equal distances from each other along the polyproline backbone;

wherein optionally the non-saccharide molecules are attached along more than one different faces of the polyproline backbone;

wherein optionally the non-saccharide molecules are attached along three faces and project from the polyproline backbone;

wherein optionally the non-saccharide molecules are attached along the same face of the polyproline backbone; and

wherein optionally the non-saccharide glycosaminoglycan mimetic molecule comprises more than one non-saccharide molecules.