Specificity studies on the heparin lyases from Flavobacterium-heparinum Biochemistry. 1993;32:8140-5.; Shriver Z, Sundaram M, Venkataraman G, Fareed J, Linhardt R, Biemann K, et al. Cleavage of the antithrombin III binding site in heparin by heparinases and its implication in the generation of low molecular weight heparin. Proc Natl Acad Sci U S A. 2000;97:10365-70) using the *Flavobacterium heparinium* enzymes heparinase I, II and III to cleave the glycosidic bonds. More than 90 % depolymerization of heparin or HS is possible when all 3 heparinases are used in combination (Karamanos NK, Vanky P, Tzanakakis GN, Tsegenidis T, Hjerpe A. Ion-pair high-performance liquid chromatography for determining disaccharide composition in heparin and heparan sulphate. J Chromatogr A. 1997;765:169-79.; Vynios DH, Karamanos NK, Tsiganos CP. Advances in analysis of glycosaminoglycans: its application for the assessment of physiological and pathological states of connective tissues. J Chromatogr B. 2002;781:21-38.). The resulting disaccharide mixtures can be analyzed by PAGE (Hampson IN, Gallagher JT. Separation of radiolabeled glycosaminoglycan oligosaccharides by polyacrylamide-gel electrophoresis Biochem J. 1984;221:697-705.), SAX-HPLC (Skidmore M AA, Yates E and Turnbull JE. Labelling heparan sulfate saccharides with chromophore, fluorescence and mass tag for HPLC and MS separations. Methods in Molecular biology. 2009;534:157-69.), or highly sensitive capillary electrophoresis (CE) (Lamari F, Militsopoulou M, Gioldassi X, Karamanos NK. Capillary electrophoresis: a superior miniaturized tool for analysis of the mono-, di-, and oligosaccharide constituents of glycan moieties in proteoglycans. Fresenius J Anal Chem. 2001;371:157-67.; Karamanos NK, Vanky P, Tzanakakis GN, Hjerpe A. High performance capillary electrophoresis method to characterize heparin and heparan sulfate disaccharides. Electrophoresis. 1996;17:391-5.; Sudhalter J, Folkman J, Svahn CM, Bergendal K, Damore PA. Importance of size, sulfation, and anticoagulant activity in the potentiation of acidic fibroblast growth-factor by heparin J Biol Chem. 1989;264:6892-7.; Militsopoulou M, Lamari FN, Hjerpe A, Karamanos NK. Determination of twelve heparin- and heparan sulfate-derived disaccharides as 2-aminoacridone derivatives by capillary zone electrophoresis using ultraviolet and laser-induced fluorescence detection. Electrophoresis. 2002;23:1104-9) by comparison to known disaccharide standards.

Summary

In one aspect, the present invention refers to an isolated or substantially purified heparan sulphate HS16, wherein the HS16 is capable of binding a peptideconsisting of the amino acid sequence RKDLGWKWIHEPKGYH (SEQ ID NO: 1). In another aspect, the present invention refers to a composition comprising isolated or substantially purified heparan

sulphate HS16 according to the present invention, optionally further comprising a growth factor, preferably TGFβ1. I still another aspect, the present invention refers to a cosmetic composition, pharmaceutical composition or medicament comprising isolated or substantially purified heparan sulphate HS16 according to the present invention, optionally wherein the pharmaceutical composition or medicament further comprises TGFβ1 protein and/or mesenchymal stem cells. In yet another aspect, the present invention refers to a pharmaceutical composition or medicament of the present invention for use in a method of medical treatment. In one other aspect, the present invention refers to an isolated or substantially purified heparan sulphate HS16 according to the present invention for use in a method of medical treatment. In another aspect, the present invention refers to the use of isolated or substantially purified Heparan sulphate HS16 according to the present invention in the manufacture of a medicament for the treatment of a disease, condition or injury to tissue, wherein the method involves the repair and/or regeneration of tissue, preferably connective tissue. In another aspect, the present invention refers to a biocompatible implant or prosthesis comprising a biomaterial and isolated or substantially purified heparan sulphate HS16 according to the present invention for use in a method of treating a disease, condition or injury to tissue in a patient, the method comprising surgically implanting the biocompatible implant or prosthesis into tissue of the patient at or surrounding the site of the disease, condition or injury leading to repair and/or regeneration of the tissue. In another aspect, the present invention refers to a biocompatible implant or prosthesis comprising a biomaterial and isolated or substantially purified heparan sulphate HS16 according to the present invention. In another aspect, the present invention refers to a method of forming a biocompatible implant or prosthesis, the method comprising the step of coating or impregnating a biomaterial with isolated or substantially purified heparan sulphate HS16 according to the present invention. In another aspect, the present invention refers to a method of culturing stem cells *in vitro*, the method comprising culturing stem cells *in vitro* in contact with isolated or substantially purified heparan sulphate HS16 according to the present invention. In another aspect, the present invention refers to a culture media comprising isolated or substantially purified heparan sulphate HS16 according to the present invention, optionally further comprising TGFβ1. In another aspect, the present invention refers to a kit of parts, the kit comprising a predetermined amount of isolated or substantially purified heparan sulphate HS16 according to the present invention and a predetermined amount of TGFβ1. In another aspect, the present invention refers to products containing therapeutically effective amounts of: (i) isolated or substantially purified heparan sulphate HS16 according to the present invention; and one or both of (ii)

TGFβ1 protein; (iii) mesenchymal stem cells, or cells in the fibroblastic lineage, for simultaneous, separate or sequential use in a method of medical treatment. In another aspect, the present invention refers to a method of increasing the stability of a growth factor, preferably TGFβ1, the method comprising contacting a growth factor, preferably TGFβ1, with isolated or substantially purified heparan sulphate HS16 according to the present invention. In another aspect, the present invention refers to a cosmetic method comprising administering heparan sulphate HS16 to a subject. In another aspect, the present invention refers to a preparation comprising a blood derived product and a predetermined quantity of heparan sulphate HS16. In another aspect, the present invention refers to a method of preserving biological material, the method comprising contacting biological material with a predetermined quantity of heparan sulphate HS16.

The present disclosure concerns a heparan sulphate species and heparan sulphate preparations comprising or consisting of the heparan sulphate species. The heparan

sulphate species is called HS16. HS16 refers to a novel class of structurally and functionally related isolated heparan sulphate.

HS16 has been found to bind TGFβ1, enhance the thermal stability of TGFβ1 and potentiate TGFβ1 signaling and thus the chondrogenic differentiation of mesenchymal stem cells.

In one example a heparan sulphate HS16 is provided. HS16 may be provided in isolated form or in substantially purified form. This may comprise providing a composition in which the heparan sulphate component is at least 80% HS16, more preferably one of at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%.

In preferred examples, HS16 is capable of binding a peptide or a polypeptide having the amino acid sequence of RKDLGWKWIHEPKGYH (SEQ ID NO: 1). The peptide may have one or more additional amino acids at one or both ends of this sequence. For example, the peptide may have any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acids at one or both end of this sequence.

In other examples the polypeptide is a TGFβ1 protein. In some examples HS16 binds to a peptide having or consisting of the amino acid sequence of SEQ ID NO:1 or a TGFβ1 protein with a K_D of less than 100μM, more preferably less than one of 50μM, 40μM, 30μM, 20μM, 10μM, 1μM, 500nM, 100nM, 50nM, 10nM or 1nM.

HS16 may be obtained, identified, isolated or enriched according to the inventors' methodology described herein, which may comprise the following steps:

(i) providing a solid support having polypeptide molecules adhered to the support, wherein the polypeptide comprises a heparin-binding domain having the amino acid sequence of RKDLGWKWIHEPKGYH;

(ii) contacting the polypeptide molecules with a mixture comprising glycosaminoglycans such that polypeptide-glycosaminoglycan complexes are allowed to form;

(iii) partitioning polypeptide-glycosaminoglycan complexes from the remainder of the mixture;

(iv) dissociating glycosaminoglycans from the polypeptide-glycosaminoglycan complexes;

(v) collecting the dissociated glycosaminoglycans.

Optionally, the method may further comprise a size fractionation step, e.g. after step (iv) or (v). Size fractionation may be used to remove heparan sulphate chains smaller than a selected threshold, e.g. one of dp4, dp6, dp8, dp10, dp12, dp14, dp16, dp18, dp20, dp22, or dp24.

In the inventors' methodology the mixture may comprise glycosaminoglycans obtained from commercially available sources. One suitable source is a heparan sulphate fraction, e.g. a commercially available heparan sulphate. One suitable heparan sulphate fraction can be obtained during isolation of heparin from porcine intestinal mucosa, another is heparan sulphate from porcine mucosa [HS^{PM}] (e.g. from Celsus Laboratories Inc. – sometimes called "Celsus HS").

Other suitable sources of heparan sulphate include heparan sulphate from any mammal (human or non-human), particularly from the kidney, lung or intestinal mucosa. In some examples the heparan sulphate is from pig (porcine) or cow (bovine) intestinal mucosa, kidney or lung.

In another example a composition comprising HS16 according to any one of the examples above and TGFβ1 protein is provided.

In one example a pharmaceutical composition or medicament is provided comprising HS16 in accordance with the examples described above. The pharmaceutical composition or medicament may further comprise a pharmaceutically acceptable carrier, adjuvant or diluent.

In some examples the pharmaceutical composition is for use in a method of treatment, the method comprising the repair and/or regeneration of tissue, e.g. connective tissue (cartilage, bone, tendon, ligament, skin, cornea) or a broken bone. In some examples the pharmaceutical composition or medicament may further comprise TGFβ1 protein. In some examples the pharmaceutical composition or medicament may further comprise mesenchymal stem cells.

In another example HS16 is provided for use in a method of medical treatment. The method of medical treatment may comprise a method of wound healing *in vivo*, the repair and/or regeneration of tissue, e.g. the repair and/or

regeneration of connective tissue (cartilage, bone, tendon, ligament, skin, cornea). Such repair and/or regeneration of may be in a mammal or a human.

In a related example the use of HS16 in the manufacture of a medicament for use in a method of medical treatment is provided. In some examples the method of medical treatment comprises the repair and/or regeneration of tissue as described above.

In a further example a biocompatible implant or prosthesis comprising a biomaterial and HS16 is provided. In some examples the implant or prosthesis is coated with HS16. In some examples the implant or prosthesis is impregnated with HS16. The implant or prosthesis may be further coated or impregnated with TGFβ1 protein and/or with mesenchymal stem cells.

In another example a method of forming a biocompatible implant or prosthesis is provided, the method comprising the step of coating or impregnating a biomaterial with HS16. In some examples the method further comprises coating or impregnating the biomaterial with one or both of TGFβ1 protein and mesenchymal stem cells.

In some examples a method may comprise administering HS16 and mesenchymal stem cells to a patient. In such methods at least two of HS16, TGFβ1 protein and mesenchymal stem cells may be formulated in a pharmaceutical composition comprising at least two of the HS16, TGFβ1 protein and mesenchymal stem cells and a pharmaceutically acceptable carrier, adjuvant or diluent.

Preferably, the HS16, TGFβ1 protein and mesenchymal stem cells are respectively provided in therapeutically effective amounts. In some examples the method further comprises the step of formulating therapeutically effective amounts of HS16, and/or TGFβ1 protein and/or mesenchymal stem cells as a pharmaceutical composition comprising the HS16, and/or TGFβ1 protein and/or mesenchymal stem cells and a pharmaceutically acceptable carrier, adjuvant or diluent, wherein the pharmaceutical composition is administered to the patient.

In another example a method of treating a patient is provided, the method comprising surgically implanting a biocompatible implant or prosthesis, which

implant or prosthesis comprises a biomaterial and HS16, into tissue of the patient at or surrounding the site of fracture.

In some examples the implant or prosthesis is coated with HS16. In some examples the implant or prosthesis is impregnated with HS16. In some examples the implant or prosthesis is further impregnated with one or both of TGFβ1 protein and mesenchymal stem cells.

In a further example culture media is provided, the culture media comprising HS16.

In another example the use of HS16 in cell culture *in vitro* is provided. In a related example the use of HS16 in the growth of connective tissue *in vitro* is provided. In another related example a method for growing connective tissue *in vitro* is provided, the method comprising culturing mesenchymal stem cells in contact with exogenously added HS16.

In yet a further example a method for the repair, replacement or regeneration of tissue, e.g. connective tissue, in a human or animal patient in need of such treatment is provided, the method comprising:

(i) culturing mesenchymal stem cells *in vitro* in contact with HS16 for a period of time sufficient for said cells to form tissue;
(ii) collecting said tissue;
(iii) implanting said tissue into the body of the patient at a site of injury or disease to repair, replace or regenerate tissue in the patient.

The tissue may be connective tissue, e.g. bone, cartilage, tendon, skin or fat. In some examples the method further comprises contacting the mesenchymal stem cells in culture with exogenous TGFβ1 protein.

In another example tissue obtained by *in vitro* culture of mesenchymal stem cells in the presence of HS16 is provided. In some examples the tissue is obtained by *in vitro* culture of mesenchymal stem cells in the presence of HS16 and TGFβ1 protein.

In a further example a method of culturing stem cells, e.g. mesenchymal stem cells is provided, the method comprising culturing stem cells in contact with HS16.

In some examples a method of culturing stem cells *in vitro* is provided, the method comprising culturing stem cells *in vitro* in contact with heparan sulphate HS16. The HS16 is preferably exogenous and isolated, and added to the culture as a supplement, e.g. as part of the culture media.

In yet a further example a kit of parts is provided, the kit comprising a predetermined amount of HS16 and a predetermined amount of TGFβ1. The kit may comprise a first container containing the predetermined amount of HS16 and a second container containing the predetermined amount of TGFβ1. The kit may further comprise a predetermined amount of mesenchymal stem cells. The kit may be provided for use in a method of medical treatment. The method of medical treatment may comprise a method of wound healing *in vivo*, the repair and/or regeneration of tissue, such as connective tissue (e.g. cartilage, bone, tendon, ligament, skin, cornea). The repair and/or regeneration may be in a mammal or a human. The kit may be provided together with instructions for the administration of the HS16, TGFβ1 protein and/or mesenchymal stem cells separately, sequentially or simultaneously in order to provide the medical treatment.

In a further example products are provided, the products containing therapeutically effective amounts of:

(i) HS16; and one or both of

(ii) TGFβ1 protein;

(iii) Mesenchymal stem cells,

for simultaneous, separate or sequential use in a method of medical treatment. The method of medical treatment may comprise a method of wound healing *in vivo*, the repair and/or regeneration of connective tissue. The repair and/or regeneration may be in a mammal or a human. The products may optionally be formulated as a combined preparation for co-administration.

As shown herein, HS16 has the property of stabilising TGFβ1, and thereby prolonging its action. HS16 prevents TGFβ1 from degradation in culture medium. This can be usefully applied to the storage of TGFβ1 preparations and the preparation of TGFβ1 containing culture media.

As such, in one example a composition comprising a growth factor and isolated HS16 is provided. The growth factor may be a protein growth factor, and is preferably TGFβ1. The composition may comprise isolated TGFβ1 and isolated HS16. In some examplesthe composition may be a culture media. In other examples the composition may be a pharmaceutical composition or medicament containing TGFβ1.

The composition may be an TGFβ1 preparation comprising TGFβ1 and isolated HS16 in a container. A suitable container may be a bottle, vial, tube or syringe.

A method of increasing the stability of a growth factor is also provided, the method comprising contacting a growth factor with isolated HS16.

The stability of the growth factor may be measured in terms of its half-life, i.e. the amount of time taken for half of the growth factor in a given composition to be degraded and/or lose its activity. The growth factor is preferably a protein growth factor, more preferably TGFβ1. HS16 acts to maintain and prolong TGFβ1 half-life. The method may involve contacting isolated HS16 with the growth factor (e.g. TGFβ1) *in vitro*, e.g. as part of preparation of a growth factor (e.g. TGFβ1) composition, its storage or transport. Alternatively, the method may involve contacting isolated HS16 with the growth factor (e.g. TGFβ1) *in vivo*, e.g. by administering isolated HS16 to tissue in which the growth factor (e.g. TGFβ1) [naturally occurring in the tissue or exogenously added to the tissue] is present. The method may also comprise the step of adding exogenous growth factor (e.g. TGFβ1) to the tissue.

The stability of TGFβ1 in a given composition or tissue that contains isolated HS16 (or to which isolated HS16 has been added) may be compared against a comparable composition not containing HS16 (or to which isolated HS16 has not been added.
In the composition and method described above the HS16 may be purified, as described herein. The TGFβ1 may be isolated and/or purified, non-isolated or partially isolated, e.g. part of an extracellular matrix material, or present in a composition of cells. Isolated or purified TGFβ1 may be recombinant TGFβ1. Recombinant TGFB1 is commercially available from a number of commercial manufacturers.

In some examples HS16 is used as a preserving agent and/or preservative during the production of blood-derived products. In some examples the blood-derived products

include platelets, platelet products, platelet lysates and platelet-rich plasma (PRP). The blood-derived products may be isolated from blood or serum, and optionally enriched or partitioned from other components of blood and/or serum.

In some examples a preparation of blood derived product(s) is provided, the preparation comprising a blood derived product and a predetermined quantity of HS16. The HS16 is preferably in isolated or purified form and is preferably exogenous to the blood derived product(s), being added to the blood derived product(s). The preparation may be a platelet preparation, e.g. platelets, platelet products, platelet lysates or platelet-rich plasma (PRP), to which HS16 has been added.

In accordance with the above, a method of preserving biological material is provided, preferably biological material comprising TGFβ1, the method comprising contacting the biological material with a predetermined quantity of HS16. In some examples the biological material may be selected from cellular material, tissue, blood-derived products, cells, or stem cells.

In another example HS16 is provided for use during isolation and/or processing of stem cells. In some examples, HS16 is provided as a reagent for use during culture and/or expansion of stem cells. Accordingly, a method of isolating, processing, culturing or expanding stem cells may be provided, the method comprising contacting the stem cells with a predetermined quantity of HS16. The stem cells may optionally express TGFβ1.

Optionally, aspects and embodiments of the present do not include an HS as described in Manton et al (Journal of Cellular Physiology 209:219-229 (2006)).

Description

The inventors have used a sequence-based affinity chromatography platform to exploit the heparin-binding domain of TGFB1. This allowed the enrichment of a TGFB1-binding heparan sulphate (HS) fraction.

The terms "sulphate", "sulphated", and "sulphation" are used interchangeably with "sulfate", "sulfated" and "sulfation" respectively.

HS16

Claims:

1. Isolated or substantially purified heparan sulphate HS16, wherein the HS16 is capable of binding a peptideconsisting of the amino acid sequence RKDLGWKWIHEPKGYH (SEQ ID NO: 1).

2. Isolated or substantially purified heparan sulphate HS16 according to claim 1, wherein following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to HPLC analysis the heparan sulphate HS16 has a disaccharide composition comprising:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	14.75±3.0
ΔUA,2S-GlcNS	4.58±2.0
ΔUA-GlcNS,6S	12.98±3.0
ΔUA-GlcNS	22.24±3.0
ΔUA,2S-GlcNAc	0.56±0.5
ΔUA-GlcNAc,6S	12.63±3.0
ΔUA-GlcNAc	32.26±3.0

3. Isolated or substantially purified heparan sulphate HS16 according to claim 1, wherein following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to HPLC analysis the heparan sulphate HS16 has a disaccharide composition comprising:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	14.75±1.0
ΔUA,2S-GlcNS	4.58±0.4
ΔUA-GlcNS,6S	12.98±1.0
ΔUA-GlcNS	22.24±1.6
ΔUA,2S-GlcNAc	0.56±0.4
ΔUA-GlcNAc,6S	12.63±1.0
ΔUA-GlcNAc	32.26±1.6

4. Isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 3 obtained by a method comprising:

(i) providing a solid support having polypeptide molecules adhered to the support, wherein the polypeptide comprises a heparin-binding domain consisting of the amino acid sequence RKDLGWKWIHEPKGYH (SEQ ID NO:1);

(ii) contacting the solid support with a mixture comprising glycosaminoglycan, preferably a heparan sulphate preparation, such that polypeptide-glycosaminoglycan complexes are allowed to form;

(iii) partitioning polypeptide-glycosaminoglycan complexes from the remainder of the mixture;

(iv) dissociating glycosaminoglycans, preferably heparan sulphate species, from the polypeptide-glycosaminoglycan complexes;

(v) collecting the dissociated glycosaminoglycans, preferably one or more heparan sulphate species.

5. The isolated or substantially purified heparin sulphate HS16 obtained by the method of claim 4, wherein the mixture comprising glycosaminoglycans is a heparan sulphate preparation obtained from porcine mucosa (HS^{PM}).

6. A composition comprising isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5, optionally further comprising a growth factor, preferably TGFβ1.

7. A cosmetic composition, pharmaceutical composition or medicament comprising isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5, optionally wherein the pharmaceutical composition or medicament further comprises TGFβ1 protein and/or mesenchymal stem cells.

8. The pharmaceutical composition or medicament of claim 7 for use in a method of medical treatment.

9. Isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5 for use in a method of medical treatment.

10. Isolated or substantially purified heparan sulphate HS16 for use according to claim 9 wherein the method of medical treatment comprises a method of wound healing

in vivo; or wherein the method of medical treatment comprises the repair and/or regeneration of tissue, preferably connective tissue, optionally wherein the method comprises administration of a therapeutically effective amount of isolated or substantially purified heparan sulphate HS16 to the patient leading to repair and/or regeneration of the tissue, preferably connective tissue.

11. Isolated or substantially purified heparan sulphate HS16 for use according to claim 9 or 10 wherein the method of medical treatment further comprises administration of TGFß1 protein to the patient.

12. Use of isolated or substantially purified Heparan sulphate HS16 according to any one of claims 1 to 5 in the manufacture of a medicament for the treatment of a disease, condition or injury to tissue, wherein the method involves the repair and/or regeneration of tissue, preferably connective tissue.

13. A biocompatible implant or prosthesis comprising a biomaterial and isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5 for use in a method of treating a disease, condition or injury to tissue in a patient, the method comprising that the biocompatible implant or prosthesis is to be surgically implanted into tissue of the patient at or surrounding the site of the disease, condition or injury leading to repair and/or regeneration of the tissue.

14. A biocompatible implant or prosthesis comprising a biomaterial and isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5.

15. A method of forming a biocompatible implant or prosthesis, the method comprising the step of coating or impregnating a biomaterial with isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5.

16. A method of culturing stem cells *in vitro*, the method comprising culturing stem cells *in vitro* in contact with isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5.

17. Culture media comprising isolated or substantially purified heparan sulphate HS16 according to any of claims 1 to 5, optionally further comprising TGFβ1.

18. A kit of parts, the kit comprising a predetermined amount of isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5 and a predetermined amount of TGFβ1.

19. Products containing therapeutically effective amounts of:

(i) Isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5; and one or both of

(ii) TGFβ1 protein;

(iii) Mesenchymal stem cells, or cells in the fibroblastic lineage,

for simultaneous, separate or sequential use in a method of medical treatment.

20. A method of increasing the stability of a growth factor, preferably TGFβ1, the method comprising contacting a growth factor, preferably TGFβ1, with isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5.

21. A cosmetic method comprising administering heparan sulphate HS16 to a subject.

22. A preparation comprising a blood derived product and a predetermined quantity of heparan sulphate HS16.

23. The preparation of claim 22, wherein the preparation is a platelet preparation.

24. A method of preserving biological material, the method comprising contacting biological material with a predetermined quantity of heparan sulphate HS16.

25. The cosmetic method, preparation or method of any one of claims 21 to 24 wherein the heparan sulphate HS16 is isolated or substantially purified heparan sulphate HS16 according to any one of claims 1 to 5.