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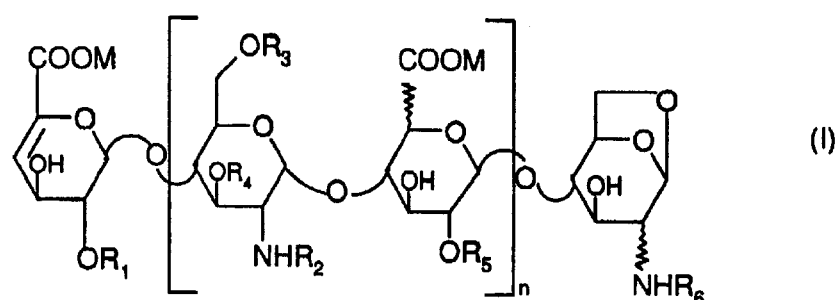
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PCT/FR00/02897

NOVEL OLIGOSACCHARIDES, THEIR PREPARATION AND
PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

The present invention relates to
oligosaccharides of formula:

5



or mixtures thereof, to diastereoisomers thereof, to a
process for their preparation and to pharmaceutical
10 compositions containing them.

Disaccharide sulfates containing a
1,6-anhydro structure at the reducing end have been
described by H.P. Wessel, J. Carbohydrate Chemistry,
11(8), 1039-1052 (1992); no pharmacological activity is
15 mentioned for these products.

Trisaccharide sulfates comprising a
1,6-anhydro unit have also been described in patent
EP 84999 and by Y. Ichikawa et al., Carbohydr. Res, 141,
273-282 (1985) as intermediates for preparing higher
20 oligosaccharides. These trisaccharides have low anti-Xa
activity.

In formula (I) n is an integer from 0 to 25,
R₁, R₃, R₄ and R₅, which may be identical or different,



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represent a hydrogen atom or a radical SO_3M , R_2 and R_6 , which may be identical or different, represent a hydrogen atom or a radical SO_3M or COCH_3 and M is sodium, calcium, magnesium or potassium.

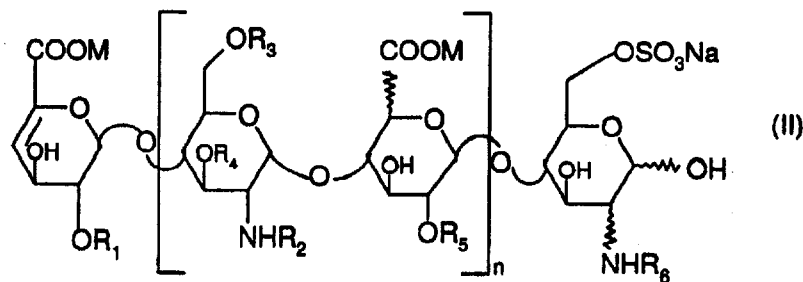
5 These oligosaccharides thus comprise an even number of saccharides.

In formula (I), R_4 is preferably a hydrogen atom.

10 Preferably, n is an integer from 0 to 10 and in particular from 0 to 6 and even more particularly from 1 to 6.

The oligosaccharides of formula (I) can be prepared by the action of an alkali metal or quaternary ammonium hydroxide on oligosaccharides of formula:

15



in which n is an integer from 0 to 25, R_1 , R_3 , R_4 and R_5 , which may be identical or different, represent a hydrogen atom or a radical SO_3M , R_2 and R_6 , which may be identical or different, represent a hydrogen atom or a radical SO_3M or COCH_3 and M is sodium, calcium, magnesium or potassium, or a mixture thereof.

20

This reaction is carried out in aqueous

medium, at a temperature of from 40 to 80°C, at a pH of from 10 to 13.

As alkali metal hydroxides which can be used, mention may be made of sodium hydroxide, potassium
5 hydroxide, lithium hydroxide and cesium hydroxide.

As a quaternary ammonium hydroxide which may be used, mention may be made of tetrabutylammonium hydroxide.

The amount of alkali metal or quaternary
10 ammonium hydroxide must be sufficient for the pH of the reaction medium to remain stable throughout the reaction. It is thus necessary to add the alkali metal or quaternary ammonium hydroxide continuously throughout the reaction.

15 Preferably, the alkali metal or quaternary ammonium hydroxide is in the form of an aqueous 1 to 5% solution.

Preferably, the reaction is carried out at a temperature of from 60 to 70°C.

20 Advantageously, the reaction pH is from 11 to 12.5.

The reaction is stopped by acidifying the reaction medium, for example by addition of acidic resin such as Amberlite IR120[®] resin (Fluka).

25 The oligosaccharides of formula (I) may optionally be purified by gel permeation chromatography of polyacrylamide-agarose type, such as that sold under the brand name Ultrogel ACA202[®] (Biosepra) according to

the protocol described below for the separation of the intermediate oligosaccharides of formula (II). The oligosaccharides of formula (I) for which n is 0 or 1 may also optionally be purified on a column of alumina with a water-ethanol mixture as eluent.

The intermediate oligosaccharides of formula (II) and mixtures thereof can be obtained by chromatographic separation on gel of a mixture of oligosaccharides (III) obtained by enzymatic depolymerization of heparin or basic depolymerization of the benzyl ester of heparin or of a benzyl ester of semi-synthetic heparin.

This chromatography is carried out on columns filled with gel of polyacrylamide-agarose type such as the gel sold under the brand name Ultrogel ACA202[®] (Biosepra). Preferably, an array of polyacrylamide agarose gel columns is used. The number of columns used is adapted as a function of the volume, of the gel and of the oligosaccharides to be separated. The mixture is eluted with a solution containing a phosphate buffer and sodium chloride. Preferably, the phosphate buffer is a solution containing 0.02 mol/l of $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ (pH 7) containing 0.1 mol/l of sodium chloride. The detection of the various fractions is carried out by UV spectrometry (254 nm) and ionic spectrometry (IBF). The fractions thus obtained can then be optionally purified, for example by SAX (strong anion exchange) chromatography according to the methods known to those

skilled in the art and in particular according to the methods described by K.G. Rice and R.J. Linhardt, Carbohydrate Research 190, 219-233 (1989), A. Larnkjaer, S.H. Hansen and P.B. Ostergaard,

5 Carbohydrate Research, 266, 37-52 (1995) and in patent WO 90/01501 (Example 2). The fractions are then freeze-dried, after which they are desalified on a column filled with gel such as a column of Sephadex G10[®] gel (Pharmacia Biochemicals).

10 When the purification is not carried out by SAX chromatography, the lyophilizates can be optionally purified by simple or fractional precipitation according to the methods known to those skilled in the art and in particular according to the method described
15 in patent FR 2 548 672. In general, the process is performed according to the following procedure:

 The lyophilized fraction to be purified is dissolved at 25°C in about ten volumes of distilled water. On adding methanol or ethanol, the desired
20 oligosaccharide is precipitated, while monitoring its enrichment by HPLC chromatography (high performance liquid chromatography). The addition of methanol or ethanol is determined as a function of the desired yield and purity of the said oligosaccharide.

25 Similarly, this operation can be carried out in several successive steps starting with the initial solution of lyophilizate. For this, more of the insolubilizing agent (methanol or ethanol) is added portionwise and

the precipitate obtained after each addition is isolated. The precipitates thus prepared are analyzed by HPLC chromatography. Depending on the desired yield and purity, the suitable fractions of precipitate are
5 combined.

For the intermediates of formula (II) for which $n = 0, 1$ or 2 , it is preferable to start with a mixture (III) obtained by enzymatic depolymerization.

This depolymerization is carried out by means
10 of heparinase I (EC 4.2.2.7), in a pH 7 phosphate buffer solution, in the presence of sodium chloride and BSA (bovine serum albumin), at a temperature of between 10 and 18°C , and preferably 15°C , for 8 to 10 days, and preferably 9 days. The depolymerization is stopped, for
15 example, by heating the reaction medium at 100°C for 2 minutes, and the mixture is recovered by lyophilization. It is preferable to use 7 IU of heparinase I per 25 g of heparin. The phosphate buffer solution generally comprises 0.05 mol/l of
20 $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ (pH 7) in the presence of 0.1 mol/l of sodium chloride. The BSA concentration is generally 2% .

For the intermediates of formula (II) for which $n = 0, 1, 2, 3$ or 4 , it is preferable to start with a mixture (III) obtained by depolymerizing a
25 benzyl ester of heparin.

The benzyl ester of heparin can be prepared according to the methods described in patents US 5 389 618, EP 40 144 and FR 2 548 672. The degree of

esterification will preferably be between 50 and 100%. Preferentially, it will be between 70 and 90%.

The depolymerization is carried out in aqueous medium, by means of an alkali metal hydroxide (for example lithium hydroxide, sodium hydroxide, potassium hydroxide or cesium hydroxide) or of a quaternary ammonium hydroxide (for example tetrabutylammonium hydroxide), preferably at a molarity of between 0.1 and 0.2 mol/l, at a temperature of between 40 and 80°C, for 5 to 120 minutes. In one preferred mode, the process is performed for 5 to 15 minutes, at a temperature of between 60 and 70°C, with a 0.15 mol/l sodium hydroxide solution. The depolymerization reaction is stopped by neutralization by addition of an acid such as acetic acid. After addition of 10% by weight per volume of sodium acetate, the oligosaccharide mixture is precipitated by adding methanol, preferably 2 volumes per 1 volume of reaction medium, and filtered.

According to one preferred aspect of the invention, the oligosaccharide mixture obtained after chemical depolymerization, in the form of an aqueous solution, is enriched by ultrafiltration through membranes with a suitable nominal cutoff threshold (of the Pellicon type made with regenerated cellulose, sold by Millipore); the type of membrane being adapted as a function of the type of enriched oligosaccharides to be recovered. For the oligosaccharides (II) for

which $n = 0$, a membrane with a nominal cutoff threshold of 1 kDa will be used, for the oligosaccharides (II) for which $n = 1$, a 1 kDa or 3 kDa membrane will be used, for the oligosaccharides (II) for which $n = 2$, a 3 kDa membrane will be used, and for the oligosaccharides (II) for which $n = 3$ or 4, a 5 kDa membrane will be used. During this operation, the permeate is recovered and the retentate is discarded. Thus, the fraction of enriched product can represent from 50 to 10% of the initial oligosaccharide mixture while at the same time conserving at least 80% of the desired oligosaccharide.

For the intermediates of formula (II) for which $n = 0$ to 25, it is preferable to start with a mixture (III) obtained by depolymerizing a benzyl ester or semi-synthetic polysaccharide sulfate. The benzyl ester of semi-synthetic polysaccharide sulfate is prepared from semi-synthetic polysaccharide sulfates obtained from polysaccharide K5 and according to the methods described in the patents WO 94/29352 and WO 96/14425. The esterification, depolymerization and recovery conditions are the same as those described above for the benzyl ester of heparin.

In all the preceding processes, the initial heparin can be of porcine, ovine, caprine or bovine origin and can be obtained from the mucus, lungs or hides of the animals. Preferably, a heparin from porcine or ovine mucus or from bovine lung is used, and

even more preferably from porcine mucus.

The oligosaccharides of formula (I) have anti-inflammatory properties and can thus be used for preventing or treating diseases associated with an inflammatory process involving the production of cytotoxic substances such as nitrogen monoxide (NO) whose inducible form is released in particular by neutrophils or macrophages when the latter migrate and are activated in a tissue. The migration, activation and adhesion of neutrophils takes place in ischemic tissue regions following an occlusion or spasm of an artery vascularizing this tissue. These ischemias can arise either in the brain (cerebrovascular accident) or in the myocardium (myocardial infarction) or in the lower limbs (so-called peripheral ischemias). The oligosaccharides of formula (I) can thus be used for the prevention and/or treatment of neurodegenerative diseases for which the cerebral inflammation plays a deleterious role which can lead to death, among which mention may be made of cerebral ischemias, cardiac ischemias (myocardial infarction), peripheral ischemias, traumas of the central nervous system and in particular cranial, spinal and craniospinal traumas, multiple sclerosis, neuropathic pain and peripheral neuropathies, motor neuron diseases including amyotrophic lateral sclerosis, neuro-AIDS, Alzheimer's disease, Parkinson's disease and Huntington's chorea and certain forms of osteoarthritis, in particular of

articular localization.

The anti-inflammatory activity of these products is demonstrated in vivo in the test of production of NO_x (nitrite and nitrate) induced by a lipopolysaccharide (LPS) obtained from *E. coli* according to the protocol described by M. Yamashita et al., *Eur. J. Pharmacol*, 338, 2, 151-158 (1997) or J.E. Shellito et al., *Am. J. Respir. Cell Mol. Biol.*, 13, 1, 45-53 (1995).

0.5 mg/kg of the oligosaccharide are injected into male CD1 mice (Charles River, 25-35 g) at T0 via intravenous bolus, and 1 or 2 mg/kg of the oligosaccharide are injected subcutaneously at T+15 minutes. At T+30 minutes, 100 mg/kg of lipopolysaccharide (LPS) obtained from *E. coli* (Sigma L3129, serotype 0127:B8). A further 1 or 2 mg/kg of the oligosaccharide are injected subcutaneously at T+3 hours. At T+5 hours 30 minutes, a blood sample is collected by ocular puncture and the concentrations of NO_x (nitrite and nitrate) in the plasma are determined by the Griess colorimetric method after reduction of the nitrate to nitrite with nitrate reductase in the following way: 12 µl of the plasma sample are mixed with 88 µl of deionized water and incubated in the dark for 1 hour at room temperature with 40 µl of phosphate buffer (0.31 M, pH 7.5), 20 µl of β-NADPH (reduced nicotinamide adenine dinucleotide phosphate) (0.86 mM), 20 µl of FDA (flavin adenine dinucleotide) (0.11 mM)

and 20 μ l of nitrate reductase (2 U/ml) (Boehringer Mannheim). 10 μ l of ZnSO_4 (1M) are added to precipitate the proteins and, after mixing, the samples are centrifuged at $20,000 \times g$ for 5 minutes. Finally, 50 μ l of the supernatant are incubated for 10 minutes at room temperature with 100 μ l of the Griess reagent (1% sulfanilamide in a phosphoric acid/0.1% naphthylethylenediamine mixture in deionized water (V/V)). The optical densities are read at 540 nm with a microplate spectrophotometer; each point being determined twice. KNO_3 and NaNO_2 are used as standards for the colorimetric method.

In this test, the oligosaccharides according to the invention inhibit the formation of NO_x by more than 50%.

Among the oligosaccharides of formula (I) which are preferred, mention may be made in particular of the oligosaccharides for which:

- n is equal to 0, R_1 and R_6 represent an SO_3Na radical and M is sodium, and the mixture of its diastereoisomers
- n is equal to 1, R_1 , R_2 , R_3 , R_5 and R_6 represent an SO_3Na radical, R_4 represents a hydrogen atom and M is sodium, and the mixture of its diastereoisomers
- n is equal to 2, R_1 , R_2 , R_3 , R_5 and R_6 represent an SO_3Na radical, R_4 represents a hydrogen atom and M is sodium, and the mixture of its diastereoisomers
- n is equal to 2, R_1 , R_2 , R_3 and R_6 represent an SO_3Na

radical, R₅ represents a hydrogen atom or an SO₃Na radical, R₄ represents a hydrogen atom and M is sodium, and the mixture of its diastereoisomers (1,6-anhydro ΔIs-Is-IIIs derivative).

5 The examples which follow are representative of the preparation of the oligosaccharides of formula (I) and of the intermediates.

In these examples, the abbreviations have the following meanings:

- 10 ΔIs: (4-deoxy-2-O-sulfo-α-L-threo-hex-enopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfoamino-6-O-sulfo-α-D-glucopyranose, tetrasodium salt, or ΔUAp2S-(1→4)-α-D-GlcNp2S6S
- Is: (2-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfoamino-6-O-sulfo-α-D-glucopyranose, tetrasodium salt, or α-L-IdoAp2S-(1→4)-α-D-GlcNp2S6S
- 15 IIIs: (α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfoamino-6-O-sulfo-α-D-glucopyranose, trisodium salt, or α-IdoAp-(1→4)-α-D-GlcNp2S6S
- 20 IIIs: (2-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfoamino-α-D-glucopyranose, trisodium salt, or α-L-IdoAp2S-(1→4)-α-D-GlcNp2S
- IdoAp: idopyranosyluronic acid
- GlcNp: 2-deoxy-2-aminoglucopyranose
- 25 ΔUap: 4-deoxy-α-L-threo-hex-enopyranosyluronic acid
- S: sulfate

EXAMPLES OF PREPARATION OF THE MIXTURES OF FORMULA (II)

EXAMPLE A - preparation of the oligosaccharides of formula (II) for which $n = 0, 1$ and 2 by enzymatic depolymerization and separation

5 25g of heparin are dissolved in 250 ml of a phosphate buffer solution containing 0.05 mol/l of $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ ($\text{pH} = 7$), 0.2 mol/l of sodium chloride and 2% of BSA (bovine serum albumin). 7 IU of heparinase I (EC 4.2.2.2.7) are introduced into the
10 mixture and the solution obtained is cooled to 15°C and then kept at this temperature throughout the depolymerization reaction. The reaction progress is monitored by taking aliquot samples at intervals, which are analyzed by gel permeation chromatography. After 9
15 days, the enzymatic reaction is stopped by heating the reaction medium at 100°C for 2 minutes. The cooled mixture is then lyophilized. An oligosaccharide mixture (III) is thus obtained.

 The oligosaccharide mixture (III) obtained is
20 then chromatographed according to the following method: the chromatography is carried out on columns filled with polyacrylamide-agarose gel known under the name Ultrogel ACA 202 and the mixture is eluted with a solution containing a phosphate buffer (0.02 ml/l
25 $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$) $\text{pH} = 7$ and 0.1 mol/l of sodium chloride. The detection is performed by UV spectrometry (254 nm) and ionic spectrometry (IBF). The products can be optionally purified by SAX (strong anion exchange)

chromatography or by fractional precipitation according to the method described in patent FR 2 548 672. The fractions of product recovered are lyophilized and then desalified on a column filled with Sephadex G10[®] gel (Pharmacia Biochemicals).

By this method, 3 g of disaccharide Is and 1100 mg of a hexasaccharide mixture typically containing 55% of Δ Is-Is-Is derivative, 35% of Δ Is-Is-IIIs and 10% of Δ Is-Is-IIIs derivative are obtained. The latter mixture can be purified according to the methods known to those skilled in the art in order to separate each of the constituents therefrom, or can be used in its current state for conversion into the 1,6-anhydro derivatives of formula (I). It should be noted that, during this conversion, the hexasaccharide Δ Is-Is-IIIs cannot lead to the formation of compounds of formula (I).

EXAMPLE B - preparation of the oligosaccharides of formula (II) for which $n = 0, 1, 2, 3$ or 4 by depolymerization of the benzyl ester of heparin and separation

a - Preparation of the benzyl ester of heparin

The benzyl ester of heparin is prepared according to Example 3 of US patent 5 389 618.

b - Depolymerization

100 g of benzyl ester of heparin are dissolved in 1.9 l of demineralized water. The mixture

is brought to 60°C with stirring. After obtaining the homogeneous solution, about 35 ml of a 23% sodium hydroxide solution are introduced in a single portion. After reaction for 10 minutes, the solution is then
5 cooled and neutralized with 80 ml of an approximately 2 N acetic acid solution. 10% by weight/volume of sodium acetate is added to this solution. The oligosaccharide mixture is precipitated by adding about 2 volumes of methanol. The precipitate is isolated by filtration,
10 washed twice with methanol and then dried under reduced pressure at 50°C. After drying, 73.8 g of an oligosaccharide mixture (II) is obtained.

c- Enrichment with oligosaccharide for which $n = 1$

30g of the oligosaccharide mixture obtained
15 above are dissolved in about 35 volumes of water. This solution is ultrafiltered through a 3 kDa membrane (Pellicon). When 600 ml of permeate have been drawn, the retentate is diluted with 500 ml of water. The operation is continued until an additional 450 ml of
20 permeate have been drawn. The two fractions of permeate are combined and then concentrated to dryness under reduced pressure. 6.1 g of a yellowish-white solid are obtained. Analysis of the solid by gel permeation chromatography indicates that it contains about 30% of
25 oligosaccharide of formula (II) for which $n = 1$.

d - Fractionation of the ultrafiltered oligosaccharide mixtures

The enriched mixture is fractionated on

columns filled with polyacrylamide-agarose gel known under the name Ultrogel ACA 202 (4 columns in series of diameter 10 cm and length 50 cm are used). 5 g of the mixture enriched by ultrafiltration are dissolved in 25 ml of water and then eluted with a 0.2 mol/l sodium chloride solution at a rate of 5 ml/min. 25-ml fractions are collected at the bottom of the column. The products are detected by UV spectrometry (254 nm) and ionic spectrometry (IBF). The fractions of product for which $n = 1$ are recovered, lyophilized and then desalified on a column filled with Sephadex G10 gel. After lyophilization, 1 g of tetrasaccharide typically containing 70% of Δ Is-Is derivative of formula II (R_1 , R_2 , R_3 , R_5 and $R_6 = \text{SO}_3\text{Na}$; $R_4 = \text{H}$ and $M = \text{Na}$) is obtained. The Δ Is-Is derivative can be optionally purified by SAX (strong anion exchange) chromatography or, according to a preferred aspect, by fractional precipitation according to the method described in patent FR 2 548 672.

EXAMPLE 1

5 ml of a 0.0063 mol/l sodium hydroxide solution are introduced into a reactor maintained at 66°C. The pH of the solution is then measured and taken as the target value (pH = 11.35). 30 mg of the oligosaccharide of formula (II) for which n is equal to 0, R_1 and R_6 represent an SO_3Na radical and M is sodium are added in a single portion, with stirring. The pH is then adjusted and maintained at pH 11.35 by continuous

addition of a 0.5 mol/l sodium hydroxide solution. After 10 hours, the addition of sodium hydroxide is stopped and the reaction mixture is cooled to 25°C. The pH of the solution is then brought to between 6 and 7 by addition of Amberlite IR120 resin. The mixture is filtered through a Whatman GF/B membrane and then concentrated to dryness under reduced pressure (2.7 kPa) at a temperature in the region of 25°C. The product is taken up in 0.5 ml of distilled water and then lyophilized. 29 mg of a mixture of diastereoisomers of the oligosaccharide of formula (I) for which n is equal to 0, R₁ and R₆ represent an SO₃Na radical and M is sodium are thus obtained [(4-deoxy-2-O-sulfo-α-L-threo-hex-4-enopyranosyluronic acid (1→4)-1,6-anhydro-2-deoxy-2-sulfoamino-β-D-mannopyranose, trisodium salt): proton spectrum in D₂O, 400 MHz, T = 298 K, δ in ppm: 3.15 (1H, s, H₂), 3.75 (2H, m, H₆ and H₃), 3.88 (1H, m, H₄), 4.20 (1H, d, J = 8 Hz, H₆), 4.22 (1H, t, J = 5 Hz, H₃'), 4.58 (1H, m, H₂'), 4.75 (1H, m, H₅), 5.53 (1H, s, H₁), 5.60 (1H, dd, J = 6 and 1 Hz, H₁'), 6.03 (1H, d, J = 5 Hz, H₄'); (4-deoxy-2-O-sulfo-α-L-threo-hex-4-enopyranosyluronic acid-(1→4)-1,6-anhydro-2-deoxy-2-sulfoamino-β-D-glucopyranose, trisodium salt): proton spectrum in D₂O, 400 MHz, T = 298 K, δ in ppm: 3.34 (1H, dd, J = 7 and 2 Hz, H₂), 3.72 (1H, t, J = 8 Hz, H₆), 3.90 (1H, m, H₃), 4.03 (1H, s, H₄), 4.20 (1H, d, J = 8 Hz, H₆), 4.23 (1H, t, J = 5 Hz, H₃'), 4.58 (1H, m, H₂'), 4.78 (1H, m, H₅),

5.50 (1H, s, H1), 5.60 (1H, dd, J = 6 and 1 Hz, H1'),
6.03 (1H, d, J = 5 Hz, H4')].

EXAMPLE 2

5 33.3 ml of a 0.0063 mol/l sodium hydroxide
solution are introduced into a reactor maintained at
62°C. The pH of the solution is then measured and taken
as the target value (pH = 11.15). 200 mg of the
oligosaccharide of formula (II) for which n is equal to
10 1, R₁, R₂, R₃, R₅ and R₆ represent an SO₃Na radical, R₄
represents a hydrogen atom and M is sodium are added in
a single portion, with stirring. The pH is then
adjusted and maintained at pH 11.15 by continuous
addition of a 0.5 mol/l sodium hydroxide solution.
15 After 12 hours, the addition of sodium hydroxide is
stopped and the reaction mixture is cooled to 25°C. The
pH of the solution is then brought to between 6 and 7
by addition of Amberlite IR120 resin. The mixture is
filtered through a Whatman GF/B membrane and then
20 concentrated to dryness under reduced pressure
(2.7 kPa) at a temperature in the region of 25°C. The
product is taken up in 3 ml of distilled water and then
lyophilized. 230 mg of the oligosaccharide of formula
(I) for which n is equal to 1, R₁, R₂, R₃, R₅ and R₆
25 represent an SO₃Na radical, R₄ represents a hydrogen
atom and M is sodium are thus obtained in the form of a
mixture of diastereoisomers [(4-deoxy-2-O-sulfo- α -L-
threo-hex-4-enopyranosyluronic acid-(1 $\rightarrow$ 4)-2-deoxy-2-

sulfoamino-6-O-sulfo- α -D-glucopyranosyl-(1 $\rightarrow$ 4) 2-O-sulfo- α -L-idopyranosyluronic acid-(1 $\rightarrow$ 4)-1,6-anhydro-2-deoxy-2-sulfoamino- β -D-mannopyranose, heptasodium salt): proton spectrum in D₂O, 400 MHz, T = 298 K, δ in ppm: 3.15 (1H, s, H2), 3.25 (1H, m, H2''), 3.60 (1H, m, H3''), between 3.70 and 4.70 (14H, broad peak, H3/H4/H6, H2'/H3'/H4'/H5', H4''/H5''/H6'', H2'''/H3'''), 4.75 (1H, m, H5), between 5.20 and 5.40 (2H, m, H1' and H1''), 5.45 (1H, m, H1'''), 5.56 (1H, m, H1), 5.94 (1H, d, J=5 Hz, H4); (4-deoxy-2-O-sulfo- α -L-threo-hex-4-enopyranosyluronic acid, -(1 $\rightarrow$ 4)-2-deoxy-2-sulfoamino-6-O-sulfo- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfo- α -L-idopyranosyluronic acid -(1 $\rightarrow$ 4)-1,6-anhydro-2-deoxy-2-sulfoamino- β -D-glucopyranose, heptasodium salt): proton spectrum in D₂O, 400 MHz, T = 298 K, δ in ppm: 3.25 (1H, m, H2''), 3.42 (1H, dd, J=4 and 1 Hz, H2), 3.60 (1H, m, H3''), between 3.70 and 4.70 (14H, broad peak, H3/H4/H6, H2'/H3'/H4'/H5', H4''/H5''/H6'', H2'''/H3'''), 4.75 (1H, m, H5), between 5.20 and 5.40 (2H, m, H1' and H1''), 5.45 (1H, m, H1'''), 5.52 (1H, m, H1), 5.94 (1H, d, J=5 Hz, H4)].

EXAMPLE 3

16.7 ml of a 0.0063 mol/l sodium hydroxide solution are introduced into a reactor maintained at 62°C. The pH of the solution is then measured and taken as the target value (pH = 11.7). 100 mg of the oligosaccharide of formula (II) for which n is equal to

2, R₁, R₂, R₃, R₅ and R₆ represent an SO₃Na radical, R₄ represents a hydrogen atom and M is sodium are added in a single portion, with stirring. The pH is then adjusted and maintained at pH 11.7 by continuous
5 addition of a 0.5 mol/l sodium hydroxide solution. After 10 hours, the addition of sodium hydroxide is stopped and the reaction mixture is cooled to 25°C. The pH of the solution is then brought to between 6 and 7 by addition of Amberlite IR120 resin. The mixture is
10 filtered through a Whatman GF/B membrane and then concentrated to dryness under reduced pressure (2.7 kPa) at a temperature in the region of 25°C. The product is taken up in 3 ml of distilled water and then lyophilized. 108 mg of the oligosaccharide of formula
15 (I) for which n is equal to 2, R₁, R₂, R₃, R₅ and R₆ represent an SO₃Na radical, R₄ represents a hydrogen atom and M is sodium are thus obtained in the form of a mixture of diastereoisomers. The sugars constituting the hexasaccharides are noted from A to F, A being the
20 1,6-anhydro residue and F being the unsaturated uronic acid residue. [(4-deoxy-2-O-sulfo- α -L-threo-hex-4-enopyranosyluronic acid-(1 $\rightarrow$ 4)-2-deoxy-2-sulfoamino-6-O-sulfo- α -D-glucopyranosyl-(1 $\rightarrow$ 4)- 2-O-sulfo- α -L-idopyranosyluronic acid -(1 $\rightarrow$ 4)- 2-deoxy-2-sulfoamino-6-O-sulfo- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfo- α -L-idopyranosyluronic acid -(1 $\rightarrow$ 4)-1,6-anhydro-2-deoxy-2-sulfoamino- β -D-mannopyranose, undecasodium salt: proton
25 spectrum in D₂O, 600 MHz, T = 298 K, δ in ppm: 3.15 (1H,

s, H2(A)), 3.25 (2H, m, H2(C+E)), 3.60 (2H, m, H3(C+E)), between 3.65 and 4.50 (19H, broad peak, H2(B+D)/H3(A+B+D+F)/H4(A+B+C+D+E)/H5(C+E)/H6(A+C+E)), 4.60 (1H, s, H2(F)), 4.80 (3H, m, H5(A+B+D)), 5.18 (1H, s, H1(D)), 5.30 (1H, s, H1(B)), 5.34 (1H, d, H1(C)), 5.36 (1H, d, H1(E)), 5.46 (1H, s, H1(F)), 5.57 (1H, s, H1(A)), 5.95 (1H, d, J=5 Hz, H4(F)); (4-deoxy-2-O-sulfo- α -L-threo-hex-4-enopyranosyluronic acid-(1 $\rightarrow$ 4)-2-deoxy-2-sulfoamino-6-O-sulfo- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfo- α -L-idopyranosyluronic acid-(1 $\rightarrow$ 4)-2-deoxy-2-sulfamino-6-O-sulfo- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-O-sulfo- α -L-idopyranosyluronic acid-(1 $\rightarrow$ 4)-1,6-anhydro-2-deoxy-2-sulfoamino- β -D-glucopyranose, undecasodium salt):
proton spectrum in D₂O, 600 MHz, T = 298 K, δ in ppm:

3.25 (2H, m, H2(C+E)), 3.42 (1H, m, H2(A)), 3.60 (2H, m, H3(C+E)), between 3.65 and 4.50 (19H, broad peak, H2(B+D)/H3(A+B+D+F)/H4(A+B+C+D+E)/H5(C+E)/H6(A+C+E)), 4.60 (1H, s, H2(F)), 4.80 (3H, m, H5(A+B+D)), 5.18 (1H, s, H1(D)), 5.31 (1H, s, H1(B)), 5.34 (1H, d, H1(C)), 5.36 (1H, d, H1(E)), 5.46 (1H, s, H1(F)), 5.52 (1H, s, H1(A)), 5.95 (1H, d, J=5 Hz, H4(F))].

EXAMPLE 4

4 ml of a 0.0316 mol/l sodium hydroxide solution are introduced into a reactor maintained at 62°C. The pH of the solution is then measured and taken as the target value (pH = 11.8). 100.8 mg of an oligosaccharide mixture of formula (II) for which n is

equal to 2, comprising 55% of Δ Is-Is-Is derivative (R_1 , R_2 , R_3 , R_5 and R_6 represent an SO_3Na radical, R_4 represents a hydrogen atom and M is sodium), 35% of Δ Is-Is-IIIs (R_1 , R_2 , R_3 and R_6 represent an SO_3Na radical, R_5 represents either an SO_3Na radical or a hydrogen atom, R_4 represents a hydrogen atom and M is sodium) and 10% of Δ Is-Is-IIIs (R_1 , R_2 , R_3 , R_5 and R_6 represent an SO_3Na radical, R_4 represents a hydrogen atom and M is sodium, the function SO_3M of carbon C6 being replaced with a hydrogen) are added in a single portion with stirring. The pH is then adjusted to and maintained at pH 11.8 by continuous addition of a 0.5 mol/l sodium hydroxide solution. After 11 hours, the addition of sodium hydroxide is stopped and the reaction mixture is cooled to 25°C. The pH of the solution is then brought to between 6 and 7 by addition of Amberlite IR120 resin. The mixture is filtered through a Whatman GF/B membrane and then concentrated to dryness under reduced pressure (2.7 kPa) at a temperature in the region of 25°C. The product is taken up in 1.5 ml of distilled water and then lyophilized. 110 mg of an oligosaccharide mixture of formula (I) for which n is equal to 2, in particular containing the 1,6-anhydro Δ Is-Is-Is derivative (R_1 , R_2 , R_3 , R_5 and R_6 represent an SO_3Na radical, R_4 represents a hydrogen atom and M is sodium) and the 1,6-anhydro Δ Is-Is-IIIs derivative (R_1 , R_2 , R_3 and R_6 represent an SO_3Na radical, R_5 represents either an SO_3Na radical or a hydrogen atom, R_4

represents a hydrogen atom and M is sodium) are thus obtained. HPLC (high performance liquid chromatography) analysis in ion-pair mode makes it possible to monitor the conversion into derivatives of formula (I). In this case, the HPLC assay shows that the conversion is achieved for the Δ Is-Is-Is and Δ Is-Is-IIIs derivatives.

EXAMPLE 5

8.6 ml of a 0.025 mol/l lithium hydroxide solution are placed in a reactor maintained at 66°C. The pH of the solution is then measured and taken as the target value (pH = 11.68). 50 mg of the oligosaccharide of formula (II) for which n is equal to 0, R_1 and R_6 represent an SO_3Na radical and M is sodium, are then added in a single portion with stirring. The pH is then adjusted and maintained at pH 11.68 by continuous addition of 0.466 mol/l lithium hydroxide solution. After 8 hours, the addition of lithium hydroxide is stopped and the reaction mixture is cooled to 25°C. HPLC (High Performance Liquid Chromatography) analysis in ion-pair mode makes it possible to monitor the conversion into derivative of formula (I) for which n is equal to 0, R_1 and R_6 represent an SO_3Na radical and M is sodium or lithium. In this case, the HPLC assay shows that the conversion achieved is 100%. The yield by external calibration is 81.2%.

EXAMPLE 6

8.3 ml of a 0.0063 mol/l potassium hydroxide solution are placed in a reactor maintained at 66°C. The pH of the solution is then measured and taken as the target value (pH = 11.1). 50 mg of the oligosaccharide of formula (II) for which n is equal to 0, R₁ and R₆ represent an SO₃Na radical and M is sodium, are then added in a single portion with stirring. The pH is then adjusted and maintained at pH 11.1 by continuous addition of 0.515 mol/l potassium hydroxide solution. After 24 hours, the addition of potassium hydroxide is stopped and the reaction mixture is cooled to 25°C. HPLC (High Performance Liquid Chromatography) analysis in ion-pair mode makes it possible to monitor the conversion into derivative of formula (I) for which n is equal to 0, R₁ and R₆ represent an SO₃Na radical and M is sodium or potassium. In this case, the HPLC assay shows that the conversion achieved is 100%. The yield by external calibration is 75.6%.

20

EXAMPLE 7

8.3 ml of a 0.0063 mol/l cesium hydroxide solution are placed in a reactor maintained at 66°C. The pH of the solution is then measured and taken as the target value (pH = 10.75). 50 mg of the oligosaccharide of formula (II) for which n is equal to 0, R₁ and R₆ represent an SO₃Na radical and M is sodium, are then added in a single portion with stirring. The

pH is then adjusted and maintained at pH 10.75 by continuous addition of 0.476 mol/l cesium hydroxide solution. After 20 hours, the addition of cesium hydroxide is stopped and the reaction mixture is cooled to 25°C. HPLC (High Performance Liquid Chromatography) analysis in ion-pair mode makes it possible to monitor the conversion into derivative of formula (I) for which n is equal to 0, R_1 and R_6 represent an SO_3Na radical and M is sodium or cesium. In this case, the HPLC assay shows that the conversion achieved is 90.3%. The yield by external calibration is 73%.

EXAMPLE 8

8.3 ml of a 0.0063 mol/l tetrabutylammonium hydroxide solution are placed in a reactor maintained at 66°C. The pH of the solution is then measured and taken as the target value (pH = 10.95). 50 mg of the oligosaccharide of formula (II) for which n is equal to 0, R_1 and R_6 represent an SO_3Na radical and M is sodium, are then added in a single portion with stirring. The pH is then adjusted and maintained at pH 10.95 by continuous addition of 0.521 mol/l tetrabutylammonium hydroxide solution. After 16 hours, the addition of cesium hydroxide is stopped and the reaction mixture is cooled to 25°C. HPLC (High Performance Liquid Chromatography) analysis in ion-pair mode makes it possible to monitor the conversion into derivative of formula (I) for which n is equal to 0, R_1 and R_6

represent an SO_3Na radical and M is sodium or tetrabutylammonium. In this case, the HPLC assay shows that the conversion achieved is 96.7%. The yield by external calibration is 65%.

5 The medicinal products according to the invention comprise, as active principle, at least one oligosaccharide of formula (I) or an oligosaccharide mixture of formula (I), in the form of a composition in which it is combined with any other pharmaceutically
10 compatible product, which can be inert or physiologically active. The medicinal products according to the invention can be used via the intravenous, subcutaneous, oral, rectal, topical or pulmonary (inhalation) route.

15 The sterile compositions for intravenous or subcutaneous administration are generally aqueous solutions. These compositions can also contain adjuvants, in particular wetting agents, tonicity agents, emulsifiers, dispersants and stabilizers. The
20 sterilization can be carried out in several ways, for example by aseptic filtration, by incorporating sterilizing agents into the composition or by irradiation. They can also be prepared in the form of sterile solid compositions which can be dissolved, at
25 the time of use, in sterile water or any other injectable sterile medium.

Solid compositions for oral administration which can be used are tablets, pills, powders (gelatin

capsules or cachets) or granules. In these compositions, the active principle is mixed with one or more inert diluents, such as starch, cellulose, sucrose, lactose or silica, under a stream of argon.

- 5 These compositions can also comprise substances other than diluents, for example one or more lubricants such as magnesium stearate or talc, an agent for promoting oral absorption, a dye, a coating (dragees) or a varnish.

- 10 Liquid compositions for oral administration which can be used are pharmaceutically acceptable solutions, suspensions, emulsions, syrups and elixirs containing inert diluents such as water, ethanol, glycerol, plant oils or liquid paraffin. These
15 compositions can comprise substances other than diluents, for example wetting products, sweeteners, thickeners, flavourings or stabilizers.

- The compositions for rectal administration are suppositories or rectal capsules which contain,
20 besides the active product, excipients such as cocoa butter, semi-synthetic glycerides or polyethylene glycols.

- The compositions for topical administration can be, for example, creams, lotions, eye drops, throat
25 sprays, nasal drops or aerosols.

 The doses depend upon the desired effect, the duration of the treatment and the route of administration used; they are generally between 0.5 mg

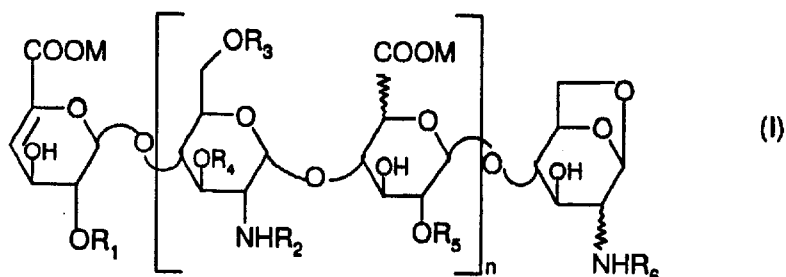
and 10 mg per kg per day subcutaneously, i.e. 3 to 60 mg per day for a 60 kg adult.

In general, the doctor will determine the appropriate dosage as a function of the age and weight
5 and all the other personal factors of the individual to be treated.

The invention also relates to the method for preventing or treating diseases associated with an inflammatory process involving the production of
10 cytotoxic substances such as nitrite oxide (NO). The oligosaccharides of formula (I) can thus be used for preventing and/or treating neurodegenerative diseases for which cerebral inflammation plays a deleterious role which can lead to death, among which mention may
15 be made of ischemias of the central nervous system, cerebral ischemias, ischemias of the retina and the cochlea, cardiac ischemias (myocardial infarction), peripheral ischemias, traumas of the central nervous system and in particular cranial, spinal and
20 craniospinal traumas, traumas of the retina and cochlea, multiple sclerosis, neuropathic pains and peripheral neuropathies, motor neuron diseases including amyotrophic lateral sclerosis, neuro-AIDS, Alzheimer's disease, Parkinson's disease and
25 Huntington's chorea and certain forms of osteoarthritis, in particular of articular localization.

CLAIMS

1. An oligosaccharide of formula:



5 in which n is an integer from 0 to 25, R_1 , R_3 , R_4 and R_5 , which may be identical or different, represent a hydrogen atom or a radical SO_3M , R_2 and R_6 , which may be identical or different, represent a hydrogen atom or a radical SO_3M or COCH_3 and M is sodium, calcium,
 10 magnesium or potassium, a mixture of these oligosaccharides and diastereoisomers thereof.

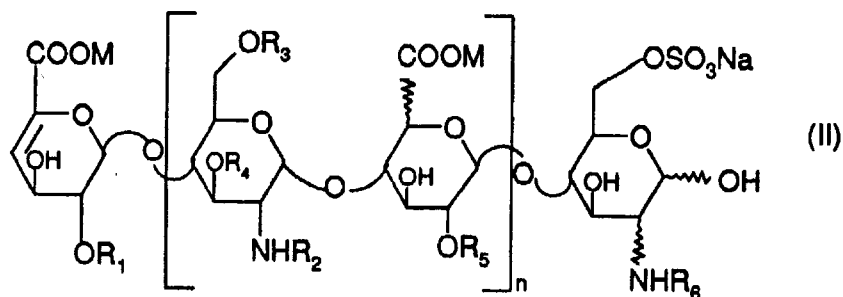
2. The oligosaccharide of formula (I) as claimed in claim 1, for which R_4 represents a hydrogen atom, a mixture of these oligosaccharides and
 15 diastereoisomers thereof.

3. The oligosaccharide of formula (I) as claimed in either of claims 1 and 2, for which n is an integer from 0 to 10, a mixture of these oligosaccharides and diastereoisomers thereof.

20 4. The oligosaccharide of formula (I) as claimed in either of claims 1 and 2, for which n is an integer from 0 to 6, a mixture of these oligosaccharides and diastereoisomers thereof.

5. The oligosaccharide of formula (I) as claimed in either of claims 1 and 2, for which n is an integer from 1 to 6, a mixture of these oligosaccharides and diastereoisomers thereof.

6. A process for preparing the oligosaccharide of formula (I) as claimed in claim 1, characterized in that an alkali metal hydroxide or quaternary ammonium hydroxide is reacted with oligosaccharides of formula:



in which n is an integer from 0 to 25, R_1 , R_3 , R_4 and R_5 , which may be identical or different, represent a hydrogen atom or a radical SO_3M , R_2 and R_6 , which may be identical or different, represent a hydrogen atom or a radical SO_3M or $COCH_3$, and M is sodium, calcium, magnesium or potassium, or a mixture thereof, and the oligosaccharides or mixtures thereof are isolated.

7. The process as claimed in claim 6, characterized in that the reaction is carried out in aqueous medium, at a temperature of from 40 to 80°C and at a pH of from 10 to 13.

8. The process as claimed in either of claims 6 and 7, characterized in that an aqueous

solution of from 1 to 5% alkali metal hydroxide or quaternary ammonium hydroxide is used.

9. The process as claimed in one of claims 6 to 8, characterized in that the reaction is carried out at a temperature of from 60 to 70°C.

10. The process as claimed in one of claims 6 to 9, characterized in that the reaction pH is from 11 to 12.5.

11. The process as claimed in claims 6 to 10, characterized in that the alkali metal hydroxide or quaternary ammonium hydroxide is sodium hydroxide, potassium hydroxide, lithium hydroxide, cesium hydroxide or tetrabutylammonium hydroxide.

12. A pharmaceutical composition containing, as active principle, at least one oligosaccharide as claimed in claim 1.

13. A pharmaceutical composition containing, as active principle, at least one oligosaccharide as claimed in one of claims 1 to 5.

14. The use of the oligosaccharide of formula (I) as claimed in one of claims 1 to 5, for the preparation of a medicinal product which is useful for preventing or treating diseases associated with an inflammatory process involving the production of nitrite oxide (NO).

15. The use of the oligosaccharide of formula (I) as claimed in claim 14 for the preparation of medicinal products which are useful for preventing

and treating cerebral, cardiac or peripheral vascular ischemias, osteoarthritis, traumas of the central nervous system, cranial, spinal or craniospinal traumas, multiple sclerosis, neuropathic pains and
5 peripheral neuropathies, motor neuron diseases, amyotrophic lateral sclerosis, neuro-AIDS, Alzheimer's disease, Parkinson's disease and Huntington's chorea.