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PROCESO PARA LA PREPARACION DE DERIVADOS DE N-ACIL-(EPI)KS-
AMINA-S-SULFATO Y PRODUCTOS ASI OBTENIDOS

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Milan, Italia.

74. APODERADO

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C.C. 438.019 de Usaquén.

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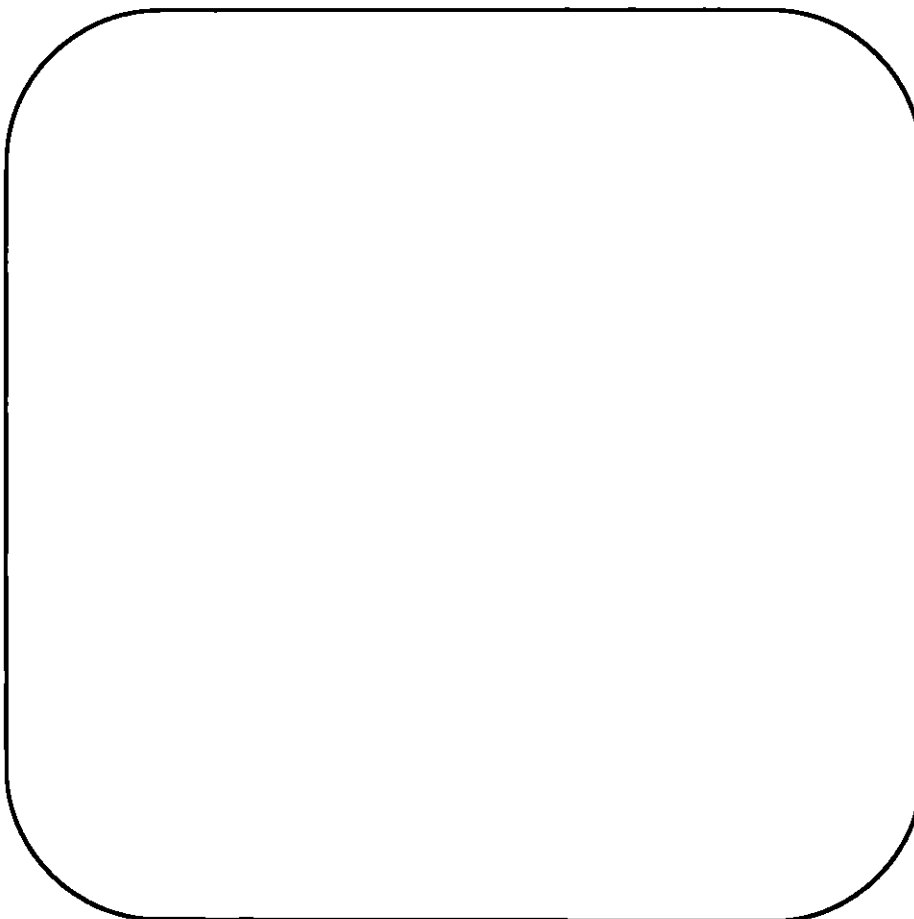
1	SOLICITUD DE:			
☒ Patente de invención.		☐ Patente de Modelo de Utilidad.		
☐ Capítulo I.		☒ Capítulo II.		
2	SOLICITANTE (71)	Nombre: GLYCORES 2000 S.r.l. sociedad constituida y existente bajo las leyes de Italia Dirección: Via Mac Mahon, 43 I-20155 Milan, Italia Domicilio: Milan, Italia	IDENTIFICACION C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número: _____	
3	REPRESENTANTE O APODERADO	Nombre: EMILIO FERRERO Dirección: Carrera 4ª. No. 72-35 Teléfono: 347 36 11 Fax: 217 92 11 E-mail: <u>clientes@camvp.com.</u> Domicilio: Bogotá, Colombia.	IDENTIFICACION C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Cual _____ Número: <u>438.019</u> T.P. <u>31.929</u>	
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Anexos:

- ☒ Comprobante de pago de la tasa de presentación de la solicitud por \$ 422.000.00.
- ☐ Comprobante de pago por reivindicación de prioridad.
- ☐ Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☐ Poderes, si fuere el caso.
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones.
- ☒ Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Copia del examen preliminar efectuado por la IPEA.
- ☒ Traducción del examen preliminar efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ De ser el caso, certificado de depósito del material biológico.
- ☐ Arte final 12x12
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.
- ☒ Otros: Formato PCT/IB/306 mediante el cual la Oficina Internacional de la OMPI notifica el registro del cambio de solicitante de los Inventores a la compañía GLYCORES 2000 S.r.l.

11

FIGURA CARACTERISTICA:

12

Solicito la concesión de la patente,

NOMBRE: EMILIO FERRERO

FIRMA:

C.C. 438.019 de Usaquén T.P. 31.929

DLM

PATENT COOPERATION TREATY

RICEVUTO

17 NOV 2004

PCT

MARIETTI, GISLON e TRUPIANO S.r.l.

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

SANTORO, Tiziana
Marietti, Gislone e Trupiano S.r.l.
Via Larga, 16
I-20122 Milan
ItalyDate of mailing (day/month/year)
05 November 2004 (05.11.2004)Applicant's or agent's file reference
03422E45

IMPORTANT NOTIFICATION

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17 June 2003 (17.06.2003)

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address

State of Nationality
**State of Residence
IT

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒ the person ☐ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address

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Via Mac Mahon, 43
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ItalyState of Nationality
**State of Residence
IT

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

Applicant indicated in box 2 has been added for "all designated states except US" and applicants ORESTE, Pasqua, Anna and ZOPPETI, Giorgio become applicant/inventor for US only.

4. A copy of this notification has been sent to:

☒ the receiving Office ☒ the designated Offices concerned
☐ the International Searching Authority ☐ the elected Offices concerned
☐ the International Preliminary Examining Authority ☐ other:The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

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ning of each regular issue of the PCT Gazette.

(54) Title: PROCESS FOR THE MANUFACTURE OF N-ACYL-(EPI)K5-AMINE-O-SULFATE-DERIVATIVES AND PROD-
UCTS THUS OBTAINED

(57) Abstract: A new method is described for the oversulfation of (epi)K5-N-sulfates to obtain (epi)K5-amine-O-oversulfates at
extremely high degree of sulfation and for the transformation of these intermediates into new N-acyl-(epi)K5-amine-O-oversulfates
basically free of activity on the coagulation parameters and useful in the cosmetic or pharmaceutical field. Also described are phar-
maceutical compositions containing, as one of their active ingredients, an (epi)K5-amine-O-oversulfate.

WO 2003/106505 A1

Process for the manufacture of N-acyl-(epi)K5-amine-O-sulfate-derivatives and products thus obtained.

SUBJECT MATTER OF THE INVENTION

The present invention concerns new derivatives of K5 polysaccharide with a high degree of sulfation, a process for their preparation, new highly O-sulfated intermediates useful in their synthesis and pharmaceutical compositions containing said derivatives of K5 polysaccharide as active ingredients basically free of activity on coagulation.

In particular, the invention refers to derivatives of K5 polysaccharid obtained starting with a K5 polysaccharide, previously N-deacetylated, N-sulfated, optionally depolymerized or optionally at least 20% C5-epimerized and optionally depolymerized, by O-oversulfation in suitable conditions and subsequent N-acylation of th free amine.

BACKGROUND OF THE INVENTION

The glycosaminoglycans such as heparin, heparan sulfate, dermatan sulfate, chondroitin sulfate and hyaluronic acid are biopolymers extracted irdustrially from various animal organs.

In particular, heparin, mainly obtained by extraction from the intestinal mucous membrane of pigs or from bovine lung, is a polydispersed copolymer with a molecular weight distribution from approximately 3,000 to approximately 30,000 D consisting ofa chain mixture basically consisting of a uronic acid (glucuronic acid or iduronic acid) and of an amino sugar (glucosamine) linked by bonds α -1 $\rightarrow$ 4 or β -1 $\rightarrow$ 4. In heparin, the uronic unit can be O-sulfated in position 2 and the glucosamine unit is N-acetylated or N-sulfated, 6-O-sulfated and 3-O-sulfated in approximately 0.5% of the glucosamine units present.

The properties and the natural biosynthesis of heparin in mammals

have been described by Lindahl et al., 1986 in Lane, D. and Lindahl, U. (Editors) "Heparin. Chemical and Biological Properties; Clinical Applications", Edward Arnold, London, Pages 159-190, by Lindahl, U., Feingold D. S. and Rodén L. 1986 TIBS, 11, 221-225 and by Conrad H. E. "Heparin Binding Proteins", Chapter 2: Structure of Heparinoids. Academic Press, 1998. The biosynthesis of heparin occurs starting with its N-acetyl-heparosan precursor consisting of a chain mixture consisting of the repetitive glucuronyl- β -1 $\rightarrow$ 4-N-acetylglucosamine disaccharide unit. Said precursor undergoes enzymatic modifications which partially hydrolyse the N-acetyl group, substituting it with an SO₃ group, epimerize the carboxy in position 5 of a part of the glucuronic units transforming them into iduronic units and introducing O-sulfate groups to get a product which, once extracted industrially, has approximately double the number of sulfate groups as regards carboxy groups per disaccharide unit. These enzymatic modifications lead, i.e. to the formation of the pentasaccharide region of a bond to antithrombin III (ATIII), called active pentasaccharide, which is the structure necessary for the high affinity bond of heparin to ATIII and fundamental for anticoagulant and antithrombotic activity of the heparin itself. This pentasaccharide, present inside only some of the chains which form heparin, contains a sulfated glucosamine unit in position 3 and a glucuronic acid spaced out between disaccharides containing iduronic acids.

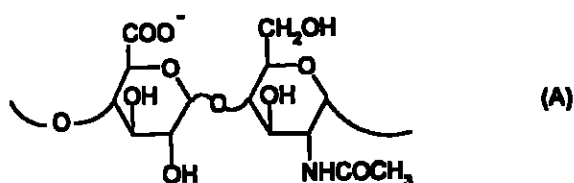
In nature, the formation of the active pentasaccharide is made possible by the epimerization reaction of the carboxy of a part of the glucuronic units into iduronic units carried out by the glucuronyl-C5-epimerase (C5-epimerization) and by suitable sulfation which also leads to the introduction of a sulfate group on the hydroxyl in position 3 of the glucosamine. More particularly, in nature the formation of the

active pentasaccharide is made possible by the fact that C5-epimerization occurs in clusters, i.e. on portions of chains, and extensively which leads to a product which contains more iduronic units than glucuronic ones. In fact, commercial heparin contains approximately 70% of iduronic units and 30% of glucuronic units.

Alongside the main anticoagulant and antithrombotic activities, heparin also exercises antilipaemic, antiproliferative, antiviral, antitumorous and antimetastatic activities, but its use as a drug is hindered by the side effects due to the anticoagulant action which can cause bleeding.

PRIOR ART

It is known that the capsular K5 polysaccharide isolated from *Escherichia coli*, described by Vann W. F. et al., in European Journal of Biochemistry, 1981, 116, 359-364 ("Vann 1981"), is consisting of a chain mixture consisting of the repetitive disaccharide unit glucuronyl- β -1 $\rightarrow$ 4-N-acetyl glucosamine and therefore shows the same repetitive sequence (A)



of N-acetyl-heparosan precursor of heparin. The capsular K5 polysaccharide, referred to hereafter as "K5 polysaccharide" or more simply "K5", was chemically modified by Lorneau et al. as described in US 5,550,116 and by Casu et al. as described in Carbohydrate Research, 1994, 263, 271-284. K5-O-sulfates having antitumorous, antimetastatic, antiviral, in particular anti-HIV activities are described in EP 333243 and WO 98/34958. The K5 was also chemically and enzymatically modified in order to obtain products having in vitro

biological activity on coagulation of the same type as that of heparin as extracted from animal organs (extractive heparin).

The attainment of the products having an activity on coagulation of the same type as that of extractive heparin occurs by processes which imitate that occurring in nature and envisage the entire key step of C5-epimerization with D-glucuronyl C5 epimerase.

The processes described in IT 1230785, WO 92/17507, WO 96/14425 and WO 97/43317 utilize K5 as the starting material. The K5 originating from fermentation is subjected to N-deacetylation followed by N-sulfation and on the K5-N-sulfate thus obtained C5-epimerization with C5-epimerase in solution is performed, obtained either by chromatography of a solution of microsomal enzymes from mouse mastocytoma (IT 1230 785) or from bovine liver (WO 92/17507, WO 96/14425 and WO 97/43317).

The D-glucuronyl C5 epimerase from bovine liver was purified by Campbell, P. et al. in J. Biol. Chem., 1994, 269/43, 26953-26958 ("Campbell 1994") who also provided its composition in amino acids and described its use in solution for the transformation of a K5-N-sulfat into the corresponding 30% epimerized product, demonstrating the formation of iduronic acid by HPLC method followed by total nitrous depolymerization to disaccharide.

The document WO 98/48006 describes the DNA sequence which codes for the D-glucuronyl C5 epimerase and a recombinant D-glucuronyl C5 epimerase, obtained from a recombinant expression vector containing said DNA, afterwards purified by Campbell et al. as shown by Jin-Ping L. et al. in J. Biol Chem. 2001, 276, 20069-20077 ("Jin-Ping 2001").

The complete sequence of the C5-epimerase was described by Crawford B. E. et al. in J. Biol. Chem., 2001, 276(24), 21538-21543

(Crawford 2001)

The document WO 01/72848 describes a method for the preparation of N-deacetylated N-sulfate derivatives of K5 polysaccharide, at least 40% epimerized of iduronic acid as regards the total of the uronic acids, having a molecular weight from 2000 to 30,000, containing from 25 to 50% of high affinity chains for ATIII and having an anticoagulant and antithrombotic activity expressed as HCII/antiXa ratio from 1.5 to 4. Said document describes the oversulfation of a 40-60% epimerized K5-N-sulfate and shows that the product obtained, whose ¹³C-RMN is illustrated, has a sulfate group content per disaccharide unit of 2-3.5. Repeating the aforesaid oversulfation in the conditions described and examining the ¹³C-RMN it is ascertained that the product obtained is actually a free amine whose content of 6-O-sulfate is 80-95%, that of 3-O-sulfate on the amino sugar is 30%, but whose sulfation degree is 3.2. It is also ascertained that in the conditions of oversulfation described in WO 01/72848 a sulfation degree of more than 3.2 is not obtained.

The patent application M12001A/00397 (WO 02/068477), describes K5-N,O-oversulfated having a sulfation degree of more than 3.2, obtained starting with a K5 free of lipophilic substances or one of its moieties of molecular weight of approximately 5,000 by (a) N-deacetylation/N-sulfation, (b) O-oversulfation and, optionally, (c) N-resulfation.

None of the aforesaid documents describes LMW-K5-N-sulfates, optionally 40-60% epimerized, in which NH₂ or acetyl groups are virtually absent.

In order to standardize the terminology and render the text more comprehensible, in the present description conventional terms or expressions will be used, in the singular or plural. In particular:

- by "K5" or "K5 polysaccharide" is meant the capsular

polysaccharide from *Escherichia coli* obtained by fermentation, i.e. a chain mixture consisting of disaccharide units (A) optionally containing a double bond at the non-reducing end as shown above, in any case prepared and purified according to the methods described in literature, in particular according to Vann 1981, according to Manzoni M. et al., Journal of Bioactive Compatible Polymers, 1996, 11, 301-311 ("Manzoni 1996") or according to the method described in WO 01/72848 and in WO 02/068447; it is obvious for a person skilled in the art that what is shown hereafter can be applied to any N-acetylheparosan;

- by "C5-epimerase" is meant the D-glucuronyl C-5 epimerase, extractive or recombinant, in any case prepared, isolated and purified, in particular as described in Campbell 1994, in WO 98/48006, in Jin-Ping L. et al. in J. Biol Chem. 2001, 276, 20069-20077 (Jin-Ping 2001") or in Crawford 2001;
- by K5-amine is meant at least 95% N-deacetylated K5, but generally in which acetyl groups are undetectable by a current NMR apparatus;
- by "K5-N-sulfate" is meant at least 95% N-deacetylated and N-sulfate K5 as described hereafter, but in which acetyl groups are undetectable with a normal NMR apparatus;
- by "epiK5" is meant the K5 and its derivatives in which 20-60% of the glucuronic units are C5-epimerized to iduronic units;
- by "epiK5-N-sulfate" is meant the K5-N-sulfate in which 20-60% of the glucuronic units is C5-epimerized to iduronic units of the type described in WO 92/17507 or WO 01/72848;
- by "epiK5-amine-O-oversulfate" is meant an O-sulfated epiK5-amine with a sulfation degree of at least 3.4;
- by "N-acyl-epiK5-amine-O-oversulfate" is meant an N-acylated epiK5-amine O-oversulfate, with a sulfation degree of at least 3.4;

- by "K5-amine-O-oversulfate" is meant an O-sulfated K5-amine with a sulfation degree of at least 2.2;

- by "N-acyl-K5-amine-O-oversulfate" is meant an N-acylated N-acyl-K5-amine-O-oversulfate with a sulfation degree of at least 2.2;

In addition:

- the conventional terms and expressions herein defined above refer to K5 as isolated after fermentation, generally with a molecular weight distribution from approximately 1,500 to approximately 50,000 with a mean molecular weight of 10,000- 25,000, advantageously of 15,000- 25,000;

- excepting specific designation of the molecular weight, the conventional terms and expressions herein defined above, when preceded by the acronym "LMW" (low molecular weight), for example LMW-K5-N-sulfate, LMW-epiK5-N-sulfate indicate low molecular weight products, having a mean molecular weight of up to 12,000;

- the conventional terms and expressions as herein defined above, when followed by "-derivative" indicate as a whole both the derivatives from native K5 and those of a low molecular weight;

- the prefix "(epi)", which precedes "K5" in conventional terms and expressions as herein defined above, indicates as a whole both the products from native K5 and those 20-60% epimerized as defined above, i.e. chain mixtures all consisting of glucuronic acid or glucuronic acid and, 20-60%, of iduronic acid and is denoted in the formulae by the symbol "ε";

- by the term "approximately" referring to the molecular weight is meant the molecular weight [± the theoretical weight of a disaccharide unit, including the weight of the sodium, calculated as 461 in the case of an (epi)K5-N-sulfate-derivative] measured by

viscometry according to Johnson et al. Carb. Res. 1976, 51, 119-127 utilizing samples whose molecular weight was calculated by HPLC as the standard;

- by the expression "preponderant species", is meant the compound which, in the mixture constituting a lmw-(epi)K5-N-sulfate, a lmw-(epi)K5-amine-oversulfate or a lmw-N-acyl-(epi)K5-amine-O-oversulfate, is the most represented type, determined by the peak of the curve of the molecular weight measured by HPLC;

- unless otherwise specifically stated, by "degree of sulfation" is meant the $\text{SO}_3^-/\text{COO}^-$ ratio, expressible also as the number of sulfate groups per disaccharide unit, measured with the conductometric method described by Casu B. et al. in Carbohydrate Research, 1975, 39, 168-176 (Casu 1975), the same utilized in WO 01/72848;

- by "conditions of O-oversulfation" is meant an extreme O-sulfation performed for example according to the method C described by B. Casu et al. in Carbohydrate Research, 1994, 263, 271-284 (Casu 1994);

- by the term "alkyl" is meant a linear or branched alkyl, whereas the term "tetrabutylammonium" indicates the tetra-n-butylammonium.

SUMMARY OF THE INVENTION

It has now been found that, starting with an epiK5-N-sulfate, it is possible to obtain an epiK5-amine-O-sulfate with a degree of sulfation greater than that of every other epiK5-amine-O-sulfate described in literature, for example in WO 01/72848, preparing the salt with a tertiary or quaternary organic base of said epiK5-N-sulfate-derivative taking care to let the reaction mixture to stand for a time period of 30-60 minutes maintaining the pH at approximately 7 with the same organic base and then treating the salt obtained with an O-sulfation reagent in the conditions of O-oversulfation. Also a lmw-epiK5-N-sulfate, when subjected to the same method of sulfation and of O-

a

oversulfation, gives a 1mw-epiK5-amine-O-sulfate with an extremely high degree of sulfation. In the case of such epi-K5-amine-oversulfate-derivatives the degree of sulfation is more than 3.2.

Similarly, it was found that, applying the conditions of O-oversulfation described above to a 1mw-K5-N-sulfate a 1mw-K5-amine-O-sulfate is obtained at a high degree of sulfation. Also a K5-N-sulfate, when subjected to the same method of salification and of O-oversulfation, gives a K5-amine-O-sulfate with a high degree of sulfation. In the case of such K5-amine-oversulfate-derivatives the degree of sulfation is more than 2.2.

In fact, it was surprisingly found that, preparing the tetrabutylammonium salt of an (epi)K5-N-sulfate-derivative in the aforesaid conditions, the salification becomes complete and, during the subsequent O-oversulfation, favours the total displacement of the sulfate group from the nitrogen atom in position 2 of the glucosamine to the oxygen atoms of the glucosamine itself, in particular to the oxygen atom in position 3, thus giving rise to (epi)K5-amine-O-sulfate-derivatives with a high degree of sulfation in which the NH₂ group is strictly free. The quantitative difference in sulfation degree of the derivatives epiK5 and K5 depends on their configuration which favours the sulfation of the epimerized derivatives.

It has also been found that, subjecting an (epi)K5-amine-O-oversulfate-derivative thus obtained to N-acylation, new N-acyl-(epi)K5-amine-O-sulfate-derivatives are obtained free of activity on coagulation and useful for the preparation of pharmaceutical or cosmetic compositions. By this new and versatile process it is thus possible to prepare in a reproducible manner epiK5-O-sulfate-derivatives, their N-acyl analogues, a K5-O-sulfate having a precise degree of sulfation and, above all, its derivatives of low molecular

weight and their N-acyl-analogues.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the NMR proton spectrum of K5-NH₂ O-sulfate with a sulfate/carboxy ratio of 2.87 (obtained according to Example 1);

Figure 2 shows the NMR proton spectrum of epiK5-N-acetylate, O-sulfate with a sulfate/carboxy ratio of 4.26 (obtained according to Example 2);

Figure 3 shows the NMR proton spectrum of K5-N-acetylate, O-sulfate with a sulfate/carboxy ratio of 2.87 (obtained according to Example 3).

DETAILED DESCRIPTION

Thus, according to one of its aspects, the present invention provides a process for the preparation of N-acyl-(epi)K5-amine-O-oversulfate-derivatives, characterized in that

(a) an (epi)K5-N-sulfate-derivative, in acidic form, is treated with a tertiary or quaternary organic base, letting the reaction mixture to stand for a time period of 30-60 minutes, maintaining the pH of the solution at a value of approximately 7 and its salt is isolated with said organic base;

(b) said salt of organic base of said (epi)K5-N-sulfate-derivative is treated with an O-sulfation reagent in the conditions of O-oversulfation;

(c) the product thus obtained is treated with a functional derivative of a (C₂-C₄) carboxylic acid, the N-acyl-(epi)K5-amine-O-oversulfate-derivative thus obtained is isolated.

Generally the N-acyl-(epi)K5-amine-O-oversulfate-derivative is isolated in sodium salt form and optionally said sodium salt is transformed into another chemically or pharmaceutically acceptable salt.

In this context, the term "chemically acceptable" refers to a cation



usable in chemical synthesis, such as the ions sodium, ammonium, (C₁-C₄)tetraalkylammonium, or for the purification of the product, whereas "pharmaceutically acceptable" is self-explanatory.

Advantageous cations are those derived from alkaline metals, alkaline-earth metals, ammonium, (C₁-C₄)tetraalkylammonium, aluminum and zinc. Preferred cations are the sodium, calcium and tetrabutylammonium ions.

According to an advantageous procedure, the step (a) is carried out by passing a solution of the sodium salt of the(epi)K5-N-sulfate-derivative, i.e. of K5 polysaccharide, previously N-deacetylated, N-sulfated preferably 100%, optionally 20-60% C5-epimerized and optionally depolymerized with nitrous acid, having a mean molecular weight from approximately 1,000 to approximately 25,000, advantageously from approximately 1,500 to approximately 25,000, through an acid ionic exchange resin, for example of the type IR-120 H⁺, collecting the eluate including also the washing water of the resin and neutralizing the eluate with tertiary or quaternary organic base, preferably with an aqueous solution of tetrabutylammonium hydroxide. The solution is let to stand for 1 hour, maintaining its pH at approximately 7 (i.e. trying to maintain neutrality) by addition of the same tertiary or quaternary organic base, preferably an aqueous solution of tetrabutylammonium hydroxide, and the salt thus obtained is recovered by lyophilization.

In step (b), the O-oversulfation occurs utilizing an excess of O-sulfating agent and working at a temperature from 20 to 70°C for a time period of up to 24 hours in an aprotic polar solvent.

Advantageously, the salt with a tertiary or quaternary organic base of the(epi)K5-N-sulfate-derivative, i.e. of K5 polysaccharide, previously N-deacetylated, N-sulfated preferably 100%, optionally 20-60% C5-

epimerized and optionally depolymerized with nitrous acid, having a mean molecular weight from approximately 1,500 to approximately 25,000 as isolated in step (a), is dissolved in dimethylformamide and treated with 2 – 10 moles of an O-sulfation reagent for every free hydroxyl at a temperature of 40-60°C for 10-20 hours. As the O-sulfation reagent is advantageously used the pyridine.SO₃ adduct in a quantity of 2.5 – 5 moles, preferably 2.5 – 4 moles per free hydroxyl per disaccharide and the reaction is advantageously carried out at 50-60°C, preferably at 55°C overnight. The product obtained upon termination of the reaction is isolated by the addition of 0.1-1 volume of water and neutralization, preferably with sodium hydroxide, precipitation with a saturated sodium chloride solution in acetone, filtration and possible ultrafiltration.

The product thus obtained is generally the sodium salt of an (epi)K5-amine-O-oversulfate-derivative having a glucuronic and iduronic acids content as shown above. When the oversulfation reaction is performed on the salt of the tertiary or quaternary base, preferably on the tetrabutylammonium salt of an epiK5-N-sulfate-derivative having a mean molecular weight from approximately 1,000 to approximately 25,000, advantageously from approximately 1,500 to approximately 25,000 in which the iduronic acid is 20-60% of the total of the uronic acids, an epiK5-amine-O-oversulfate-derivative is obtained having a mean molecular weight from approximately 3,500 to approximately 40,000, advantageously from approximately 4,500 to approximately 40,000, and a sulfation degree of at least 3.4, advantageously of at least 3.5, more advantageously from 3.55 to 4, preferably from 3.55 to 3.8.

When the oversulfation reaction is performed on the salt of the tertiary or quaternary base, preferably on the tetrabutylammonium salt of a

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K5-N-sulfate-derivative having a mean molecular weight from approximately 1,500 to approximately 25,000, a K5-amine-O-oversulfate-derivative is obtained having a mean molecular weight from approximately 3,500 to 40,000, advantageously from approximately 4,500 to approximately 40,000 and a sulfation degree of at least 2.2, advantageously from 2.2 to 3 or from 2.3 to 3, more advantageously from 2.5 to 3, preferably from 2.7 to 2.9. Preferably, the product thus obtained comes from a lmw-K5-N-sulfate and is preferably the sodium salt of a lmw-K5-amine-O-oversulfated having a degree of sulfation from 2.3 to 3. The mean molecular weight of such product can be from approximately 3,500 to approximately 11,000.

The sodium salt thus obtained can be converted into another salt. By way of example, calcium ionic exchange can be performed working with an ultrafiltration membrane.

In step (c), the (epi)K5-amine-O-oversulfate-derivative with a high degree of sulfation is N-acylated utilizing the known methods in literature.

The N-acylation is performed by making the (epi)K5-amine-O-oversulfate-derivative originating from step (b) react with a functional derivative of a mono or dicarboxylic acid containing from 2 to 4 carbon atoms in hydroalcoholic solution at a temperature of approximately 4°C. As functional derivatives of said (C₂-C₄) carboxylic acids, preferably of acetic, propionic, malonic, or succinic acid or of mono esters, in particular methylic or ethylic, of the latter, one can use the anhydride, the chloride, a mixed anhydride or an active ester. The product obtained, an N-(C₂-C₄)acyl-(epi)K5-amine-O-oversulfate-derivative, is neutralized with a base, preferably sodium hydroxide, and then isolated by ultrafiltration and precipitation with a saturated sodium chloride solution in acetone. Optionally the step of N-acylation

is repeated until obtaining total substitution or however more than 95%. Are thus obtained the N-acyl-(epi)K5-amine-O-oversulfates, in particular the N-acetyl-, N-(2-carboxy)acetyl-, N-(2-methoxycarbonyl)acetyl-, N-(2-ethoxycarbonyl)acetyl-, N-propionyl-, N-(3-carboxy)propionyl-, N-(3-methoxycarbonyl)propionyl-, N-(3-ethoxycarbonyl)propionyl-(epi)K5-amine-O-oversulfates.

The new N-acyl-(epi)K5-amine-O-oversulfate-derivatives thus obtained are generally in their sodium salt forms. Said sodium salt can be converted into another chemically or pharmaceutically acceptable salt. Particularly advantageous salts are those of alkaline metals, alkaline-earth metals, of ammonium, (C₁-C₄)tetraalkylammonium, aluminum and zinc. The salts of sodium, calcium and tetrabutylammonium are preferred.

Unlike the process described in WO 98/34958, which consists in a sulfation in heterogeneous phase of the K5 sodium salt to obtain K5-O-sulfates (according to the terminology used herein "N-acetyl-K5-amine-O-sulfates") with a degree of sulfation from 0.5 to 4 and which has to resort to the use of chlorosulfonic acid to obtain derivatives of low molecular weight in an uncontrolled manner, the process of the present invention is extremely versatile. In fact, it allows the preparation of N-acyl-K5-amine-O-oversulfates of every type in very high yields, with easily controllable reactions. In particular, the process of the present invention allows the preparation of N-acyl-K5-amine-O-oversulfates otherwise unobtainable, like the epimerized C-5 derivatives, the N-acylated derivatives with a different acyl from the acetyl present in the native K5 and the derivatives of low molecular weight. Besides, unlike the method described in WO 98/34958, the process of the present invention allows the attainment of LMW-K5-O-sulfates in controlled manner in order to obtain the mean molecular

weight desired and, above all, a well defined sulfation degree which can range from 2.3 to 3 but which can regularly be 2.7-2.9. Finally, all the products of the process of the present invention, in particular the free intermediate amines and the N-acyl-K5-amine-O-oversulfates are useful active ingredients for pharmaceutical or cosmetic compositions.

The starting materials of step (a) are (epi)K5-N-sulfate-derivatives known in literature or their moieties, or LMW-(epi)K5-N-sulfates prepared by nitrous depolymerization of the corresponding (epi)K5-sulfates.

The starting (epi)K5-N-sulfate-derivatives have a mean molecular weight from approximately 1,000 to approximately 25,000, advantageously from approximately 1,500 to approximately 25,000. Preferably, as starting material is used an (epi)K5-N-sulfate having a mean molecular weight between 10,000 and 25,000 or LMW-(epi)K5-N-sulfate having a mean molecular weight from approximately 1,500 to approximately 12,000, advantageously from approximately 1,500 to approximately 8,000, preferably from approximately 1,500 and approximately 7,500.

A lmwepiK5-N-sulfate having an iduronic units content of approximately 20%, obtained by N-deacetylation, N-sulfation and C5-epimerization of a moiety of K5 having a mean molecular weight of 5,000 is described in WO 92/17507, but this product contains a high percentage of acetyl groups.

An epiK5-N-sulfate particularly advantageous as starting material is that obtained by epimerization of a K5-N-sulfate virtually free of acetyl groups in turn prepared from particularly pure K5, in particular not containing lipophilic substances, described in WO 02/068477.

The low molecular weight C5-epimerized K5-N-sulfates having a higher

content of iduronic units, in particular 40-60%, preferably 50-55%, are instead particularly advantageous new products as starting materials in the preparation of the N-substituted epiK5-amine-O-oversulfat derivatives of the present invention.

The LMW-epiK5-N-sulfates can also be obtained by C5-epimerization of the corresponding LMW-K5-N-sulfates, preferably when their mean molecular weight is more than 4,000. The LMW-K5-N-sulfates virtually free of acetyl groups are all new products preparable by fractionation or, preferably, by depolymerization of a K5-N-sulfate free of acetyl groups. The K5-N-sulfate is well known in literature and is described in documents cited herein above to illustrate the state of the art. The aforesaid starting material is invariably obtained by N-deacetylation of K5 and subsequent N-sulfation of the K5-amine thus obtained. However, it was ascertained that the preparation of a K5-N-sulfate virtually free of acetyl or NH₂ groups is helped if the K5 from which it is prepared is particularly pure, in particular if it does not contain lipophilic substances. It is therefore preferred, according to the present invention, to use a starting K5-N-sulfate prepared from a purified K5 as described in WO 02/068477. Said K5-N-sulfate, whose ¹³C-RMN spectrum does not show traces of N-acetyl or NH₂ groups is also described in WO 02/068477.

The new LMW-epiK5-N-sulfates as shown above are prepared by a process characterized in that a K5-N-sulfate is subjected, in any order,

- (i) to C5-epimerization with a D-glucuronyl C5-epimerase that is isolated, purified and in solution or immobilized on a solid support, at a pH of approximately 7, at a temperature of approximately 30°C and for a time period of 12-24 hours in the presence of at least one bivalent ion selected among calcium, magnesium, barium and manganese; and

(ii) to nitrous depolymerization optionally followed by reduction, normally with sodium borohydride.

The expression "in any order" shows that the process can be indifferently carried out both in the direction (i)-(ii), i.e. in the sequence above indicated, and in the reverse direction, i.e. also in the direction (ii)-(i), subjecting the K5-N-sulfate at first to the nitrous depolymerization reaction, optionally followed by reduction with sodium borohydride, and afterwards to the C5-epimerization in the aforesaid conditions. The preferred order is in the direction (i)→(ii). The sequence (ii)-(i) is preferably utilized starting with LMW-K5-N-sulfates having a mean molecular weight of more than 4000, preferably starting with approximately 6,000. For example, one can determine the amounts of sodium nitrite which, starting with 1 g of epiK5-N-sulfate, allow the attainment of a lmw-epiK5-N-sulfate with a molecular weight of more than 4,000, in particular of at least 6,000, so as to obtain useful intermediates for the preparation of LMWepiK5-N,O-oversulfates. In fact, in this case, in step (ii) the percentage of optimum epimerization is obtained.

According to a preferential aspect of the invention, the C5-epimerase is immobilized on an inert solid support.

The C5-epimerase, preferably recombinant, isolated and purified for example according to Campbell 1994, WO 98/48006, Jin-Ping 2001 or Crawford 2001, is immobilized on an inert support in the presence of the substrate, i.e. in the presence of starting K5-N-sulfate-derivative or in the presence of LMW-K5-N-sulfate, advantageously having a mean molecular weight of more than 4,000, preferably of at least 6,000. The immobilization is performed according to conventional methods, for example as described in WO 01/72848.

The C-5epimerization reaction is carried out by recirculating 20-1,000

ml of a solution of 25 mM HEPES at a pH of approximately 7 containing 0.001-10 g of substrate (K5-N-sulfate or LMW-K5-N-sulfate, preferably with a molecular weight of more than 4,000, in particular of at least 6,000) and a cation selected among calcium, magnesium, barium and manganese at a concentration between 10 and 60 mM through a column containing from 1.2×10^7 to 3×10^{11} cpm of the immobilized enzyme, maintaining the pH at approximately 7 at approximately 30°C, at a flow of 30-220 ml/hour for a time period of 12-24 hours, advantageously of 15-24 hours.

Preferably the said solution is recirculated at a flow of approximately 200 ml/hour overnight (15-20 hours). The product obtained is purified and separated according to known methods, for example by ultrafiltration and precipitation with ethanol. The product thus obtained is comprised either of epiK5-N-sulfate (and in such case is dissolved in water and subjected to depolymerization) or of LMW-epiK5-N-sulfate (in such case it constitutes the end product). The percentage of epimerization, in practice the amount of iduronic units as regards the glucuronic ones, is calculated with $^1\text{H-RMN}$ according to the method described in WO 96/4425.

The nitrous depolymerization reaction is carried out according to known methods by the depolymerization of heparin, for example according to the method described in EP 37319, in WO 82/03627 or according to the method by depolymerization of a K5-N-sulfate described in EP 544592, but starting with a K5-N-sulfate or an epiK5-N-sulfate containing from 0 to no more than 10%, preferably no more than 5%, of acetyl groups. Preferably, the depolymerization, performed with sodium nitrite and hydrochloric acid on an epiK5-N-sulfate virtually free of acetyl groups, is followed by *in situ* reduction with sodium borohydride.

In practice, a cold aqueous solution of epiK5-N-sulfate is brought to acid pH (approximately 2) with hydrochloric acid and, still cold, treated with sodium nitrite maintaining the temperature (approximately 4°C) and the pH (approximately 2) constant and, upon termination of depolymerization (approximately 15 - 30 minutes) the solution is neutralized with sodium hydroxide and treated, still at approximately 4°C, with an aqueous solution of sodium borohydride. Upon termination of the reduction (approximately 4 hours) the excess sodium borohydride is destroyed with hydrochloric acid, the solution is neutralized with sodium hydroxide and the depolymerized (and reduced) product is isolated according to known methods, for example by straightforward precipitation with ethanol or acetone. The product obtained upon termination of the depolymerization can be either a lmw-epiK5-N-sulfate (in such case it constitutes the end product) or a lmw-K5-N-sulfate (and in such case is directly subjected to C5-epimerization as shown herein above, after isolation or also in solution without being previously isolated), in particular when it has a mean molecular weight of more than 4,000, preferably of at least 6,000, or is utilized to prepare LMW-K5-N,O-oversulfated of antiangiogenetic and antiviral activity. By appropriately controlling the depolymerization reaction, in particular utilizing different amounts of sodium nitrite/hydrochloric acid, LMW-K5-N-sulfates or LMW-epiK5-N-sulfates are obtained having a mean molecular weight in the entire interval from approximately 1,500 to approximately 12,000, advantageously from approximately 1,500 to approximately 10,000, preferably from approximately 1,500 to approximately 7,500, calculated at the ^{13}C -RMN spectrum through the integration of the signal attributed to the C2 of the 2,5-anhydromannitol with that of the anomeric carbon of the glucosamine inside the polysaccharide chain.

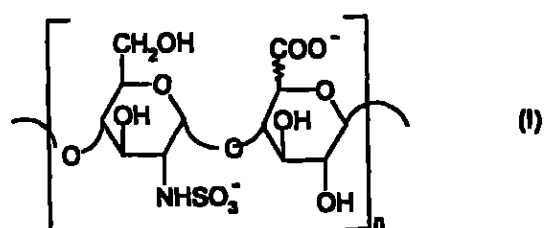
According to a general procedure starting for example with 1 g of K5-N-sulfate or of epiK5-N-sulfate, the starting product is dissolved in 100-200 ml of deionized water and thermostated at 4 °C. Then an amount of sodium nitrite is added so as to obtain the mean molecular weight desired, for example from approximately 2,000 to approximately 4,000. Therefore, starting with an epiK5-N-sulfate having a molecular weight of 20,000 measured with HPLC method equipped with a BioRad BioSil 250 column and utilizing a heparin standard of known molecular weight, will require the addition of from 330 to 480 mg of sodium nitrite dissolved in a 0.2% aqueous solution. The solution containing the epiK5-N-sulfate and the sodium nitrite, kept at 4°C, is brought to pH 2 through addition of 0.1 N HCl cooled to 4°C. It is reacted under slow agitation for 20-40 minutes, then is neutralized with 0.1 N NaOH. The product obtained is brought to room temperature and treated with reducing agent such as for example sodium borohydride (250-500 mg dissolved in 50-100 ml of water) and reacted for 4-8 hours. The excess sodium borohydride is eliminated bringing the pH to 5-5.5 with 0.1 N HCl and let to stand for a further 2-4 hours. In the end it is neutralized with 0.1 N NaOH and the product is recovered by precipitation with acetone or ethanol after having concentrated the product through evaporation at reduced pressure.

Similarly, the amounts of sodium nitrite can be determined which, starting with 1 g of K5-N-sulfate or of epiK5-N-sulfate, allow the attainment of a lmw-K5-N-sulfate or a lmw-epiK5-N-sulfate with a mean molecular weight from approximately 4,000 to approximately 12,000, advantageously from approximately 4,000 to approximately 7,500, in particular of 6,000-7,500.

The LMW-epiK5-N-sulfates thus obtained, with an Iduronic acid content from 20 to 60%, advantageously from 40 to 60%, preferably of 50-55%

and virtually free of NH_2 and N-acetyl groups, having a mean molecular weight from approximately 1,500 to approximately 12,000, advantageously from approximately 1,500 to approximately 10,000, preferably from approximately 1,500 to approximately 7,500 and their chemically or pharmaceutically acceptable salts constitute new products useful as starting materials in the preparation of particularly interesting LMW- epiK5-N,O-oversulfates, but also themselves useful as active ingredients of pharmaceutical or cosmetic compositions and constitute an additional aspect of the present invention.

Advantageously, the starting materials in the preparation of the N-acetyl-(epi)K5-amine-O-oversulfate-derivatives of the present invention are (epi)K5-N-sulfate-derivatives consisting of a chain mixture in which at least 90% of said chains have the formula I



in which the glucuronic units/iduronic units ratio is from 100/0 to 40/60, n is a integer from 2 to 100, advantageously from 3 to 100, and the corresponding cation is chemically or pharmaceutically acceptable.

In particular, said starting (epi)K5-N-sulfate-derivatives are consisting of a chain mixture in which at least 90% of said chains have the aforesaid formula I, with a glucuronic units content from 100 to 40%, advantageously with a glucuronic acid content of 100% or 80-40% of glucuronic acid and 20-60% of iduronic acid, n is a integer from 2 to 100, advantageously from 3 to 100 and the corresponding cation is chemically acceptable. Preferably, the glucuronic acid content is 100% or out of the total of uronic acids 40-60% is glucuronic acid and

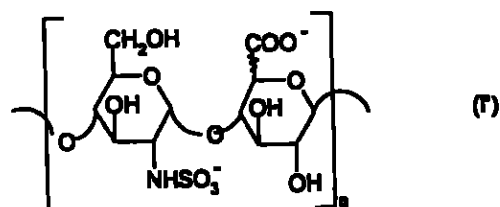
60-40% is iduronic acid.

Preferred starting materials are LMW-(epi)K5-N-sulfates consisting of a chain mixture in which at least 90% of said chains have the formula I in which the uronic units are all consisting of glucuronic acid or are 40-60% comprised, preferably 50-55%, of iduronic acid, n is a integer from 2 to 20, advantageously from 3 to 15 and the corresponding cation is chemically acceptable. The epiK5-N-sulfates, prepared by C5-epimerization of K5-N-sulfates, are well known and widely described in literature. Their preparation by C-5 epimerization of the glucuronic unit of K5-N-sulfate with a D-glucuronyl C5 epimerase was described in documents cited herein above to illustrate the state of the art, for example in WO 92/17507, WO 98/14425, WO 97/43317, WO 01/72848 and US 2002/0062019. However, according to a preferential manner of procedure, by epimerization a K5-N-sulfate is used obtained from a K5 free of lipophilic substances as described in WO 02/068477 and the C5 epimerization is performed with a D-glucuronyl C5-epimerase isolated, purified and immobilized on a solid support, at a pH of approximately 7, at a temperature of approximately 30°C and for a time period of 12-24 hours in the presence of at least one bivalent ion selected among calcium, magnesium, barium and manganese as shown above. Also the K5-N-sulfate was shown herein above.

The completely N-sulfated (epi)K5-N-sulfates of low molecular weight, in particular those in which the uronic units are all in glucuronic acid and those having an iduronic units content of 40-60%, preferably 50-55%, are instead all particularly advantageous new products as starting materials in the preparation of the N-acyl-(epi)K5-amine-O-oversulfate-derivatives according to the present invention. Said starting materials are LMW-(epi)K5-N-sulfates having an iduronic acid content of 0% or 40-60% as regards the total of the uronic acids and a

mean molecular weight from approximately 1,500 to approximately 12,000, advantageously from approximately 1,500 to approximately 8,000, preferably from approximately 1,500 to approximately 7,500; and their chemically or pharmaceutically acceptable salts. The distribution can range from approximately 1,000 to approximately 10,000.

Said LMW-(epi)K5-N-sulfates are advantageously consisting of a chain mixture in which at least 90% of said chains have the formula I'

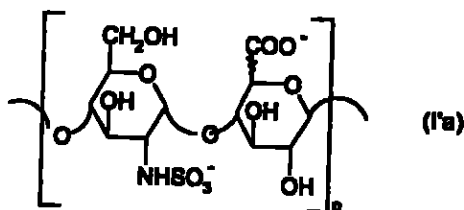


in which the uronic units are 100% consisting of glucuronic acid or 60-40% of glucuronic acid and 40-60% of iduronic acid, q is a integer from 2 to 20 and the corresponding cation is chemically or pharmaceutically acceptable.

In this context, the term "chemically" refers to a cation usable in chemical synthesis, such as the ions sodium, ammonium, (C₁-C₄)tetraalkylammonium, or for the purification of the product.

Advantageous cations are those derived from alkaline metals, alkaline-earth metals, ammonium, (C₁-C₄)tetraalkylammonium, aluminum and zinc. Preferred cations are the sodium, calcium and tetrabutylammonium ions.

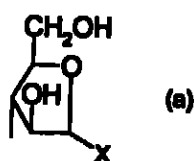
Preferred starting materials are the new LMW-(epi)K5-N-sulfates consisting of a chain mixture in which the preponderant species has the formula I'a



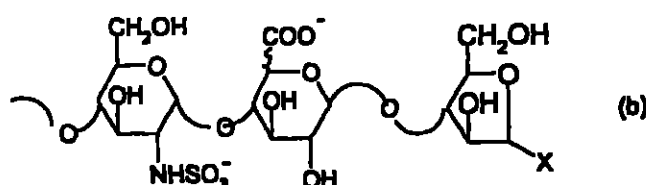
in which the uronic units are 100% consisting of glucuronic acid or 60-40% glucuronic and 40% to 60% of iduronic acid, p is a integer from 4 to 8. The mean molecular weight of these products is from approximately 2000 to approximately 4000 and the corresponding cation is chemically or pharmaceutically acceptable.

In particular the new LMW-(epi)K5-N-sulfates are useful starting materials consisting of a chain mixture in which at least 90% of said chains have the formula I' herein above, obtained by nitrous depolymerization of the corresponding (epi)K5-N-sulfates shown above and subsequent possible reduction for example with sodium borohydride.

The origin of these (epi)K5-N-sulfates from a step of nitrous depolymerization involves, at the reducing end of the majority of the chains in said chain mixture, the presence of a 2,5-anhydromannos unit or, in case of reduction with for example sodiumborohydride, of 2,5-anhydromannitol of structure (a)



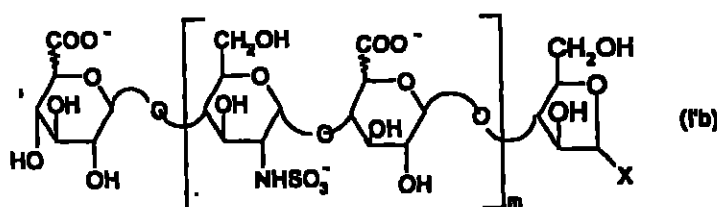
in which X represents a formyl group or a hydroxymethyl group. Therefore, the reducing end of the majority (60-70%) of the chains is actually represented by the structure (b)



in which X is as defined above.

The presence of the structure (a) does not have any influence on the chemical characteristics of the (epi)K5-N-sulfates and their derivatives since any sulfations would involve a possible introduction of one or two sulfate groups which would not significantly change however the sulfation degree of the O-sulfated derivatives. Besides, the presence of the structure (a) does not influence biological activity of the products, as demonstrated by Østergaard P. B. et al. in *Thrombosis Research*, 1987, 45, 739-749 (Østergaard 1987) for heparins of low molecular weight.

Particularly advantageous LMW-(epi)K5-N-sulfates according to the present invention are consisting of chain mixtures in which the preponderant species is a compound of formula I'b



in which X is formyl or hydroxymethyl, m is 4, 5 or 6, the corresponding cation is one chemically or pharmaceutically acceptable ion, the uronic units are all of glucuronic acid or the glucuronic and iduronic units are present alternately, starting with a glucuronic or iduronic unit. In such case the Glucuronic/iduronic ratio is from 45/55 to 55/45, i.e. approximately 50/50.

All the LMW-(epi)K5-N-sulfates herein shown above are new products which constitute an additional object of the present invention.

The LMW-(epi)K5-N-sulfates, i.e. the LMW-K5-N-sulfates shown above and, unlike other epiK5-N-sulfates, for example as indicated in WO 92/17507, also the LMW-epiK5-N-sulfates of the present invention, being virtually free of N-acetyl groups, do not have anticoagulant activity and have an interesting activity against the free radicals. Thanks to their low toxicity, the new LMW-K5-N-sulfates and LMW-epiK5-N-sulfates are active ingredients for the preparation of pharmaceutical compositions utilizable as adjuvants in the treatment of ischemic cardiopathy and for the treatment of radiation dermatitis or of cosmetic compositions useful as anti-ageing of the skin.

When the LMW-(epi)K5-N-sulfates shown above are used as starting materials for the O-oversulfation reaction, it is in any case preferred that, in their preparation by nitrous depolymerization as shown above, said depolymerization is followed by the reduction for example with sodium borohydride to give LMW-K5-N-sulfates characterized by terminal units (a) in which X is hydroxymethyl, since according to the process of the present invention said LMW-(epi)K5-N-sulfates are subjected to reactions of sulfation and acylation whose influence, of the 2,5-anhydromannose radical of structure (a) is unknown on the formyl group, in which X represents formyl.

Said starting materials are preferably used in sodium salt form, unless a salt is already available with a tertiary or quaternary organic base prepared according to step (a) shown above, preferably the tetrabutylammonium salt.

According to the present invention, the starting (epi)-K5-N-sulfate-derivatives, preferably 100% N-sulfated, are subjected to the aforesaid steps (a) and (b), upon termination of which are isolated the corresponding, new (epi)K5-amine-O-oversulfate-derivatives, in which the amine is not substituted, normally in sodium salt form, which can

be transformed into another chemically or pharmaceutically acceptable salt.

Thus, according to another of its aspects, the present invention refers to new (epi)K5-amine-O-oversulfate-derivatives and their chemically or pharmaceutically acceptable salts, obtainable by a process characterized in that

(a) an (epi)K5-N-sulfate-derivative, in acidic form, is treated with a tertiary or quaternary organic base, letting the reaction mixture to stand for a time period of 30-60 minutes, maintaining the pH of the solution at a value of approximately 7 by addition of said tertiary or quaternary organic base and its salt is isolated with said organic base;

(b) said salt of organic base of said (epi)K5-N-sulfate-derivative is treated with an O-sulfation reagent in the conditions of O-oversulfation; and the (epi)K5-amine-O-oversulfate-derivative is isolated.

The derivative thus obtained is generally an (epi)K5-amine-O-oversulfate-derivative having a mean molecular weight from approximately 4,500 to approximately 40,000, isolated in sodium salt form which can be transformed into another chemically or pharmaceutically acceptable salt. Particularly advantageous salts are those of alkaline metals, alkaline-earth metals, of ammonium, (C₁-C₄)tetraalkylammonium, aluminum and zinc and, among these, the salts of sodium, calcium and tetrabutylammonium are preferred.

As already herein shown above, the degree of sulfation of the (epi)K5-amine-O-oversulfate-derivatives as obtained above depends on the configuration of the starting (epi)K5-N-sulfate-derivative, since the volume in the disaccharide chain of K5-N-sulfate-derivative, in which the uronic units are comprised exclusively of glucuronic acid, allows a oversulfation which is less than that occurring with the epiK5-N-sulfate-derivative, especially if the epimerization degree of the latter is 40-60%

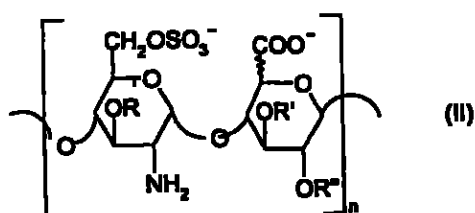
of glucuronic acid and 60-40% of iduronic acid.

In fact it has been ascertained that, subjecting a K5-N-sulfate-derivative to the aforesaid steps (a) and (b) a K5-amine-O-oversulfate-derivative is obtained having a sulfation degree from 2.2 to 3, advantageously from 2.5 to 3, preferably from 2.7 to 2.9.

Subjecting an epiK5-N-sulfate-derivative to the aforesaid steps (a) and (b) in which the iduronic acid content is 20-60%, preferably 40-60% of the total of the uronic acids, an epiK5-amine-O-oversulfate-derivative is obtained having a sulfation degree of at least 3.4, advantageously of at least 3.5, more advantageously from 3.55 to 4, preferably from 3.55 to 3.8.

These (epi)K5-amine-O-oversulfated-derivatives are useful products as intermediates in the preparation of their N-(C₂-C₄)acylated derivatives and as active ingredients basically free of activity on the coagulation parameters but having other interesting pharmacobiological properties, useful for the preparation of pharmaceutical compositions. For use of the epiK5-amine-O-oversulfate-derivatives of the present invention as active ingredients of pharmaceutical compositions, it is advantageous to prepare both derivatives of low molecular weight, with a mean molecular weight from approximately 3,000 to approximately 11,500, preferably from approximately 4,500 to approximately 8,500, with a molecular weight distribution of between approximately 1,000 and approximately 15,000, preferably between approximately 2,000 and approximately 10,000 and derivatives of high molecular weight, originating from the unfractionated K5, with a mean molecular weight from approximately 15,000 to approximately 45,000, preferably between approximately 20,000 and approximately 45,000, with a molecular weight distribution from approximately 2,000 to approximately 70,000.

Thus, according to another of its aspects the invention provides new active ingredients of pharmaceutical compositions consisting of (epi)K5-amine-O-oversulfate-derivatives consisting of chain mixtures in which at least 90% of said chains have the formula II

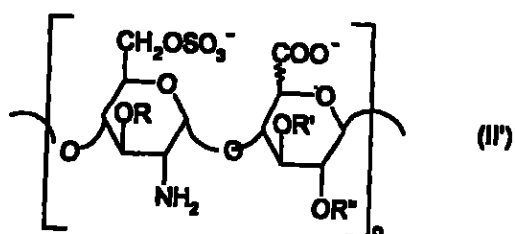


in which n is a integer from 2 to 100, preferably from 3 to 100, R , R' and R'' are hydrogen or SO_3^- , the uronic units are all of glucuronic acid, for a sulfation degree of at least 2.2, advantageously from 2.2 to 3 or from 2.3 to 3, more advantageously from 2.5 to 3, preferably from 2.7 to 2.9; or are 20-60% consisting of iduronic acid, for a sulfation degree of at least 3.4, advantageously of at least 3.5, more advantageously from 3.55 to 4, preferably from 3.55 to 3.8 and the corresponding cation is chemically or pharmaceutically acceptable.

Advantageous epiK5-amine-O-oversulfated-derivatives of extremely high degree of sulfation are consisting of a chain mixture in which at least 90% of said chains have the formula II in which the uronic units are 40-60% consisting of iduronic acid, n is a integer from 2 to 100, preferably from 3 to 100, with a mean molecular weight from approximately 2,000 to approximately 40,000, advantageously from approximately 4,500 to approximately 40,000, R is at least 40%, preferably 50-80% SO_3^- , R' and R'' are both SO_3^- or one is hydrogen and the other is 5-10% SO_3^- in monosulfate glucuronic acid and 10-15% SO_3^- in monosulfate iduronic acid, the degree of sulfation is more than 3.4 and the corresponding cation is chemically or pharmaceutically acceptable.

Preferred epiK5-amine-O-oversulfate-derivatives of very high degree

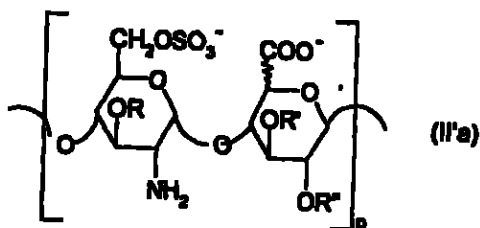
of sulfation are the LMW-epiK5-amine-O-oversulfates consisting of a chain mixture in which at least 90% of said chains have the formula II'



in which q is a integer from 2 to 20, R , R' and R'' are hydrogen or SO_3^- , the uronic units are 20-60% comprised, preferably 40-60%, of iduronic acid, for a degree of sulfation from 3.55 to 4, and the corresponding cation is one chemically or pharmaceutically acceptable ion.

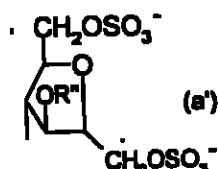
Are particularly interesting the chain mixtures of formula II' in which the uronic units are 40-60% comprised, preferably 50-55%, of iduronic acid, R is at least 40%, advantageously 50-80%, preferably approximately 65% SO_3^- , R' and R'' are both SO_3^- or one is hydrogen and the other is 5-10% SO_3^- in glucuronic acid and 10-15% SO_3^- in iduronic acid, n is a integer from 3 to 15, with a mean molecular weight from approximately 4,000 to approximately 8,000 and the corresponding cation is chemically or pharmaceutically acceptable.

Among these LMW-epiK5-amine-O-oversulfates are preferred those consisting of a chain mixture in which the preponderant species has the formula II'a



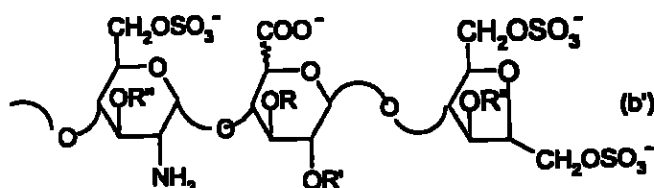
in which p is a integer from 4 to 8, R , R' and R'' are as defined above, the degree of sulfation is from 3.55 to 4 and the corresponding cation is chemically or pharmaceutically acceptable.

The origin of the new LMW-epiK5-amine-O-oversulfated from LMW-epiK5-N-sulfates obtained by nitrous depolymerization and subsequent reduction with, for example, sodium borohydride, involves, at the reducing end of the majority of the chains in said chain mixture, the presence of a sulfated 2,5-anhydromannitol unit of structure (a')



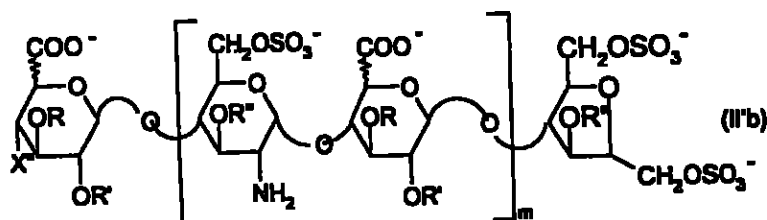
in which R'' represents hydrogen or SO₃.

Thus, the reducing end of the majority of the chains in said chain mixture is represented by the structure (b')



in which the uronic unit can be glucuronic or iduronic.

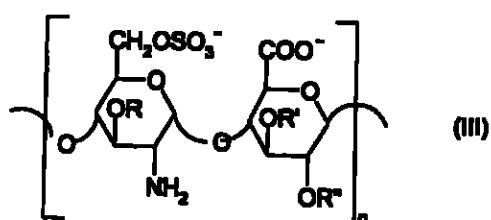
Among the aforesaid new LMW-epiK5-amine-O-oversulfates, are preferred those consisting of mixtures in which the preponderant species is a compound of formula II'b



in which R, R' and R'' are hydrogen or SO₃, X'' is OH or OSO₃, m is 4, 5 or 6, the uronic units are 40-60% consisting of iduronic acid, for a degree of sulfation from 3.55 to 4, the iduronic units being present alternately, starting with a glucuronic or iduronic unit, and the corresponding cation is one chemically or pharmaceutically

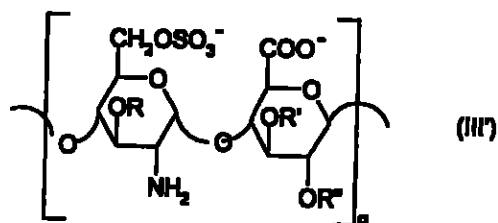
acceptable ion.

According to another of its aspects the invention provides new K5-amine-O-oversulfate derivatives consisting of chain mixtures in which at least 90% of said chains have the formula III



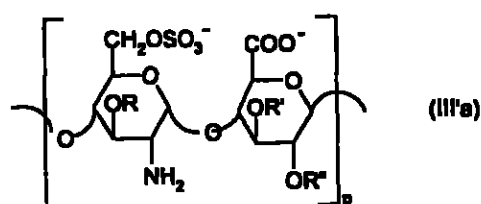
in which n is a integer from 2 to 100, preferably from 3 to 100, R , R' and R'' are hydrogen or SO_3^- , the degree of sulfation is at least 2.2, advantageously from 2.2 to 3 or from 2.3 to 3, more advantageously from 2.5 to 3, preferably from 2.7 to 2.9 and the corresponding cation is chemically or pharmaceutically acceptable.

Preferred K5-amine-O-oversulfates are the LMW-K5-amine-O-oversulfates consisting of chain mixtures in which at least 90% of said chains have the formula III'



in which q is a integer from 2 to 20, R , R' and R'' represent hydrogen or an SO_3^- group, for a sulfation degree of at least 2.2, advantageously from 2.2 to 3 or from 2.3 to 3, more advantageously from 2.5 to 3, preferably from 2.7 to 2.9 and the corresponding cation is one chemically or pharmaceutically acceptable ion.

Among these LMW-K5-amine-O-oversulfates are preferred those consisting of a chain mixture in which the preponderant species has the formula III'a

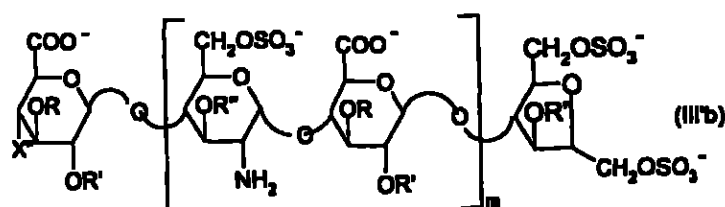


in which p is a integer from 4 to 8, R , R' and R'' are as defined above, the degree of sulfation is at least 2.2, advantageously from 2.2 to 3 or from 2.3 to 3, more advantageously from 2.5 to 3, preferably from 2.7 to 2.9 and the corresponding cation is chemically or pharmaceutically acceptable.

The origin of the new LMW-K5-amine-O-oversulfated from LMW-K5-sulfates obtained by nitrous depolymerization and subsequent reduction with, for example, sodium borohydride, involves, at the reducing end of the majority of the chains in said chain mixture, the presence of a sulfated 2,5-anhydromannitol unit of structure (a') as shown above, in which R'' represents hydrogen or SO_3^- .

Thus, the reducing end of the majority of the chains in said chain mixture is represented by the structure (b') as shown above, in which the uronic unit is still of glucuronic acid.

Among the aforesaid new LMW-K5-amine-O-oversulfates, are preferred those consisting of mixtures in which the preponderant species is a compound of formula III'b



in which R , R' and R'' are hydrogen or SO_3^- , X'' is OH or OSO_3^- , for a sulfation degree of at least 2.2, advantageously from 2.2 to 3 or from 2.3 to 3, more advantageously from 2.5 to 3, preferably from 2.7 to 2.9, m is 4, 5 or 6 and the corresponding cation is one chemically or

pharmaceutically acceptable ion.

These LMW-K5-amine-O-oversulfated are new products useful as intermediates in the preparation of their N-sulfated or N-acylated derivatives but themselves have interesting pharmacological properties, in particular as anti-free radicals and microbicides and, as such, constitute new active ingredients of pharmaceutical compositions which constitute therefore an additional aspect of the present invention.

In particular, according to another of its aspects, the invention concerns the use of the aforesaid (epi)K5-amine-O-oversulfate-derivatives with a high degree of sulfation for the preparation of new N-substituted epiK5-amine-O-oversulfate-derivatives.

The N-acyl-(epi)K5-amine-O-oversulfate-derivatives according to the present invention are obtained by subjecting the (epi)K5-amine-O-oversulfate-derivatives to the step (c) of the process of the present invention.

Thus, according to one of its additional aspects, the present invention provides new N-acyl(epi)K5-amine-O-oversulfate-derivatives obtainable by a process characterized in that

- (a) an (epi)K5-N-sulfate-derivative, in acidic form, is treated with a tertiary or quaternary organic base, letting the reaction mixture to stand for a time period of 30-60 minutes, maintaining the pH of the solution at a value of 7 by addition of said tertiary or quaternary organic base and its salt is isolated with said organic base;
- (b) said salt of organic base of said (epi)K5-N-sulfate-derivative is treated with an O-sulfation reagent in the conditions of O-oversulfation;
- (c) the product thus obtained is treated with a functional derivative of a (C₂-C₄) carboxylic acid, the N-acyl-(epi)K5-amine-O-oversulfate-

derivative thus obtained is isolated. Generally the N-acyl-(epi)K5-amine-O-oversulfate-derivative is isolated in sodium salt form and optionally said sodium salt is transformed into another chemically or pharmaceutically acceptable salt.

As occurs for the (epi)K5-amine-O-oversulfate-derivatives, also the degree of sulfation of the N-acyl-(epi)K5-amine-O-oversulfate-derivatives as obtained above depends on the configuration of the starting (epi)K5-N-sulfate-derivative, since the volume in the disaccharide chain of K5-N-sulfate-derivative, in which the uronic units are comprised exclusively of glucuronic acid, allows a oversulfation which is less than that occurring with the epiK5-N-sulfate-derivative, especially if the epimerization degree of the latter is 40-60% of glucuronic acid and 60-40% of iduronic acid.

In fact, it has been ascertained that, subjecting a K5-N-sulfate-derivative to the aforesaid steps (a)-(c) an N-acyl-K5-amine-O-oversulfate-derivative is obtained having a degree of sulfation from 2.2 to 3, advantageously from 2.5 to 3, preferably from 2.7 to 2.9.

Subjecting an epiK5-N-sulfate-derivative to the aforesaid steps (a), (b) and (c) in which the iduronic acid content is 20-60% of the total of the uronic acids, N-acyl-epiK5-amine-O-oversulfate-derivatives are obtained having a sulfation degree of at least 3.4, advantageously of at least 3.5, more advantageously from 3.55 to 4, preferably from 3.55 to 3.8.

Thus, according to another of its aspects, the present invention provides new N-acyl-(epi)K5-amine-O-oversulfate-derivatives in which the acyl is a (C₂-C₄) carboxylic acid, having a mean molecular weight from approximately 2,000 to approximately 45,000, preferably between approximately 4,500 and approximately 40,000, a degree of sulfation as indicated above, said derivatives being basically inactive

on the coagulation parameters but useful active ingredients of pharmaceutical or cosmetic compositions.

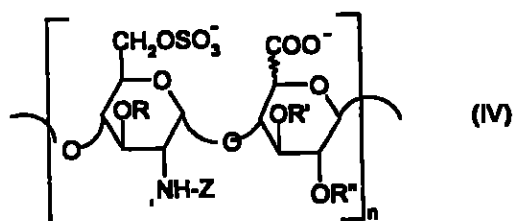
For use of the N-acyl-epiK5-amine-O-oversulfate-derivatives of the present invention as pharmaceutical or cosmetic products it is advantageous to prepare both derivatives of low molecular weight, with a mean molecular weight from approximately 3,000 to approximately 11,500, preferably from approximately 4,500 to approximately 8,500, with a molecular weight distribution of between approximately 1,000 and approximately 15,000, preferably between approximately 2,000 and approximately 10,000 and derivatives of high molecular weight, originating from the unfractionated K5, with a mean molecular weight from approximately 15,000 to approximately 45,000, preferably between approximately 20,000 and approximately 45,000, with a molecular weight distribution from approximately 2,000 to approximately 70,000.

In the N-acyl-epiK5-amine-O-oversulfate-derivatives of the present invention, the degree of sulfation is very high, since on the 4 free hydroxyls available per disaccharide unit, at least 3.4, preferably from 3.5 to 3.8, are sulfated, whereas the nitrogen of the glucosamine is virtually 100% acylated. Besides, the N-acyl-epiK5-amine-O-oversulfate-derivatives are 100% 6-O-sulfated and 50-80% 3-O-sulfated in their glucosamine units, 5-10% 3-O-monosulfated in glucuronic units, 10-15% O-monosulfated in iduronic units and 2,3-di-O-sulfated in the remaining uronic units, considering that the degree of sulfation is at least 3.4.

The N-acyl-K5-amine-O-oversulfate-derivatives which, as indicated above, have a degree of sulfation from 2.2 to 3, are at least 90% 6-O-sulfated.

According to another of its aspects, the invention provides new N-

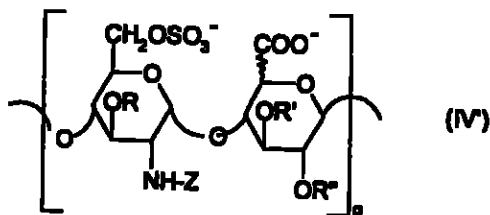
acyl-epiK5-amine-O-oversulfate-derivatives consisting of chain mixtures in which at least 90% of said chains have the formula IV



in which the uronic units are 20-60% consisting of iduronic acid, n is a integer from 2 to 100, preferably from 3 to 100, R , R' and R'' are hydrogen or SO_3^- , Z is $(\text{C}_2\text{-C}_4)$ acyl, the degree of sulfation is at least 3.4, advantageously of at least 3.5, more advantageously from 3.55 to 4, preferably from 3.55 to 3.8 and the corresponding cation is chemically or pharmaceutically acceptable.

Advantageous N-acyl-epiK5-amine-O-oversulfate-derivatives of extremely high degree of sulfation are consisting of a chain mixture in which at least 90% of said chains have the formula IV in which the uronic units are 40-60% consisting of iduronic acid, n is a integer from 2 to 100, preferably from 3 to 100, with a mean molecular weight from approximately 2,000 to approximately 45,000, advantageously from approximately 4,500 to approximately 45,000, R is at least 40%, preferably 50-80% SO_3^- , R' and R'' are both SO_3^- or one is hydrogen and the other is 5-10% SO_3^- in monosulfate glucuronic acid and 10-15% SO_3^- in monosulfate iduronic acid, the degree of sulfation is more than 3.4 and the corresponding cation is chemically or pharmaceutically acceptable.

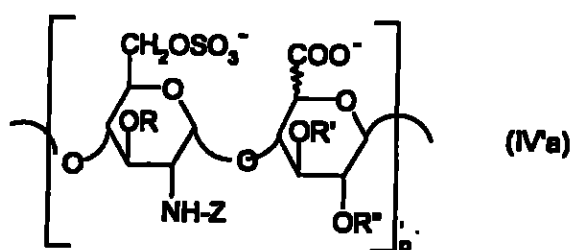
The N-acyl-epiK5-amine-O-oversulfate-derivatives of extremely high degree of sulfation of particular interest are N-acyl-LMW-epiK5-amine-O-oversulfates consisting of a chain mixture in which at least 90% of said chains have the formula IV'



in which q is a integer from 2 to 20, R , R' and R'' represent hydrogen or an SO_3 group for a degree of sulfation from 3.55 to 4, Z is $(\text{C}_2\text{-C}_4)\text{acyl}$, and the corresponding cation is one chemically or pharmaceutically acceptable ion.

Chain mixtures of formula IV' in which the uronic units are 40-60% comprised, preferably 50-55%, of iduronic acid, R is at least 40%, advantageously 50-80%, preferably approximately 65% SO_3 , R' and R'' are both SO_3 or one is hydrogen and the other is 5-10% SO_3 in glucuronic acid and 10-15% SO_3 in iduronic acid, q is a integer from 3 to 15, with a mean molecular weight from approximately 4,500 to approximately 9,000 and the corresponding cation is chemically or pharmaceutically acceptable are preferred.

Other particularly interesting N-acyl-LMW-epiK5-amine-O-oversulfat s are those of very low molecular weight, consisting of a chain mixture in which the preponderant species is a compound of formula IV'a

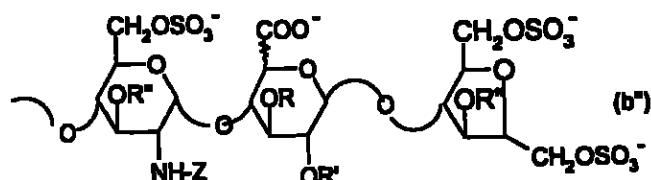


in which p is a integer from 4 to 8, R , R' and R'' are hydrogen or an SO_3 group for a degree of sulfation from 3.55 to 4, Z is $(\text{C}_2\text{-C}_4)\text{acyl}$, and the corresponding cation is one chemically or pharmaceutically acceptable ion.

The origin of the new N-acyl-LMW-epiK5-amine-O-oversulfat s from

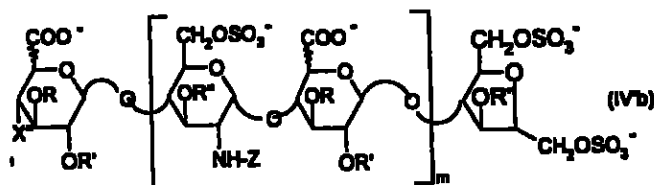
LMW-epiK5-sulfates obtained by nitrous depolymerization and subsequent reduction with, for example, sodium borohydride, involves, at the reducing end of the majority of the chains in said chain mixture, the presence of a sulfated 2,5-anhydromannitol unit of structure (a') defined above, in which R'' represents hydrogen or SO₃.

Thus, the reducing end of the majority of the chains in said chain mixture is represented by the structure (b'')



in which Z represents (C₂-C₄)acyl and the uronic unit can be glucuronic or iduronic.

Among the aforesaid new N-acyl-LMW-epiK5-amine-O-oversulfates, are preferred those consisting of mixtures in which the preponderant species is a compound of formula IV'b



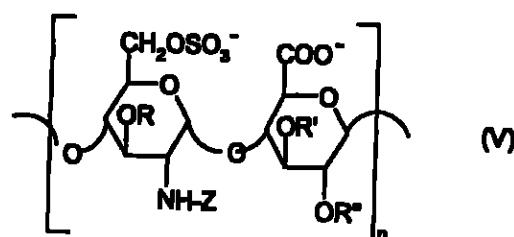
in which R, R' and R'' are hydrogen or SO₃, Z is (C₂-C₄)acyl, X'' is OH or OSO₃, m is 4, 5 or 6, for a degree of sulfation from 3.55 to 4, the uronic units are present alternately, starting with a glucuronic or iduronic unit, and the corresponding cation is one chemically or pharmaceutically acceptable ion.

Said cations are advantageously those of alkaline metals, alkaline-earth metals, of ammonium, (C₁-C₄)tetraalkylammonium, aluminum and zinc and, among these, preferably the salts of sodium, calcium and tetrabutylammonium.

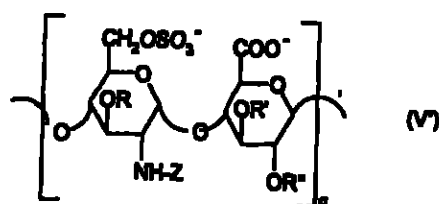
In all the N-acyl-epiK5-amine-O-oversulfates shown above, in

particular those of formula IV, IV', IV'a, IV'b, as also in the structure b'', the acyl group (Z) is preferably a (C₂-C₄)acyl selected among the group consisting of acetyl, (2-carboxy)acetyl, (2-methoxycarbonyl)acetyl, (2-ethoxycarbonyl)acetyl, propionyl, (3-carboxy)propionyl, N-(3-methoxycarbonyl)propionyl and (3-ethoxycarbonyl)propionyl.

According to another of its aspects, the invention provides new N-acyl-K5-amine-O-oversulfate-derivatives consisting of chain mixtures in which at least 90% of said chains have the formula V



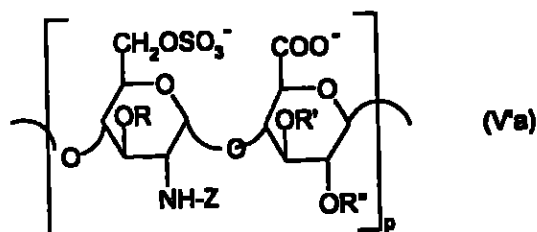
in which n is a integer from 2 to 100, preferably from 3 to 100, Z is (C₂-C₄)acyl, R, R' and R'' are hydrogen or SO₃⁻, the degree of sulfation is at least 2.2, advantageously from 2.2 to 3 or from 2.3 to 3, more advantageously from 2.5 to 3, preferably from 2.7 to 2.9 and the corresponding cation is chemically or pharmaceutically acceptable. Particularly interesting are new N-acyl-LMW-K5-amine-O-oversulfates consisting of chain mixtures in which at least 90% of said chains have the formula V'



in which q is a integer from 2 to 20, Z is (C₂-C₄)acyl, R, R' and R'' represent hydrogen or an SO₃⁻ group for a sulfation degree of at least 2.2, advantageously from 2.2 to 3 or from 2.3 to 3, more

advantageously from 2.5 to 3, preferably from 2.7 to 2.9 and the corresponding cation is one chemically or pharmaceutically acceptable ion.

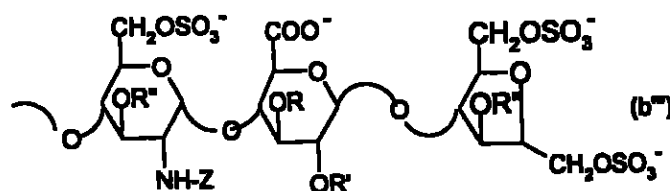
Among these N-acyl-LMW-K5-amine-O-oversulfates are preferred those consisting of a chain mixture in which the preponderant species has the formula V'a



in which p is a integer from 4 to 8, Z is (C_2-C_4) acyl, R, R' and R'' are as defined above, the degree of sulfation is at least 2.2, advantageously from 2.2 to 3 or from 2.3 to 3, more advantageously from 2.5 to 3, preferably from 2.7 to 2.9 and the corresponding cation is chemically or pharmaceutically acceptable.

The origin of the new N-acyl-LMW-K5-amine-O-oversulfates from LMW-K5-sulfates obtained by nitrous depolymerization and subsequent reduction with, for example, sodium borohydride, involves, at the reducing end of the majority of the chains in said chain mixture, the presence of a sulfated 2,5-anhydromannitol unit of structure (a') as shown above, in which R'' represents hydrogen or SO_3^- .

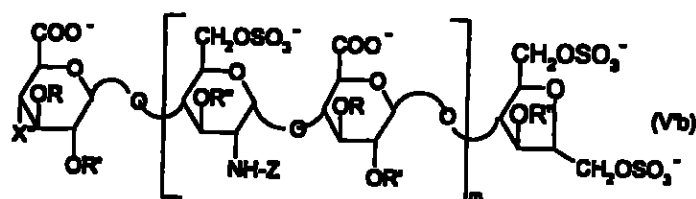
Thus, the reducing end of the majority of the chains in said chain mixture is represented by the structure (b''')



in which Z, R, R' and R'' are as defined above.

Among the aforesaid new N-acyl-LMW-K5-amine-O-oversulfates, are

preferred those consisting of mixtures in which the preponderant species is a compound of formula V'b



in which Z is (C₂-C₄)acyl, R, R' and R'' are hydrogen or SO₃⁻, X'' is OH or OSO₃⁻, for a sulfation degree of at least 2.2, advantageously from 2.2 to 3 or from 2.3 to 3, more advantageously from 2.5 to 3, preferably from 2.7 to 2.9, m is 4, 5 or 6 and the corresponding cation is one chemically or pharmaceutically acceptable ion.

In all the N-acyl-K5-amine-O-oversulfates shown above, in particular those of formula V, V', V'a, V'b, as also in the structure b'', the acyl group (Z) is preferably a (C₂-C₄)acyl selected among the group consisting of acetyl, (2-carboxy)acetyl, (2-methoxycarbonyl)acetyl, (2-ethoxycarbonyl)acetyl, propionyl, (3-carboxy)propionyl, N-(3-methoxycarbonyl)propionyl and (3-ethoxycarbonyl)propionyl.

Particularly interesting are the N-acyl-K5-amine-O-oversulfates shown above in which Z is a (C₂-C₄)acyl different from acetyl. Also interesting and particularly active are the N-acyl-K5-amine-O-oversulfates shown above, in which Z is acetyl, having a degree of sulfation of 2.7-2.9, preferably of approximately 2.8.

The new N-acyl-(epi)K5-amine-O-oversulfate-derivatives, especially in their salts form, are highly anionic products able to capture the free radicals. These can be used in the cosmetics industry as coadjuvants against hair loss or to prepare "anti-ageing" creams, but are above all useful in the pharmaceutical industry, as products for the treatment of dermatitis and as microbicides.

Thus, according to one of its additional aspects, the present invention

provides pharmaceutical compositions including, as one of their active ingredients, a pharmacologically active amount of an N-acyl-(epi)K5-amine-O-oversulfate-derivative as shown above or of one of its pharmaceutically acceptable salts, in mixture with a pharmaceutical excipient.

In the pharmaceutical compositions of the present invention for oral, subcutaneous, intravenous, transdermal or topical administration, the active ingredients are preferably administered in the form of dosage units, in mixture with the classic pharmaceutical excipients or vehicles. The dose regimen can vary widely depending on the age, the weight and health condition of the patient. This dose regimen includes the administration of a dose from 1 to 1000 mg, advantageously from 10 to 750 mg, preferably 250 to 500 mg from one to three times a day by intravenous, subcutaneous, oral, transdermal or topical administration. The present invention also provides a cosmetic composition including an effective amount of an N-acyl-(epi)K5-amine-O-oversulfated-derivative or one of its pharmaceutically acceptable salts, in mixture with a cosmetic excipient.

Besides, the present invention provides a pharmaceutical composition including, as one of its active ingredients, an (epi)K5-amine-O-oversulfate-derivative obtainable according to steps (a) and (b) of the process described above, or one of its pharmaceutically acceptable salts, in mixture with a pharmaceutical excipient. Advantageously, said (epi)K5-amine-O-oversulfate-derivative is consisting of a chain mixture in which at least 90% of said chains have the formula II, II', III or III' or in which the preponderant species is a compound of formula IIa, II'a, III'a or III'b. Preferred active ingredient is a 1mw-K5-amine-O-oversulfate having a degree of sulfation from 2.2 to 3, advantageously having a mean molecular weight from

approximately 3,500 to approximately 11,000, more advantageously from approximately 3,500 to approximately 5,200 and basically free of N-acetyl and N-sulfate groups.

In the pharmaceutical compositions of the present invention for oral, subcutaneous, intravenous, transdermal or topical administration, the active ingredients (epi)K5-amine-O-oversulfate-derivatives are preferably administered in the form of dosage units, in mixture with the classic pharmaceutical excipients or vehicles. The dose regimen can vary widely depending on the age, the weight and health condition of the patient. This dose regimen includes the administration of a dose of an (epi)K5-amine-O-oversulfate-derivative from 1 to 1000 mg, advantageously from 10 to 750 mg, preferably 250 to 500 mg from one to three times a day by intravenous, subcutaneous, oral, transdermal or topical administration.

The pharmaceutical compositions including an (epi)K5-amine-O-oversulfate-derivative such as those shown above are formulated with the classic excipients suitable for different ways of administration. Particularly advantageous are the formulations in the form of creams, ointments, liniments, gels, foams, balsams, vaginal pessaries, suppositories, solutions or suspensions suitable for local administration. Finally, the present invention provides a pharmaceutical composition containing, as one of its active ingredients, a pharmacologically active amount of a low-molecular-weight-(epi)K5-N-sulfate, i.e. of a low-molecular-weight-K5-N-sulfate or of a low-molecular-weight-epiK5-N-sulfate as shown above or of one of their pharmaceutically acceptable salts, in mixture with a pharmaceutical excipient.

In the pharmaceutical compositions of the present invention for oral, subcutaneous, intravenous, transdermal or topical administration, the active ingredients are preferably administered in the form of dosage

units, in mixture with the classic pharmaceutical excipients or vehicles. The dose regimen can vary widely depending on the age, the weight and health condition of the patient. This dose regimen includes the administration of a dose from 1 to 1000 mg, advantageously from 10 to 750 mg, preferably 250 to 500 mg from one to three times a day by intravenous, subcutaneous, oral, transdermal or topical administration. According to another of its aspects, the present invention also provides a cosmetic composition containing, as one of its active ingredients, an effective amount of a lmw-K5-N-sulfate or LMW-epiK5-N-sulfate or of one of its pharmaceutically acceptable salts, in mixture with a cosmetic excipient.

Advantageously, said LMW-(epi)K5-N-sulfate-derivative is consisting of a chain mixture in which at least 90% of said chains have the formula I, or I', or in which the preponderant species is a compound of formula I'a, or I'b. Preferred active ingredient is a lmw-(epi)K5-N-sulfate-derivative having a mean molecular weight from approximately 1,000 to approximately 12,000, advantageously from approximately 1,500 to approximately 8,000, preferably from approximately 1,500 to approximately 7,500 and basically free of N-acetyl groups.

A salt selected from the group consisting of salts of alkaline metals or alkaline-earth metals, of ammonium, (C₁-C₄)tetraalkylammonium, aluminum or zinc, in particular the salt of sodium, potassium, calcium, magnesium, aluminum or zinc constitutes an effective active ingredient of the compositions of the present invention.

The following examples illustrate the invention without however limiting it.

PREPARATION I

LMW-epiK5-N-sulfate

a) Epimerization of K5-N-sulfate

2 g of K5 N-sulfate, obtained as described in Example 1, steps (i) and (ii), of WO 02/068477, are dissolved in 120 ml of 25 mM HEPES buffer, pH 7, containing 50 mM CaCl_2 . The solution obtained is made to recirculate through a 50 ml column filled with the resin containing the immobilized enzyme obtained as described in WO 96/14425. This operation is carried out at 30°C with a flow of 200 ml/h for 24 hours. The product obtained is purified through ultrafiltration over a 1000 D membrane and passage over an ionic exchange column IR 120 H^+ , neutralizing the eluate with 1N NaOH. The sample is recovered by precipitation with ethanol or acetone.

An epimerized product is obtained with an iduronic 'acid/glucuronic acid ratio of 55/45 against a ratio of 0 /100 of the starting product. The percentage of epimerization was calculated with ^1H -RMN according to the method described in WO 96/14425. The yield, calculated by measuring the content of uronic acids against a standard with the carbazole method (Bitter and Muir Anal. Biochem. 39, 88-92-1971) is 90%.

b) Depolymerization of epi-K5-N-sulfate

1 g of product obtained in step (a) is depolymerized by the degradation method with nitrous acid and subsequent reduction of the aldehyde which forms. In particular one continues by dissolving the product in 25 ml of distilled water and adding it with 230 mg of sodium nitrite dissolved in 115 ml of distilled water. The solution is then brought to 4°C and the pH to 2 with 0.1 N HCl and maintained for 30 minutes. At the end of the reaction the solution is brought to room temperature and the pH to 7 with 0.1 M NaOH. The solution is then added with 450 mg. of NaBH_4 and reacted for 4 hours. The product is recovered by precipitation with 3 volumes of acetone at 4°C, filtration with filtering funnel and dried at 40° C in a vacuum oven. 900 mg of

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LMW-epiK5-N-sulfate are obtained with a molecular weight distribution measured with HPLC method which ranges from 1,000 to 4,000 and with a glucuronic unit content of 45% and iduronic unit content of 55%.

PREPARATION II

LMW-K5-N-sulfate

The product obtained as described in Example 1, steps (i) and (ii), of WO 02/068477 is depolymerized by the degradation method with nitrous acid and subsequent reduction of the aldehyde which forms. One continues by dissolving 1 g of K5-N-sulfate in 200 ml of distilled water and adding it with 480 mg of sodium nitrite dissolved in 240 ml of distilled water. The solution is then brought to 4°C and the pH to 2 with 0.1 N HCl and maintained for 30 minutes. At the end of the reaction the solution is brought to pH 7 with 0.1 M NaOH and then to room temperature. The solution is then added with 450 mg. of NaBH₄ and reacted for 4 hours. The excess NaBH₄ is eliminated with HCl bringing the pH to 5-6. The product, neutralized with 0.1 M NaOH, is recovered by precipitation with 3 volumes of acetone at 4°C, filtration with filtering funnel and dried at 40° C in a vacuum oven. 900 mg of LMW-K5-N-sulfate are obtained with a mean molecular weight of approximately 2,000, consisting of a chain mixture in which the preponderant species is a compound of formula I'b in which m is 4 and the uronic units are those of glucuronic acid.

PREPARATION III

(a) Depolymerization of K5-N-sulfate

2 g of K5-N-sulfate obtained as described in Example 1, steps (i) and (ii), of WO 02/068477 is depolymerized as described in PREPARATION II, utilizing 100 mg of sodium nitrite and 300 mg. of sodium borohydride. 1.8 g. of LMW-K5-N-sulfate are obtained with a mean molecular weight of 5,000

(b) Epimerization of LMW-K5-N-sulfate

1 g of LMW-K5 N-sulfate obtained in step (a) is treated as described in step (a) of the Example 1. An epimerized product is obtained with an iduronic acid/glucuronic acid ratio of 44/56 against a ratio of 0/100 of the starting product, with a molecular weight distribution from 2,000 to 10,000 and with a mean molecular weight of 5,000 D. The yield, calculated by measuring the content of uronic acids against a standard with the carbazole method (Bitter and Muir Anal. Biochem. 39, 88-92-1971) is 90%.

PREPARATION IV

Preparation of an epiK5-N-sulfate

A solution of 10 g of K5-N-sulfate obtained as described in Example 1, steps (i) and (ii), of WO 02/068477 in 600 ml of 25 mM HEPES buffer at pH 7, containing CaCl_2 at a concentration of 50 mM is made to recirculate through a 50 ml column filled with Sepharose 4B resin containing 5 g of recombinant C5-epimerase (WO 96/14425) immobilized as described in WO 01/72848. The reaction is carried out at 30°C at pH 7 with a flow of 200 ml/h for 24 hours. The product obtained is purified by ultrafiltration and precipitation with ethanol. Thus an epiK5-N-sulfate is obtained whose iduronic acid content is 54%.

PREPARATION V

Preparation of a lmw-epiK5-N-sulfate

1 g of product obtained in PREPARATION IV is depolymerized by the degradation method with nitrous acid and subsequent reduction of the aldehyde which forms. In particular one continues by dissolving the product in 25 ml of distilled water and adding it with 230 mg of sodium nitrite dissolved in 115 ml of distilled water. The solution is then brought to 4°C and the pH to 2 with 0.1 N HCl and maintained for 30

minutes. At the end of the reaction the solution is brought to room temperature and the pH to 7 with 0.1 M NaOH. The solution is then added with 450 mg. of NaBH₄ and reacted for 4 hours. The product is recovered by precipitation with 3 volumes of acetone at 4°C, filtration with filtering funnel and dried at 40° C in a vacuum oven. 900 mg of LMW-epiK5-N-sulfate are obtained with a molecular weight distribution measured with HPLC method which ranges from 1,000 to 4,000.

EXAMPLE 1

EpiK5-amine-O-oversulfate

(a) Tetrabutylammonium salt of epi K5-N-sulfate

A solution of 400 mg of epiK5-N-sulfate with an iduronic acid content of 54% ds obtained in PREPARATION IV in 40 ml of water is thermostated at 4°C, then passed over an ionic exchange resin IR 120 H⁺ preconditioned with water at 4°C. The eluate obtained, consisting of 100 ml of a solution at pH 1.94, is neutralized with a solution of 15% tetrabutylammonium hydroxide and left at room temperature for one hour, maintaining the pH at 7 by addition of 15% tetrabutylammonium hydroxide and finally is lyophilized. Thus 805 mg of tetrabutylammonium salt of epiK5-N-sulfate are obtained.

(b) Epi-K5-amine-O-oversulfate

To a solution containing the 805 mg of the salt thus obtained in 30 ml of dimethylformamide is set at 55°C and treated with 30 ml of dimethylformamide containing 2.26 g of pyridine.SO₃ adduct. The reaction at 55°C is continued overnight then 60 ml of water are added to the mixture. After neutralization with 1N NaOH, the product is precipitated with 3 volumes of acetone saturated with NaCl and set at 4°C overnight. The precipitate is recovered by filtration on guch G4 and then ultrafiltered with 1000 D Millipore TFF system and dried at reduced pressure. 550 mg of epi-K5-amine-O-oversulfated are

obtained having a content of iduronic acid of 54%, of glucosamin -6-O-sulfate of 100%, of glucosamine 3-O-sulfate of 60%, of monosulfate glucuronic acid of 10%, of monosulfate iduronic acid of 15%, the rest of the uronic units being disulfated, with a sulfation degree of 3.55 measured with the conductometric method according to Casu et al. 1975.

EXAMPLE 2

N-Acetyl-epiK5-amine-O-oversulfate

To a solution of 250 mg of epi-K5-amine-O-oversulfate obtained in Example 1 in 75 ml of water and cooled to 4°C are added 7.5 ml of methanol and 3.75 ml of acetic anhydride. The reaction is kept at 4°C for 2 hours maintaining the pH at a value of 7 with 5M NaOH. After ultrafiltration on 1,000 D membrane are added 3 volumes of acetone saturated with sodium chloride and the precipitate is recovered by centrifugation at 5,000 rpm for 5'. Thus 249 mg of N-acetyl-epiK5-amine-O-oversulfated are obtained with an iduronic acid content of 54%, glucosamine-6-O-sulfate of 100%, N-acetyl of 100%, glucosamine 3-O-sulfate of 60%, monosulfate glucuronic acid of 10%, monosulfate iduronic acid of 15%, the rest of the uronic units being disulfated, and whose sulfation degree is 3.5 measured with the conductometric method according to Casu et al. 1975.

EXAMPLE 3

N-acetyl-K5-amine-O-oversulfate

1 gr of K5 N,O oversulfate obtained in Example 2 of WO/02068477 is dissolved in 200 ml of water and the solution is brought to pH 2 with 1 N HCl and thermostated at 50°C. The reaction is kept at 50 °C for 3 hours and then cooled to room temperature and neutralized with 1N NaOH. The sample is then cooled to 4°C and 20 ml of MeOH and 10 ml of acetic anhydride are added to the reaction which is left at 4°C for 2

hours maintaining the pH constantly at 7. At the end of the reaction the sample is purified from salts by ultrafiltration on a membrane with a 1000 D cut off and then recovered by dried at reduced pressure. A 100% N-acetylated product is obtained, 100% 6-O sulfate, 30% monosulfate and 70% disulfate on the glucuronic acid is obtained.

CLAIMS

1. Process for the preparation of N-acyl-(epi)K5-amine-O-oversulfate-derivatives and of its chemically or pharmaceutically acceptable salts, characterized in that
 - (a) an (epi)K5-N-sulfate-derivative, in acidic form, is treated with a tertiary or quaternary organic base, letting the reaction mixture to stand for a time period of 30-60 minutes, maintaining the pH of the solution at a value of approximately 7 and its salt is isolated with said organic base;
 - (b) said salt of organic base of said (epi)K5-N-sulfate-derivative is treated with an O-sulfation reagent in the conditions of O-oversulfation;
 - (c) the (epi)K5-amine-O-oversulfate-derivative thus obtained is treated with a functional derivative of a (C₂-C₄) carboxylic acid, the N-acyl-(epi)K5-amine-O-oversulfate-derivative is isolated.
2. Process according to claim 1, characterized in that said N-acyl-(epi)K5-N,O-oversulfate-derivative is isolated in sodium salt form and optionally transformed into another chemically or pharmaceutically acceptable salt.
3. Process according to anyone of claims 1 and 2, characterized in that, in step (a) tetrabutylammonium hydroxide is used as an organic base.
4. Process according to anyone of claims from 1 to 3, characterized in that, in step (b) the O-oversulfation is carried out in dimethylformamide utilizing 2-4 moles of O-sulfation reagent per available OH per disaccharide at a temperature of 40-60°C for 15-20 hours.
5. Process according to anyone of claims from 1 to 4, characterized in that as starting material an (epi)K5-N-sulfate-derivative is used having

a mean molecular weight from approximately 1,000 to approximately 25,000.

6. Process according to anyone of claims from 1 to 5, characterized in that said starting material is 40-60% C5-epimerized.

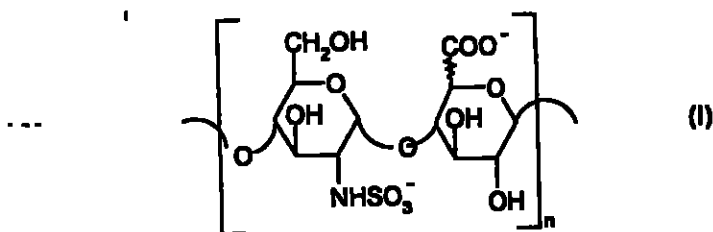
7. Process according to anyone of claims from 1 to 6, characterized in that said starting material has a mean molecular weight from approximately 1,500 to approximately 25,000.

8. Process according to claim 7, characterized in that said starting material has a mean molecular weight between 10,000 and 25,000.

9. Process according to claim 7, characterized in that said starting material has a mean molecular weight from approximately 1,500 to approximately 12,000.

10. Process according to claim 9, characterized in that said starting material has a mean molecular weight from approximately 1,500 to approximately 8,000.

11. Process according to claim 5, characterized in that as starting material an (epi)K5-N-sulfate-derivative is used consisting of a chain mixture in which at least 90% of said chains have the formula I



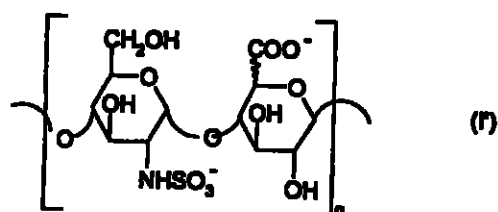
in which the glucuronic units/iduronic units ratio is from 100/0 to 40/60, n is a integer from 2 to 100 and the corresponding cation is chemically or pharmaceutically acceptable.

12. Process according to claim 11, characterized in that said starting material is consisting of a chain mixture in which at least 90% of said chains have the formula I, in which the uronic units are 40-60%

consisting of iduronic acid.

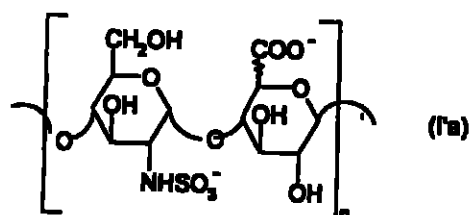
13. Process according to claim 11, characterized in that said starting material is a lmw-(epi)K5-N-sulfate consisting of a chain mixture in which at least 90% of said chains have the formula I in which the uronic units are all consisting of glucuronic acid or are 40-60% consisting of iduronic acid, n is a integer from 3 to 15 and the corresponding cation is chemically acceptable.

14. Process according to claim 11, characterized in that said starting material is a lmw-(epi)K5-N-sulfate consisting of a chain mixture in which at least 90% of said chains have the formula I'



in which the uronic units are 100% consisting of glucuronic acid or 60-40% of glucuronic acid and 40-60% of iduronic acid, q is a integer from 2 to 20 and the corresponding cation is chemically or pharmaceutically acceptable.

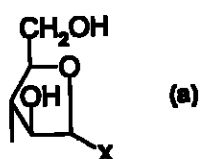
15. Process according to claim 11, characterized in that said starting material is a lmw-(epi)K5-N-sulfate consisting of a chain mixture in which the preponderant species has the formula I'a



in which the uronic units are 100% consisting of glucuronic acid or 60-40% glucuronic and 40% to 60% of iduronic acid, p is a integer from 4 to 8.

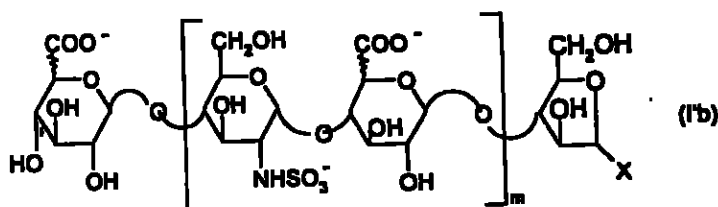
16. Process according to anyone of claims from 5 to 15, characterized in that said starting material is a lmw-(epi)K5-N-sulfate obtained by nitrous depolymerization of the corresponding (epi)K5-N-sulfate and subsequent reduction.

17. Process according to claim 16, characterized in that said starting LMW-(epi)K5-N-sulfate contains, at the reducing end of the majority of the chains in said chain mixture, a 2,5-anhydromanno unit of structure (a)



in which X' represents a hydroxymethyl group.

18. Process according to anyone of claims 16 and 17, characterized in that as starting material a lmw-(epi)K5-N-sulfate is used consisting of chain mixtures in which the preponderant species is a compound of formula I'b

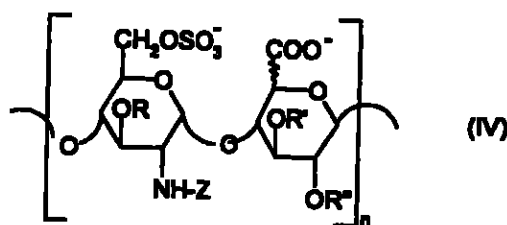


in which X is hydroxymethyl, m is 4, 5 or 6, the corresponding cation is one chemically or pharmaceutically acceptable ion, the uronic units are all of glucuronic acid or the glucuronic and iduronic units are present alternately, starting with a glucuronic or iduronic unit.

19. Process according to anyone of claims from 1 to 18, characterized in that said starting (epi)K5-N-sulfate-derivative is utilized in sodium salt form.

20. An N-acyl-(epi)K5-amine-O-oversulfate-derivative obtainable according to the process of claims from 1 to 19.

21. An N-acyl-(epi)K5-amine-O-oversulfate-derivative according to claim 20 in salt form of an alkaline metal or alkaline-earth metal, of ammonium, (C₁-C₄)tetraalkylammonium, aluminum or zinc.
22. An N-acyl-epiK5-amine-O-oversulfate-derivative, in which acyl is a (C₂-C₄)acyl, having an iduronic acid content of 20-60%, a mean molecular weight from approximately 2,000 to approximately 45,000 and a sulfation degree of at least 3.4, or one of its chemically or pharmaceutically acceptable salts.
23. An N-acyl-epiK5-amine-O-oversulfate-derivative according to claim 22, whose mean molecular weight is between approximately 15,000 and approximately 45,000.
24. An N-acyl-epiK5-amine-O-oversulfate-derivative according to claim 22, whose mean molecular weight is between approximately 4,500 and approximately 8,500.
25. An N-acyl-epiK5-amine-O-oversulfate-derivative according to anyone of claims from 22 to 24, characterized in that said degree of sulfation is from 3.4 to 3.8.
26. An N-acyl-epiK5-amine-O-oversulfate-derivative consisting of chain mixtures in which at least 90% of said chains have the formula IV

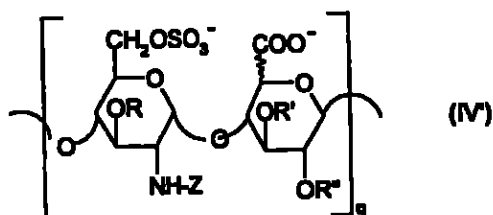


in which the uronic units are 20-60% consisting of iduronic acid, n is a integer from 2 to 100, R, R' and R'' are hydrogen or SO₃, Z is (C₂-C₄)acyl, the degree of sulfation is at least 3.4 and the corresponding cation is chemically or pharmaceutically acceptable.

27. An N-acyl-epiK5-amine-O-oversulfate-derivative according to

claim 26, characterized in that it consists of a chain mixture in which at least 90% of said chains have the formula IV in which the uronic units are 40-60% consisting of iduronic acid, n is a integer from 3 to 100, with a mean molecular weight from approximately 2,000 to approximately 45,000, R is at least 40% SO_3^- , R' and R'' are both SO_3^- or one is hydrogen and the other is 5-10% SO_3^- in monosulfate glucuronic acid and 10-15% SO_3^- in monosulfate iduronic acid and the corresponding cation is chemically or pharmaceutically acceptable.

28. An N-acyl-epiK5-amine-O-oversulfate-derivative according to anyone of claims 26 and 27, characterized in that it is a lmw-N-acyl-epiK5-O-oversulfate consisting of a chain mixture in which at least 90% of said chains have the formula IV'

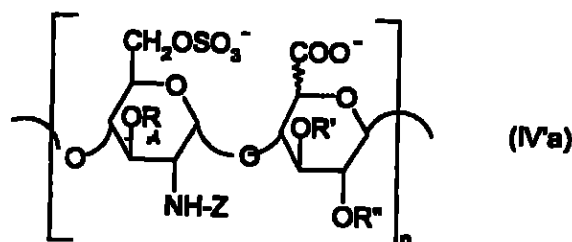


in which q is a integer from 2 to 20, R, R' and R'' represent hydrogen or an SO_3^- group for a degree of sulfation from 3.55 to 4, Z is $(\text{C}_2\text{-C}_4)\text{acyl}$, and the corresponding cation is chemically or pharmaceutically acceptable.

29. A lmw-N-acyl-epiK5-O-oversulfate according to claim 28, characterized in that in said chain mixture of formula IV' the uronic units are 50-55% consisting of iduronic acid, R is at least 40% SO_3^- , R' and R'' are both SO_3^- or one is hydrogen and the other is 5-10% SO_3^- in glucuronic acid and 10-15% SO_3^- in iduronic acid, q is a integer from 3 to 15, with a mean molecular weight from approximately 4,500 to approximately 9,000 and the corresponding cation is chemically or pharmaceutically acceptable.

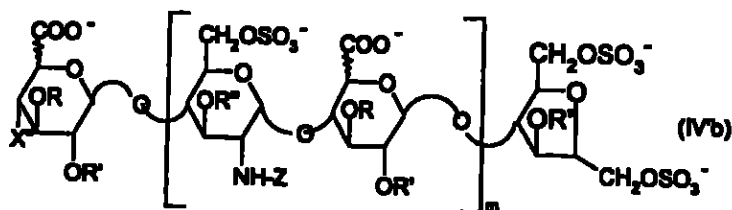
30. A lmw-N-acyl-epiK5-O-oversulfate according to anyone of claims

28 and 29, characterized in that it consists of a chain mixture in which the preponderant species is a compound of formula IV'a



in which p is a integer from 4 to 8, R, R' and R'' are hydrogen or an SO₃⁻ group for a degree of sulfation from 3.55 to 4, Z is (C₂-C₄)acyl, and the corresponding cation is chemically or pharmaceutically acceptable.

31. A 1mw-N-acyl-epiK5-O-oversulfate according to claim 30, characterized in that said preponderant species is a compound of formula IV'b



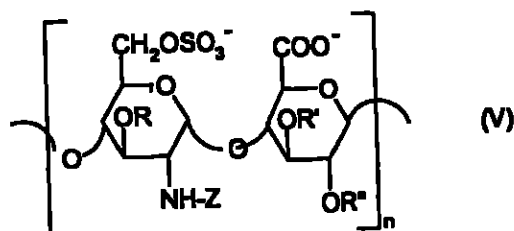
in which R, R' and R'' are hydrogen or SO₃⁻, Z is (C₂-C₄)acyl, X is OH or OSO₃⁻, m is 4, 5 or 6, for a degree of sulfation from 3.55 to 4, the uronic units are present alternately, starting with a glucuronic or iduronic unit, and the corresponding cation is chemically or pharmaceutically acceptable.

32. A 1mw-N-acyl-epiK5-O-oversulfate according to any of claims from 22 to 31 in which the substituent (C₂-C₄)acyl is selected from the group consisting of acetyl, (2-carboxy)acetyl, (2-methoxycarbonyl)acetyl, (2-ethoxycarbonyl)acetyl, propionyl, (3-carboxy)propionyl, N-(3-methoxycarbonyl)propionyl and (3-ethoxycarbonyl)propionyl.

33. An N-acyl-epiK5-amine-O-oversulfate-derivative according to anyone of claims from 22 to 32, characterized in that said salt or

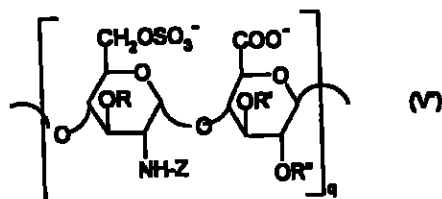
corresponding cation is an alkaline metal or alkaline-earth metal, of ammonium, (C₁-C₄)tetraalkylammonium, aluminum or zinc.

34. An N-acyl-K5-amine-O-oversulfate-derivative consisting of a chain mixture in which at least 90% of said chains have the formula V



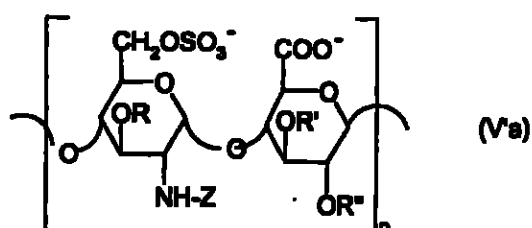
in which n is a integer from 2 to 100, Z is (C₂-C₄)acyl, R , R' and R'' are hydrogen or SO₃, the degree of sulfation is at least 2.2, and the corresponding cation is chemically or pharmaceutically acceptable.

35. An N-acyl-K5-amine-O-oversulfate-derivative according to claim 34, characterized in that it is a lmw-N-acyl-K5-amine-O-oversulfate consisting of a chain mixture in which at least 90% of said chains have the formula V'



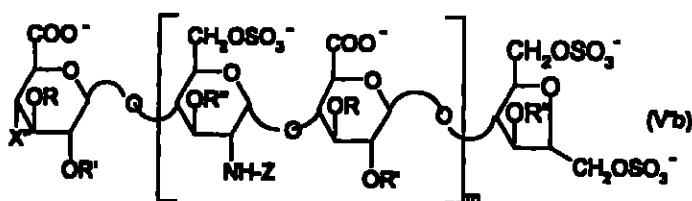
in which q is a integer from 2 to 20, Z is (C₂-C₄)acyl, R , R' and R'' represent hydrogen or an SO₃ group for a degree of sulfation from 2.2 to 3, and the corresponding cation is chemically or pharmaceutically acceptable.

36. A lmw-N-acyl-K5-amine-O-oversulfate according to claim 35, characterized in that it consists of a chain mixture in which the preponderant species is a compound of formula V'a



in which p is a integer from 4 to 8, Z is (C_2-C_4) acyl, R , R' and R'' represent hydrogen or an SO_3^- group for a degree of sulfation from 2.2 to 3, and the corresponding cation is chemically or pharmaceutically acceptable.

37. A lmw-N-acyl-K5-amine-O-oversulfate according to claim 36, characterized in that said preponderant species is a compound of formula V'b



in which Z is (C_2-C_4) acyl, R , R' and R'' are hydrogen or SO_3^- , X'' is OH or OSO_3^- , for a degree of sulfation from 2.2 to 3, m is 4, 5 or 6 and the corresponding cation is one chemically or pharmaceutically acceptable ion.

38. An N-acyl-K5-amine-O-oversulfate-derivative according to anyone of claims from 34 to 37, characterized in that said degree of sulfation is from 2.3 to 3.

39. An N-acyl-K5-amine-O-oversulfate-derivative according to claim 38, characterized in that said degree of sulfation is from 2.5 to 3.

40. An N-acyl-K5-amine-O-oversulfate-derivative according to claim 39, characterized in that said degree of sulfation is from 2.7 to 2.9.

41. An N-acyl-K5-amine-O-oversulfate-derivative according to anyon of claims from 34 to 40, in which the substituent (C_2-C_4) acyl is different

from acetyl.

42. An N-acyl-K5-amine-O-oversulfate-derivative according to anyone of claims from 34 to 40, in which the substituent (C₂-C₄)acyl is acetyl, having a degree of sulfation of 2.7-2.9.

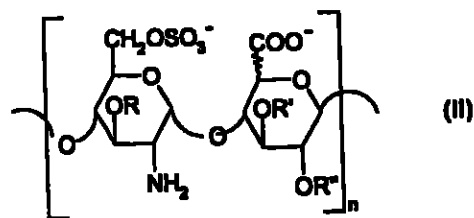
43. An N-acyl-K5-amine-O-oversulfate-derivative according to claim 42, having a degree of sulfation of approximately 2.8.

44. An N-acyl-K5-amine-O-oversulfate-derivative according to anyone of claims from 34 to 43, characterized in that said salt or corresponding cation is an alkaline metal or alkaline-earth metal, of ammonium, (C₁-C₄)tetraalkylammonium, aluminum or zinc.

45. Pharmaceutical composition including, as one of its active ingredients, an (epi)K5-amine-O-oversulfate-derivative or one of its pharmaceutically acceptable salts, obtainable by the process according to steps (a) and (b) of the claim 1, isolated in sodium salt form and optionally transformed into another pharmaceutically acceptable salt, in mixture with a pharmaceutical excipient.

46. Composition according to claim 45, characterized in that said active ingredient is an (epi)K5-amine-O-oversulfate-derivative having a mean molecular weight from approximately 4,500 to approximately 40,000.

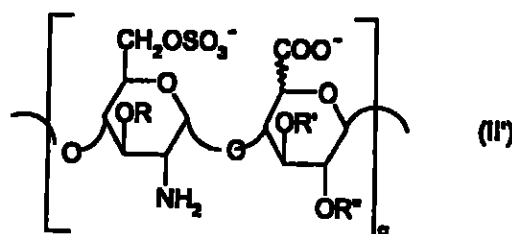
47. Pharmaceutical composition according to anyone of claims 45 and 46, in which said active ingredient is an (epi)K5-amine-O-oversulfate-derivative consisting of a chain mixture in which at least 90% of said chains have the formula II



in which n is a integer from 2 to 100, R, R' and R'' are hydrogen or SO₃⁻,

the uronic units are all of glucuronic acid, for a degree of sulfation from 2.2 to 3, or are 20-60% consisting of iduronic acid, for a sulfation degree of at least 3.4, and the corresponding cation is pharmaceutically acceptable.

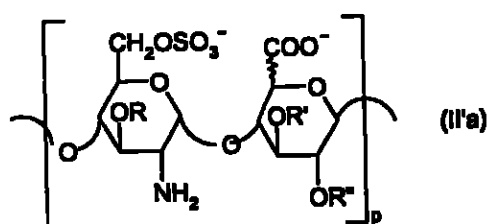
48. Pharmaceutical composition according to claim 47, characterized in that said active ingredient is a LMW-epiK5-amine-O-oversulfate consisting of a chain mixture in which at least 90% of said chains have the formula II'



in which q is a integer from 2 to 20, R , R' and R'' are hydrogen or SO_3^- , the uronic units are 20-60% comprised, preferably 40-60%, of iduronic acid, for a degree of sulfation from 3.55 to 4.

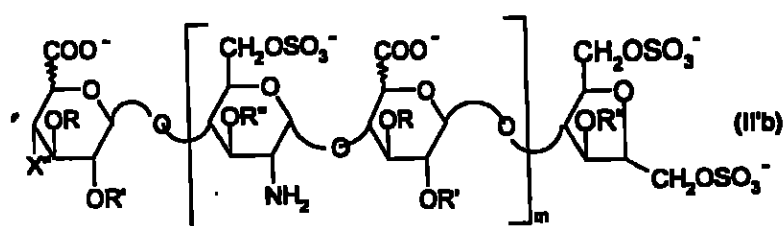
49. Pharmaceutical composition according to claim 48, characterized in that, in said chain mixture of formula II', the uronic units are 40-60% consisting of iduronic acid, R is at least 40% SO_3^- , R' and R'' are both SO_3^- or one is hydrogen and the other is 5-10% SO_3^- in glucuronic acid and 10-15% SO_3^- in iduronic acid, n is a integer from 3 to 15, with a mean molecular weight from approximately 4,000 to approximately 8,000.

50. Pharmaceutical composition according to anyone of claims 48 and 49, characterized in that said LMW-epiK5-amine-O-oversulfate is consisting of a chain mixture in which the preponderant species has the formula II'a



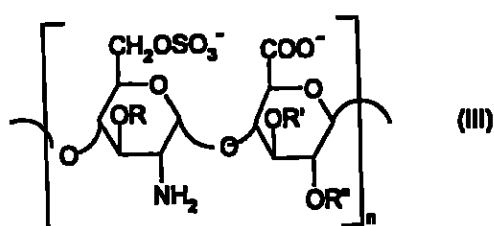
in which p is a integer from 4 to 8, R , R' and R'' are as defined above, the degree of sulfation is from 3.55 to 4 and the corresponding cation is pharmaceutically acceptable.

51. Pharmaceutical composition according to claim 50, characterized in that said preponderant species is a compound of formula II'b



in which R , R' and R'' are hydrogen or SO_3^- , X'' is OH or OSO_3^- , m is 4, 5 or 6, the uronic units are 40-60% consisting of iduronic acid, for a degree of sulfation from 3.55 to 4, the iduronic units being present alternately, starting with a glucuronic or iduronic unit, and the corresponding cation is one pharmaceutically acceptable ion.

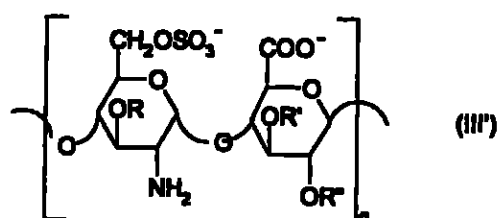
52. Pharmaceutical composition according to claim 45 including, as one of its active ingredients, a K5-amine-O-oversulfate-derivative consisting of a chain mixture in which at least 90% of said chains have the formula III



in which n is a integer from 2 to 100, R , R' and R'' are hydrogen or SO_3^- , the degree of sulfation is at least 2.2, and the corresponding cation is

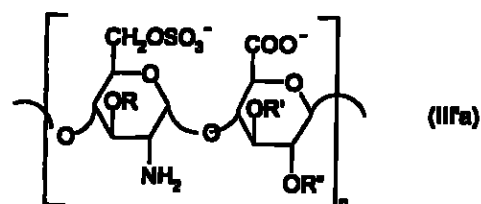
pharmaceutically acceptable.

53. Pharmaceutical composition according to claim 52, characterized in that said active ingredient is a lmw-K5-amine-O-oversulfate consisting of a chain mixture in which at least 90% of said chains have the formula III'



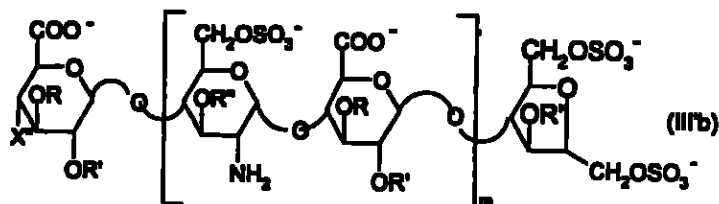
in which q is a integer from 2 to 20, R, R' and R'' represent hydrogen or an SO₃ group for a sulfation degree of at least 2.2.

54. Pharmaceutical composition according to claim 53, characterized in that said LMW-K5-amine-O-oversulfate is consisting of a chain mixture in which the preponderant species has the formula III'a



in which p is a integer from 4 to 8, R, R' and R'' are as defined above, the degree of sulfation is from 2.2 to 3.

55. Pharmaceutical composition according to claim 50, characterized in that said preponderant species is a compound of formula III'b



in which R, R' and R'' are hydrogen or SO₃, X'' is OH or OSO₃, for a degree of sulfation from 2.2 to 3, m is 4, 5 or 6 and the corresponding

cation is one pharmaceutically acceptable ion.

56. Pharmaceutical composition according to anyone of claims from 45 to 55, characterized in that said pharmaceutically acceptable salt or cation is sodium, potassium, calcium, magnesium or zinc.

57. Pharmaceutical composition according to anyone of claims from 45 to 56, characterized in that it is in the form of cream, ointment, liniment, gel, foam, balsam, vaginal pessary, suppository, solution or suspension for local administration.

58. A pharmaceutical composition containing, as one of its active ingredients, a pharmacologically active amount of a lmw-(epi)K5-N-sulfate basically free of acetyl groups, or of one of its pharmaceutically acceptable salts, in mixture with a pharmaceutical excipient.

59. A cosmetic composition containing an effective amount of a lmw-(epi)K5-N-sulfate according to anyone of claims from 1 to 10 and from 25 to 37, in which said salt or cation is pharmaceutically acceptable, in mixture with a cosmetic excipient.

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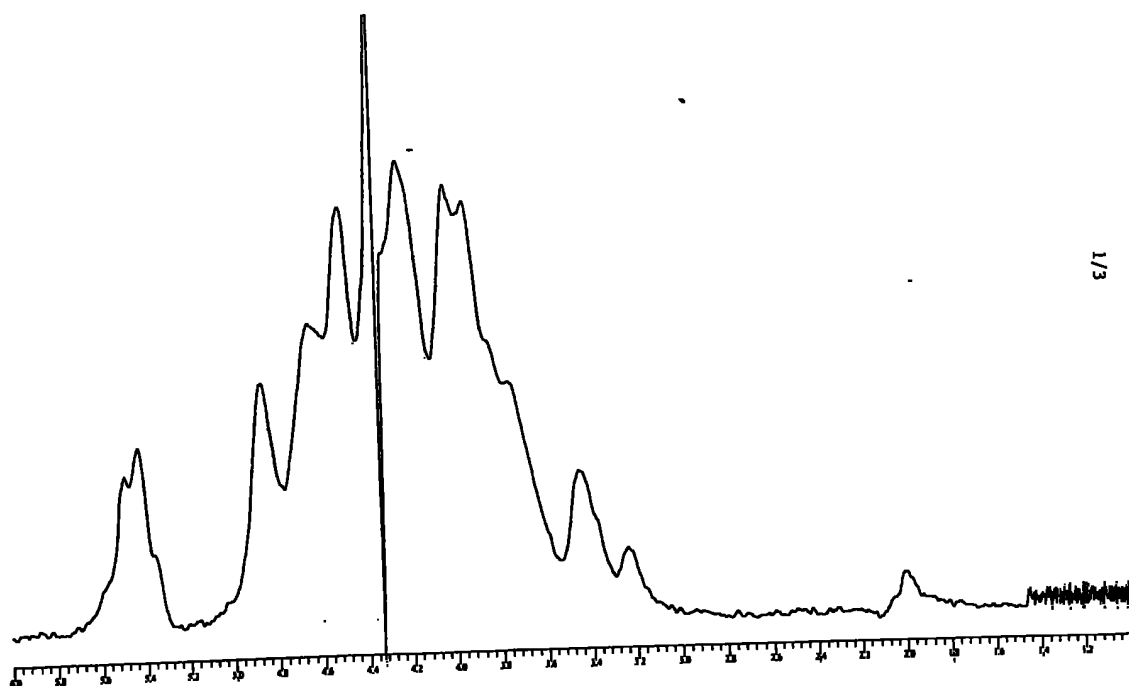


Fig. 1

2/3

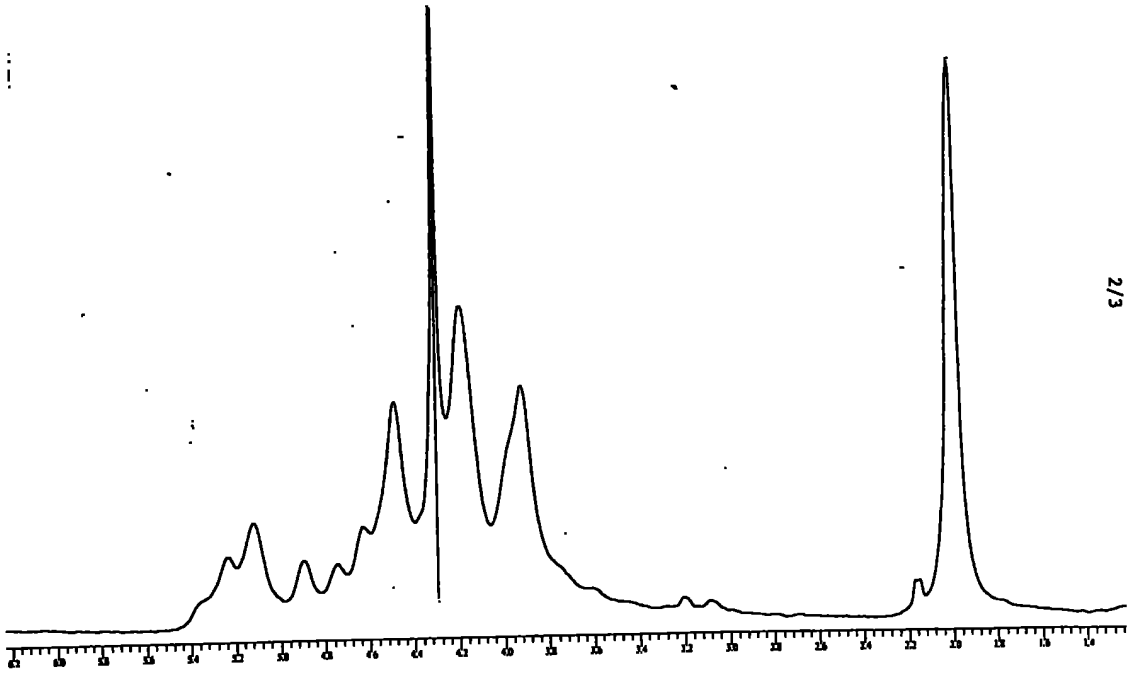


Fig. 2

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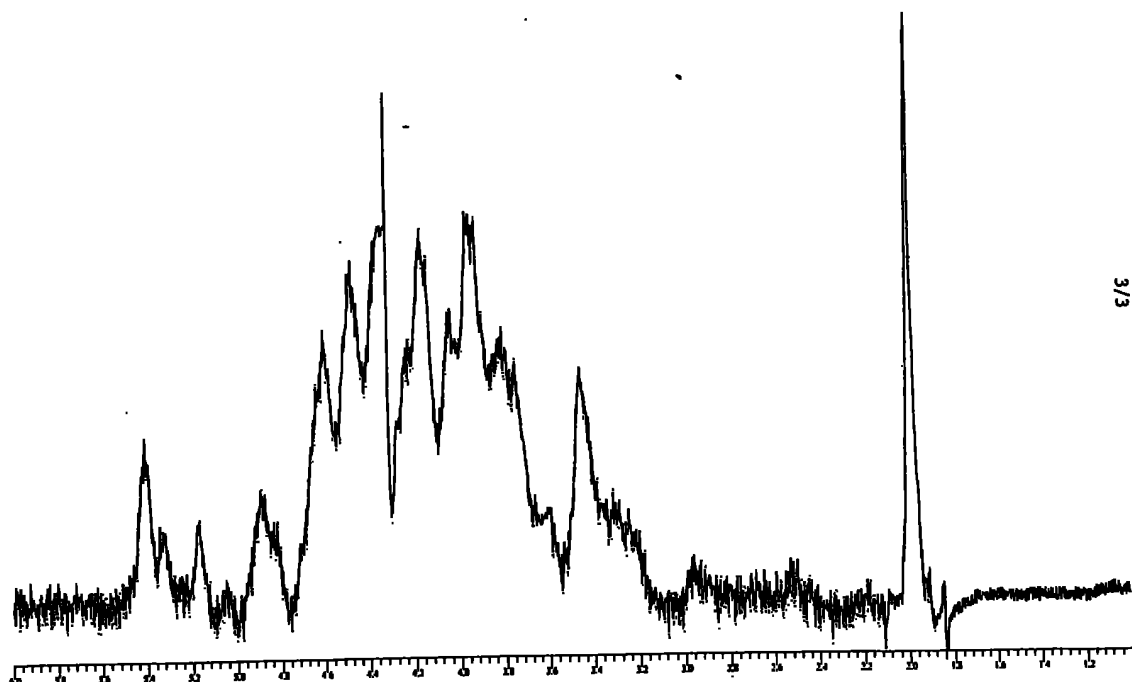


Fig. 3

[Handwritten signature]

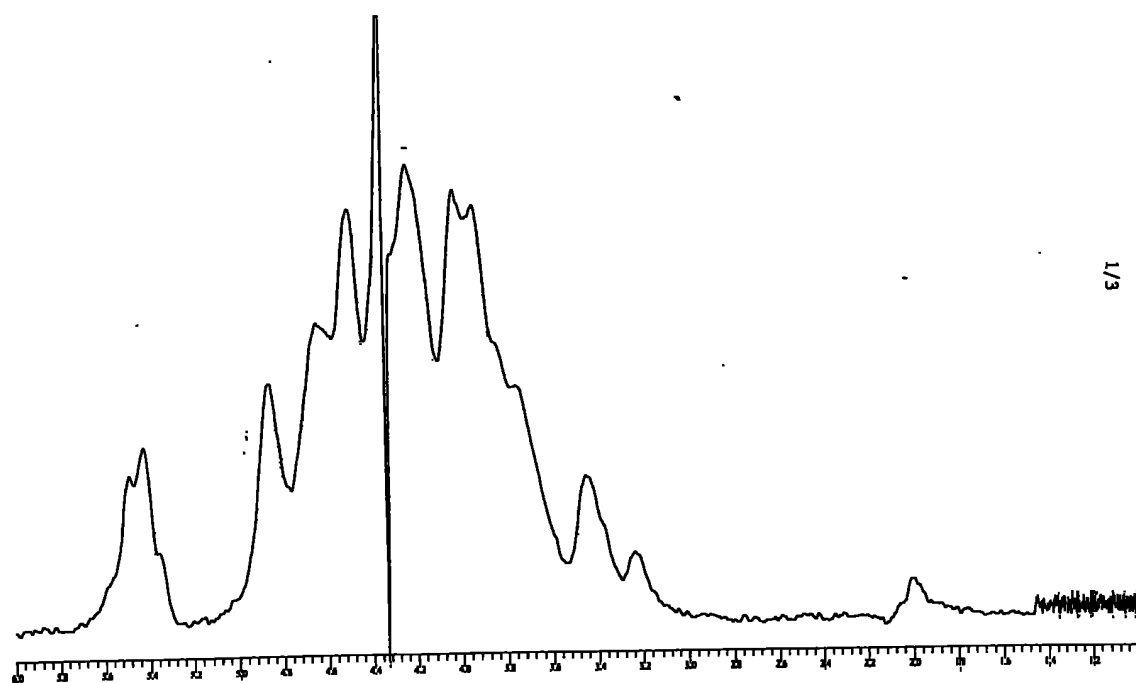
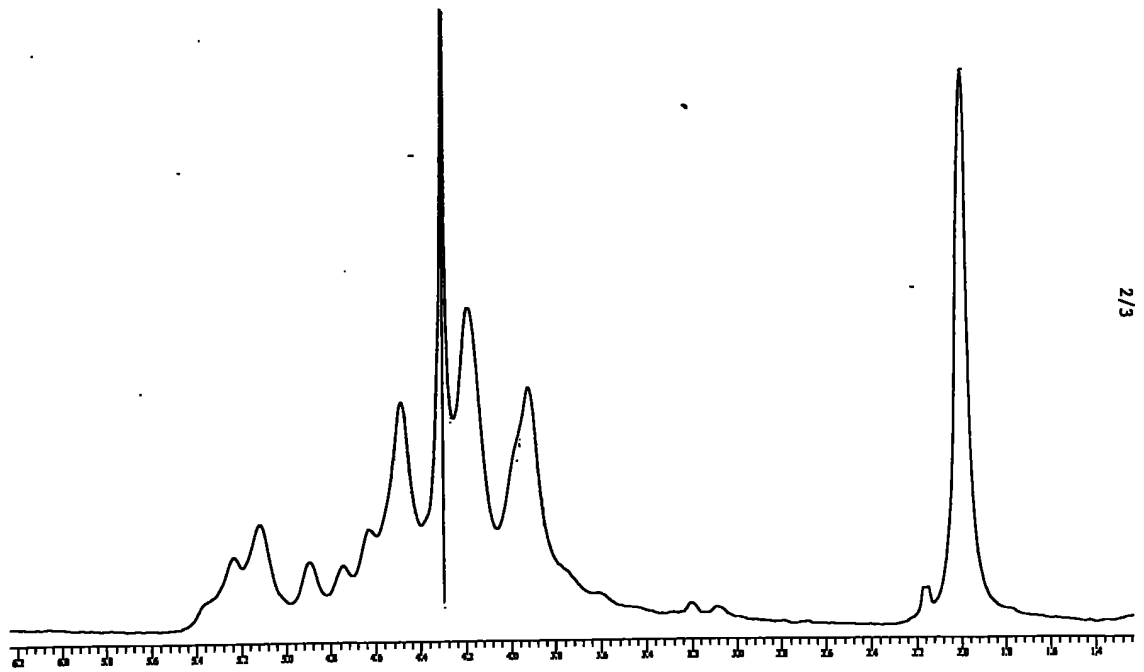
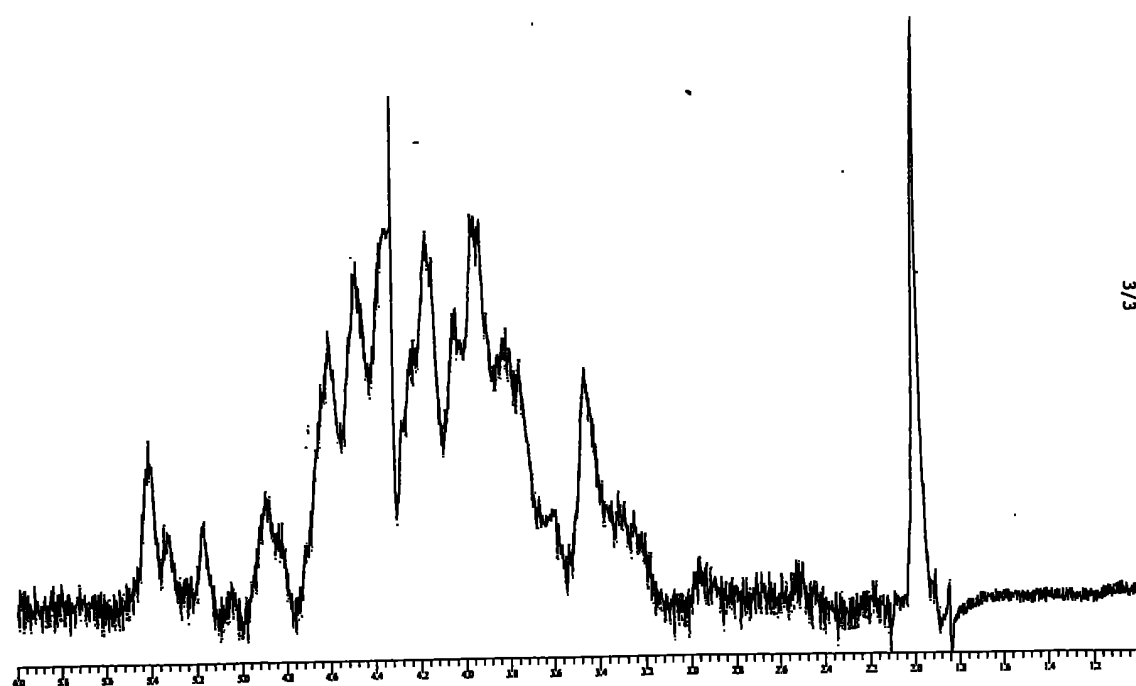


Fig. 1



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Fig. 2



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Fig. 3

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C08B37/00 C08B37/08 C08B37/10 A61K31/726 A61K31/727
A61K31/737

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 544 592 A (SANOFI ELF) 2 June 1993 (1993-06-02) cited in the application abstract; claims page 10, line 42 -page 12, line 21 page 21, line 42 -page 22, line 60 examples 4,5	1,11, 14-16, 20,26, 28,34, 45-58
A	WO 01 72848 A (CIPOLLETTI GIOVANNI ;PASQUA ORESTE (IT); INALCO SPA (IT); ZOPPETTI) 4 October 2001 (2001-10-04) cited in the application page 9, line 30 -page 10, line 4 claims; examples -/-	1,45,58

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

g document member of the same patent family

Date of the actual completion of the international search

21 October 2003

Date of mailing of the international search report

05/11/2003

Name and mailing address of the ISA

European Patent Office, P.O. 5816 Patentean 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 851 epo nl,
Fax: (+31-70) 340-3018

Authorized officer

Mazet, J-F

INTERNATIONAL SEARCH REPORT

PCT/IB 03/02339

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 43317 A (CIPOLLETTI GIOVANNI ;PASQUA ORESTE (IT); INALCO SPA (IT); ZOPPETTI) 20 November 1997 (1997-11-20) cited in the application page 6, line 27 -page 7, line 2 claims	1, 45, 58
A	WO 98 09636 A (TORRI GIANGIACOMO ;NAGGI ANNAMARIA (IT); ISTITUTO SCIENT DI CHIMIC) 12 March 1998 (1998-03-12) page 3, line 27 -page 5, line 20; example 1 claims	45-57
A	WO 98 34958 A (CIPOLLETTI GIOVANNI ;PASQUA ORESTE (IT); INALCO SPA (IT); ZOPPETTI) 13 August 1998 (1998-08-13) cited in the application page 1, line 25 -page 2, line 22	22-27, 34, 38-40, 42, 43
A	US 5 550 116 A (LORMEAU JEAN CLAUDE ET AL) 27 August 1996 (1996-08-27) cited in the application examples 6, 7 column 34, line 27 -column 35, line 65	1
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A	NAGGI A ET AL: "TOWARD A BIOTECHNOLOGICAL HEPARIN THROUGH COMBINED CHEMICAL AND ENZYMATIC MODIFICATION OF THE ESCHERICHIA COLI K5 POLYSACCHARIDE" SEMINARS IN THROMBOSIS AND HEMOSTASIS, STUTTGART, DE, vol. 27, no. 5, 2001, pages 437-443, XP008004483 ISSN: 0094-6176 the whole document	1

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WO 9743317	A	20-11-1997	IT MI960956 A1	10-11-1997
			AT 210154 T	15-12-2001
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			DE 69708862 T2	14-08-2002
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PCT/IB 03/02339

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INTERNATIONAL SEARCH REPORT

PCT/IB 03/02339

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PATENT COOPERATION TREATY

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From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT
OR THE DECLARATION

(PCT Rule 44.1)

To:
MARIETTI, GISLON E TRUPIANO S.R.L.
Attn. Santoro, Tiziana
Via Larga, 16
I-20122 Milan
ITALY

Date of mailing
(day/month/year) 05/11/2003

Applicant's or agent's file reference
03422E45

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.
PCT/IB 03/02339

International filing date
(day/month/year) 17/06/2003

Applicant

ORESTE, Pasqua, Anna

1. ☒ The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 18:

The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after 18 months from the priority date, the International application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the International application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the completion of the technical preparations for International publication.

Within 18 months from the priority date, a demand for International preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 18 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority

 European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3018

Authorized officer

Alicja Van der Heijden

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the letter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been filed, see below.

How?

Either by canceling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

- (i) the claim is unchanged;
- (ii) the claim is cancelled;
- (iii) the claim is new;
- (iv) the claim replaces one or more claims as filed;
- (v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Office, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 03422E45	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, Item 5 below.	
International application No. PCT/IB 03/ 02339	International filing date (day/month/year) 17/06/2003	(Earliest) Priority Date (day/month/year) 18/06/2002
Applicant ORESTE, Pasqua, Anna		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 06 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the International search was carried out on the basis of the International application in the language in which it was filed, unless otherwise indicated under this item.

☐ the International search was carried out on the basis of a translation of the International application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the International application, the International search was carried out on the basis of the sequence listing:

☐ contained in the International application in written form.

☐ filed together with the International application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the International application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (see Box II).

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 03/02339

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C08B37/00 C08B37/08 C08B37/10 A61K31/726 A61K31/727
A61K31/737

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 544 592 A (SANOFI ELF) 2 June 1993 (1993-06-02) cited in the application abstract; claims page 10, line 42 -page 12, line 21 page 21, line 42 -page 22, line 60 examples 4,5	1,11, 14-16, 20,26, 28,34, 45-58
A	WO 01 72848 A (CIPOLLETTI GIOVANNI ;PASQUA ORESTE (IT); INALCO SPA (IT); ZOPPETTI) 4 October 2001 (2001-10-04) cited in the application page 9, line 30 -page 10, line 4 claims; examples	1,45,58
	-/-	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the International filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the International filing date but later than the priority date claimed

T later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the International search

21 October 2003

Date of mailing of the International search report

05/11/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentplan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Mazet, J-F

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 03/02339

52

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 43317 A (CIPOLLETTI GIOVANNI ;PASQUA ORESTE (IT); INALCO SPA (IT); ZOPPETTI) 20 November 1997 (1997-11-20) cited in the application page 6, line 27 -page 7, line 2 claims	1,45,58
A	WO 98 09636 A (TORRI GIANGIACOMO ;NAGGI ANNAMARIA (IT); ISTITUTO SCIENT DI CHIMIC) 12 March 1998 (1998-03-12) page 3, line 27 -page 5, line 20; example 1 claims	45-57
A	WO 98 34958 A (CIPOLLETTI GIOVANNI ;PASQUA ORESTE (IT); INALCO SPA (IT); ZOPPETTI) 13 August 1998 (1998-08-13) cited in the application page 1, line 25 -page 2, line 22	22-27, 34, 38-40, 42,43
A	US 5 550 116 A (LORMEAU JEAN CLAUDE ET AL) 27 August 1996 (1996-08-27) cited in the application examples 6,7 column 34, line 27 -column 35, line 65	1
A	EP 0 489 647 A (SANOFI ELF) 10 June 1992 (1992-06-10)	
A	NAGGI A ET AL: "TOWARD A BIOTECHNOLOGICAL HEPARIN THROUGH COMBINED CHEMICAL AND ENZYMATIC MODIFICATION OF THE ESCHERICHIA COLI K5 POLYSACCHARIDE" SEMINARS IN THROMBOSIS AND HEMOSTASIS, STUTTGART, DE, vol. 27, no. 5, 2001, pages 437-443, XP008004483 ISSN: 0094-6176 the whole document	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 03/02339

53

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0544592	A	02-06-1993	FR 2684385 A1 AT 176484 T CA 2083576 A1 DE 69228362 D1 DE 69228362 T2 DK 544592 T3 EP 0544592 A2 ES 2129037 T3 HU 64087 A2 JP 5271305 A MX 9206783 A1 US 5384398 A US 5314876 A	04-06-1993 15-02-1999 29-05-1993 18-03-1999 09-09-1999 20-09-1999 02-06-1993 01-06-1999 29-11-1993 19-10-1993 31-05-1994 24-01-1995 24-05-1994
WO 0172848	A	04-10-2001	IT MI20000665 A1 AU 4651001 A CA 2404478 A1 CN 1422283 T WO 0172848 A1 EP 1268559 A1 US 2003023079 A1 US 2002062019 A1	01-10-2001 08-10-2001 04-10-2001 04-06-2003 04-10-2001 02-01-2003 30-01-2003 23-05-2002
WO 9743317	A	20-11-1997	IT MI960956 A1 AT 210154 T AU 3026597 A DE 69708862 D1 DE 69708862 T2 DK 897393 T3 WO 9743317 A1 EP 0897393 A1 ES 2167748 T3 US 6162797 A	10-11-1997 15-12-2001 05-12-1997 17-01-2002 14-08-2002 02-04-2002 20-11-1997 24-02-1999 16-05-2002 19-12-2000
WO 9809636	A	12-03-1998	IT MI961840 A1 AT 241367 T AU 715868 B2 AU 4456197 A DE 69722450 D1 WO 9809636 A1 EP 0956027 A1 JP 2000517328 T NZ 334566 A PL 331798 A1 RU 2176915 C2	06-03-1998 15-06-2003 10-02-2000 26-03-1998 03-07-2003 12-03-1998 17-11-1999 26-12-2000 28-01-2000 02-08-1999 20-12-2001
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INTERNATIONAL SEARCH REP RT

Information on patent family members

International Application No

PCT/IB 03/02339

54

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9834958	A	PT 958307 T US 6288044 B1	28-06-2002 11-09-2001
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		EP 0489647 A2	10-06-1992
		ES 2099145 T3	16-05-1997
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		NZ 240838 A	25-11-1993
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		DE 69125109 T2	28-08-1997
		DK 489647 T3	08-09-1997
		EP 0489647 A2	10-06-1992
		ES 2099145 T3	16-05-1997
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		NZ 240838 A	25-11-1993

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 03/02339

55

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0489647	A	PL 292624 A1	08-03-1993
		PT 99671 A ,B	30-10-1992
		SI 9111886 A ,B	28-02-1998
		SK 280642 B6	16-05-2000
		RU 2099353 C1	20-12-1997
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		ZA 9109518 A	26-08-1992

PATENT COOPERATION TREATY

RICE & CO

- 2 FEB 2004

MARIETTI, GISLON & TRUPIANO S.R.L.

56

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

Santoro, Tiziana
MARIETTI, GISLON & TRUPIANO S.R.L.
Via Larga, 16
I-20122 Milan
ITALIE

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year) 30/01/2004Applicant's or agent's file reference
03422E45

IMPORTANT NOTIFICATION

International application No.
PCT/IB03/02339International filing date (day/month/year)
17/06/2003Priority date (day/month/year)
18/06/2002

Applicant

ORESTE, Pasqua, Anna

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

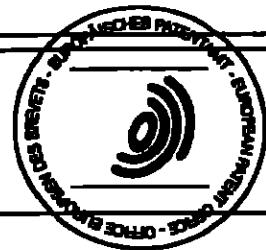
Name and mailing address of the IPEA/



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D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer

CHURCHER G
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

(Rationalised Report according to the Notice of the President of the EPO published in the OJ11/2001)

Applicant's or agent's file reference 03422E45	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/IB03/02339	International filing date (day/month/year) 17/06/2003	Priority date (day/month/year) 18/06/2002
International Patent Classification (IPC) or national classification and IPC C08B37/00		
Applicant: ORESTE, Pasqua, Anna		

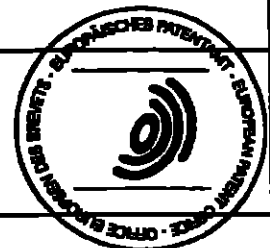
- This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 2 sheets, including this cover sheet.
☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of _____ sheets.

- This report contains indications relating to the following items:
 - ☒ Basis of the report
 - ☐ Priority
 - ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - ☐ Lack of unity of invention
 - ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - ☐ Certain documents cited
 - ☐ Certain defects in the international application
 - ☐ Certain observations on the international application

Date of submission of the demand 29/12/2003	Date of completion of this report 27/01/2004
Name and mailing address of the IPEA/  European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer VAN GOETHEM G A J Tel. (+49-89) 2399 2828

Form PCT/IPEA/409 (cover sheet) P20476 (October 2002)



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No.

PCT/IB03/02339

I. Basis of the report

The basis of this international preliminary examination is the application as originally filed.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability

In light of the documents cited in the international search report, it is considered that the invention as defined in the claims meets the criteria mentioned in Article 33 (1) PCT, i.e. it appears to be novel, to involve an inventive step and to be industrially applicable.

TRATADO DE COOPERACIÓN DE PATENTES
PCT
INFORME DE EXAMEN PRELIMINAR INTERNACIONAL
(Artículo 36 y Regla 70 del PCT)

Referencia del expediente del agente o solicitante 03422E45	PARA ACTUACIONES ADICIONALES Ver notificación de Transmisión del Reporte de Examen Preliminar (Formato PCT/IPEA416)	
Solicitud Internacional No. PCT/IB03/02339	Fecha de presentación internacional (día/mes/año) 17 de junio de 2003	Fecha de prioridad (día/mes/año) 18 de junio de 2002
Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC C08B 37/00		
Solicitante ORESTE, Pasqua Anna		

1. Este informe de examen preliminar internacional ha sido preparado por esta Administración encargada del Examen Preliminar Internacional y se envía al solicitante en concordancia con el artículo 36.

2. Este INFORME consta de una total de 2 incluyendo la portada

☐ Este informe también se acompaña de ANEXOS, esto es documentos de la descripción, reivindicaciones y/o dibujos que han sido corregidos y que son la base de este Informe y/o documentos que contienen rectificaciones realizadas ante esta Administración (Ver Regla 70.16 y Sección 607 de las Instrucciones Administrativas según el PCT)

Estos anexos constan de un total de hojas.

3. Este informe contiene Indicaciones relativas a los siguientes aspectos:

I	☒	Fundamentos del Informe
II	☐	Prioridad
III	☐	No se establece concepto relativo a la novedad, nivel inventivo y aplicación industrial
IV	☐	Carencia de unidad de la invención
V	☒	Declaración razonada según el artículo 35(2), en relación con la novedad, nivel inventivo o aplicación industrial; citaciones y explicaciones que soportan dicha declaración
VI	☐	Ciertos documentos citados
VII	☐	Ciertos defectos de la solicitud internacional
VIII	☐	Ciertas observaciones respecto a la solicitud internacional

Fecha de presentación de la demanda 29 de diciembre de 2003	Fecha de terminación de este reporte 27 de enero de 2004
Nombre y dirección de correo de la autoridad encargada del examen preliminar Oficina de Patentes Europeas, D-80288 Munich Tel.: (+49-89) 2399-0, Tx: 523658 epmu d Fax: (+49-89) 2399-4485	Funcionario Autorizado VAN GOETHEM G A J Teléfono No. (+49-89) 2399 2826

Formato PCT/IPEA409 (portada) P20476 (OCTUBRE 2002)

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I. Base del informe

La base para el informe de examen preliminar internacional es la solicitud como se presentó originalmente.

V. Declaración motivada según la Regla 66.2(a)(II) en cuanto a la novedad, el nivel inventivo y la aplicación Industrial

A la luz de los documentos citados en el informe de búsqueda internacional, se considera que la invención como se define en las Reivindicaciones, cumple con los criterios mencionados en el artículo 33(1) del PCT, es decir, parece ser novedosa, comprender un nivel inventivo y tener aplicabilidad industrial.

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only		
Identification of IPEA		Date of receipt of DEMAND
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference 03422E45
International application No. PCT/IB03/02339	International filing date (day/month/year) 17 June 2003 (17.06.03)	(Earliest) Priority date (day/month/year) 18 June 2002 (18.06.02)
Title of invention "Process for the manufacture of N-acyl-(epi)K5-amln-O-sulfate-derivatives and products thus obtained"		
Box No. II APPLICANT(S)		
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) ORESTE, Pasqua Anna Via Mac Mahon, 107 I-20155 Milan Italy		Telephone No. +39-02-33101771
		Facsimile No. +39-02-34534495
		Teleprinter No.
		Applicant's registration No. with the Office
State (that is, country) of nationality: Italian		State (that is, country) of residence: Italy
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) ZOPPETTI, Giorgio Via Mac Mahon, 107 I-20155 Milan Italy		
State (that is, country) of nationality: Italian		State (that is, country) of residence: Italy
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.) 		
State (that is, country) of nationality:		State (that is, country) of residence:
☐ Further applicants are indicated on a continuation sheet.		

Sheet No. 2

International application No.
PCT/IB03/02339

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representativeand ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.Name and address: (Family name followed by given name; for a legal entity, full official designation.
The address must include postal code and name of country.)SANTORO, Tiziana
MARIETTI, GISLON e TRUPIANO S.r.l.
Via Larga, 16
I-20122 Milan
Italy

Telephone No.

+39-02-86464387

Facsimile No.

+39-02-86463303

Teleprinter No.

Agent's registration No. with the Office

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filedthe description ☒ as originally filed☐ as amended under Article 34the claims ☒ as originally filed☐ as amended under Article 19 (together with any accompanying statement)☐ as amended under Article 34the drawings ☒ as originally filed☐ as amended under Article 342. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). (This check-box may be marked only where the time limit under Article 19 has not yet expired.)

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English.....

☐ which is the language in which the international application was filed.☐ which is the language of a translation furnished for the purposes of international search.☒ which is the language of publication of the international application.☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States (that is, all States which have been designated and which are bound by Chapter II of the PCT)

excluding the following States which the applicant wishes not to elect:

Sheet No. 3.

International application No.
PCT/IB03/02339

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | |
|--|---|--------|
| 1. translation of international application | : | sheets |
| 2. amendments under Article 34 | : | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | sheets |
| 5. letter | : | sheets |
| 6. other (specify) | : | sheets |

For International Preliminary Examining Authority use only

received	not received
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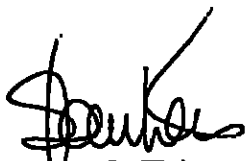
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| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |
| ☐ | ☐ |

The demand is also accompanied by the item(s) marked below:

- | | |
|--|---|
| 1. ☒ fee calculation sheet | 5. ☐ statement explaining lack of signature |
| 2. ☐ original separate power of attorney | 6. ☐ sequence listings in computer readable form |
| 3. ☐ original general power of attorney | 7. ☐ tables in computer readable form related to sequence listings |
| 4. ☐ copy of general power of attorney; reference number, if any: | 8. ☐ other (specify): |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).


SANTORO, Tiziana
(Agent)

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.

4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.

5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only	
International Application No.	
International Filing Date	
Name of receiving Office and "PCT International Application"	
Applicant's or agent's file reference (if desired) (12 characters maximum) 03422E45	

Box No. I TITLE OF INVENTION	
Process for the manufacture of N-acyl-(epi)K5-amine-O-sulfate-derivatives and products thus obtained	
Box No. II APPLICANT ☒ This person is also inventor	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	
ORESTE, Pasqua, Anna Via Mac Mahon, 43 20155 Milano Italy	
Telephone No. +39-02-33101771	
Facsimile No. +39-02-34534495	
Teleprinter No.	
Applicant's registration No. with the Office	
State (that is, country) of nationality: IT	
State (that is, country) of residence: IT	
This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)	
ZOPPETTI, Giorgio Via Mac Mahon, 43 20155 MILANO Italy	
This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.)	
Applicant's registration No. with the Office	
State (that is, country) of nationality: IT	
State (that is, country) of residence: IT	
This person is applicant for the purposes of: ☒ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)	
SANTORO, Tiziana MARIETTI, GISLON e TRUPIANO S.r.l. Via Larga, 16 I-20122 Milan Italy	
Telephone No. +39-02-86464387	
Facsimile No. +39-02-86463303	
Teleprinter No.	
Agent's registration No. with the Office	
☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	

Box No. V DESIGNATION OF STATES

Mark the applicable check-boxes below; at least one must be marked.

The following designations are hereby made under Rule 4.9(a):

Regional Patent

- ☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZM Zambia, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT (if other kind of protection or treatment desired, specify on dotted line)
- ☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP European Patent: AT Austria, BE Belgium, BG Bulgaria, CH & LI Switzerland and Liechtenstein, CY Cyprus, CZ Czech Republic, DE Germany, DK Denmark, EE Estonia, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, SI Slovenia, SK Slovakia, TR Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GQ Equatorial Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | | |
|---|--|---|
| ☒ AE United Arab Emirates | ☒ GM Gambia | ☒ NZ New Zealand |
| ☒ AG Antigua and Barbuda | ☒ HR Croatia | ☒ OM Oman |
| ☒ AL Albania | ☒ HU Hungary | ☒ PH Philippines |
| ☒ AM Armenia | ☒ ID Indonesia | ☒ PL Poland |
| ☒ AT Austria | ☒ IL Israel | ☒ PT Portugal |
| ☒ AU Australia | ☒ IN India | ☒ RO Romania |
| ☒ AZ Azerbaijan | ☒ IS Iceland | ☒ RU Russian Federation |
| ☒ BA Bosnia and Herzegovina | ☒ JP Japan | ☒ SC Seychelles |
| ☒ BB Barbados | ☒ KE Kenya | ☒ SD Sudan |
| ☒ BG Bulgaria | ☒ KG Kyrgyzstan | ☒ SE Sweden |
| ☒ BR Brazil | ☒ KP Democratic People's Republic of Korea | ☒ SG Singapore |
| ☒ BY Belarus | ☒ KR Republic of Korea | ☒ SK Slovakia |
| ☒ BZ Belize | ☒ KZ Kazakhstan | ☒ SL Sierra Leone |
| ☒ CA Canada | ☒ LC Saint Lucia | ☒ TJ Tajikistan |
| ☒ CH & LI Switzerland and Liechtenstein | ☒ LK Sri Lanka | ☒ TM Turkmenistan |
| ☒ CN China | ☒ LR Liberia | ☒ TN Tunisia |
| ☒ CO Colombia | ☒ LS Lesotho | ☒ TR Turkey |
| ☒ CR Costa Rica | ☒ LT Lithuania | ☒ TT Trinidad and Tobago |
| ☒ CU Cuba | ☒ LU Luxembourg | ☒ TZ United Republic of Tanzania |
| ☒ CZ Czech Republic | ☒ LV Latvia | ☒ UA Ukraine |
| ☒ DE Germany | ☒ MA Morocco | ☒ UG Uganda |
| ☒ DK Denmark | ☒ MD Republic of Moldova | ☒ US United States of America |
| ☒ DM Dominica | ☒ MG Madagascar | ☒ UZ Uzbekistan |
| ☒ DZ Algeria | ☒ MK The former Yugoslav Republic of Macedonia | ☒ VC Saint Vincent and the Grenadines |
| ☒ EC Ecuador | ☒ MN Mongolia | ☒ VN Viet Nam |
| ☒ EE Estonia | ☒ MW Malawi | ☒ YU Yugoslavia |
| ☒ ES Spain | ☒ MX Mexico | ☒ ZA South Africa |
| ☒ FI Finland | ☒ MZ Mozambique | ☒ ZM Zambia |
| ☒ GB United Kingdom | ☒ NO Norway | ☒ ZW Zimbabwe |
| ☒ GD Grenada | | |
| ☒ GE Georgia | | |
| ☒ GH Ghana | | |

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

- | | | |
|---|--------------------------------|--------------------------------|
| ☒ NI Nicaragua | ☐ | ☐ |
| ☒ PG Papua New Guinea | ☐ | ☐ |

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)

Box No. VI PRIORITY CLAIM				
The priority of the following earlier application(s) is hereby claimed:				
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1) 18 June 2002 (18-06-2002)	MI2002A001345	IT		
item (2) 18 June 2002 (18-06-2002)	MI2002A001346	IT		
item (3) 27 August 2002 (27-08-2002)	MI2002A001854	IT		
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office) identified above as:

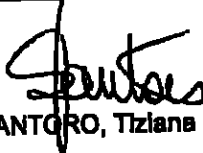
☐ all items
 ☐ item (1)
 ☐ item (2)
 ☐ item (3)
 ☐ item (4)
 ☐ item (5)
 ☐ other, see Supplemental Box

* Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):

Box No. VII INTERNATIONAL SEARCHING AUTHORITY	
Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):	
ISA / EPO	
Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):	
Date (day/month/year)	Number Country (or regional Office)

Box No. VIII DECLARATIONS		
The following declarations are contained in Boxes Nos. VIII (i) to (v) (mark the applicable check-boxes below and indicate in the right column the number of each type of declaration):		Number of declarations
☐ Box No. VIII (i)	Declaration as to the identity of the inventor	:
☐ Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:
☐ Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:
☐ Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:
☐ Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	:

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Box No. IX CHECK LIST; LANGUAGE OF FILING		
This international application contains: (a) in paper form, the following number of sheets : request (including declaration sheets) : 4 description (excluding sequence listings and/or tables related thereto) : 42 claims : 13 abstract : 1 drawings : 3 Sub-total number of sheets : 63 sequence listings : : tables related thereto : : <i>(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)</i> Total number of sheets : 63 (b) ☐ only in computer readable form (Section 801(a)(i)) (i) ☐ sequence listings (ii) ☐ tables related thereto (c) ☐ also in computer readable form (Section 801(a)(ii)) (i) ☐ sequence listings (ii) ☐ tables related thereto Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the ☐ sequence listings: ☐ tables related thereto: <i>(additional copies to be indicated under items 9(ii) and/or 10(ii), in right column)</i>	This international application is accompanied by the following item(s) (mark the applicable check-boxes below and indicate in right column the number of each item): 1. ☒ fee calculation sheet : 1 2. ☐ original separate power of attorney WILL FOLLOW : : 3. ☐ original general power of attorney : : 4. ☐ copy of general power of attorney; reference number, if any: : : 5. ☐ statement explaining lack of signature : : 6. ☐ priority document(s) identified in Box No. VI as item(s): will follow : : 7. ☐ translation of international application into (language): English will follow : : 8. ☐ separate indications concerning deposited microorganism or other biological material : : 9. ☐ sequence listings in computer readable form (indicate type and number of carriers) (i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application) : : (ii) ☐ (only where check-box (b)(i) or (c)(i) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter : : (iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listings mentioned in left column : : 10. ☐ tables in computer readable form related to sequence listings (indicate type and number of carriers) (i) ☐ copy submitted for the purposes of international search under Section 802(b-quater) only (and not as part of the international application) : : (ii) ☐ (only where check-box (b)(ii) or (c)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-quater) : : (iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column : : 11. ☐ other (specify): : :	Number of items
Figure of the drawings which should accompany the abstract:	Language of filing of the international application: Italian	
Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE <i>Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).</i>  SANTORO, Tiziana		

For receiving Office use only	
1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only
Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

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PCT

NOTIFICATION OF RECEIPT OF
RECORD COPY

(PCT Rule 24.2(a))

From the INTERNATIONAL BUREAU

To:

SANTORO, Tiziana
Marietti, Gislone e Trupiano S.r.l.
Via Larga, 16
I-20122 Milan
Italy

Date of mailing (day/month/year) 25 July 2003 (25.07.03)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 03422E45	International application No. PCT/IB03/02339

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

ORESTE, Pasqua, Anna et al(all designated States)

International filing date : 17 June 2003 (17.06.03)
Priority date(s) claimed : 18 June 2002 (18.06.02)
18 June 2002 (18.06.02)
27 August 2002 (27.08.02)

Date of receipt of the record copy
by the International Bureau : 17 July 2003 (17.07.03)

List of designated Offices :

AP : GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW
EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
EP : AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR
OA : BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
National : AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ,
EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 338-7080	Authorized officer: Aysha FLEYFEL Telephone No. (41-22) 338 8301
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Continuation of Form PCT/IB/301

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NOTIFICATION OF RECEIPT OF RECORD COPY

Date of mailing (day/month/year) 25 July 2003 (25.07.03)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 03422E45	International application No. PCT/IB03/02339

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- ☒ time limits for entry into the national phase - see updated important information (as of April 2002)
- ☐ confirmation of precautionary designations (if applicable)
- ☒ requirements regarding priority documents (if applicable)

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

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INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated on the cover sheet of this Notification by paying national fees and furnishing translations, as prescribed by Articles 22 and 39 and the applicable national laws. In addition, the applicant may also have to comply with other special requirements applicable in certain Offices. It is the applicant's responsibility to ensure the necessary steps to enter the national phase are taken in a timely fashion. Most Offices do not issue reminders to applicants in connection with the entry into the national phase.

The applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be **30 MONTHS** from the priority date, not only in respect of any elected Office where a demand for international preliminary examination is filed before the expiration of 18 months from the priority date (see Article 38(1)), but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see PCT Gazette No. 44/2001 of 1 November 2001, pages 19928, 19932 and 19934, as well as the PCT Newsletter, October and November 2001 and February 2002 issues.

In practice, time limits other than the 30-month time limit will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For regular updates on the applicable time limits (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the PCT Gazette ("Section IV" part published on a weekly basis), to the PCT Newsletter (on a monthly basis) and to the relevant National Chapters in Volume II of the PCT Applicant's Guide (the paper version of which is updated usually twice a year and the Internet version of which is updated usually on a weekly basis). Finally, a cumulative table of all applicable time limits for entering the national phase is available from WIPO's Internet site, via links from various pages the site including those of the Gazette, Newsletter and Guide, at <http://www.wipo.int/pct/en/index.html>.

Information about the requirements for filing a demand for international preliminary examination is set out in the PCT Applicant's Guide, Volume IA, Chapter IX. Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date (this time limit may not be extended). If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. The Notice of confirmation and payment must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 18 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 18-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 18-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within the time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 18-month time limit is the filing date of the earliest application whose priority is claimed.

RICEVUTO

PATENT COOPERATION TREATY

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21 AGO 2003

PCT

MARLETTI, GISLON e TRUPIANO S.r.l.

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

From the INTERNATIONAL BUREAU

To:

SANTORO, Tiziana
Marletti, Gislone e Trupiano S.r.l.
Via Larga, 16
I-20122 Milan
Italy

Date of mailing (day/month/year) 13 August 2003 (13.08.03)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference 03422E45	
International application No. PCT/IB03/02339	International filing date (day/month/year) 17 June 2003 (17.06.03)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 18 June 2002 (18.06.02)
Applicant ORESTE, Pasquà, Anna et al	

- The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
- This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
- An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
- The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
18 June 2002 (18.06.02)	MI2002A001345	IT	07 Augu 2003 (07.08.03)
18 June 2002 (18.06.02)	MI2002A001346	IT	07 Augu 2003 (07.08.03)
27 Augu 2002 (27.08.02)	MI2002A001854	IT	07 Augu 2003 (07.08.03)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Aysha FLEYFEL
Facsimile No. (41-22) 338-7080	Telephone No. (41-22) 338 8301

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RICEVUTO

PATENT COOPERATION TREATY

23 MAR 2004

MARIETTI, GISLON e TRUPIANO S.r.l.

PCT

From the INTERNATIONAL BUREAU

To:

SANTORO, Tiziana
Marietti, Gislone e Trupiano S.r.l.
Via Larga, 16
I-20122 Milan
Italy

INFORMATION CONCERNING ELECTED
OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

Date of mailing (day/month/year) 19 March 2004 (19.03.2004)		
Applicant's or agent's file reference 03422E45		IMPORTANT INFORMATION
International application No. PCT/IB2003/002339	International filing date (day/month/year) 17 June 2003 (17.06.2003)	
Priority date (day/month/year) 18 June 2002 (18.06.2002)		
Applicant ORESTE, Pasqua, Anna et al		

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

EP : AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE,
SI, SK, TR
National : BG, CA, CN, DE, GB, IL, JP, KP, KR, MN, NO, PL, RO, RU, SK, US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

AP : GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW
EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
OA : BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
National : AE, AG, AL, AM, AT, AU, AZ, BA, BB, BR, BY, BZ, CH, CO, CR, CU, CZ, DK, DM, DZ, EC,
EE, ES, FI, GD, GE, GH, GM, HR, HU, ID, IN, IS, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MW, MX, MZ, NI, NZ, OM, PG, PH, PT, SC, SD, SE, SG, SL, TJ, TM, TN, TR, TT, TZ, UA, UG,
UZ, VC, VN, YU, ZA, ZM, ZW

3. The applicant is reminded that he must enter the "national phase" before the expiration of 30 months from the priority date before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 38(3)(b) and Rule 74.1).

Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

The entry into the European regional phase is postponed until 31 months from the priority date for all States designated for the purposes of obtaining a European patent.

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Proceso para la preparación de derivados de
N-acil-(epi)K5-amina-O-sulfato-
y productos así obtenidos

Materia Objeto de la Invención

La presente invención se relaciona con nuevos derivados del polisacárido K5 con un alto grado de sulfatación, un proceso para su preparación, nuevos intermedios altamente o-sulfatados útiles en su síntesis y composiciones farmacéuticas que contienen dichos derivados de polisacáridos K5 como ingredientes activos básicamente libres de actividad sobre la coagulación.

En particular, la invención se refiere a derivados de polisacárido K5 obtenidos partiendo con un polisacárido K5, previamente los N-desacetilados, N-sulfatados, opcionalmente despolimerizados u opcionalmente al menos 20 % C5-epimerizados y opcionalmente despolimerizados, mediante la O-sobresulfuración en condiciones adecuadas y la subsecuente N-acilación de la amina libre.

Antecedentes de la Invención

Los glicosaminoglicanos tales como la heparina, el sulfato de heparan, el sulfato de dermatan, el sulfato de condrotina y el ácido hialurónico son biopolímeros extraídos industrialmente de varios órganos animales.

En particular, la heparina, principalmente obtenida mediante la extracción de membranas de mucosa intestinal de cerdos o de el pulmón de bovinos, es un copolímero de polidispersado con una distribución de peso molecular de aproximadamente de 3000 a aproximadamente 30000 D que consiste de una mezcla de cadena que consiste básicamente de un ácido urónico (ácido glucurónico o ácido idurónico) y de un azúcar amino (glucosamina) ligado mediante enlaces α -1 $\rightarrow$ 4 o β -1 $\rightarrow$ 4. En la heparina, la unidad urónica puede ser O-sulfatada en la posición 2 y la unidad de glucosamina es N-acetilada o N-sulfatada, 6-O-sulfatada y 3-O-sulfatada en aproximadamente 0,5% de la unidades de glucosamina presentes.

Las propiedades y la biosíntesis natural de heparina en mamíferos ha sido descrita por Lindahl et al., 1986 en

lene, D. Y Lindahl, U. (editores) "heparin. Chemical and biological properties; clinical applications", Edward Arnold, London, pages 159-190, by Lindahl, U, Feingold D.S. and Rodén L, 1986 TIBS, 11, 221-225 y por Conrad H.E "Heparin Binding proteins", Chapter 2: Structure of Heparinoids. Academic Press, 1998. La biosíntesis de heparina ocurre partiendo con su precursor N-acetil-heparosán que consiste en una mezcla de cadena que consisten de unidades de disacárido glucurónil- β -1 $\rightarrow$ 4-N-acetilglucosamina repetitivas. Dicho precursor sufre modificaciones enzimáticas que ionizan parcialmente el grupo N-acetilo, sustituyéndolo con un grupo SO₃, epimeriza el carboxilo en la posición 5 de una parte de las unidades glucurónicas transformándolas en unidades idurónicas e introduciendo grupos O-sulfato para conseguir un producto del cual una vez extraído industrialmente tiene aproximadamente el doble del número de sulfato con relación a los grupos carboxy por unidad de disacárido. Estas modificaciones enzimáticas conducen, por ejemplo, a la formación de la región pentasacárido de un enlace de antitrombina III (ATIII), denominada pentasacárido activo, que es la estructura necesaria para la alta unión de afinidad de heparina al ATIII y

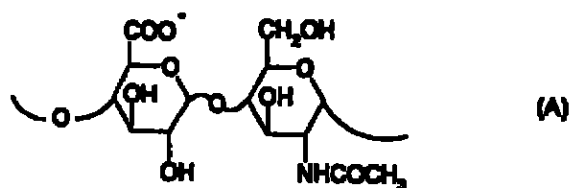
fundamental para la actividad anticoagulante y antitrombótica de la heparina misma. Este pentasacárido, presente solamente dentro de alguna de las cadenas las cuales forman heparina, contienen una unidad de glucosamina sulfatada en la posición 3 y un ácido glucurónico espaciado entre disacáridos que contienen ácidos idurónicos.

En la naturaleza, la formación de los pentasacáridos activos se hace posible por medio de la reacción de epimerización del carboxilo de una parte de las unidades glucurónicas en unidades idurónicas llevadas a cabo por glucuronil-C5-epimerasa (epimerización-C5) y mediante la sulfatación adecuada que también conduce a la introducción de un grupo sulfato sobre el hidroxilo de la posición 3 de la glucosamina. más particularmente, en la naturaleza la formación del pentasacárido activo se hace posible por en echo de que la epimerización C5 ocurre en grupos, es decir, sobre porciones de cadenas, y extensivamente la cual conduce a un producto que contiene más unidades idurónicas que aquellas glucurónicas. De echo, la heparina comercial contiene 70% de unidades idurónicas y 30 %.

A lo largo de las actividades anticoagulantes y antitrombóticas principales, la heparina también ejerce actividades antilipaémico, antiproliferativas, antivirales, antitumorosas, y antimestastáticas, pero su uso como una droga impedido por los efectos colaterales debidos a la acción anticoagulante que pueden originar sangrado.

TÉCNICA ANTERIOR

Es conocido que el polisacárido K5 capsular aislado de *Escherichia coli*, descrito por Vann W. F. Et al., en la revista Europea de bioquímica 1981, 116, 359-364 ("Vann 1981"), es consistente de una mezcla de cadena que consiste de la unidad de disacárido repetitiva glucuronil- β -1 $\rightarrow$ 4-N-acetil glucosamina y por lo tanto muestra la misma secuencia repetitiva (A)



Del precursor N-acetil-heparosan de la heparina. El polisacárido K5 capsular, denominado en lo sucesivo como "polisacárido K5" o más simplemente "K5", que químicamente modificado por Lormeau et al. Como se describió en la US 5,550, 116 y por Casu et al. Como se describió en Carbohydrate Research, 1994, 263, 271-284. Los K5-O-sulfatos que tienen actividades antitumorosas, antimetastáticas, antivirales, en particular anti-HIV son descritos en la EP 333243 y WO 98/34958. El K5 fue también química y enzimáticamente modificado con el fin de obtener productos que tienen actividad biológica in Vitro sobre la coagulación del mismo tipo que aquella de la heparina como se extrae de los órganos animales (heparina extractiva).

La obtención de los productos que tienen una actividad sobre la coagulación del mismo tipo como aquella de la heparina extractiva ocurre mediante procesos que imitan aquellos que ocurren en la naturaleza y prevé una etapa clave completa de la epimerización C5 con D-glucuronil C5 epimeraza.

Los procesos descritos en la IT 1230785, WO 92/17507, WO 96/14425 y WO 97/43317 utiliza K5 como el material de

partida. El K5 que se origina de la fermentación esta sometido a N-desacetilación seguida por N-sulfatación y sobre el K5-sulfato así obtenido epimerización C5 con epimerasa C5 en solución se desarrolla, obtenida mediante cromatografía de una solución de enzimas microsomales de mastositoma de ratón (IT 12030 785) o de hígado de bovino (WO 92/1750, WO 96/14425, y WO 97/43317).

La D-glúcuronil C5 hepimerasa proveniente de hígado de bovino que se purifico por Campbell, P. et al. En J. Biol. Chem., 1994, 269/43, 26953-26958 ("Campbell 1994") quien también suministro su composición en amino ácidos y describió su uso y solución para la transformación de un K5-N-sulfato en el producto 30% epimerizado correspondiente, demostrando una formación de ácido idurónico mediante el método HPLC seguido por despolimerización nitrosa total a disacáridos.

El documento WO 98/48006 describe la secuencia de ADN que codifica para el D-glucuronil C5 epimerasa y un D-glucuronil C5 epimerasa recombinante, obtenido de un vector de expresión recombinante que contiene dicho AND, posteriormente purificado por Campbell et al. Como lo

mostró Shown by Jin-Ping L. Et al. In J. Biol. Chem. 2001, 276, 20069-20077 ("Jin-Ping 2001")

La secuencia completa de la epimerasa C5 se describió por Crawford B. E. Et al. In J. Biol. Chem., 2001, 276(24), 21538-21546 (Crawford 2001)

El documento WO 01/72848 describe un método para la preparación de derivados de polisacáridos K5 N-desacetilados N-sulfatados al menos epimerizados el 40% de ácido idurónico con relación al total de los ácidos urónicos, que tienen un peso molecular desde 2.000 a 30.000, que contienen desde 25 hasta 50% de cadenas de alta afinidad para ATIII y que tienen un índice de actividad anticoagulante y antitrombótica expresada como HCII/antiXa de 1,5 a 4. Dicho documento describe la sobre sulfatación de K5-sulfato epimerizado 40-60% y muestra que el producto obtenido, cuyo ^{13}C -RMN es ilustrado, tiene un contenido de grupo sulfato por unidad de disacárido 2-3.5. Repitiendo la sobre sulfatación anteriormente mencionada en las condiciones descritas y examinando el ^{13}C -RMN se asegura que el producto obtenido es de echo una amina libre cuyo contenido de 6-O-sulfato es de 80-95%,

de el 3-O-sulfato sobre el azúcar a 30%, pero cuyo grado de sulfatación es 3.2. También se establece que en las condiciones de sobre sulfatación descritas en la WO 01/72848 no se obtiene un grado de sulfatación de más de 3.2.

La solicitud de patente MI2001A/00397 (WO 02/068477), describe K5-n, O-sobresulfatado que tienen un grado de sobre sulfatación de más de 3.2, obtenido partiendo con un K5 libre de sustancias lipofílicas o una de sus porciones de peso molecular de aproximadamente de 5.000 mediante (a) N-desacetilación/N-sulfatación, (b) O-sobresulfatación y, opcionalmente, (c) N-resulfatación.

Ninguno de los documentos mencionados anteriormente describe el LMW-K5-N-sulfatos, opcionalmente 40-60% epimerizados en los cuales NH_2 o los grupos acetilo están virtualmente ausentes.

Con el fin de estandarizar la tecnología y hacer el texto más comprensible, en la presente descripción los términos convencionales y expresiones son utilizados en singular o en plural. En particular:

- por "K5" o "polisacárido K5" significa el polisacárido capsular de *Escherichia coli* obtenido por fermentación, es decir, una mezcla de cadenas que consiste de unidades de disacáridos (A) contienen opcionalmente un doble enlace en el extremo no reducido como si se mostraba anteriormente, en cualquier caso preparado y purificado de acuerdo con los métodos descritos en la literatura, en particular de acuerdo a Vann 1981, de acuerdo con Manzoni M. Et al., Journal of Bioactive Compatible Polymers, 1996, 11, 301-311 ("Manzoni 1996") de acuerdo con el método descrito en WO 01/72848 y en WO 02/068447; es obvio para una persona experta en la técnica que lo que se muestra en lo sucesivo se puede aplicar a cualquier N-acetilheparosan;
- por "epimerasa C5" significa la D-glucoronil C-5 epimerasa, extraída o recombinante, en cualquier caso preparada, aislada y purificada, en particular, como se describió en Campbell 1994, en la WO 98/48006, en la Jin-Ping L. Et al. En J. Bio

Chem. 2001, 276, 20069-20077 (Jin-ping 2001) en Crawford 2001;

- -por amina K5 se significa al menos K5 N-desacetilada 95% pero generalmente en la cual los grupos acetilos son indetectables por un aparato RMN habitual;
- por "K5-N-sulfato" que significa al menos K5 N-desacetilada y N-sulfatada 95% como se describe en los sucesivo, pero en la cual los grupos acetilos son indetectables con un aparata de PMN normal;
- - por, "epik5" significa que el K5 y sus derivados en los cuales 20-60% de las unidades glucurónicas son C5-epimerizadas a unidades idurómicas;
- - por "epik5-N-sulfato" que significa el K5-N-sulfato en el cual 20-60% de las unidades glucurónicas C5-epimerizadas a unidades idurónicas del tipo descrito en la WO 92/17507 o la WO 01/72848;

- - por "epik5-amina-O-sobresulfato" que significa una epik5-amina O-sulfatada con un grado de sulfatación de al menos 3.4;
- - por "N-acetil-epik5-amina-O-sobresulfato" que significa una épica5-amina O-sobresulfato, N-acilada, con un grado de sulfatación de al menos 3.4;
- por "K5-amina-O-sobresaturado" que significa una K5-amina O-sulfatada con un grado de sulfatación de al menos 2.2;
- por "N-acil-k5-amina-O-sobresulfato" que significa un K5-amina-O-sobresulfato N-acilada con un grado de sulfatación de al menos 2.2;
- además:
- los términos convencionales y expresiones definidos aquí anteriormente se refieren al K5 como aislado después de fermentación, generalmente con una distribución de peso molecular desde 1500 hasta

aproximadamente 50000 con un peso molecular medio de 10000-250000, ventajosamente de 15000-25000;

- - exceptuando la designación específica del peso molecular, los términos y expresiones convencionales de fluidos aquí anteriormente, cuando están presididos por el acrónimo "LMW" (bajo peso molecular), por ejemplo LMW-K5-N-sulfato, LMW-epik5-N-sulfato indican productos de bajo peso molecular, que tienen un peso molecular medio de hasta 12000;
- - los términos convencionales y expresión como se designo de la aquí anteriormente cuando son seguidos por el indicativo "bien derivado" como un todo tanto los derivados del K5 nativo como aquellos de el bajo peso molecular;
-
- el prefijo "(epi)", que precede a "K5" en términos convencionales y de expresiones como se diseñaron aquí anteriormente, indica como un todo tanto a los productos del K5 nativo como aquellos 20-60% epimerizados como se definió anteriormente, es

decir las mezclas de cadena todas consisten de ácido glucurónico, y 20-60% de ácido idurónico y se notan en las fórmulas por medio del símbolo " " [‡]

- por el término "aproximadamente" en referencia al peso molecular que significa el peso molecular [$\pm$ el peso teórico de una unidad de disacárido, incluyendo el peso del sodio, calculado como 461 en el caso de un (epi) K5-N-sulfato-derivado] medido mediante disco simetría de acuerdo a Johnson et al. Carb. Res. 1979, 51, 119-127 utilizando muestras cuyo peso molecular fue calculado mediante HPLC como el estándar;
- y ante la expresión "especies preponderantes", que significa el compuesto el cual, en la mezcla que constituye un Imw-(epi)K5-N-sulfato, o un Imw-(epi)K5-amina-O-sobresulfato, es el tipo más representado, determinado, por el pico de la curva del peso molecular medido mediante HPLC;
- a menos que se establezca específicamente otra cosa, el "grado de sulfatación" significa la

SO₃/COO, expresable también como el número de grupos sulfato por unidad de disacárido, medida con el método conducto métrico descrito por Casu B. Et al. En Carbohydrate Research, 1975, 39,, 168-176 (Casu 1975), el mismo utilizado en la WO 01/72848;

- por "condiciones de O-sobresulfatación" se significa una O-sulfatación extrema desarrollada por ejemplo de acuerdo con el método C descrito por B. Casu et al. En corbohydrare Research, 1994, 263, 271-284 (Casu 1994);
- por el termino "alquilo" que significa un alquilo lineal o ramificado, mientras que el termino "tetrabutylamino" indica el tetra-n-butylamonio.

RESUMEN DE LA INVENCION

Se ha encontrado ahora que, partiendo de un epik5-N-sulfato, es posible obtener un epik5-amina-O-sulfato con un grado de sulfatación mayor que aquel de epik5-amina-O-sulfato descritos en la literatura, por ejemplo en la WO 01/72848, preparando la sal con una base orgánica

terciaria o cuaternaria de dicho derivado epik5-N-sulfato teniendo cuidado de permitirle a la mezcla de reacción permanecer durante un periodo de tiempo de 30-60 minutos manteniendo el pH a aproximadamente 7 con la misma base orgánica y luego tratando la sal obtenida con un reactivo de O-sulfatación en las condiciones de O-sobresulfatación. También un IMW-epik5-N-sulfato, cuando es sometido al mismo método de salificación y de O-sobre sulfatación, da un Imw-epik5-amina-O-sulfato con un extremadamente alto grado de sulfatación. En el caso de tales derivados epi-K5-amina-sobresulfato el grado de sulfatación es más de 3,2.

Similarmente, se encontró que aplicando las condiciones de O-sobre sulfatación descritas anteriormente a un Imw-K5-sulfato un Imw-K5-amina-O-sulfato se obtiene a un alto grado de sulfatación. También a K5-N-sulfato cuando es sometido al mismo método de salificación o de O-sobre sulfatación, da un K5-amina-O-sulfato con un alto grado de sulfatación. En el caso de tales derivados de K5-N-amina-O-sobre sulfato el grado de sulfatación es más de 22.

De echo, se encontró de manera sorprendente que, preparando la sal de tetrabutylamonio de un derivado (epi) K5-N-sulfato-derivado en las condiciones anteriormente dichas, la salificación se completaba y, durante la O-sobre sulfatación subsiguiente, se favorece en desplazamiento total del grupo sulfato desde el átomo de nitrógeno 2 en la glucosalina a los átomos de oxígeno de la glucosalina misma, en particular a el átomo de oxígeno en la posición 3, dando origen así a derivados de (epi)K5-amina-O-sulfato con un alto grado de sulfatación en el cual el grupo NH_2 es estrictamente libre. La diferencia cuantitativa en el grado de sulfatación del derivado de epik5 y K5 depende de su configuración que favorece la sulfatación de los derivados epimerizados.

También se a encontrado que sometiendo un derivado de (epi)K5-amina -O-sobre sulfato obtenido así a N-acilación, son obtenidos nuevos derivados de N-asil-(epi)K5-amina-O-sulfato libres de actividad sobre la coagulación y útiles para la preparación de composiciones farmacéuticas o cosméticas. Por este nuevo proceso versátil es posible así preparar de una manera reproducible derivados de epik5-O-sulfato, sus análogos N-acilo un K5-sulfato que tiene un grado de preciso de

sulfatación y, por encima de todo, sus derivados de bajo peso molecular y sus N-acilos análogos.

BREVE DESCRIPCIÓN DE LAS FIGURAS

Figura 1 muestra el espectro del protón NMR de K5-NH₂ O-sulfato con un proporción sulfato/carboxi de 2.87 (obtenida de acuerdo al ejemplo 1);

La figura 2 muestra el espectro de protón RMN de epik5-N-cetilato, O-sulfato con una proporción sulfato/carboxi de 4.26 (obtenida de acuerdo con el ejemplo 2);

La figura 3 muestra el espectro de protón RMN de K5-N-acetilato, O-sulfato con la proporción de sulfato/carboxi de 2.87 obtenida de acuerdo al ejemplo 3.

DESCRIPCIÓN DETALLADA

Así, de acuerdo a uno de sus aspectos, la presente invención suministra un proceso para preparación de N-acil-(epi)K5-amina-O-sobre sulfato, caracterizados por

- (a) un derivado de (epi)K5-N-sulfato, y en forma ácida, es tratado con un base terciara u orgánica cuaternaria, permitiéndole a la mezcla de reacción permanecer durante un periodo de tiempo de 30-60 minutes, manteniendo el pH de la solución a un valor de aproximadamente 7 y su sal es aislada con dicha base orgánica;
- (b) dicha sal de base orgánica de dicho derivado (epi)K5-N-sulfato se trata con un reactivo de O-sulfatación en las condiciones de O-sobre sulfatación
- (c) el producto así obtenido es tratado con un derivado funcional de un ácido carboxílico (C₂-C₄), el derivado de N-acil-(epi)K5-amina-O-sobre sulfato así obtenido es aislado.

De manera general el derivado de N-acilo-(epi)K5-amina-O-sobre sulfato es aislado en forma de sal sodio y opcionalmente dicha sal de sodio es transformada en otra sal química o farmacéuticamente aceptable.

En este contexto, el termino "químicamente aceptable" se refiere a un catión utilizable en síntesis química, tal como los iones sodio, amonio, (C₁-C₄) o para la

purificación del producto, mientras "farmacéuticamente aceptable" es auto explicativo.

Los cationes ventajosos sin aquellos derivados de metales alcalinos, metales alcalinos tales como, amonio, tetraalquilamonio (C_1-C_4), aluminio y zinc. Los cationes preferidos son los iones de calcio, sodio y tetrabutylamonio.

De acuerdo con un procedimiento ventajoso, la etapa (a) es llevada a cabo al pasar una solución de la sal de sodio del derivado (epi)K5-N-sulfato, es decir, que el polisacárido K5, previamente viene bien N-desacetilado, N-sulfatado, preferiblemente el 100% opcionalmente 20-60% C5-epimerizado y opcionalmente despolimerizado con ácido nitroso, teniendo un peso molecular medio aproximadamente 1.000 a aproximadamente 25.000 ventajosamente y aproximadamente 1500 a aproximadamente 25.000, a través de una resina de intercambio iónico ácido, por ejemplo del tipo IR-120H⁺, que recolecta el elevado que incluye también el agua de desperdicio de la resina y neutralizar el elevado con base orgánica terciaria o cuaternaria, preferiblemente con una solución acuosa de hidróxido de

tetrabutylamonio. La solución se le permite permanecer durante 1 hora, manteniendo su pH en aproximadamente 7 es decir (tratando de mantener la neutralidad) mediante la adición de la misma base orgánica terciaria o cuaternaria, preferiblemente una solución acuosa de hidróxido de tetrabutylamonio, y la sal así obtenida se recupera mediante liofilización.

En la etapa (b) la O-sobre sulfuración ocurre utilizando un exceso de agente O-sulfatante y trabajando a una temperatura de 20 a 70°C durante un periodo de tiempo de hasta 24 horas en un solvente polar aprótico.

Ventajosamente, la sal con una base orgánica terciaria o cuaternaria del derivado (epi)K5-N-sulfato, es decir, del polisacárido k5, previamente N-desacetilado, N-sulfatado preferiblemente 100%, u opcionalmente 20-60% C5-epimerizado y opcionalmente despolimerizado en ácido nitroso, tiene un peso molecular aproximadamente de 1500 a aproximadamente 25000 como se dio en la etapa (a), es disuelto en dimetilformamida y tratado con 2-10 moles de un reactivo de O-sulfatación para cada hidroxilo libre a una temperatura de 40-60°C durante 10-20 horas. Como el

reactivo de O-sulfatación es ventajosamente utilizada la piridina. SO_3 en una cantidad de 2.5-5 moles preferiblemente 2.5-4 moles por hidroxilo libre por sacárido y la reacción es ventajosamente llevada a cabo 50-60°C, preferiblemente a 55°C durante toda la noche. El producto obtenido luego de la terminación de la reacción es aislado mediante la adición de 0.1-1 un volumen de agua y neutralización, preferiblemente con el hidróxido de sodio, precipitación con solución de cloruro de sodio saturado en acetona, filtración y posible auto filtración.

El producto así obtenido es generalmente la sal de sodio de un derivado de (epi)K5-amina-O-sobre sulfato que tiene un contenido de ácidos glucurónico y idurónico como se mostró anteriormente. Cuando la reacción de sobre sulfatación es desarrollada sobre la sal de la base terciaria o cuaternaria, preferiblemente sobre la sal de tetrabutilamonio de un derivado de epik5-N-sulfato que tiene un peso molecular medio de aproximadamente 1.000 a aproximadamente 25.000, ventajosamente desde aproximadamente 1.500 a aproximadamente 25.000 en el cual el ácido idurónico es 20-60% del total de los ácidos

urónicos, un derivado de epik5-amina-O-sobre sulfato se obtiene teniendo un peso molecular medio de aproximadamente 3.500 a aproximadamente 40.000, ventajosamente y aproximadamente 4.500 a aproximadamente 40000 y un grado de sulfatación de al menos 3,4, ventajosamente de al menos 3,5, más ventajosamente de más de 3,55 a 4, preferiblemente de 3,55 a 3,8.

Cuando la reacción de sobre sulfatación es desarrollada sobre la sal de la base terciaria o cuaternaria, preferiblemente sobre la sal de tetralbutilamonio de un derivado de K5-N-sulfato- que tiene un peso molecular medio de aproximadamente 1500 a aproximadamente 25000, se obtiene un derivado de K5-amina-O-sobre sulfato que tiene un peso molecular medio de aproximadamente 3500 a 40000. Ventajosamente y aproximadamente de 4500 a aproximadamente 40.000 y un grado de sulfatación de al menos 2,2, ventajosamente de 2,2 a 3 o de 2,3 a 3 más ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9. Preferiblemente, el producto así obtenido viene de un Imw-K5-amina-O-sulfato y es preferiblemente la sal de sodio de un Imw-K5-amina-O-sobre sulfato que tiene un grado de sulfatación de 2,3 a 3. El peso molecular medio

de tal producto puede ser aproximadamente 3.500 a aproximadamente 11.000.

La sal de sodio así obtenida se puede convertir en otra sal. Por vía de ejemplo, el intercambio iónico de calcio se puede desarrollar trabajando con una membrana de ultra filtración.

En la etapa (C) el derivado de (epi)K5-amina-O-sobre sulfato con un alto grado de sulfatación N-acilado utilizando los métodos conocidos en la literatura.

La N-acilación es desarrollada al elaborar el derivado (epi)K5-amina-O-sobre sulfato originado de la etapa (b) reacciona con un derivado funcional de un ácido mono o dicarboxílico que contiene de dos a cuatro átomos de carbono en solución hidroalcohólica a una temperatura de aproximadamente 4°C. Como derivados funcionales de dichos ácidos carboxílicos (C₂-C₄), previamente de ácido acético, propiónico, malónico, o succínico o de mono ésteres, en particular metílico o etílico, del último, uno puede utilizar el anhídrido, el cloruro, o una mezcla de anhídrido y un extractivo. El producto obtenido, un derivado de N-(C₂-C₄) acil-(epi)K5-amina-O-sobre sulfato,

es neutralizado con una dosis, preferiblemente hidróxido de sodio y luego aislado mediante ultra filtración y precipitación con una solución de cloruro de sodio saturado en acetona. Opcionalmente la etapa de N-acilación es repetida hasta que obtiene la sustitución total o sin embargo más del 95%. Son así obtenido en N-acil-(epil)K5-amina-O-sobre sulfatos, en particular el N-acetil, N-(2-carboxilo)acetil, N-(2-metoxicarbonil)acetil, N-(2-etoxicarbonil)acetil, N-propianil, N-(3-carboxi) propianil, N-(3-metoxicarbonil)propianil, N-(3-etoxicarbonil)propionil, - (epi)K5-amina-O-sobre sulfatos.

Los nuevos derivados de N-acil-(epi)K5-amina-O-sobre sulfato así están generalmente en sus formas de sal de sodio. Dicha sal de sodio se puede convertir en otra sal química o farmacéuticamente aceptable. Las sales particularmente ventajosas son aquellas de metales alcalinos, de metales alcalinotérreos, de amonio, el tetraaquilamonio (C₁-C₄), aluminio y zinc. Las sales de sodio, calcio y tetrabutilamonio son las preferidas.

A diferencia en los procesos descritos en la WO 98/34958, que consiste de una sulfatación de fase heterogénea de la sal de sodio K5 para obtener sulfatos K5-O- (de acuerdo con la tecnología utilizada aquí "N-acetil-K5-amina-O-sulfatos") formulados sulfatación de 0,5 a 4 y que tienen que concurrir al uso de ácido clorosulfónico para obtener derivados debajo peso molecular de una manera no controlada, el proceso de la presente invención es extremadamente versátil. De hecho, este permite la preparación de N-amina-K5-O-sobre sulfatados de cada tipo en cada alto rendimiento con reacciones fácilmente controlables. En particular, el proceso de la presente invención permite la preparación de N-acil-K5-amina-O-sobre sulfatos de otra manera no obtenibles, como los derivados C-5 epimerizados, los derivados N-acilados con un acilo diferente del acilo presente en el K5 nativo y los derivados de bajo peso molecular. Además, en el método descrito en la WO 98/34958, el proceso de la presente invención permite lograr los LMW-K5-O-sulfatos de manera controlada con el fin de obtener el peso molecular medio deseado y por encima de todo un grado de sulfatación bien definido que puede variar desde los 2,3 a 3 pero que puede regularmente ser 2,7-2,9. Finalmente todos los productos del proceso de la presente invención,

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en particular las aminas intermedios libres y los N-acil-K5-amina-O-sobre sulfatos son ingredientes activos útiles para composiciones farmacéuticas o cosméticas.

Los materiales de partida (epi)K5-N-sulfato conocidos en la literatura o sus porciones, o LMN-(epi)K5-N-sulfatos preparados mediante despolimerización nitrosa de los sulfatos (epi)K5-sulfatos correspondientes.

Los derivados de partida (epi)K5-N-sulfato- tienen un peso molecular medio de aproximadamente 1.000 a aproximadamente 25.000, ventajosamente desde aproximadamente 1.500 a aproximadamente 25.000. Preferiblemente, como material de partida se utiliza un (epi)K5-sulfato que tiene un peso molecular medio entre 10.000 o 25.000 o LMW-(epi)K5-N-sulfato que tiene un peso molecular medio de aproximadamente 1.500 a aproximadamente 12.000, ventajosamente aproximadamente 1.500 a aproximadamente 8.000, preferiblemente aproximadamente 1.500 a 7.500.

Un IMWCepik5-N-sulfato que tiene un contenido de unidades idurónicas de aproximadamente 20% obtenidas mediante N-

desacetilación, N-sulfatación, y C5-epimerización de una porción de K5 que tiene un peso molecular de 5000 de describe en la WO 92/17507, en este producto contiene un alto porcentaje de grupos acetilo.

Un epik5-N-fulfato particularmente ventajoso como material de partida es aquel obtenido mediante la epimerización de un K5-N-sulfato virtualmente libre de grupos acetilo a su vez preparados de K5 particularmente puro, en particular que no contienen sustancias lipofílicas, descritas en la WO 02/068477.

K5-N-sulfatos C5-epimerizados de bajo peso molecular que tienen un mayor contenido de unidades idurónicas, en partículas 40-60%, preferiblemente 50-55% son al contrario nuevos productos particularmente ventajosos como materiales de partida en la preparación de derivados epik5-amina-O-sobre sulfatados N-sustituidos de la presente invención.

Los LMW-epiK5-N-sulfatos, también se pueden obtener mediante la epimerización C5 de los correspondientes LMW-K5-sulfatos, preferiblemente cuando su peso molecular

medio es más de 4000. Los LMW-K5-N-sulfatos virtualmente libres de grupos acetilos son todos nuevos productos preparables por fraccionamiento o, preferiblemente, por despolimerización de un K5-N-sulfato libre de grupos acetilo. El K5-N-sulfato es bien conocido en la literatura y es descrito en el documento citado aquí anteriormente para ilustrar el estado de la técnica. El material de partida anteriormente mencionado es invariablemente obtenido mediante N-desacetilación de K5 y la subsiguiente N-sulfatación de la K5-amina si obtenida. Sin embargo, se halló que la preparación de un K5-N-sulfato virtualmente libre de acetilo en los grupos NH_2 se ayuda si el K5 del cual este es preparado es particularmente puro, en particular si este no contiene sustancias lipofílicas. Se prefiere por lo tanto de acuerdo a la presente invención, utilizar un K5-N-sulfato de partida preparado de un K5 purificado como se describió en la WO 02/068477. Dicho K5-N-sulfato, cuyo espectro ^{13}C -RMN no muestra tasas de N-acetilo o NH_2 también se describen en la WO 02/068477.

Los nuevo LMW-epiK5-N-sulfatos como se mostró anteriormente son preparados por un proceso caracterizado por que un K5-N-sulfato es sometido, en cualquier orden,

- (i) a epimerización C5 con una D-glucuronil C5-epimerasa que es aislada, purificada y en solución o inmovilizada sobre un soporte sólido, ha un PH de aproximadamente 7, a una temperatura de aproximadamente 30% y por un periodo de 12-24 en la presencia de al menos un ión bivalente seleccionado entre calcio, magnesio, bario, y manganeso; y
- (ii) a despolimerización nitrosa opcionalmente seguida por reducción, donde normalmente con boro hidruro de sodio.

La expresión (en cualquier orden) muestra que el proceso puede indiferentemente llevar a cabo tanto en la dirección (i)-(ii), es decir en la secuencia anteriormente indicada, como en la dirección inversa, es decir también en la dirección (ii)-(i), sometiendo a K5-N-sulfato a primero la reacción despolimerización nitrosa, opcionalmente seguida por la reducción con borohidruro de sodio y posteriormente a la epimerización C5 en la condiciones anteriormente mencionadas. El orden preferido es en la dirección (i)→(ii). La secuencia

(ii)-(i) es preferiblemente utilizada partiendo con LMW-K5-N-sulfatos que tienen un peso molecular medio de más 4.000, preferiblemente partiendo con aproximadamente 6.000. Por ejemplo, uno puede determinar las cantidades de nitrito de sodio el cual, partiendo con un grama de epiK5-N-sulfato, permite lograr un Lmw-epiK5-N-sulfato con un peso molecular de más de 4.000, en partícula de al menos 6.000, con el fin de obtener intermedios útiles para la preparación de LMWepiK5-N, O-sobre sulfatos. De hecho, en este caso en la etapa (ii) se obtiene el porcentaje de epimerización óptima.

De acuerdo con un aspecto preferido de la invención, la epimerasa C5 se inmoviliza sobre un soporte sólido inerte.

La epimerasa C5, preferiblemente recombinante, aislada, y purificada por ejemplo de acuerdo a Campbell 1994, Wo 98/48006, Jin-Ping 2001 o Crawford 2001, se inmoviliza sobre un soporte inerte en la presencia del sustrato, es decir, en la presencia del derivado de partida K5-N-sulfato o en la presencia LMW-K5-N-sulfato, que ventajosamente tiene un peso molecular medio de más de 4.000, preferiblemente de al menos 6.000. La

inmovilización de desarrolla de acuerdo a métodos convencionales, por ejemplo, como se describió en la WO 01/72848.

La reacción de epimerización C5 es llevada a cabo al circular 20-1000 ml de una solución de 25 mM de HEPES a un PH de aproximadamente de 7 que contiene 0.001-10 g de sustrato (K5-N-sulfato o LMW-K5-N-sulfato, preferiblemente con un peso molecular de más de 4000, en partícula de al menos de 6000) y un catión seleccionado entre calcio, magnesio, bario y manganeso a una concentración entre 10 y 60 mM a través de una Columna que contiene de 1.2×10^7 a 3×10^{11} cpm de la enzima desmovilizada, manteniendo el pH aproximadamente 7 a aproximadamente 30°C, en un flujo 30-220ml/hora durante un periodo de 12-24 horas, ventajosamente de 15-24 horas.

Preferiblemente la dicha solución es recirculada en un flujo de aproximadamente 200 ml/hora durante la noche (15-20 horas). El producto obtenido se purifica y se separa de acuerdo a métodos conocidos, por ejemplo, mediante micro filtración y precipitación con el calor. El producto así obtenido está comprendido o de epiK5-N-sulfato (y en tal caso es disuelto en agua y sometido a

despolimerización) o de LMW-epik5-N-sulfato (en tal caso este constituye el producto final). El porcentaje de epimerización, en la práctica en la cantidad de unidades idurónicas con relación a aquellas glucurónicas, se calculan con ^1H -RMN de acuerdo al método descrito en WO 96/4425.

La reacción de despolimerización nitrosa se lleva acabo de acuerdo a métodos conocidos mediante la despolimerización de heparina, por ejemplo de acuerdo al método descrito en la EP 373119, en la WO 82/03627 o de acuerdo al método mediante la despolimerización de un K5-N-sulfato descrito en la EP544592, pero partiendo con un K5-N-sulfato o un epik5-N-sulfato que contiene de 0 a no más de un 10% preferiblemente a no más del 5% de grupos acetilo. Preferiblemente, la despolimerización, desarrollada con nitrilo de sodio y ácido clorhídrico sobre un epik5-N-sulfato virtualmente libre de grupos acetilo, es seguida por reducción in situ con boro hidruro de sodio.

En la practica, una solución acuosa fría de epik5-N-sulfato es llamada a un PH ácido (aproximadamente 2) con

ácido clorhídrico y, aún fría tratada con nitrilo de sodio manteniendo la temperatura (aproximadamente 4°C) y el PH (aproximadamente 2) constante y, luego de la terminación de la despolimerización (aproximadamente 15-30 minutos) la solución se neutraliza con hidróxido de sodio y se trata a una temperatura aproximadamente 4°C, con una solución acuosa de boro hidruro de sodio. Luego de la terminación de la reducción (aproximadamente 4 horas) el exceso de borohidruro de sodio se destruye con ácido clorhídrico, la solución se neutraliza con hidróxido de sodio y se despolimeriza (y se reduce) el producto es aislado de acuerdo con métodos conocidos por ejemplo mediante precipitación directa con etanol o acetona. El producto obtenido luego de la terminación de la despolimerización puede ser un Lmw-K5-N-sulfato (en tal caso este constituye el producto final) o un Lmw-K5-N-sulfato (en tal caso es directamente sometido a epimerización C5 como se mostró aquí anteriormente, después del aislamiento o también en solución sin ser previamente aislado), en particular cuando este tiene un peso molecular medio de las de 4000, preferiblemente de algunos 6000, o se utiliza para preparar LMN-K5-N, O-sobresulfatado de actividad antiangiogénica y anti viral. Mediante la reacción de despolimerización apropiadamente

controlante, en particular utilizando diferentes cantidades de nitrilo de sodio/acido clorhídrico, LMW-K5-N-sulfatos o los LMW-epiK5-N-sulfatos son obtenidos teniendo un peso molecular medio en intervalo completo de aproximadamente 1.500 a aproximadamente 12.000, ventajosamente desde aproximadamente 1.500 a aproximadamente 10.000, preferiblemente desde aproximadamente 1.500 a aproximadamente a 7.500, calculado en el espectro ^{13}C -RMN ha traves de la integración de la señal atribuida a C2 del 2,5-anhidromanitol con aquel del carbono anomérico de la glucosamina dentro de la cadena de polisacáridos.

De acuerdo con un procedimiento general partiendo por ejemplo con un gramo de K5-N-sulfato a de epiK5-N-sulfato, el producto de partida es disuelto en 100-200 ml de agua desionizada y termo establecida a 4°C. Luego una cantidad de nitrilo de sodio se agrega con el fin de obtener el peso molecular medio deseado, por ejemplo de aproximadamente 2.000 a aproximadamente 4.000. Por lo tanto, partiendo de un epiK5-N-sulfato que tiene un peso molecular de 20.000 medido con el método HPLC equipada con una columna BioRad BioSil 250 y utilizando un estándar de heparina de peso molecular conocido, se

requerirá la adición de 330 a 480 mg de nitrilo de sodio disuelto en una solución acuosa al 0,2%. La solución que contiene el epik5-N-sulfato y el nitrilo de sodio, mantenida a 4°C, es llevada a un PH 2 a través de la adición de HCL 0,1 N enfriada a 4°C. Esta reacciona bajo agitación lenta durante 20-40 minutos, entonces es neutralizada con NaOH 0,1 N. el producto obtenido es llevado a temperaturas ambiente de cuarto y tratado con agente reductor tal como por ejemplo borohidruro de sodio (250-500 mg disueltos en 50-100 ml de agua) y echa a reaccionar durante 4-8 horas, el exceso de borohidruro de sodio se elimina elevando el PH a 5-5,5 con HCL 0,1 N y se le permite permanecer durante 2-4 horas adicionales. Al final este es neutralizado con NaOH 0,1 N y el producto es recuperado mediante precipitación con acetona o etanol después de haber concentrado el producto a través de evaporación a presión reducida.

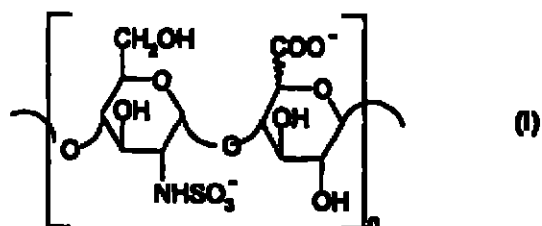
Generalmente, las cantidades de nitrito de sodio se pueden determinar las cuales, partiendo con 1 g de K5-N-sulfato o de epik5-N-sulfato, permiten el logro de un Lmw-K5-N-sulfato o un Lmw- epik5-N-sulfato con un peso molecular medio de aproximadamente 4.000 a

aproximadamente 12.000, ventajosamente desde aproximadamente 4.000 a aproximadamente 7.500, en particular de 6.000-7.500.

Los LMW-epiK5.N-sulfatos así obtenidos, con un contenido de ácido idurónico desde 20 a 60% ventajosamente desde 40 a 60% preferiblemente de 50-55% y virtualmente libre de grupos NH_2 y N-acetilo, que tiene un peso molecular medio de aproximadamente 1500 a aproximadamente 12.000, ventajosamente desde aproximadamente 1.500 a aproximadamente 10.000, preferiblemente desde aproximadamente 1.500 a aproximadamente 7.500 y sus sales químicamente o farmacéuticamente aceptables constituyen nuevos productos útiles como materiales de partida en la preparación de LMW-epiK5-N, O-sobre sulfatos particularmente interesantes, pero también ellos mismos útiles como ingredientes activos de composiciones farmacéuticas o cosméticas y constituye un aspecto adicional de la presente invención.

Ventajosamente los materiales de partida en la preparación de derivados de N-aceti-(epi)K5-amina-O-sobre sulfato de la presente invención son derivados de

(epi)K5-N-sulfato que consisten de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tienen la fórmula 1.



En la cual la proporción de unidades glucurónicas/unidades idurónicas es de 100/0 a 40/60, n es un entero de 2 a 100, ventajosamente de 3 a 100, y el catión correspondiente es química o farmacéuticamente aceptable.

En particular dichos derivados de (epi)K5-N-sulfato de partida son consistentes de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tienen la fórmula 1 anteriormente mencionada, con un contenido de unidades glucurónicas de 100 a 40%, ventajosamente con un contenido de ácido glucurónico de 100% u 80 - 40% de ácido glucurónico y 20 - 60% de ácido idurónico, n es un entero 2 a 100, ventajosamente de 3 a 100, y el catión correspondiente es químicamente aceptable.

Preferiblemente, el contenido de ácido glucurónico es 100% del total de ácidos urónicos 40-60% es ácido glucurónico y 60-40% es ácido idurónico.

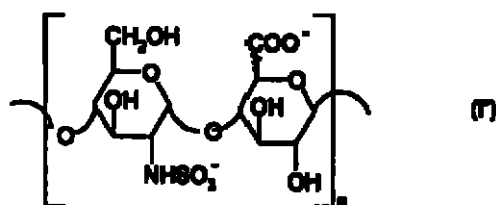
Los materiales de partida preferidos son LMW-(epi)K5-N-sulfatos que consisten de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tiene la fórmula I en la cual las unidades urónicas son todas consistentes de ácido glucurónico o son 40-60% comprendidas, preferiblemente 50-55%, de ácido idurónico, n es un entero de 2 a 20, ventajosamente de 3 a 15 y el catión correspondiente es químicamente aceptable. Los epiK5-N-sulfatos preparados mediante epimerización C5 de epiK5-N-sulfatos son bien conocidos y ampliamente descritos en la literatura. Su preparación mediante epimerización C5 de la unidad glucurónica de K5-N-sulfato con un C5 D glucuronil epimerasa se describió en los documentos citados aquí anteriormente para ilustrar el estado de la técnica, por ejemplo, en la WO 92/17507, WO 98/14425, WO 97/43317, WO 01/72848 y US 2002/0062019. sin embargo de acuerdo a una manera preferencial del procedimiento, mediante la epimerización se utiliza un K5-N-sulfato obtenido de un K5 libre de sustancias lipofílicas como se describió en la WO 02/068477 y la epimerización C5 es

desarrollada con un D-glucuronil C5 epimerasa aislado, purificado e inmovilizado sobre un soporte sólido, a un pH de aproximadamente 7, a una temperatura de aproximadamente 30° C' y durante un periodo de tiempo de 12 - 24 horas en la presencia de al menos un ión bivalente seleccionado entre calcio, magnesio, bario y manganeso, como se mostró anteriormente. También el K5-N-sulfato fue mostrado aquí anteriormente.

Los (epi)K5-N-sulfatos N-sulfatados de bajo peso molecular, en particular aquellos en los cuales las unidades urónicas están todas en el ácido glucurónico y aquellas que tienen un contenido de unidades idurónicas de 40-60%, preferiblemente 50-55% son al contrario todos nuevos productos particularmente ventajosos como materiales de partida en la preparación de derivados de N-acil-(epi)K5-amino-O-sobresulfato de acuerdo con la presente invención. Dichos materiales de partida son LMW-(epi)K5-N-sulfatos que tienen un contenido de ácido idurónico de 0% a 40-60% con relación al total de los ácidos urónicos y un peso molecular medio de aproximadamente 1.500 a aproximadamente 12.000, ventajosamente de aproximadamente 1.500 a aproximadamente 8.000, preferiblemente de aproximadamente 1.500 a

aproximadamente 7.500; y sus sales química o farmacéuticamente aceptables. La distribución puede variar desde aproximadamente 1.000 a aproximadamente 10.000.

Dicho LMW-(epi)K5-N-sulfatos son ventajosamente consistentes de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tienen la fórmula I'



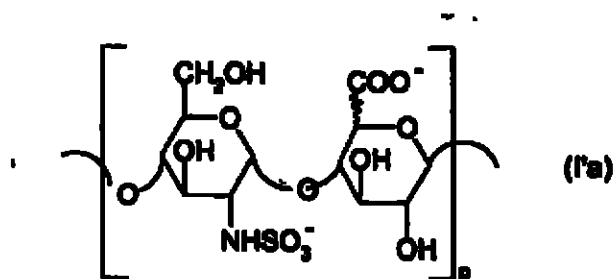
En la cual, las unidades urónicas son 100% consistentes de ácido glucurónico o de 60-40% de ácido glucurónico y 40-60% de ácido idurónico, q es un entero de 2 a 20 y el catión correspondiente es química o farmacéuticamente aceptable.

En este contexto, el término "químicamente" se refiere a un catión utilizable en síntesis química, tal como iones de sodio, amonio, tetralquilamonio (C₁-C₄), o para la purificación del producto.

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Los cationes ventajosos son aquellos derivados de los metales alcalinos, metales alcalinotérreos, amonio, tetralquilamonio (C_1-C_4), aluminio y zinc. Los cationes preferidos son los iones de sodio, calcio y tetrabutilamonio.

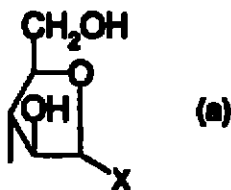
Los materiales de partida preferidos son los nuevos LMW-(epi)K5-N-sulfatos consistentes de una mezcla de cadena en la cual las especies preponderantes tienen la fórmula I'a



En la cual las unidades urónicas son 100% consistentes de ácido glucurónico o 60-40% glucurónico y 40 a 60% de ácido idurónico, p es un entero de 4 a 8, el peso molecular medio de estos productos va de aproximadamente 2.000 a aproximadamente 4.000 y el catión correspondiente es química o farmacéuticamente aceptable.

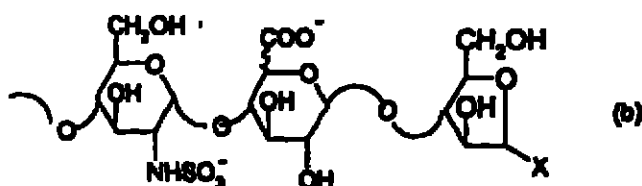
En particular los nuevos LMW(epi)K5-N-sulfatos son materiales de partida útiles que consisten de una mezcla de cadena en la cual al menos el 95% de dichas cadenas tienen la fórmula I' mostrada aquí anteriormente obtenida por despolimerización nitrosa del correspondientes (epi)K5-N-sulfatos mostrados anteriormente y la subsecuente posible reducción por ejemplo con borohidruro de sodio.

El origen de estos (epi)K5-N-sulfatos provenientes de una etapa de despolimerización nitrosa involucra, en el extremo reductor de la mayoría de las cadenas en dicha mezcla de cadena, la presencia de una unidad de 2,5-anhidromanosa o, en el caso de reducción con por ejemplo sodio boro hidruro, de 2,5-anhidromanitol de estructura (a)



En la cual X representa un grupo formilo o un grupo hidroximetilo. Por lo tanto, el extremo reductor de la

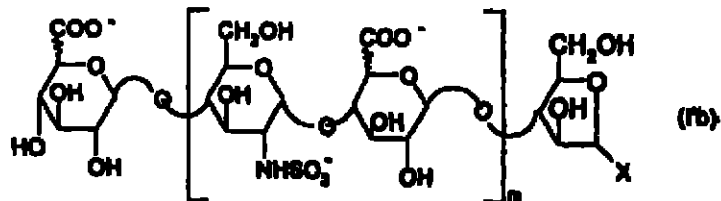
mayoría (60 - 70%) de las cadenas está actualmente representada por la estructura (b)



En la cual X es como se definió anteriormente.

La presencia de la estructura (a) no tiene ninguna influencia sobre las características químicas de los (epi)K5-N-sulfatos y sus derivados en razón a que cualquiera situaciones involucrarían una posible introducción de 1 o 2 grupos sulfato lo cual sin embargo no cambiaría significativamente el grado de sulfatación de los derivados O-sulfatados. Adicionalmente, la presencia de la estructura (a) no influencia la actividad biológica de los productos, como se demostró por (Østergaard 1987) P.B. et al. en Trombosis Research, 1987, 45, 739-749 (Østergaard 1987) para heparinas de bajo peso molecular.

Los LMW(epi)K5-N-sulfatos particularmente ventajosos de acuerdo con la presente invención son consistentes de mezclas de cadena en los cuales las especies preponderantes son un compuesto de fórmula I'b



En la cual X es formilo o hidroximetilo, m es 4, 5, o 6, el catión correspondiente es un ión química o farmacéuticamente aceptable. Las unidades urónicas son todas de ácido glucurónico o las unidades glucurónicas e idurónicas están presentes alternativamente, partiendo con una unidad glucurónica o idurónica, en tal caso la proporción glucurónica/idurónica va de 45/55 a 55/45 es decir aproximadamente 50/50.

Todos los LMW(epi)K5-N-sulfatos aquí mostrados anteriormente son nuevos productos que constituyen un objeto adicional de la presente invención.

Los LMW-(epi)K5-N-sulfatos, es decir los LMW-K5-N-sulfatos, mostrados anteriormente y, a diferencia de otros epoK5-N-sulfatos, por ejemplo como se indicó en la WO 92/17507, también los LMW-(epi)K5-N-sulfatos de la presente invención, estando virtualmente libres de grupos N-acetilo, no tienen actividad anticoagulante y tienen una interesante actividad contra los radicales libres. Gracias a su baja toxicidad, los nuevos LMW-K5-N-sulfatos, y los LMW-(epi)K5-N-sulfatos son ingredientes activos para la preparación de composiciones farmacéuticas utilizables como adyuvantes en el tratamiento de cardiopatía isquémica y para el tratamiento de dermatitis por radiación o de composiciones útiles como antienvejecimiento de la piel.

Cuando los LMW-(epi)K5-N-sulfatos mostrados anteriormente son utilizados como materiales de partida para la reacción de O-sobresulfatación, se prefiere en cualquier caso que, en su preparación por despolimerización nitrosa como se mostró anteriormente, dicha despolimerización sea seguida por la reducción por ejemplo con borohidruro de sodio para dar el LMW-K5-N-sulfatos caracterizados por unidades terminales (a) en las cuales X es hidroximetilo, en razón a que de acuerdo con el proceso de la presente

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invención dichos LMW-(epi)K5-N-sulfatos son sometidos a reacciones de sulfatación y acilación cuya influencia, del radical 2,5-anhidromanosa de la estructura (a) es desconocida sobre el grupo formilo, en el cual X representa formilo.

Dichos materiales de partida son preferiblemente utilizados en forma de sal de sodio, a menos que una sal ya esté disponible con una base terciaria u orgánica cuaternaria preparada de acuerdo con la etapa (a) mostrada anteriormente, preferiblemente la sal de tetrabutilamonio.

De acuerdo con la presente invención, los derivados de (epi)K5-N-sulfato de partida, preferiblemente 100% N-sulfatados, son sometidos a las etapas anteriormente mencionadas (a) y (b), luego de la terminación de la cual son aislados los correspondientes nuevos derivados de (epi)K5-amino-O-sobresulfato, en los cuales la amina no es sustituida, normalmente en la forma de sal de sodio, la cual se puede transformar en otra sal química o farmacéuticamente aceptable.

Así, de acuerdo con otros de sus aspectos, la presente invención se refiere a nuevos derivados de (epi)K5-amino-O-sobresulfato y sus sales química o farmacéuticamente aceptables obtenibles' mediante un proceso caracterizado porque (a) un derivado de (epi)K5-N-sulfato, en forma ácida, es tratada con una base terciaria u orgánica cuaternaria que permite que la mezcla de reacción permanezca durante un periodo de 30-60 minutos, manteniéndolo el pH de la solución en un valor de aproximadamente 7 mediante la adición de dicha base terciaria u orgánica cuaternaria y su sal se aisle con dicha base orgánica; (b) dicha sal de base orgánica de dicho derivado (epi)K5-N-sulfato es tratado con un reactivo de O-sulfatación en las condiciones de O-sobresulfatación y el derivado de (epi)K5-amino-O-sobresulfato es aislado.

El derivado así obtenido es generalmente un derivado de (epi)K5-amino-O-sobresulfato que tiene un peso molecular medio de aproximadamente 4.500 a aproximadamente 40.000, aislado en forma de sal de sodio que se puede transformar en otra sal química o farmacéuticamente aceptable. Las sales particularmente ventajosas son aquellas de metales alcalinos, metales alcalinotérreos, de amonio,

tetraalquilamonio (C_1-C_4) aluminio y zinc, y entre estos, las sales de sodio, calcio y tetrabutylamonio son las preferidas.

Como ya se mostró aquí anteriormente, el grado de sulfatación de los derivados de (epi)K5-amino-O-sobresulfato como se obtuvieron anteriormente dependen de la configuración del derivado de (epi)K5-N-sulfato de partida, en razón a que el volumen de la cadena de disacárido del derivado K5-N-sulfato en la cual las unidades urónicas están comprendidas exclusivamente de ácido glicurónico, permiten una sobresulfatación que es menor de aquella que ocurre con el derivado de (epi)K5-N-sulfato, especialmente si el grado de epimerización del último es 40-60% de ácido glucurónico y 60-40% de ácido idurónico.

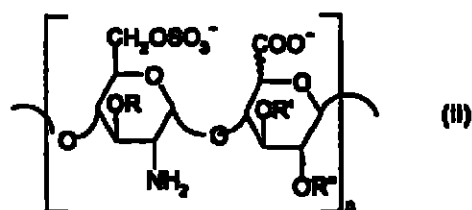
De hecho se ha hallado que, sometiendo un derivado de K5-N-sulfato a las etapas anteriormente mencionadas (a) y (b) un derivado de K5-amino-O-sobresulfato se obtiene teniendo un grado de sulfatación de 2,2 a 3, ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9.

Sometiendo a un derivado de epiK5-N-sulfato a las etapas anteriormente mencionada (a) y (b) en las cuales el contenido de ácido idurónico es 20 - 60% preferiblemente 40 - 60% del total de los ácidos urónicos, se obtiene un derivado de epiK5-amina-O-sobresulfato que tiene un gado de sulfatación de al menos 3,4, ventajosamente de al menos 3,5, más ventajosamente de 3,55 a 4, preferiblemente de 3,55 a 3,8.

Estos derivados de (epi)K5-amina-O-sobresulfatado son productos útiles como intermedios en la preparación de sus derivados N-(C₂-C₄)acilados y como ingredientes activos básicamente libres de actividad sobre los parámetros de coagulación pero que tienen otras propiedades farmacológicas interesantes, útiles para la preparación de composiciones farmacéuticas. Para el uso de los derivados de (epi)K5-amina-O-sobresulfato de la presente invención como ingredientes activos de composiciones farmacéuticas, es ventajoso preparar tanto los derivados de bajo peso molecular, con un peso molecular medio de aproximadamente 3.000 a aproximadamente 11.500, preferiblemente desde aproximadamente 4.500 a aproximadamente 8.500, con una distribución de peso molecular de entre aproximadamente

1.000 y aproximadamente 15.000, preferiblemente entre aproximadamente 2.000 y aproximadamente 10.000 y derivados de alto peso molecular, que se originan de un K5 no fraccionado, con un peso molecular medio de aproximadamente 15.000 a aproximadamente 45.000, preferiblemente entre aproximadamente 20.000 y aproximadamente 45.000, con una distribución de peso molecular desde aproximadamente 2.000 a aproximadamente 70.000.'

Así, de acuerdo con otros de sus aspectos la invención suministra nuevos ingredientes activos de composiciones farmacéuticas que consisten de derivados de (epi)K5-amina-O-sobresulfato que consisten de mezcla de cadena en las cuales al menos el 90% de dichas cadenas tienen la fórmula II



En la cual n es un entero de 2 a 100, preferiblemente de 3 a 100, R, R' y R'' son hidrógeno o SO₃⁻, las unidades

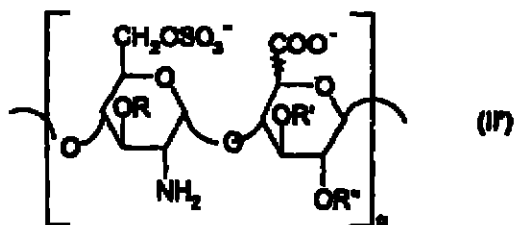
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urónicas son todas de ácido glucurónico para un grado de sulfatación de al menos 2,2, ventajosamente de 2,2 a 3 o de 2,3 a 3, más ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9, o son 20-60% consistentes de ácido idurónico, para un grado de sulfatación de al menos 3,4, ventajosamente de al menos 3,5, más ventajosamente de 3,55 a 4, preferiblemente de 3,55 a 3,8 y el catión correspondiente es química o farmacéuticamente aceptable.

Los derivados de (epi)K5-amina-O-sobresulfatados ventajosos de grado extremadamente alto de sulfatación son consistentes de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tienen la fórmula II en la cual las unidades urónicas son 40-60% consistentes de ácido idurónico, n es un entero de 2 a 100, preferiblemente de 3 a 100, con un peso molecular medio de aproximadamente 2.000 a aproximadamente 40.000 ventajosamente de aproximadamente 4.500 a aproximadamente 40.000, R es al menos 40% preferiblemente 50-80% SO_2^- , R' y R'' son ambos SO_2^- o uno es hidrógeno y el otro es 5-10% SO_3^- , en ácido monosulfato glucurónico y 10-15% SO_3^- en ácido monosulfato idurónico, el grado de sulfatación es

más de 3,4 y el catión correspondiente es química o farmacéuticamente aceptable.

Los derivados preferidos de epiK5-amina-O-sobresulfatados de muy alto grado de sulfatación son los lmw-epiK5-amina-O-sobresulfatos que consisten de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tienen la fórmula II'

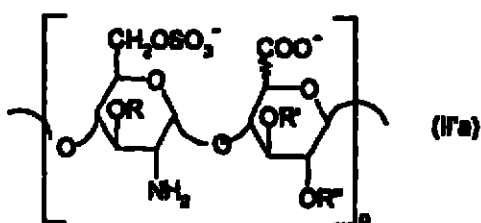


En la cual 'q' es un entero de 2 a 20, R, R' y R'' son hidrógeno o SO₃-, las unidades urónicas están 20 - 60% comprendidas, preferiblemente 40-60%, de ácido idurónico para un grado de sulfatación de 3,55 a 4, y el catión correspondiente es un ión química o farmacéuticamente aceptable.

Son particularmente interesantes las mezclas de cadena de la fórmula II' en las cuales las unidades urónicas están 40-60% comprendidas, preferiblemente 50-55% de ácido

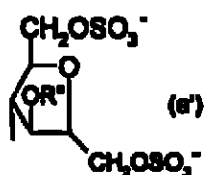
idurónico, Res al menos 40%, ventajosamente 50 - 80%, preferiblemente aproximadamente 65% SO_3^- , R' y R'' son ambos SO_3^- o uno es hidrógeno y el otro 5 - 10% de SO_3^- en ácido glucurónico y 10 - 15% de SO_3^- en ácido idurónico, N es un entero de 3 a 15, con un peso molecular medio de aproximadamente 4.000 a aproximadamente 8.000 y el catión correspondiente es química o farmacéuticamente aceptable.

Entre ' estos Lmw(epi)K5-amina-O-sobresulfatos, son preferidos aquellos que consisten de una mezcla de cadena en la cual las especies preponderantes tienen la fórmula II'a



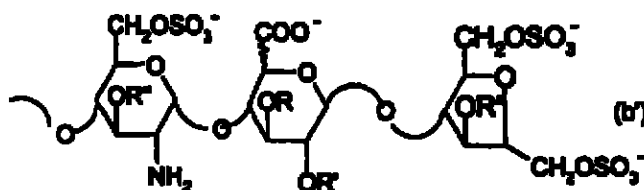
En la cual p es un entero de 4 a 8, R, R' , y R'' son como se definió anteriormente, el grado de sulfatación va de 3,55 a 4 y el catión correspondiente es química o farmacéuticamente aceptable.

El origen del nuevo LMW-epik5-amina-O-sobresulfatado proveniente del LMW-epik5-N-sulfatos provenientes de los mediante despolimeración nitrosa y subsecuente reducción con, por ejemplo, borohidruro de sodio, involucra, en el extremo reductor de las mayorías de las cadenas en dicha mezcla de cadena, la presencia de una unidad 2,5-anhidromanitol sulfatada de la estructura (a').



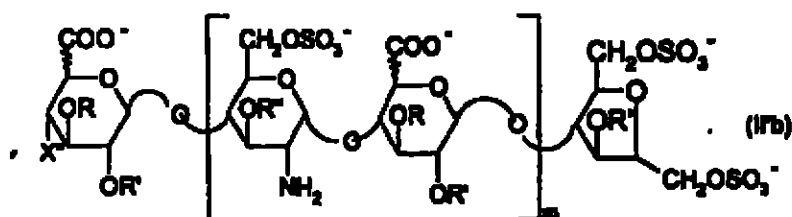
En la cual R'' representa hidrógeno SO₃.

El extremo reductor de la mayoría de las cadenas el dicha mezcla de cadenas está representado por la estructura (b').



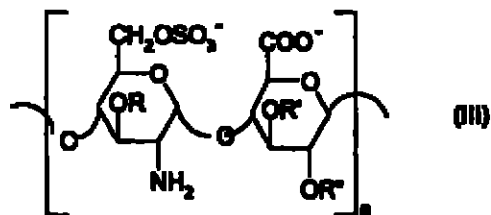
En la cual la unidad urónica puede ser glucurónica o hidrónica.

Entre los nuevos LMW-epik5-amina-O-sobresulfatados anteriormente mencionados, se prefieren aquí los que consisten de mezclas en los cuales las especies preponderantes son un compuesto de fórmula II'b.



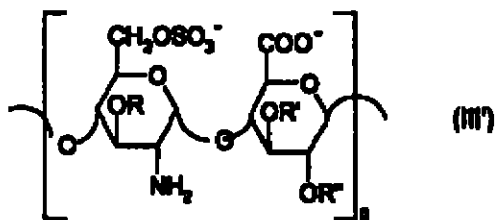
En el cual R, R' y R'' son hidrógeno o SO₃⁻, X'' es OH o OSO₃⁻, m es 4,5 o 6, las unidades urónicas son 40-60% consistentes de ácido idurónico, para un grado de sulfatación de 3,5 a 4, las unidades idurónicas estando presente alternativamente, partiendo con una unidad glucorónica o idurónica, y el catión correspondiente es uno química o farmacéuticamente aceptable.

De acuerdo con otro de sus aspectos la invención suministra nuevos derivados de K5-amina-O-sobresulfato que consisten de mezclas de cadena de los cuales al menos el 920% de dichas cadenas tiene la fórmula III

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En las cuales n es un entero de 2 a 100, preferiblemente de 3 a 100, R , R' y R'' son hidrógeno o SO_3^- , el grado de sulfatación es al menos 2,2, ventajosamente de 2,2 a 3 o desde 2,3 a 3, más ventajosamente de 2,5 a 3, preferiblemente 2,7 a 2,9 y el catión correspondiente es química o farmacéuticamente aceptable.

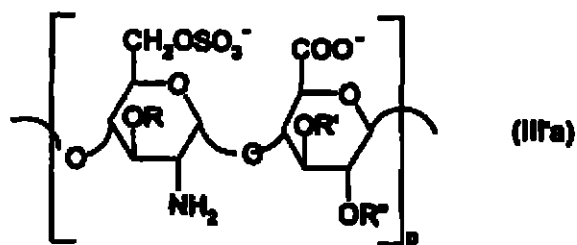
Los K5-amina-O-sobresulfatos preferidos son los LMW-K5-amina-O-sobresulfatos que consisten de mezcla de cadenas de los cuales al menos el 90% de dichas cadenas tiene la fórmula III'



En la cual q es un entero de 2 a 20, R , R' y R'' representan hidrógeno o un grupo SO_3^- , para un grado de

sulfatación de al menos 2,2, ventajosamente de 2,2 a 3 o 2,3 a 3, más ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9 y el catión correspondiente es un ión químicamente o farmacéuticamente aceptable.

Entre estos LMW-K5-amina-o-sobresulfatos son preferidos aquellos que consisten de una mezcla de cadena en los cuales las especies preponderantes tienen la fórmula III'a



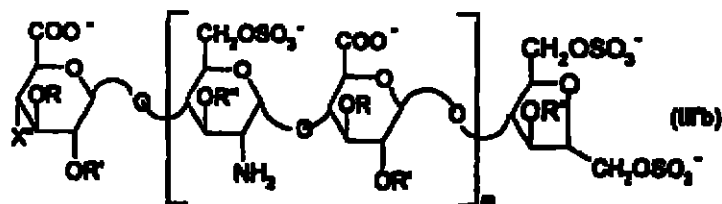
En la cual p es un entero de 4 a 8, R , R' y R'' son como se definió anteriormente, el grado de sulfatación es al menos 2,2, ventajosamente de 2,2 a 3 o 2,3 a 3, más ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9 y el catión correspondiente es químicamente o farmacéuticamente aceptable.

El origen del nuevo LMW-K5-amina-O-sobresulfatado proveniente del LMW-k5-sulfatos obtenidos mediante

despolimerización nitrosa y la subsecuente reducción como ejemplo, borohidruro de sodio, involucra en el extremo reductor de la mayoría de las cadenas en dicha mezcla de cadena, la presencia de una unidad de 2,5 -anhidromanitol sulfatada de estructura (a') como se mostró anteriormente, en la cual R'' representa hidrógeno o SO_3^-

Así, el extremo reductor de la mayoría de las cadenas en dicha mezcla de cadena que representa mediante la estructura (b') como se mostró anteriormente, en la cual la unidad urónica es todavía de ácido glucurónico.

Entre los nuevos LMW-K-amina-O-sobresulfatos anteriormente mencionados, son preferidos aquí los que consisten de mezclas en la cuales las especies preponderantes son un compuesto de fórmula III'b



En la cual R, R' y R'' son hidrógeno o SO_3^- , X'' es OH o OSO_3 , para un grado de sulfatación es al menos 2,2,

ventajosamente de 2,2 a 3 o 2,3 a 3, más ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9, m es 4, 5 o 6 y el catión correspondiente es un ión químicamente o farmacéuticamente aceptable.

El LMW-K5-amina-O-sobresulfados son nuevos productos útiles como intermedios en la preparación de sus derivados N-sulfatados o N-acilados pero ellos mismos tienen ' propiedades farmacológicas interesantes, en particular como radicales antilibre microbiocidas y, como tales, constituyen nuevos ingredientes activos o composiciones farmacéuticas que constituyen por lo tanto un aspecto adicional de la presente invención.

En particular, de acuerdo con otro de sus aspecto, la invención se relaciona con el uso de los derivados de (epi)K5-amina-O-sobresulfato anteriormente mencionados con un alta grado de sulfatación para la preparación de nuevos derivados de epiK5-amina-O-sobresulfatos N-sustituídos.

Los derivados de N-acil-(epe)K5-amina-O-sobresulfato de acuerdo con la presente invención son obtenidos al

someter los derivados de (epi)K5-amina-O-sobresulfato a la etapa © del proceso de la presente invención.

Así, de acuerdo con uno de sus aspectos adicionales, la presente invención suministra nuevos N-acil(epi)k5-amina-O-sobresulfato obtenibles mediante un proceso caracterizado por que

(a) un derivado de (epi)K5-N-sulfato, en forma ácida como es tratado con una base orgánica terciaria o cuaternaria, permitiendo que la mezcla de reacción permanezca durante un periodo de tiempo de 30-60 minutos, manteniendo el pH de la solución en un valor de 7 mediante la adición de dicha base orgánica terciaria o cuaternaria y su sal es aislada con dicha base orgánica;

(b) dicha sal de la base orgánica de dicho derivado de (epi)K5-N-sulfato es tratado con un reactivo de O-sulfatación en las condiciones de O-sobresulfatación

(c) el producto así obtenido es tratado con un derivado funcional de un ácido carboxílico (C₂-C₄), el derivado de N-acilo-(epi)K5-amina-O-sobresulfato así obtenido es aislado. Generalmente el derivado de N-acil-(epi)K5-amina-O-sobresulfato es aislado en forma de sal de sodio

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y opcionalmente dicha sal de sodio es transformada en otra sal química o farmacéuticamente aceptable.

Como ocurre para los derivados de (epi)-K5-amina-O-sobresulfato, también el grado de sulfatación de los derivados N-acil-(epi)k5-amina-O-sobresulfato como se obtuvieron anteriormente depende de la configuración del derivado de (epi)K5-N-sulfato de partida, en razón a que el volumen en la cadena de disacárido del derivado de K5-N-sulfato, en la cual las unidades urónicas están comprendidas exclusivamente de ácido glucurónico, permiten una sobresulfatación que es menor que la que ocurre con el derivado de epiK5-n-sulfato, especialmente si el grado de epimerización del último es 40-60% de ácido glucurónico y 60 y un 40% de ácido idurónico.

De hecho, se ha hallado que, sometiendo el derivado de K5-N-sulfato a las etapas anteriormente dichas (a)-(c) se obtiene un N-acil-k5-amina-O-sobresulfato se obtiene un derivado de N-acil-K5-amina-O-sobresulfato teniendo un grado de sulfatación de 2,2 a 3, ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9. m es 4, 5 o 6 y el catión correspondiente es un ión químicamente o farmacéuticamente aceptable.

Sometiendo a un derivado de epiK5-N-sulfato a las etapas anteriormente dichas (a), (b) y (c) en la cual el contenido de ácido idurónico es 20-60% del total de los ácidos urónicos, los derivados de N-acil-epiK5-amina-sobresulfato son obtenidos teniendo un grado de sulfatación de al menos 3,4, ventajosamente de al menos 3,5, más ventajosamente de 3,55 a 4, preferiblemente de 3,55 a 3,8.

Así, de acuerdo con otros de sus aspectos, la presente invención suministra nuevos derivados de N-acil-(epi)K5-amina-O-sobresulfato en el cual el acilo es un ácido carboxílico (C_2-C_4), que tiene un peso molecular medio de aproximadamente 2.000 a aproximadamente 45.000, preferiblemente entre aproximadamente 4.500 y aproximadamente 40.000, un grado de sulfatación como se indicó anteriormente, dichos derivados estando básicamente inactivos sobre los parámetros de coagulación pero los ingredientes activos útiles de las composiciones farmacéuticas o cosméticas.

Para uso de los derivados de N-acil-epiK5-amina-O-sobresulfato de la presente invención como productos

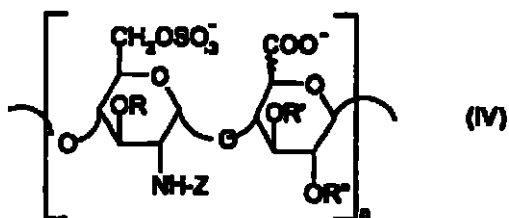
farmacéuticos o cosméticos es ventajoso preparar tanto derivados de bajo peso molecular con un peso molecular medio de aproximadamente 3.000 a aproximadamente 11.500, preferiblemente de aproximadamente 4.500 a aproximadamente 8.500, con una distribución de peso molécula de entre aproximadamente 1.000 y aproximadamente 15.000, preferiblemente entre aproximadamente 2.000 y aproximadamente 10.000 y derivados de alto peso molecular, que se originan de un K5 no fraccionado, con un peso molecular medio de aproximadamente 15.000 a aproximadamente 45.000, preferiblemente entre aproximadamente 20.000 y aproximadamente 45.000, con una distribución de peso molecular de aproximadamente 2.000 a aproximadamente 70.000.

En los derivados de N-acil-epiK5-amina-O-sobresulfato de la presente invención, grado de sulfatación es muy alto, en razón a que sobre los 4 hidroxilos libres disponibles por unidad de disacárido, al menos 3,4, preferiblemente de 3,5 a 3,8, son sulfatados mientras el nitrógeno de la glicosamina es virtualmente 100% acilado. Además, los derivados de N-acil-epiK5-amina-O-sobresulfato son 100% 6-O-sulfatados se encuentra en un 80% 3-O-sulfatados en sus unidades glucosalina, 5-10% 3-O-monosulfatados en

unidades glucurónicas, 10-15% O-monosulfatados en unidades idurónicas y 2,3-di-O-sulfatados en las unidades de urónicas restantes, que consideran que el grado de sulfatación es al menos 3,4.

Los derivados de N-acil-K5-amina-O-sobresulfato que, como se indicó anteriormente, tienen un grado de sulfatación de 2,2 a 3, son al menos 90% 6-O-sulfatados.

De acuerdo con otro de sus aspectos, la invención suministra nuevos derivados de N-acil-epiK5-amina-O-sobresulfato que consisten de mezclas de cadenas en las cuales al menos el 90% de dichas cadenas tienen la fórmula IV

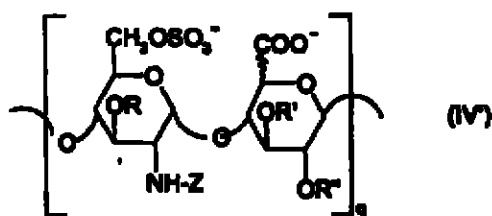


En la cual las unidades urónicas son 20-60% consistentes del ácido idurónico, n es un entero de 20 a 10, preferiblemente 3 a 100, R, R' y R'' son hidrógeno o SO₃-, Z es acilo (C₂-C₄), el grado de sulfatación es al menos

3,4, ventajosamente de al menos 3,5, más ventajosamente de 3,55 a 4, preferiblemente de 3,55 a 3,8 y el catión correspondiente químicamente farmacéuticamente aceptable.

Los derivados N-acil-epiK5-amina-O-sobresulfato ventajosos de grado extremadamente alto de sulfatación son consistentes de una mezcla de cadena en la cual al menos el 90% de dichas cadena tiene la fórmula IV en la cual las unidades urónicas son 40-60% consistentes de ácido idurónico, n es un entero de 2 a 100, preferiblemente de 3 a 100, con un peso molecular medio de aproximadamente 2.000 a 45.000, ventajosamente desde aproximadamente 4.500 a aproximadamente 45.000, R es al menos 40% preferiblemente 50-80% SO_3^- , R' y R'' son ambos SO_3^- o uno es hidrógeno y el otro es 5-10% SO_3^- en ácido monosulfato glucurónico y 10-15% SO_3^- en ácido monosulfato idurónico, el grado de sulfatación es más de 3,4 y el catión correspondiente es química o farmacéuticamente aceptable.

Los derivados de N-acil-epiK5-amina-O-sobresulfato de extremadamente alto grado de sulfatación de particular interés son N-acil-LMW-epiK5-amina-O-sobresulfatos que consisten de una cadena en la cual al menos el 90% de dichas cadena tienen la fórmula IV'

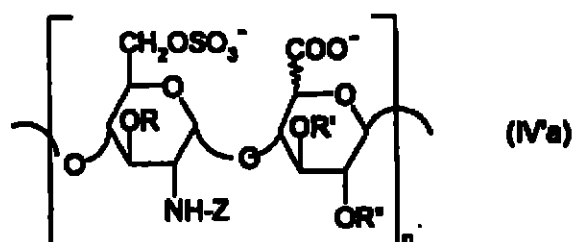


En la cual q es un entero de 2 a 20, R , R' y R'' representan hidrógeno o un grupo SO_3^- para un grado de sulfatación de 3,55 a 4, Z es acilo ($\text{C}_2\text{-C}_4$) y el catión correspondiente es un ión química o farmacéuticamente aceptable.

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Las mezclas de cadena de la fórmula IV en la cual las unidades urónicas son 40-60% comprendidas, preferiblemente 50-55%, de ácido idurónico, es al menos 40%, ventajosamente se encuentre en un 80%, preferiblemente aproximadamente 65% $\text{SO}_3\text{-R}'$ y R'' son ambas SO_3^- o uno es hidrógeno y el otro 5-10% de SO_3^- en ácido glucurónico y 10-15% SO_3^- en ácido idurónico, q es un entero de 3 a 15, con un peso molecular medio de aproximadamente 4.500 a aproximadamente 9.000 y el catión correspondiente es química o farmacéuticamente aceptable son los preferidos.

N-acil-LMW-epiK5-amina-O-sobresulfatos particularmente interesantes son aquellos de muy bajo peso molecular que consisten de una mezcla de cadena en la cual las especies de ponderantes son un 'compuesto de fórmula IV'a

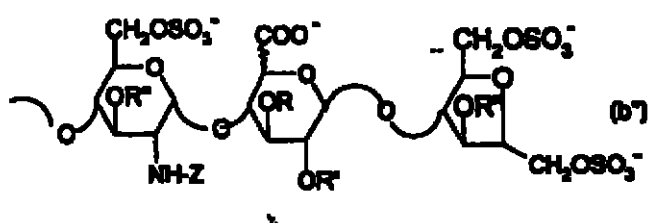


En la cual p es un entero de 4 a 8, R , R' y R'' representan hidrógeno o un grupo SO_3^- para un grado de sulfatación de 3,55 a 4, Z es acilo ($\text{C}_2\text{-C}_4$), y el catión correspondiente es un ión química o farmacéuticamente aceptable.

El origen de los nuevos N-acil-LMW-epiK5-amina-O-sobresulfatos proveniente de LMW-epiK5-sulfatos obtenidos mediante despolimerización nitrosa y la subsecuente reducción con, por ejemplo, borohidruro de sodio, involucran, en el extremo reductor de la mayorías de las cadenas en dicha mezcla de cadena, la presencia de una unidad de 2,5-anhidromanitol sulfatada de estructura (a')

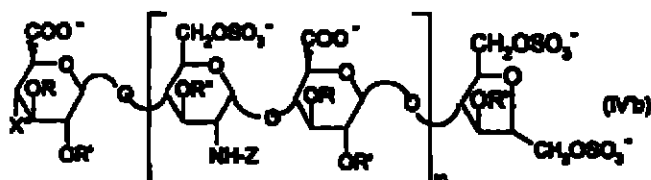
definida anteriormente, en la cual R'' representa hidrógeno o SO_3^- .

Así, el extremo reductor de la mayoría de las cadenas en dichas mezclas de cadenas es representado por la estructura (b'')



En la cual Z representa acilo ($\text{C}_2\text{-C}_4$) y la unidad urónica puede ser glucurónica o idurónica.

Entre los nuevos N-acil-LMWepiK5-amina-O-sobresulfatos anteriormente dichos son preferidos aquellos que consisten de métodos en los cuales la especie preponderante es un compuesto de fórmula IV'b



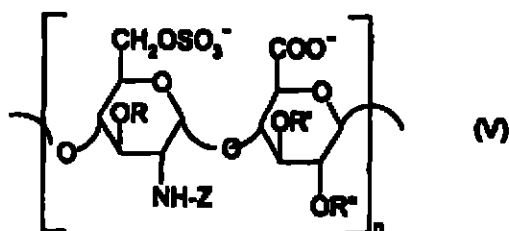
En la cual R, R' y R'' son hidrógeno o SO₃⁻ Z es acilo (C₂-C₄), x'' es OH o SO₃⁻, m es 4, 5 o 6, para un grado de sulfatación de 3,55 a 4, las unidades urónicas están presentes alternativamente, partiendo con una unidad glucurónica o idurónica y el catión correspondiente es química o farmacéuticamente aceptable.

Dichos cationes son ventajosamente aquellos de metales alcalinos, metales alcalinotérreos, de amonio, tetralquilamonio (C₁-C₄), aluminio y zinc y, entre estos, preferiblemente las sales de sodio, calcio y tetrabutylamonio.

En todos los N-acil-epiK5-amina-O-sobresulfatos mostrados anteriormente, en particular aquellos de la fórmula IV, IV', IV'a, IV'b como también en la estructura b'', el grupo acilo (Z) es preferiblemente una acilo (C₂-C₄) seleccionado del anterior grupo que consiste de acetilo, (2-carboxy)acetilo, (2-metoxicarbonil)acetilo, (2-

etoxicarbonil) acetilo, propenilo, (3-carboxi)propionilo, N-(3-metoxicarbonil)propionilo y (3-etoxicarbonil)propionilo.

De acuerdo con otro de sus aspectos, la invención suministra nuevos derivado de N-acil-K5-amina-O-sobresulfato que consiste de una mezcla de cadena en la cual al menos el 90% de dichas cadena tiene la fórmula V

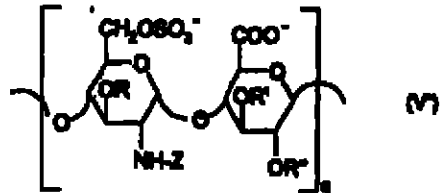


En la cual n es un entero de 2 a 100, preferiblemente de 3 a 100, Z es acilo (C_2-C_4), R, R' y R'' son hidrógeno o SO_3^- , el grado de sulfatación es al menos 2,2, ventajosamente de 2,2 a 3 o de 2,3 a 3, más ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9 y el catión correspondiente es química y farmacéuticamente aceptable.

Particularmente interesantes son los nuevos N-acil-LMW-K5-amina-O-sobresulfatos que consisten de una mezcla de

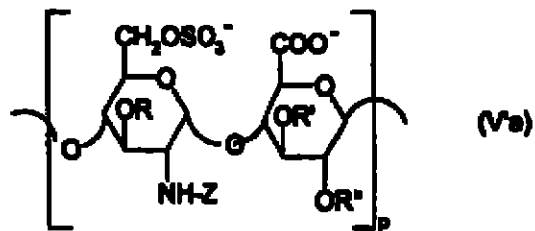
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cadena en las cuales al menos el 90% de dichas cadenas tienen la fórmula V'



En la cual q es un entero de 2 a 20, Z es acilo ($\text{C}_2\text{-C}_4$), R, R' y R'' representan hidrógeno o un grupo SO_3^- para un grado de sulfatación de al menos 2,2. Ventajosamente de 2,2 a 3 o de 2,3 a 3, más ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9 y el catión correspondiente es ion química y farmacéuticamente aceptable.

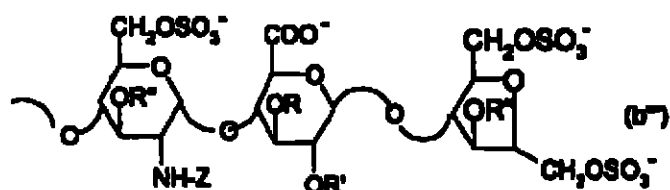
Entre estos N-acil-LMW-K5-amina-O-sobresulfatos son preferidos aquellos que consisten de una mezcla de cadena en la cual las especies preponderantes tienen la fórmula V'a



En la cual p es un entero de 4 a 8, Z es acilo (C_2-C_4), R , R' y R'' son como se definió anteriormente, el grado de sulfatación es de al menos 2,2, ventajosamente de 2,2 a 3 o de 2,3 a 3, más ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9 y el catión correspondiente es química o farmacéuticamente aceptable.

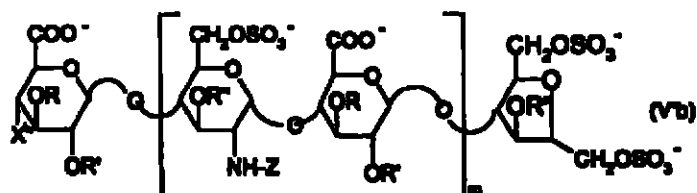
El origen de los nuevos N-acil-LMW-epiK5-amina-O-sobresulfatos proveniente de LMW-epiK5-sulfatos obtenidos mediante despolimeración nitrosa y la subsecuente reducción con, por ejemplo, borohidruro de sodio, involucran, en el extremo reductor de la mayorías de las cadenas en dicha mezcla de cadena, la presencia de una unidad de 2,5-anhidromanitol sulfatada de estructura (a') como se mostró anteriormente, en la cual R'' representa hidrógeno o SO_3^- .

Así, el extremo reductor de la mayoría de las cadenas en dichas mezclas de cadenas es representado por la estructura (b'')



En la cual Z, R, R' y R'' son como se definió anteriormente.

Entre los nuevos N-acil-LMW-K5-amina-O-sobresulfatos, anteriormente mencionados, son preferidos aquellos que consisten de mezclas en las cuales las especies preponderantes son un compuesto de fórmula V'b



En la cual Z es acilo (C_2-C_4), R, R' y R'' son hidrógeno o SO_3^- , X'' es OH o OSO_3^- para un grado de sulfatación de al menos 2,2, ventajosamente de 2,2 a 3 o de 2,3 a 3, más ventajosamente de 2,5 a 3, preferiblemente de 2,7 a 2,9, m es 4, 5 o 6 y el catión correspondiente es un ion química o farmacéuticamente aceptable.

En todos los N-acil-K5-amina-O-sobresulfatos mostrados anteriormente, en particular aquellos de la fórmula V, V', V'a, V'b, como también en la estructura de b''', el grupo acilo (Z) es preferiblemente un acilo (C₂-C₄) seleccionado de entre el grupo que consiste de acetilo, (2-carboxi)acetilo, (2-metoxycarbonil)acetilo, (2-etoxycarbonil)acetilo, propionilo, (3-carboxi)propionilo, N-3-metoxycarbonil)propionilo y (3-etoxycarbonil)propionilo.

Particularmente interesantes son los N-acil-K5-amina-O-sobresulfatos mostrados anteriormente en los cuales Z es un acilo (C₂-C₄) diferente acetilo. También interesante y particularmente activos son los N-acil-K5-amina-O-sobresulfatos mostrados anteriormente, en los cuales Z es acetilo, que tiene un grado de sulfatación de 2,7-2,9 preferiblemente de aproximadamente 2,8.

Los nuevos derivados de N-acil-(epi)K5-amina-O-sobresulfato, especialmente en su forma de sales, son productos altamente aniónicos capaces de capturar los radicales libres. Estos pueden ser utilizados en la industria de cosméticos como coadyuvantes contra la pérdida de pelo o para preparar cremas "anti-

envejecimiento", pero son todos los anteriores útiles en la industria farmacéutica, como productos para el tratamiento de dermatitis y como microbiocidas.

Así, de acuerdo con uno de sus aspectos adicionales, la presente invención suministra composiciones farmacéuticas que incluyen como uno de sus ingrediente activos, una cantidad farmacológicamente activa de un derivado de N-acil-(épi)K5-amina-O-sobresulfato como se mostró anteriormente o una de sus sales farmacéuticamente aceptables, en mezcla con excipiente farmacéutico.

En las composiciones farmacéuticas de la presente invención para administración oral, subcutánea, intravenosa, intradérmica o tópica, los ingredientes activos son preferiblemente administrados en la forma de unidades de dosis, en mezcla con excipientes o vehículos farmacéuticos clásicos. El régimen de dosis puede variar ampliamente dependiendo de la edad, el peso y la condición de salud del paciente. Este régimen de dosis incluye la administración de una dosis de 1 a 1000 mg, ventajosamente de 10 a 750 mg, preferiblemente 250 a 500 mg de una a tres veces por día mediante administración intravenosa, subcutánea, oral, transdérmica, o tópica

La presente invención también suministra una composición cosmética que incluye una cantidad efectiva de un derivado N-acil-(epi)K5-amina-O-sobresulfatado o una de sus sales farmacéuticamente aceptables, en mezcla con un excipiente cosmético.

Además, la presente invención suministra una composición farmacéutica que incluye, como uno de sus ingredientes activos un derivado (epi)K5-amina-O-sobresulfato obtenido de acuerdo con las etapas (a) y (b) del proceso descrito anteriormente, o una de sus sales farmacéuticamente aceptables, y mezcla con un excipiente farmacéutico. Ventajosamente, dicho derivado de (epi)K5-amina-O-sobresulfato es consistente de una mezcla de cadena en la cual al menos el 90% de dichas cadena tiene la fórmula II, II', III o III' o en las cuales las especies preponderantes tienen un compuesto de fórmula IIa, II'a, III'a o III'b. el ingrediente activo preferido es el LMW-K5-amina-O-sobredulfato que tienen un grado de sulfatación de 2,2 a 3, ventajosamente tiene un peso molecular medio y aproximadamente 3.500 a aproximadamente 11.000, más ventajosamente de aproximadamente 3500 a

aproximadamente 5.200 y básicamente libre de grupos N-acetil y N-sulfato.

En las composiciones farmacéuticas de la presente invención para administración oral subcutánea, intravenosa, transdérmica o tópica, los derivados de (epi)K5-amina-O-sobresulfatos ingredientes activos son preferiblemente administrados en la forma de unidades de dosis, en mezcla con los excipientes o vehículos farmacéuticos clásicos. El régimen de dosis puede variar ampliamente dependiendo de la edad, el peso y la condición de salud del paciente. Este régimen de dosis incluye la administración de una dosis de un derivado de (epi)K5-amina-O-sobresulfato de 1 a 1.000 mg, ventajosamente de 10 a 750 mg, preferiblemente 250 a 500 mg de 1 a 3 veces al día mediante administración intravenosa, subcutánea, oral, transdérmica o tópica.

La composición farmacéutica que incluye un derivado de (epi)K5-amina-O-sobresulfato tal como aquí mostrados anteriormente son formulados con los excipientes clásicos adecuados para diferentes forma de administración. Particularmente ventajosas son las formulaciones en la forma de cremas como ungüentos, linimentos, geles,

espumas, bálsamos, supositorios vaginales, supositorios, soluciones o suspensiones adecuadas para administración local. Finalmente, la presente invención suministra una composición farmacéutica que contiene, como uno de sus ingredientes activos, una cantidad farmacéuticamente activa de un LMW-(epi)K5-N-sulfato, es decir, de un LMW-K5-N-sulfato o de un LMW-epiK5-N-sulfato como se mostró anteriormente o de una de sus sales farmacéuticamente aceptables, en mezcla con excipiente farmacéutico.

En las composiciones farmacéuticas de la presente invención para administración oral, subcutánea, intravenosa, transdérmica o tópica, los ingredientes activos son preferiblemente administrados en la forma de unidades de dosis, en mezcla con excipientes o vehículos farmacéuticos clásicos.

El régimen de dosis puede variar ampliamente dependiendo de la edad, peso y condición de salud del paciente. Este régimen de dosis incluye la administración de una dosis de 1 a 1.000 mg, ventajosamente de 10 a 750 mg, preferiblemente 250 a 500 mg de 1 a 3 veces al día mediante administración intravenosa, subcutánea, oral, transdérmica o tópica.

De acuerdo con otro de sus aspectos la presente invención también suministra una composición cosmética que contiene como uno de sus ingredientes activos una cantidad efectiva de LMW-K5-N-sulfato o LMW-(epi)K5-N-sulfato de una de sus sales farmacéuticamente aceptables, en mezcla con un excipiente cosmético.

Ventajosamente, dicho derivado de LMW-(epi)K5-N-sulfato es consistente de una mezcla de cadena en la cual al menos el 90% de dichas cadena tiene la fórmula I, I', o en las cuales las especies preponderantes son un compuesto de fórmula Ia, I'b. El ingrediente activo preferido es el derivado LMW-(epi)K5-N-sulfato que tiene un peso molecular medio de aproximadamente 1 a aproximadamente 12.000, ventajosamente de aproximadamente 1.500 a aproximadamente 8.000, preferiblemente de aproximadamente 1.500 a aproximadamente 7.500 y básicamente libre de grupos N-acetilo.

Una sal seleccionada del grupo que consiste de sales de metales alcalinos o metales alcalinotérreos, de amonio, tetraalquilamonio (C₁-C₄), aluminio o zinc, en particular la sal de sodio, potasio, calcio, magnesio, aluminio o

zinc constituyen un ingrediente activo efectivo de las composiciones de la presente invención.

Los siguientes ejemplos ilustran la invención sin embargo limitarla.

PREPARACIÓN I

LMN-epiK5-N-sulfato

a) Epimerización de K5-N-sulfato

2 g de K5 N-sulfato, obtenido como se describió en el ejemplo 1, estas etapas (i) y (ii), de la WO02/068477, son disueltos en 120 ml de 25 ml de un amortiguador HEPES, pH 7, que contiene 50 mM de CaCl_2 . La solución obtenida se hace recircular a través de una columna de 50 ml llenada con la resina que contiene la enzima inmovilizada obtenida como se describió en la WO 96/14425. Esta operación es llevada a cabo a 30° C con un flujo de 200 ml/h durante 24 horas. El producto obtenido es purificado a través de ultrafiltración sobre una membrana de 1.000 D el paso sobre una columna de intercambio iónico 1R 120 H^+ , neutralizando el eluado con

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NaOH 1N. La muestra es recuperada mediante precipitación con etanol o acetona.

Un producto epimerizado se obtiene con una proporción de ácido idurónico / ácido glucurónico de 55/45 contra una promoción de 0/100 de producto o partida. El porcentaje de epimerización se calculó con ^1H -RMN de acuerdo con el método descrito en la WO 96/14425. El rendimiento, calculado mediante la revisión del contenido de ácidos urónicos contra un estándar con el método de carbazol (Bitter y Muir Anal. Biochem. 39, 88-92-1971) es 90%.

b) Despolimerización del epi-K5-N-sulfato

1 g del producto obtenido en la etapa (a) se despolimeriza mediante el método de degradación con ácido nitroso y la subsecuente reducción del aldehído que forma. En particular uno continua disolviendo el producto del 25 ml y agua destila y agregándola con 230 mg de nitrito de sodio disuelto en 115 ml de agua destilada. Las solución es luego llevada a 4° C y al pH 2 con HCl 0,1 N y mantenida durante 30 minutos. Al final de la reacción la solución es llevada a temperatura ambiente de

con NaOH 0,1 M. la solución es luego agregada con 450 mg de NaBH_4 y se hace reaccionar durante 4 horas. El producto es recuperado por precipitación con tres volúmenes de acetona a 4° C, filtración con un embudo de filtración y secada a 40° C en un horno de vacío, 900 mg del LMW-epiK5-N-sulfato son obtenidos con una distribución de peso molecular medida con el método HPLC que varía desde 1.000 a 4.000 y con un contenido unidad glucurónica de 45% y contenido de idurónica de 55%.

PREPARACIÓN II

LMW-K5-N-sulfato

El producto obtenido como se describió en el ejemplo 1, etapas (i) y (ii), de la WO02/068477 se despolimeriza mediante el método de degradación con ácido nitroso y la subsecuente reducción del aldehído que forma. Uno continua disolviendo un 1 g de K5-N-sulfato en 200 ml de agua destilada y agregándola con 480 mg de nitrito de sodio disuelta en 240 ml de agua destilada. La solución es luego llevada a 4° C y el pH a 2 con HCl 0,1 M y mantenida durante 30 minutos. Al final de la reacción la

solución es llevada a pH 7 con NaOH 0,1 M y luego a temperatura ambiente de cuarto. La solución es luego agregada con 450 mg de NaBH₄ y se hace reaccionar durante 4 horas. El exceso de NaBH₄ se elimina con HCl llevando el pH a 5-6. El producto neutralizado con NaOH 0,1 M es recuperado por precipitación con tres volúmenes de acetona a 4° C, filtración con un embudo de filtración y secada a 40° C en un horno de vacío. Son obtenidos 900 mg del LMW-K5-N-sulfato con peso molecular medio de aproximadamente 2.000, que consiste de una mezcla de cadena en la cual las especies preponderantes son un compuesto de fórmula I'b en la cual m es 4 y las unidades urónicas son aquellas de ácido glucurónico.

PREPARACIÓN III

a) Despolimerización del K5-N-sulfato

2 g de K5 N-sulfato, obtenido como se describió en el ejemplo 1, etapas (i) y (ii), de la WO02/068477 se despolimerizan como se describió en la PREPARACIÓN II, utilizando 100 mg de nitrito de sodio y 300 mg de

borohidruro de sodio. Son obtenidos 1,8 g de LMW-K5-N-sulfato con un peso molecular medio de 5.000

b) **Epimerización de LMW-K5-N-sulfato**

1 g de LMW-K5-N-sulfato obtenido en la etapa (a) se trata como se describió en la etapa (a) del ejemplo 1. Un producto epimerizado es obtenido con una proporción de ácido idurónico/ácido glucurónico de 44/56 contra una proporción de 0/100 del producto de partida, con una distribución de peso molecular de 2.000 a 10.000 y con un peso molecular medio de 5.000 D. El rendimiento calculado al medir el contenido de ácidos urónico contra un estándar con el método de carbazol (Bitter and Muir Anal. Biochem. 39,88-92-1971) es 90%.

PREPARACIÓN IV

Preparación del epiK5-N-sulfato

Una solución de 10 g de K5-N-sulfato se obtiene como describió en el ejemplo 1, etapas (i) y (ii), de la WO 02/068477 en 600 ml de 25 mM de un amortiguador HEDED a

pH 7, que contiene CaCl_2 a una concentración de 50 mM se hace recircular a través de una columna de 50 ml llena con resina 4B sefarosa que contiene 5g de la epimerasa C5-recombinante (WO '96/14425) inmovilizada como se describió en WO 01/72848. La reacción es llevada a acabo a 30° C a un pH 7 con un flujo de 200ml/h durante 24 horas. El producto obtenido se purifica mediante ultrafiltración y precipitación con etanol. Así se obtiene un epiK5-N-sulfato cuyo contenido de ácido idurónico es 54%.

PREPARACIÓN V

Preparación de LMW-epiK5-N-sulfato

Un 1 g del producto obtenido de la PREPARACIÓN IV se despolimeriza mediante el método de degradación con ácido nitroso y la subsecuente reducción del aldehído que forma. En particular uno continua disolviendo el producto 25 ml de agua destilada y agregándola con 230 mg de nitrito de sodio disuelto en 115 ml de agua destilada. La solución es luego llevada a 4° C y el pH 2 con HCl 0,1 N y mantenida durante 30 minutos. Al final de la reacción

la solución es llevada temperatura de ambiente de cuarto y el pH a 7 con NaOH 0,1 M. La solución es luego agregada con 450 mg de NaBH₄ y hecha reaccionar durante 4 horas. El producto es recuperado mediante precipitación con tres volúmenes de acetona a 4° C, la filtración con un embudo de filtración y secada a 40° C en un horno de vacío. Son obtenidos 900 mg del LMW-K5-N-sulfato con una distribución de peso molecular medida con el método HPLC que varía desde 1.000 a 4.000.

EJEMPLO 1

EpiK5-amina-O-sobresulfato

a) Sal de Teterbutilamonio de epiK5-N-sulfato

Una solución de 400 mg de epiK5-N-sulfato con un contenido de ácido idurónico del 54% como se obtuvo en la preparación IV en 40 ml de agua es termoestablecido en 4° C, luego pasado sobre una resina de intercambio iónico 1R 120 H⁺ reacondicionada con agua a 4° C. El eluado obtenido consiste de 100 ml de una solución a pH 1,94, es neutralizada con una solución de 15% de hidróxido de

tetrabutylamonio y dejada a temperatura ambiente de cuarto durante 1 hora, manteniendo el pH en 7 mediante la adición de 15% de hidróxido de tetrabutylamonio y finalmente es liofilizado. Así se obtiene 805 mg de sal de tetrabutylamonio de epiK5-N-sulfato.

b) Epi-K5-amina-O-sobresulfato

A una solución que contiene 805 mg de la sal así obtenida en 30 ml de dimetilformamida se ajusta a 45° C y se trata con 30 ml de dimetilformamida que contiene 2,26 g del aducto piridina.SO₃. la reacción a 55° C se continua durante toda la noche y luego 60 ml de agua se agregan a la mezcla. Después de la neutralización con NaOH 1N, el producto especificado con tres volúmenes de acetona saturada con NaCl y ajustada a 4° C durante la noche. El precipitado se recupera mediante filtración sobre Duch G4 y luego ultrafiltrada con un sistema Millipore TFF de 1.000 D y secada a presión reducida. Se obtienen 550 mg de epi-K5-amina-O-sobresulfada que tiene un contenido de ácido idurónico del 54%, de glucosamina 6-O-sulfato del 100% de glucosamina 3-O-sulfato del 60% de ácido mosulfaro glucurónico del 10%, de ácido monosulfato y

idurónico del 15% el resto de las unidades urónicas son disulfatadas, con un grado de sulfatación de 3,55 medido con el método conductométrico de acuerdo con Casu et al. 1975.

EJEMPLO 2

N-acetil-epiK5-amina-O-sobresulfato

A una solución 250 mg de epi-K5-amina-O-sobresulfato obtenida del ejemplo 175 ml de y enfriado a 4° C se agrega 7,5 ml de etanol y 3,75 ml de anhídrido acético. La reacción es mantenida a 4° C durante 2 horas manteniendo el pH un valor de 7 con NaOH 5M. Después de ultrafiltración sobre una membrana de 1.000 D son agregados tres volúmenes de acetona saturada con cloruro de sodio y el precipitado se recupera mediante centrifugación a 5.000 rpm durante 5 minutos. Así se obtienen 249 mg de N-acetil-epiK5-amina-O-sobresulfatada con un contenido de ácido idurónico del 54%, glucosalina-6-O-sulfato del 100%, N-acetil del 100%, glucosalina 3-O-sulfato del 30%, ácido monosulfato

glucurónico del 10%, ácido monosulfato idurónico del 15%, el resto de las unidades urónicas son disulfatadas, y cuyo grado de sulfatación es 3,5 medido con el método conductimétrico de acuerdo con Casu et al.1975.

EJEMPLO 3

N-acetil-K5-amina-O-sobresulfato

1 gr de K5 N,O sobresulfato obtenido en el Ejemplo 2 de la WO/020668477 se disuelven en 200 ml de agua y la solución es llevada a un pH 2 con HCl 1 N y termoestablecida a 50° C. La reacción es mantenida a 50° C durante 3 horas y luego enfriada a temperatura ambiente de cuarto y neutralizada con NaOH 1 N. la muestra es luego enfriada a 4° C y 20 ml de Me OH y 10 ml de anhídrido acético son agregados a la reacción que es dejada a 4° C durante 2 horas manteniendo el pH constantemente en 7. Al final de la reacción la muestra es purificada de sales mediante ultrafiltración sobre una membrana con una separación de 1.000 D y luego recuperada y secada a presión reducida. Se obtiene un producto 100%

N-acetilado, 100% 6-O sulfato, 30% monosulfato y 70% bisulfato sobre el ácido glucurónico.

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/65-**REIVINDICACIONES**

1. Proceso para la preparación de derivados N-acil-(epi)K5-amina-O-sobresulfato y de sus sales química o farmacéuticamente aceptables, caracterizados por que

(d) Un derivado derivado de (epi)K5-N-sulfato, en forma ácida, es tratado con un base orgánica terciara o cuaternaria, permitiéndole a la mezcla de reacción permanecer durante un periodo de tiempo de 30-60 minutes, manteniendo el pH de la solución a un valor de aproximadamente 7 y su sal es aislada con dicha base orgánica;

(e) Dicha sal de base orgánica de dicho derivado (epi)K5-N-sulfato es tratada con un reactivo de O-sulfatación en las condiciones de O-sobre sulfatación;

(f) El derivado (epi)K5-amina-O-sobresulfato así obtenido es tratado con un derivado funcional de un ácido carboxílico (C₂-C₄), el derivado de N-acil-(epi)K5-amina-O-sobresulfato es aislado.

2. Proceso de acuerdo con la reivindicación 1, caracterizado por que dicho derivado de N-acilo-(epi)K5-N, O-sobre sulfato es aislado en forma de sal sodio y opcionalmente dicha sal de sodio es transformada en otra sal química o farmacéuticamente aceptable.
3. Proceso de acuerdo con una cualquiera de las reivindicaciones 1 o 2, caracterizado por que, en la etapa (a) se utiliza hidróxido de tetrabutilamonio como una base orgánica.
4. Proceso de acuerdo con una cualquiera de las reivindicaciones 1 a 3, caracterizados por que, en la etapa (b) O-sobresulfatación es llevada a cabo el dimetilformamida utilizando 2-4 moles de un reactivo de O-sulfatación por OH disponible por disacárido a una temperatura de 40-60° C durante 15-20 horas.
5. Proceso de acuerdo con una cualquiera de las reivindicaciones 1 a 4, caracterizado por que

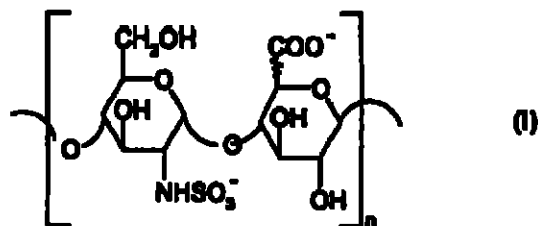
como material de partida es utilizado un (epi)K5-N-sulfato que tiene un peso molecular en medio de aproximadamente 1.000 a aproximadamente 25.000.

6. Proceso de acuerdo con una cualquiera de las reivindicaciones 1 a 5, caracterizado por que dichos materiales de partida son 40-60% C5-epimerizados.
7. Proceso de acuerdo con una cualquiera de las reivindicaciones 1 a 6, caracterizado por que dicho material de partida tiene un peso molecular medio de aproximadamente 1.500 a aproximadamente 25.000.
8. Proceso de acuerdo con la reivindicación 7, caracterizado por que dicho material de partida tiene un peso molecular medio entre 10.000 y 25.000.
9. Proceso de acuerdo con la reivindicación 7, caracterizado por que dicho material de partida

tiene un peso molecular medio de aproximadamente 1.500 a aproximadamente 12.000.

10. Proceso de acuerdo con la reivindicación 9, caracterizado por que dicho material de partida tiene un peso molecular medio de aproximadamente 1.500 a aproximadamente 8.000.

11. Proceso de acuerdo con la reivindicación 5, caracterizado por que como material de partida se utiliza un derivado de (epi)K5-N-sulfato que consiste de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tiene la fórmula I



En la cual la proporción de unidades glucurónicas/unidades idurónicas van de 100/0 a 40/60, n es un entero de 2 a 100 y el catión

correspondiente es químicamente o farmacéuticamente aceptable.

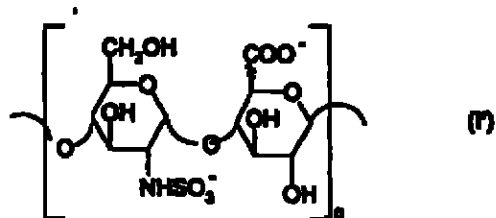
12. Proceso de acuerdo con la reivindicación 11, caracterizado por que dicho material de partida es consistente en una mezcla de cadena en la cual al menos el 90% de dichas cadenas tiene la fórmula I, en la cual las unidades urónicas son 40-60% consistentes de ácido idurónico.

13. Proceso de acuerdo con la reivindicación 11, caracterizado por que dicho material de partida es un LMN-(epi)K5-N-sulfato que consistente de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tiene la fórmula I, en la cual las unidades urónicas son todas consistentes ácido glucurónico o son 40-60% consistentes de ácido idurónico, n es un entero de 3 a 15 y el catión correspondiente es químicamente aceptable.

14. Proceso de acuerdo con la reivindicación 11, caracterizado por que dicho material de partida es un LMW-(epi)K5-N-sulfato consistente de una

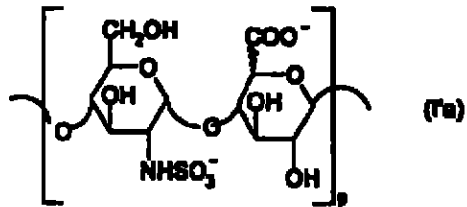
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mezcla de cadena en la cual al menos el 90% de dichas cadenas tiene la fórmula I'



En la cual las unidades urónicas son 100% consistentes de ácido glucurónico o son 60-40% de ácido glucurónico y 40-60% de ácido idurónico, q es un entero de 2 a 20 y el catión correspondiente es químicamente o farmacéuticamente aceptable.

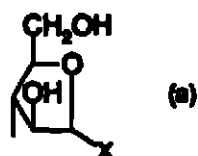
15. Proceso de acuerdo con la reivindicación 11, caracterizado por que dicho material de partida es un LMW-(epi)K5-N-sulfato consistente de una mezcla de cadena en la cual las especies preponderantes tienen la fórmula I'a



En la cual las unidades urónicas son 100% consistentes de ácido glucurónico o son 60-40% glucurónico y 40-60% de ácido idurónico, p es un entero de 4 a 8.

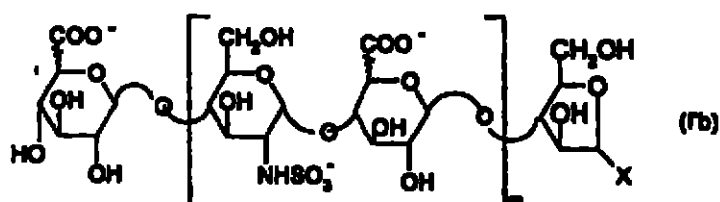
16. Proceso de acuerdo con una cualquiera de las reivindicaciones 5 a 15, caracterizado por que dicho material de partida es un LMW-(epi)K5-N-sulfato obtenido mediante despolimerización nitrosa del correspondiente (epi)K5-N-sulfato y la subsecuente reducción.

17. Proceso de acuerdo con la reivindicación 16, caracterizado por que dicho LMW-(epi)K5-N-sulfato de partida contiene, en extremo reductor de la mayoría de las cadenas en dicha mezcla de cadena una unidad 2,5-anhidromano de estructura (a)



en la cual X representa un grupo hidroximetilo.

18. Proceso de acuerdo con una cualquiera de las reivindicaciones 16 y 17, caracterizado por que como material de partida se utiliza un LMW-(epi)K5-N-sulfato que consiste de mezclas de cadenas en las cuales las especies preponderantes son un compuesto de fórmula I'b



En la cual X es hidroximetilo, m es 4, 5 o 6, el catión correspondiente es un ión química o farmacéuticamente aceptable, las unidades urónicas son todas de ácido glucurónico o las unidades glucurónica e idurónicas están presente

alternativamente, partiendo con una unidad glucurónica o idurónica

19. Proceso de acuerdo con una cualquiera de las reivindicaciones 1 a 18, caracterizada por que dicho derivado (epi)K5-N-sulfato de partida es utilizado en forma de sal de sodio.

20. Un derivado de N-acil-(epi)K5-amina-O-sobresulfato obtenible de acuerdo al proceso de la reivindicación de la 1 a la 19.

21. Un derivado de N-acil-(epi)K5-amina-O-sobresulfato de acuerdo con la reivindicación 20 en forma de sal de un metal alcalino o un metal alcalinotérreo, de amonio, de tetralquilamonio (C₁-C₄) , aluminio o zinc.

22. Un derivado de N-acil-(epi)K5-amina-O-sobresulfato en el cual el acilo es un acilo (C₂-C₄), que tiene un contenido de ácido idurónico de 20-60%, un peso molecular medio de aproximadamente 2.000 a 45.000 y un grado de de sulfatación de al menos

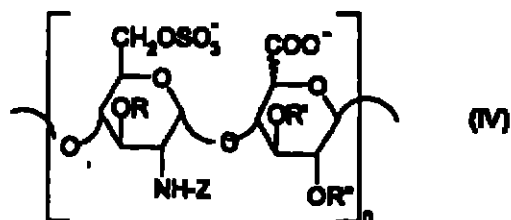
3,4, o una de sus sales química o farmacéuticamente aceptable.

23. Un derivado de N-acil-(epi)K5-amina-O-sobresulfato de acuerdo con la reivindicación 22, cuyo peso molecular medio está entre aproximadamente 15.000 y aproximadamente 45.000.

24. Un derivado de N-acil-(epi)K5-amina-O-sobresulfato de acuerdo con la reivindicación 22, cuyo peso molecular medio está entre aproximadamente 4.500 y aproximadamente 8.500.

25. Un derivado de N-acil-(epi)K5-amina-O-sobresulfato de acuerdo con una cualquiera de las reivindicaciones de la 22 a 24, caracterizado por que dicho grado de sulfatación va 3,4 a 3,8.

26. Un derivado de N-acil-(epi)K5-amina-O-sobresulfato que consiste de mezclas de cadena en las cuales al menos 90% de dichas cadenas tiene la fórmula IV

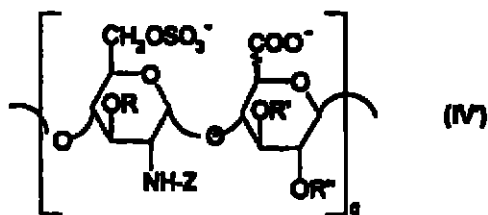
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En la cual las unidades urónicas son 20-60% consistente de ácido idurónico, n es un entero de 2 a 100, R , R' y R'' son hidrógeno o SO_3^- , Z es acilo ($\text{C}_2\text{-C}_4$), el grado de sulfatación es al menos 3,4, y el catión correspondiente es químicamente farmacéuticamente aceptable.

27. Un derivado de N-acil-(epi)K5-amina-O-sobresulfato de acuerdo con la reivindicación 26 caracterizado por que este consiste de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tiene la fórmula IV en la cual las unidades urónicas son 40-60% consistentes de ácido idurónico n es un entero de 3 a 100, con un peso molecular medio de aproximadamente 2.000 a aproximadamente 45.000, R es al menos 40% SO_3^- , R' y R'' son ambos SO_3^- o uno es hidrógeno y el otro es 5-10% SO_3^- en ácido monosulfato glucurónico y 10-15% SO_3^- en

ácido monosulfato idurónico y el catión correspondiente es química o farmacéuticamente aceptable.

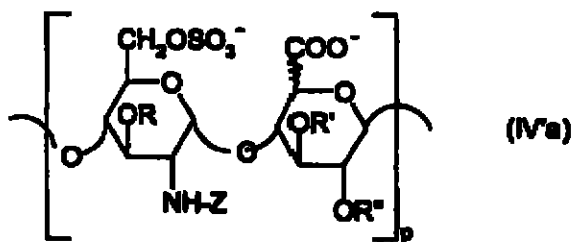
28. Un derivado de N-acil-(epi)K5-amina-O-sobresulfato de acuerdo con una cualquiera de las reivindicaciones 26 y 27, caracterizado por que un LMW-N-acilo-epiK5-O-sobresulfato que consiste de una mezcla de cadena de la cual al menos el 90% de dichas cadenas tiene la fórmula IV'



En la cual q es un entero de 2 a 20, R, R' y R'' representan hidrógeno o un grupo SO₃⁻ para un grado de sulfatación de 3,55 a 4, Z es acilo (C₂-C₄) y el catión correspondiente es química o farmacéuticamente aceptable.

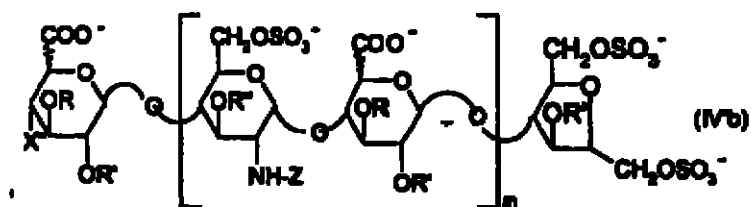
29. Un LMW-N-acil-(epi)K5-O-sobresulfato de acuerdo con la reivindicación 28, caracterizado por que en dicha mezcla de cadena de fórmula IV' las unidades urónicas son 50-55% consistente de ácido idurónico, R es al menos 40% SO₃, R' y R'' son ambas SO₃⁻ o uno es hidrógeno y el otro 5-10% de SO₃⁻ en ácido glucurónico y 10-15% SO₃⁻ en ácido idurónico, q es un entero de 3 a 15, con un peso molecular medio de aproximadamente 4.500 a aproximadamente 9.000 y el catión correspondiente es química o farmacéuticamente aceptable.

30. Un lmw-N-acilo-epiK5-O-sobresulfato de acuerdo con una cualquiera de las reivindicaciones 28 a 29, caracterizado porque este consiste de una mezcla de cadena en la cual las especies preponderantes es un compuesto de fórmula IV'a



En la cual p es un entero de 4 a 8, R , R' y R'' son hidrógeno o un grupo SO_3^- para un grado de sulfatación de 3,55 a 4, Z es acilo ($\text{C}_2\text{-C}_4$), y el catión correspondiente es química o farmacéuticamente aceptable.

31. Un $\text{lmw-N-acilo-epiK5-O-sobresulfato}$ de acuerdo con la reivindicación 30 caracterizado porque dichas especies preponderantes son un compuesto de fórmula IV'b

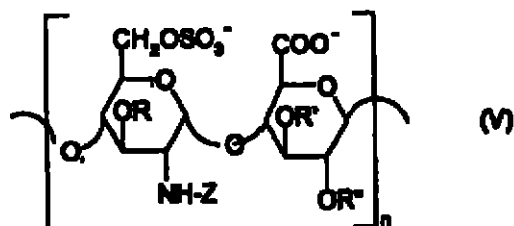


En el cual R , R' y R'' son hidrógeno o SO_3^- , Z es acilo ($\text{C}_2\text{-C}_4$), X'' es OH o OSO_3^- , m es 4, 5 o 6, para un grado de sulfatación de 3,55 a 4, las unidades urónicas están presentes alternativamente, partiendo con una unidad glucurónica o idurónica, y el catión correspondientes es química o farmacéuticamente aceptable.

32. Un $\text{lmw-N-acilo-epiK5-O-sobresulfato}$ de acuerdo con cualquiera de las reivindicaciones de la 22 a la 31 en la cual el acilo ($\text{C}_2\text{-C}_4$) sustituyente es seleccionado del grupo que consiste de un acetilo, (2-carboxi) acetilo, (2-metoxycarbonilo) acetilo, (2-etoxycarbonil) acetilo, propionilo, (3-carboxi) propionilo, N-(3-metoxycarbonil) propionilo y (3-etoxycarbonil) propionilo.

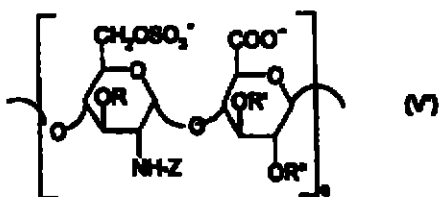
33. Un derivado $\text{lmw-N-acilo-epiK5-O-sobresulfato}$ de acuerdo con una cualquiera de las reivindicaciones 22 a 32, caracterizado porque dicha sal o un catión correspondiente es un metal alcalino o metal alcalinotérreo, y amoni, tetraalquilamonio ($\text{C}_1\text{-C}_4$), aluminio o zinc.

34. Un derivado de $\text{N-acil-K5-amina-O-sobresulfato}$ que consiste de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tienen la fórmula V



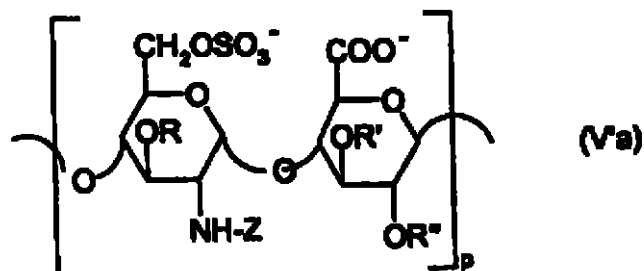
En la cual n es un entero de 2 a 100, Z es acilo ($\text{C}_2\text{-C}_4$), R , R' y R'' son hidrógeno o SO_3^- , el grado de sulfatación es al menos de 2,2, y el catión correspondiente es química o farmacéuticamente aceptable.

35. Un derivado de N-acil-K5-amina-O-sobresulfato de acuerdo con la reivindicación 34, caracterizado porque este es un lmw-N-acil-K5-amina-O-sobresulfato que consiste de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tiene la fórmula V'



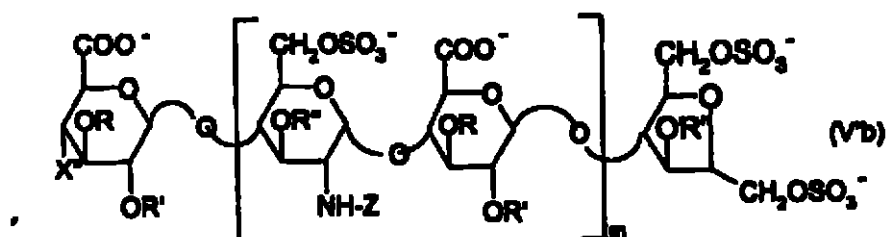
En la cual q es un entero de 2 a 20, Z es acilo(C_2-C_4), R , R' y R'' representan hidrógeno o un grupo SO_3^- para un grado de sulfatación de 2,2 a 3 y el catión correspondiente es química o farmacéuticamente aceptable.

36. Un lmw-N-acil-K5-amina-O-sobresulfato de acuerdo con la reivindicación 35, caracterizado porque éste consiste en una mezcla de cadena en la cual las especies preponderantes son un compuesto de fórmula V'a



En la cual p es un entero de 4 a 8, Z es acilo (C_2-C_4), R , R' y R'' representan hidrógeno o un grupo SO_3^- para un grado de sulfatación de 2,2 a 3 y el catión correspondiente es química o farmacéuticamente aceptable.

37. Un lmw-N-acil-K5-amina-O-sobresulfato de acuerdo con la reivindicación 36, caracterizado porque dichas especies preponderantes son un compuesto de fórmula V'b'



En la cual Z es acilo (C_2-C_4), R, R' y R'' son hidrógeno o SO_3^- , X'' es OH u OS_3^- , para un grado de sulfatación de 2,2 a 3 m es 4, 5 o 6 y el catión correspondiente es un ión química o farmacéuticamente aceptable.

38. Un derivado de N-acil-K5-amina-O-sobresulfato de acuerdo con una cualquiera de las reivindicaciones 34 a 37, caracterizado porque dicho grado de sulfatación es de 2,3 a 3.

39. Un derivado de N-acil-K5-amina-O-sobresulfato de acuerdo con la reivindicación 38, caracterizado porque dicho grado de sulfatación va de 2,5 a 3.

40. Un derivado de N-acil-K5-amina-O-sobresulfato de acuerdo con la reivindicación 39, caracterizado porque dicho grado de sulfatación va de 2,7 a 2,9.

41. Un derivado de N-acil-K5-amina-O-sobresulfato de acuerdo con una cualquiera de las reivindicaciones de la 34 a la 40, en la cual el sustituyente acilo (C_2-C_4) es diferente de acetilo.

42. Un derivado de N-acil-K5-amina-O-sobresulfato de acuerdo con una cualquiera de las reivindicaciones de la 34 a la 40, en la cual el sustituyente ha sido acilo (C_2-C_4) es acetilo, que tiene un grado de sulfatación de 2,7-2,9.

43. Un derivado de N-acil-K5-amina-O-sobresulfato de acuerdo con la reivindicación 42, que tiene un grado de sulfatación de aproximadamente 2,8.

44. Un derivado de N-acil-K5-amina-O-sobresulfato de acuerdo con una cualquiera de las

reivindicaciones de la 34 a la 43, caracterizado porque dicha sal o catión correspondientes es un metal alcalino o un metal alcalinotérreo, de amonio, tetraalquilamonio (C_1-C_4), aluminio de zinc.

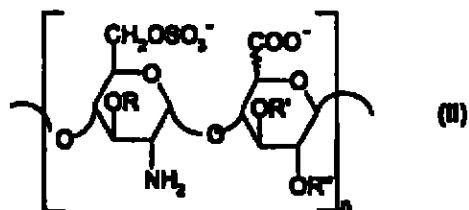
45. Composición farmacéutica que incluye, como uno de sus ingredientes activos, un derivado de (epi)k5-amina-O-sobresulfato o una de sus sales farmacéuticamente aceptables, obtenida por el proceso de acuerdo con las etapas (a) y (b) de la reivindicación 1, aislado en forma de sal de sodio y opcionalmente transformado en otra sal farmacéuticamente aceptable en mezcla con excipiente farmacéutico.

46. Composición de acuerdo con la reivindicación 45 caracterizada en que dicho ingrediente activo es un derivado de (epi)k5-amina-O-sobresulfato que tiene un peso molecular medio de aproximadamente 4.500 a aproximadamente 40.000.

47. Composición farmacéutica de acuerdo a una cualquiera de las reivindicaciones 45 y 46, en la

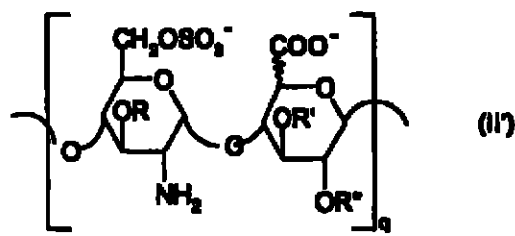
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cual dicho ingrediente activo es un derivado de (epi)k5-amina-O-sobresulfato que consiste de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tiene la fórmula II



En la cual n es un entero de 2 a 100, R, R', y R'' son hidrógeno o SO₃⁻, las unidades urónicas son todas de ácido glucurónico, para un grado de sulfatación de 2,2 a 3, o son 20-60% consistentes de ácido idurónico, para un grado de sulfatación de al menos 3,4, y el catión correspondiente es farmacéuticamente aceptable.

48. Composición farmacéutica de acuerdo con la reivindicación 47, caracterizada porque dicho ingrediente activo es un LMW-epiK5-amina-O-sobresulfato que consiste de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tiene la fórmula II'

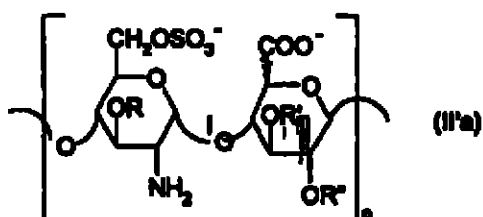


En la cual q es un entero de 2 a 20, R , R' y R'' son hidrógeno o SO_3^- las unidades urónicas son 20-60% comprendidas, preferiblemente 40 - 60% de ácido idurónico, para un grado de sulfatación de 3,55 a 4.

49. Composición farmacéutica de acuerdo con la reivindicación 48, caracterizada porque, en dicha mezcla de cadena de fórmula II', las unidades urónicas son 40-60% consistentes de ácido idurónico, R es al menos 40% SO_3^- , R' y R'' son ambos SO_3^- , o uno es hidrógeno y el otro es 5-10% SO_3^- en ácido glucurónico y 10-15% de SO_3^- en ácido idurónico, n es un entero de 3 a 15, con un peso molecular medio de aproximadamente 4.000 a aproximadamente 8.000.

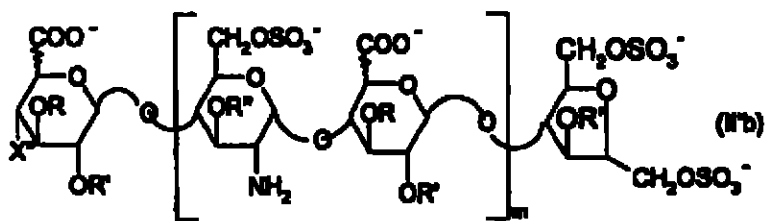
50. Composición farmacéutica de acuerdo con una cualquiera de las reivindicaciones 48 y 49,

caracterizado porque dicho LMW-epiK5-amina-O-sobresulfato que consiste de una mezcla de cadena en la cual las especies preponderantes tiene la fórmula II'a .



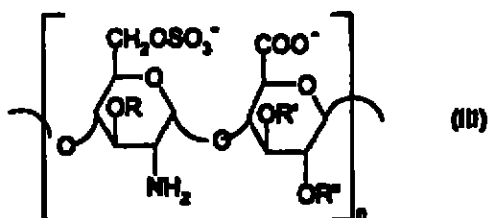
En la cual q es un entero de 4 a 8, R, R' y R'' son como se definió anteriormente, el grado de sulfatación va de 3,55 a 4 y el catión correspondiente es farmacéuticamente aceptable.

51. Composición farmacéutica de acuerdo con la reivindicación 50, caracterizado porque dichas especies preponderantes es un compuesto de fórmula II'b



En la cual R, R' y R'' son hidrógeno o SO₃⁻, X'' es OH o OS₃⁻, m es 4, 5 o 6, las unidades urónicas son 40-60% consistentes de ácido idurónico, para un grado de sulfatación de 3,55 a 4, las unidades idurónicas están presentes alternativamente, partiendo con una unidad glucurónica o idurónica, y el correspondiente es un ión farmacéuticamente 'aceptable.

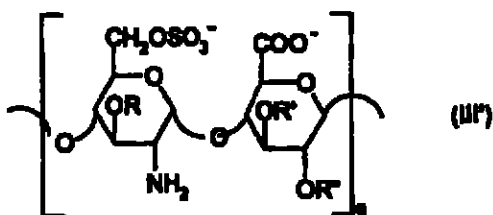
52. Composición farmacéutica de acuerdo con la reivindicación 45, que incluye como uno de sus ingredientes activos un derivado de K5-amina-O-sobresulfato consistente de una mezcla de cadena en la cual al menos el 90% de dichas cadenas tiene la fórmula III



En la cual n es un entero de 2 a 100, R, R' y R'' son hidrógeno o SO₃⁻, y el grado de sulfatación es

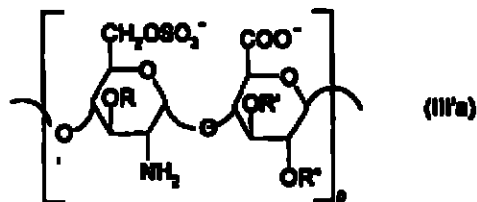
al menos 2,2, y el catión correspondiente es farmacéuticamente aceptable.

53. Composición farmacéutica de acuerdo con la reivindicación 52, caracterizado porque dicho ingrediente activo es un lmw-K5-amina-O-sobresulfato que consiste de una mezcla de cadena en la cual al menos el 90% de dichas cadenas 'tiene la fórmula III'



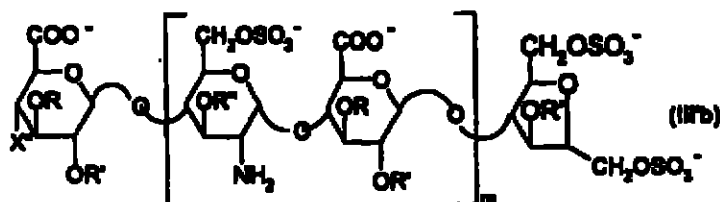
En la cual q es un entero de 2 a 20, R, R' y R'' representan hidrógeno o un grupo SO₃⁻ para un grado de sulfatación de al menos 2,2.

54. Composición farmacéutica de acuerdo con la reivindicación 53, caracterizada porque dicho lmw-K5-amina-O-sobresulfato que consiste de una mezcla de cadena en la cual las especies preponderantes tienen la fórmula III'a

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En la cual p es un entero de 4 a 8, R , R' y R'' son como se definió anteriormente, el grado de sulfatación es de 2,2 a 3.

55. Composición farmacéutica de acuerdo con la reivindicación 50, caracterizado porque dichas especies preponderantes es un compuesto de fórmula III'b



En la cual R , R' y R'' son hidrógeno o SO_3^- , X'' es OH u OSO_3^- , para un grado de sulfatación de 2,2 a 3, m es 4, 5 o 6 y el catión correspondiente es un ión farmacéuticamente aceptable.

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56. Composición farmacéutica de acuerdo con una cualquiera de las reivindicaciones de la 45 a 55 caracterizada porque dicha sal o catión farmacéuticamente aceptable son sodio, potasio, calcio, magnesio o zinc.

57. Composición farmacéutica de acuerdo con una cualquiera de las reivindicaciones de la 45 a 56 caracterizada porque este está en la forma de una crema, un ungüento, linimento, gel, espuma, bálsamo, supositorio vaginal, supositorio, solución o suspensión para administración local.

58. Una composición farmacéutica que contiene, como uno de sus ingrediente activos, una cantidad farmacológicamente activa de un $\text{lmw-(epi)k5-N-sulfato}$ básicamente libre de grupos acetilo, o de una de sus sales farmacéuticamente aceptables, en mezcla con un excipiente farmacéutico.

59. Una composición cosmética que contiene una cantidad efectiva de un $\text{lmw-(epi)k5-N-sulfato}$ de acuerdo con una cualquiera de las reivindicaciones de la 1 a la 10 y de 25 a 37, en la cual dicha sal o catión es farmacéuticamente aceptable, en mezcla con un excipiente cosmético.

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RESUMEN

Se describe un nuevo método para la sobresulfatación de (epi)K5-N-sulfatos para obtener (epi)K5-amina-O-sobresulfatos a un grado extremadamente alto de sulfatación y para la transformación de estos intermedios en nuevos N-acil-(epi)K5-amino-O-sobresulfato básicamente libres de actividad sobre los parámetros de coagulación y útiles en el campo cosmético o farmacéutico. También se describen composiciones farmacéuticas que contiene, como uno de sus ingredientes activos, un (epi)K5-amina-O-sobresulfato.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800174089-2

RECIBO OFICIAL DE CAJA : 04 - 99,402
FECHA : DICIEMBRE 27 DE 2004

SUPERINDUSTRIA Y COMERCIO
Radicacon : 04129790 00000000 Folios: 193
Fecha (AMD): 2004-12-29 10:01:26
Tramite : 011 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	32758702	27/12/2004	21,068,000.00

***** CONCEPTO *****

CANT. REMITISTICO	CONCEPTO	TOTAL CONCEPTO
1 50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	422,000.00
	TOTAL :	422,000.00

SON: CUATROCIENTOS VEINTIDOS MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

60614594-841-002-S

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE 194

	USUARIO	RADICADOR
PATENTE DE INVENCION ✓	()	() ✓
MODELO DE UTILIDAD	()	() ✓
- Indicación de que se solicita la concesión de una patente.	()	() ✓
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	()	() ✓
- Descripción de la invención.	()	() ✓
- Dibujos de ser estos pertinentes.	()	() ✓
- Comprobante de Pago de las tasas establecidas.	()	() ✓

COMPLETA () ✓	INCOMPLETA ()
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DISEÑO INDUSTRIAL ()	
- Indicación de que se solicita el registro de Diseño Industrial	() ()
- Datos de identificación del solicitante o de la persona que presenta la solicitud.	() ()
- Representación gráfica o fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	() ()
- Comprobante de pago de las tasas establecidas	() ()
COMPLETA ()	INCOMPLETA ()

ESQUEMA DE TRAZADO ()	
- Indicación de que solicita el registro de un Esquema de trazado.	() ()
- Datos de identificación del solicitante o de la persona que presenta La solicitud.	() ()
- Representación gráfica del esquema de trazado	() ()
- Comprobante de pago de las tasas establecidas.	() ()
COMPLETA ()	INCOMPLETA ()

Firma Responsable: 

Fecha:

194

195

2020
Bogotá D C

Doctor(a)
EMILIO FERRERO
Apoderado(a)
GLYCORES 2000 S. r. l.

Oficio N°
11920

ASUNTO:

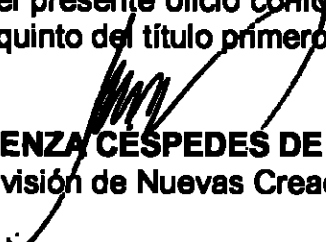
Radicación N°	04-129790
Solicitud internacional	PCTIB03/02339
Fecha inicio fase nacional	18/01/2005
Trámite	011
Evento	379
Actuación	430
Folios	001

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

- La solicitud no contiene copia del documento en que consta la cesión del derecho de patente del inventor al solicitante o a su causante (Art. 26-k) Decisión 486).
- La solicitud no contiene el poder debidamente otorgado, con el lleno de requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando éste fuere una persona jurídica.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

El anterior requerimiento fue notificado por fijación en lista de fecha,
21 ENE. 2005.

202044

✓
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