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Direction de la Propriété Intellectuelle

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(54) **AMIDES D'ACIDE HYALURONIQUE, DERIVES DE CE DERNIER ET LEURS
PROCEDE DE PREPARATION**

(30) Priorité :

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LE GOUVERNEMENT
DU GRAND-DUCHÉ DE LUXEMBOURG
Ministère de l'Économie
Et du Commerce extérieur

Direction de la Propriété intellectuelle

Demande de brevet d'invention

- Loi du 20 juillet 1992 portant modification du régime des brevets d'invention
- Règlement grand-ducal du 17 novembre 1997 - concernant la procédure et les formalités administratives en matière de brevets d'invention et de certificats complémentaires de protection - portant fixation des taxes et rémunérations à percevoir en matière de brevets et de certificats complémentaires de protection

Demande No: Date de réception: 01.02.2008 Date de dépôt: 01-Feb-2008

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A. REQUÊTE

Le(s) demandeurs(s) requièr(en)t la délivrance d'un brevet d'invention pour l'objet décrit et représenté dans les pièces annexées

1. Titre de l'invention:

AMIDES D'ACIDE HYALURONIQUE, DERIVES DE CE DERNIER ET LEURS PROCEDE DE PREPARATION

2. Demandeur

Référence (si connue):

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Etat dans lequel est situé le domicile ou siège du demandeur: IT

☐ Un (Des) demandeur(s) supplémentaire(s) est (sont) indiqué(s) sur une feuille en annexe

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☒ Le(s) demandeur(s) déclarent élire domicile auprès du (des) mandataires

Type de mandataire (cocher une seule case):

☒ Mandataire agréé

☐ Représentant commun

☐ Avocat

☐ Employé

4. Adresse postale au Grand-Duché de Luxembourg:

Le(s) demandeur(s) requièr(en)t que les communications du Service soient envoyées à:

- ☐ l'adresse du demandeur mentionnée à la rubrique 2
☒ l'adresse du (des) mandataire(s) mentionnée à la rubrique 3
☐ l'adresse suivante:
-

5. Désignation d'inventeur(s)

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- ☐ Un (Des) inventeur(s) supplémentaire(s) est (sont) mentionné(s) en annexe
☐ Une désignation d'inventeur(s) est / sera produite séparément
-

6. Déclaration de priorité:

Demande No: Date de dépôt: Pays: Déposant(s)

- ☐ Une (des) déclaration(s) de priorité supplémentaire(s) est (sont) mentionné(s) sur une feuille en annexe
-

7. ☐ La présente demande est une DEMANDE DIVISIONNAIRE de la demande suivante:

Numéro: Date de dépôt:

8. ☐ La présente demande est fondée sur la DEMANDE INTERNATIONALE (PCT) suivante:

No de publication: Date de dépôt:
Date de publication:

9. ☐ Il est demandé L'ÉTABLISSEMENT D'UN RAPPORT DE RECHERCHE D'ANTÉRIORITÉS

10. ☐ Il est demandé la VALIDATION D'UN RAPPORT DE RECHERCHE D'ANTÉRIORITÉS effectué sur la demande suivante:

Demande No: Date de dépôt: Pays: Déposant(s)

11. Abrégé

Il est demandé de publier l'abrégé de la demande de brevet accompagné de la figure No:

12. Annexes:

- | | | |
|---|---------------------------|----|
| ☒ Description de l'invention (3 exemplaires) | Nombre de pages: | 22 |
| ☒ Revendication(s) (3 exemplaires) | Nombre de revendications: | 40 |
| ☒ Dessin(s) (3 exemplaires) | Nombre de dessins: | 3 |
| ☐ Abrégé (3 exemplaires) | | |
| ☐ Dessin à publier avec l'abrégé (joindre une copie séparée) | No: | |
| ☒ Traduction des revendications | | |
| ☐ Feuille avec demandeur(s) supplémentaire(s) | | |
| ☐ Feuille avec mandataire(s) supplémentaire(s) | | |
| ☐ Feuille avec inventeur(s) supplémentaire(s) | | |
| ☐ Désignation d'inventeur(s) séparée | | |
| ☐ Document(s) de priorité (3 exemplaires) | | |
| ☐ Traduction de document(s) de priorité (3 exemplaires) | | |
| ☐ Document(s) de cession du droit de priorité | | |
| ☐ Autres: | | |

B. PROCES-VERBAL DE DEPÔT

La présente demande de brevet d'invention a été déposée au Ministère de l'Economie,
Direction de la Propriété Industrielle et des Droits Intellectuels, à Luxembourg,

en date du 01-Feb-2008

à 15.00 heures.

Le(s) déposant(s) / mandataire(s):

Pour le Ministre de l'Economie,

Stéphane SPEICH

Lex KAUFHOLD
Chargé de Direction
Direction de la Propriété Intellectuelle



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AMIDES OF HYALURONIC ACID AND THE DERIVATIVES THEREOF AND A PROCESS FOR THEIR PREPARATION

10

SUBJECT OF THE INVENTION

The present invention is directed to amides of hyaluronic acid and derivatives thereof for the preparation of pharmaceutical formulations, of biomaterials and for the coating of biomedical objects and the process for their preparation.

15

FIELD OF THE INVENTION

Hyaluronic acid is a heteropolysaccharide composed of alternate residues of D-glucuronic acid and N-acetyl-D-glycosamine. It is a straight-chained polymer the molecular weight of which varies between 50,000 and 13,000,000 Da depending on the source from which it was obtained and the methods used to obtain it. It is present in nature in pericellular gels, in the fundamental substance of the connective tissue of vertebrate organisms of which it represents one of the main components, in the synovial fluid of the joints, in the vitreous humor, in the human umbilical cord tissues and in rooster combs.

25 In recent years, numerous types of hyaluronic acid derivatives have been synthesised to obtain compounds with pharmacological properties, or compounds that can be processed in various forms of biodegradable and biocompatible biomaterials for use in various fields of medicine, surgery and tissue engineering.

30 Among the amide derivatives reported in the state of the art are known water-insoluble compositions constituted by mixtures deriving from the

reaction between the carboxyl of hyaluronic acid, a nucleophil, such as an aminic compound, and an activating agent (US 5,760,200; US 4,937,270). Such mixtures are mainly used in the prevention of post-surgical adhesions.

5 U.S. patent No. 5,733,891 describes pharmaceutical compositions containing amide derivatives of hyaluronic acid obtained by reaction of its carboxyls with basic anti-tumour agents. The purpose of these compounds is to focus the action of the active principle on the diseased tissues and to limit any harmful effects on the healthy tissues.

10 Moreover, there are known amides of glycosaminoglycans, such as hyaluronic acid, with photosensitive compounds bound by polyfunctional compounds that act as bridges in the formation of amide bonds (US 5,462,976).

Lastly, there is a known process for the preparation of insoluble amides
15 by the reaction of active esters of hyaluronic acid with amines. (WO 95/24429).

The aim of the present invention is to provide isolated and characterised amides of hyaluronic acid or derivatives thereof, obtained by reacting the carboxy groups or amino groups originating from deacetylation reactions,
20 with amines and acids of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series respectively, and without the use of spacer chains.

Said compounds can be either water soluble or insoluble, according to the acid, the amine, the percentage of amide bond or the derivative of
25 hyaluronic acid used to prepare the amide.

Therefore, the products according to the present invention are suitable for a large number of applications according to their solubility in water, their viscosity and the stability of the amide bond.

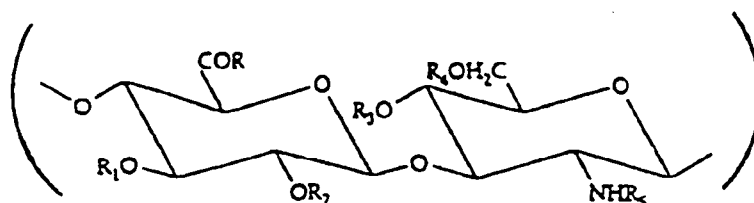
Indeed, said compounds can be used to prepare both pharmaceutical
30 compositions and biomaterials. Moreover, they have the advantage of

being able to be formed by reaction, not only with amines, but also with pharmacologically active acids.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to amides of hyaluronic acid and derivatives thereof for the preparation of pharmaceutical formulations, biomaterials and for the coating of biomedical objects and the process for their preparation.

The amides according to the present invention can be represented by the following general formula that represents the repetitive unit of the polymer:



wherein:

R = NR₆R₇, or alcoholic group of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, OH, O-, alcoholic group of hyaluronic acid, amino group of deacylated hyaluronic acid;

R₁, R₂, R₃, R₄ = H, SO₃⁻, acyl group derived from a carboxylic acid of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, -CO- (CH₂)₂-COOY; Y = negative charge, or H;

R₅ = -CO-CH₃, H, SO₃⁻, acyl group derived from a carboxylic acid of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, acylic group of hyaluronic acid;

R₆ = is H or a aliphatic, aromatic, arylaliphatic, cycloaliphatic, or heterocyclic group, substituted or unsubstituted;

R₇ = is H or a aliphatic, aromatic, arylaliphatic, cycloaliphatic, or heterocyclic group, substituted or unsubstituted;

wherein at least one of R or R₅ forms an amide group.

These are therefore amides obtained by reaction of an amine with a free carboxyl of hyaluronic acid or a derivative thereof, or by reaction of an acid with a deacylated amino group of hyaluronic acid or a derivative thereof.

Of the hyaluronic acid derivatives that can be used to prepare amides according to the present invention, the following are preferred:

- hyaluronic acid esters wherein a part or all of the carboxy functions are esterified with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series (EP 0216453 B1);
- autocross-linked esters of hyaluronic acid wherein a part or all of the carboxy groups are esterified with the alcoholic functions of the same polysaccharide chain or other chains (EP 0341745 B1);
- the cross-linked compounds of hyaluronic acid wherein a part or all of the carboxy groups are esterified with polyalcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, generating cross-linking by means of spacer chains (EP 0265116 B1);
- hemiesters of succinic acid or the heavy metal salts of the hemiester of succinic acid with hyaluronic acid or with partial or total esters of hyaluronic acid (WO 96/357207);
- the O-sulphated derivatives (WO 95/25751) or N-sulphated derivatives (PCT/EP98/01973).

Of the amides obtained by reaction of an amine on the carboxyl of hyaluronic acid or of a derivative thereof, of particular interest are the water-soluble ones.

By amide is meant a group of the formula -CON= .

- 5 Aliphatic means acyclic or pertaining to open-chain or branched carbon compounds such as alkanes, alkenes or alkynes. Examples of an aliphatic moiety include but are not limited to $\text{C}_1\text{-C}_{20}$ noncyclic hydrocarbons and their isomers such as methyl, ethyl, propyl, isopropyl, n-butyl, tert-butyl, isobutyl, pentyl, isopentyl, neopentyl, tert-pentyl, 2-
- 10 methylbutyl, 1,2-dimethylpropyl, hexyl, isohexyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 2,2-dimethylbutyl, 1,3-dimethylbutyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1-ethylbutyl, 2-ethylbutyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl,
- 15 pentadecyl, hexadecyl, cetyl, heptadecyl, octadecyl, nonadecyl, stearyl, etc.

Aromatic means an aryl moiety having one or more unsaturated rings, each ring usually having 5 to 8 members and preferably 5 to 6 members.

- Examples of the aromatic moiety include but are not limited to benzyl,
- 20 toluyl, naphthyl, anthracenyl, phenanthryl, fluorenyl, coronenyl, triphenylenyl, fluoranthenyl, benzofluoranthenyl, benzopyrenyl, and pyrenyl.

- Cycloaliphatic pertains to a carbon ring structure, usually having 3 to 8 members and preferably 5 to 6 members, that does not contain a
- 25 resonance structure. Examples of cycloaliphatic groups include but are not limited to cycloalkanes and cycloolefins such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclohexenyl (tetrahydrobenzenyl), cyclohexylidenyl, and cyclooctadienyl.

The heterocyclic series pertains to dissimilar atoms in a ring. A

- 30 heterocyclic group is a heteroaryl group usually having a 3- to 8-membered, preferably 5- to 6-membered ring or fused ring containing at

least one hetero atom (such as O, S, N, etc.) and include but are not limited to thienyl, furanyl, pyranal, 2H-pyrrolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidyl, pyridazinyl, isothiazolyl, isoxazolyl, furazanyl, benzothienyl, isobenzofuranyl, chromenyl, indolindinyl, isoindolyl, indolyl, purinyl, quinolidinyl, isoquinolyl, quinolyl, phthalazinyl, quinazolyl, carbazolyl, acridinyl, and phenanthridinyl.

An arylalkyl group is a group having both aromatic and aliphatic substituents as defined above. Examples of arylalkyl groups include but are not limited to ethylbenzenyl, isobutylbenzeneyl, benzyl, ethylbenzyl, propylbenzyl, isopropylbenzyl, butylbenzyl, isobutylbenzyl, cyclohexylbenzyl, styrenyl, and biphenyl.

An acyl group is an organic radical derived from an organic acid by the removal of a hydroxy group. Examples of acyl groups include but are not limited to formyl, acetyl, proprionayl, butyryl, valeryl, isovaleryl, pivaloyl ; aroyl such as benzenesufonyl, benzoyl, toluoyl, and naphthoyl; diacyl groups such oxalyl and succinic anhydride; and heteroaroyls such as furoyl, nicotnoyl, isonicotinoyl, etc.

Such amides can be used to advantage for the preparation of pharmaceutical compositions, for example in the form of gels, for the transport and release of drugs or biologically active substances for use in viscoelastic surgery or in ophthalmic surgery.

The amides according to the present invention can be salified with the heavy metals on the free or sulphuric carboxy groups, meaning by heavy metals the elements of the 4th, 5th and 6th periods of the periodical table such as silver, iron, cobalt, copper, zinc, arsenic, strontium, zirconium, antimony, gold, cesium, tungsten, selenium, platinum, ruthenium, bismuth, tin, titanium and mercury. Said salts can be used in dermatology, in ophthalmology, in dentistry, stomatology, rheumatology, urology, gynaecology, internal surgery, as food supplements, anti-

oxidating, anti-rheumatic, anti-tumoural, anti-inflammatory, analgesic and anti-ulcer agents.

Moreover, the amide derivatives can be obtained by reaction of carboxyl or deacylated nitrogen of hyaluronic acid or a derivative thereof with an amine or with a pharmacologically active acid respectively, or they may be salified or simply associated with said compounds.

Of the pharmacologically active substances, the following are preferred:

antibiotics, anti-infective, antimicrobial, antiviral, cytostatic, cytotoxic, anti-tumoral, anti-inflammatory and wound healing agents, anaesthetics, analgesics, vasoconstrictors, cholinergic or adrenergic agonists and antagonists, anti-thrombotic, anti-coagulant, haemostatic, fibrinolytic and thrombolytic agents, proteins and their fragments, peptides and polynucleotides.

Hereafter we report some examples of pharmacologically active substances belonging to the aforesaid classes of drugs:

- antibiotics: amino glucosides, macrolides, tetracycline, peptides such as gentamicin, neomycin, streptomycin, dihydrostreptomycin, kanamycin, amikacin, tobramycin, spectinomycin, erythromycin, oleandomycin, carbomycin, spiramycin, oxytetracycline, rolitetracycline, bacitracin, polymyxin B, gramicidin, colistin, chloramphenicol, lincomycin, vancomycin, novobiocin, ristocetin, clindamycin, amphotericin B, griseofulvin, nystatin and their salts;
- anti-infective agents: diethylcarbamazine, mebendazole, sulfamides such as sulfacetamide, sulfadiazine, sulfisoxazole;
- anti-virals and anti-tumors: idoxuridine, adenine, adenine arabinoside, trifluorothymidine, acyclovir, ethyldeoxyuridine, bromovinyldeoxyuridine, 5-iodo-5'-amino-2',5'-dideoxyuridine;
- steroid anti-inflammatory agents: dexamethasone, hydrocortisone, prednisolone, fluorometholone, medrisone and their esters;
- non-steroid anti-inflammatory agents: indomethacin, oxyphenbutazone, fluorbiprofene, dichlofenac, ibuprofen;

- anaesthetics: benoxinate, proparacaine, dibucaine, lidocaine, benzocaine, benzydamine, bupivacaine and their salts;
- cholinergic agonists: pilocarpine, methacholine, carbamylcholine, aceclidine, physostigmine, neostigmine, demecarium and their salts;
- cholinergic antagonists: atropine and its salts;
- adrenergic agonists: noradrenalin, adrenalin, naphazoline, methoxamine and their salts;
- adrenergic antagonists: propranolol, timolol, pindolol, bupranolol, atenolol, methoprolol, oxprenolol, practolol, butoxamine, sotalol, butadrine, labetalol and their salts;
- antibacterials and disinfectants: nitrofurazone, mafenide, chlorhexidine, the derivatives of 8-hydroxyquinoline and their salts;
- cytotoxics: fluorouracil, methotrexate, podophyllin.

Of particular interest are the forms for the transport and release of the above said substances and of biologically active substances such as proteins and their fragments, peptides, polynucleotides, growth factors, enzymes, vaccines, substances used in the treatment of diseases associated with genetic defects such as those depending on enzymatic hypo- or hyper-activity due to defects of the gene encoding for a given enzyme, deforming diseases and hereditary diseases.

The amide derivatives according to the present invention, in association with radioactive and non-radioactive substances, used in contrast systems, can be used as markers in *in vivo* diagnostics, for the identification and treatment of tumour tissues or damaged tissues.

One considerable advantage is represented by the possibility of processing the amide compounds and their salts in different forms of biomaterials such as sponges, films, membranes, threads, tampons, nonwoven fabric, microspheres, nanospheres, gauzes, gels and guide channels. Said biomaterials, used in one or more associated forms, may be constituted by one or more amide derivatives and their salts, optionally in association

with other natural, synthetic or semisynthetic polymers, and optionally, with biologically active substances.

Some examples of natural polymers that can be used are collagen, coprecipitates of collagen and glycosaminoglycans, cellulose, polysaccharides in the form of gels such as chitin, chitosan, pectin or pectic acid, agar, agarose, xanthane, gellan, alginic acid or the alginates, polymannans or polyglycans, starch and natural gums.

Semisynthetic polymers, for example, can be chosen from the group consisting of collagen cross-linked with agents such as aldehydes or precursors of the same, dicarboxylic acids or their halogenides, diamines, derivatives of cellulose, hyaluronic acid, chitin or chitosan, gellan, xanthane, pectin or pectic acid, polyglycans, polymannan, agar, agarose, natural gum or glycosaminoglycans.

Lastly, examples of synthetic polymers that can be used are polylactic acid, polyglycolic acid or copolymers of the same or their derivatives, polydioxanes, polyphosphazenes, polysulphonic resin, polyurethanes, PTFE.

The above said biomaterials can be used to advantage in various fields of surgery, such as in internal and osteo-articular surgery, neuro-surgery, anastomotic, viscoelastic, ophthalmic, oncological, plastic and aesthetic, otorhinolaryngological, abdominal and pelvic, urogynaecological, cardiovascular surgery, in the prevention of post-surgical adhesions and hypertrophic scarring.

Moreover, the amide compounds in association with fibrin, and optionally other biologically active substances, can be used for the preparation of surgical glues.

The biomaterials according to the present invention can be used not only in the field of surgery but also in haemodialysis, cardiology, dermatology, ophthalmology, otorhinolaryngology, dentistry, orthopaedics, gynaecology, urology, in extra-corporeal blood circulation and oxygenation, in cosmetics and in angiology.

Said biomaterials, in their various forms, can be used to advantage as scaffolds on which to grow cells such as mesenchymal cells or mature cells to obtain connective, glandular and nerve tissue.

These biopolymers can also be used in the processes of coating objects used both in the medical field and in industrial sectors, giving new biological characteristics to the surfaces of the material used as a support.

Examples of the objects that can be coated are: catheters, guide channels, probes, cardiac valves, soft tissue prostheses, prostheses of animal origin such as cardiac valves from pigs, artificial tendons, bone and cardiovascular prostheses, contact lenses, blood oxygenators, artificial kidneys, hearts, pancreas, and livers, blood bags, syringes, surgical instruments, filtration systems, laboratory instruments, containers for cultures and for the regeneration of cells and tissues, supports for peptides, proteins and antibodies.

The process of coating the surface of such objects can be performed, for example by the Plasma Coating technique, described in the international patent application by the Applicant, publication No. WO96/24392.

The process for the preparation of amides on the nitrogen of hyaluronic acid or one of its deacetylated derivatives can be summarised as the following steps:

- deacetylation reaction, for example, by reaction with hydrazine sulphate (J. Riesenfeld, *Analy. Bioch.* 1990, vol. 188, pp 383-389);
- preparation of the quaternary ammonium salt of the deacetylated compound such as the tetrabutylammonium salt;
- preparation of the acylating agent in the form of an active ester, for example, of paranitrophenylester of aliphatic, aromatic, arylaliphatic, cycloaliphatic or heterocyclic acid, chosen for the formation of the amide;

- N-acylation reaction between the quaternary ammonium salt of hyaluronic acid or of one of its deacetylated derivatives and the acylating agent.

The compound is analytically characterised by the following methods:

- 5 - *analysis of the percentage of free amino groups:*
the method described by J. Riesenfeld (Analy. Bioch. 1990, vol. 188, pp 383-389);
- *mean molecular weight:*
this is determined by GPC using a set of Shadex B-803 and B-806
10 columns, and RI and MALLS equipment;
- *IR and UV spectroscopy analysis:*
- *TLC analysis.*

The sample is hydrolysed in a 1mol. solution of sodium hydroxide for 2-4 hours at 70°C and then acidified with a 1 mol. solution of
15 hydrochloric acid. The acid that is released during hydrolysis is extracted with organic solvent. The dry organic extract is analysed by HPLC.

- *% of N-acylation (hydrolysis of the amide)*
two types of analysis are performed to measure the percentage of N-
20 acylated groups:
 - a) the method described by J. Riesenfeld (Analy. Bioch. 1990, vol. 188, pp 383-389);
 - b) the sample is hydrolysed in a 1 mol. solution of sodium hydroxide for 2-4 hours at 70°C and then acidified with a 1
25 mol. solution of hydrochloric acid. The acid that is released during hydrolysis is extracted with organic solvent. The dry organic extract is analysed by HPLC.

Preparation of the amides on the carboxyl of hyaluronic acid or a derivative thereof consists in activating the carboxy groups by reaction of
30 the same, in acid form or in the form of quaternary ammonium salt, with

an agent such as carbonyldiimidazole, which converts carboxylic acid in the reactive form of an acylating agent.

Said reaction can be performed by catalysis with hydrochloric acid or acid resin and with an amine of the aliphatic, aromatic, arylaliphatic, cycloaliphatic and heterocyclic series.

Characterisation of the compounds includes the following methods:

- *IR and UV spectroscopy:*
- *Chromatographic analysis.*

The sample is hydrolysed in a 1 mol. solution of sodium hydroxide for 2-4 hours at 70°C and the amine that is released during hydrolysis is extracted with organic solvent. The dry organic extract is analysed by HPLC.

The percentage of amidation of the product is generally in the range of about 1% to about 90%, more preferably in the range of about 5% to about 60%, and most preferably in the range of about 20% to about 50%.

Example 1

Preparation of partially N-deacetylated hyaluronic acid in the form of sodium salt (DHA/Na)

One gram of sodium hyaluronate, with a mean molecular weight of 600 Kda, is solubilised in 50 ml of a 1% solution of hydrazine sulphate in hydrazine monohydrate.

This is left to react under agitation for five days (120 hours) at 55°C, after which the reaction is stopped by adding 100 ml of ethanol.

The precipitate thus formed is filtered through a Gooch crucible, washed with ethanol and then dried at room temperature at reduced pressure.

Any hydrazide of hyaluronic acid that will probably be formed during the reaction with hydrazinolysis is destroyed by reaction with HIO₃ (iodic acid). As the reaction may be very vigorous, it is conducted while cooling the reaction container in iced water.

The product of hydrazinolysis is solubilised in 50 ml of a solution of 5% sodium acetate and reacted with 25 ml of a 0.5 M solution of iodic acid.

The reaction proceeds for 30 minutes under agitation, after which 5 ml of a 57% solution of HI is added to destroy any unreacted HIO_3 .

The iodine that has formed is extracted from the aqueous solution with at least three 30-ml aliquots of ethyl ether (until complete decolouring of the aqueous phase). The aqueous solution is brought to neutral pH by adding a solution of NaOH 0.5M followed by treatment with 100 ml of ethanol. The precipitate obtained is filtered with a Gooch crucible, washed with ethanol and then dried at room temperature and at reduced pressure.

The product obtained is characterised analytically to determine the percentage of N-deacetylated groups and the mean molecular weight.

Yield of the reaction	90%
% of N-deacetylation	26%
mean molecular weight	130 Kda

Example 2

Preparation of the salt of hyaluronic acid partially N-deacetylated with tetrabutylammonium (DHA/TBA).

One gram (2.5 mmol.) of hyaluronic acid sodium salt, partially N-deacetylated, is solubilised in 60 ml of water and the solution is percolated through a column filled with 25 ml of a sulphonic resin in the form of tetrabutylammonium salt (TBA). The sulphonic resin in H^+ form is activated with a 40% solution w/v of TBAOH.

The eluate, containing N-deacetylated hyaluronic acid TBA salt is collected and freeze-dried.

Example 3

Preparation of p-NO₂-phenylester of benzoic acid (acylating agent)

Ten grams (0.082 mol.) of benzoic acid is solubilised in 800 ml of CH_2Cl_2 , after which 11.4 g (0.082 mol.) of p-NO₂-phenol and 16.9 g (0.082 mol) of DCC (Dicyclohexylcarbodiimide) are added. The reaction proceeds for 2 hours, while the solution is boiled and refluxed.

Subsequently, the dicyclohexylurea that forms is filtered and the filtered product is dried with a rotavapor under reduced pressure. The product

thus obtained is purified by repeated crystallisation in ethyl acetate. The crystals are filtered and placed to dry at room temperature at reduced pressure.

The derivative is characterised by TLC analysis (eluent: CH₂Cl₂/ethyl acetate 90/10 and R_f=0.77) and by IR and UV spectroscopy.

Yield of the reaction 92%

Example 4

Preparation of p-NO₂-phenylester of cinnamic acid (acylating agent)

Twelve grams (0.082 mol.) of cinnamic acid is solubilised in 800 ml of CH₂Cl₂, after which 11.4 g (0.082 mol.) of p-NO₂-phenol and 16.9 g (0.082 mol) of DCC (Dicyclohexylcarbodiimide) are added. The reaction proceeds for 2 hours during which time the solution is boiled and refluxed.

Subsequently, the dicyclohexylurea is filtered and the filtered product is dried using a rotavapor at reduced pressure. The product obtained is purified by repeated crystallisation in ethanol, the crystals are filtered and left to dry at room temperature and reduced pressure.

The derivative is characterised by TLC analysis (eluent: CH₂Cl₂/ethyl acetate 90/10 and R_f=0.77) and by IR and UV spectroscopy.

Yield of the reaction 89%

Example 5

Preparation of p-NO₂-phenylester of dodecanoic acid (acylating agent)

Sixteen grams of dodecanoic acid is solubilised in 1 litre of CH₂Cl₂, after which 11.4 g (0.082 mol.) of p-NO₂-phenol and 16.9 g (0.082 mol.) of DCC (Dicyclohexylcarbodiimide) are added. The reaction proceeds for 2 hours during which time the solution is boiled and refluxed.

Subsequently, the dicyclohexylurea is filtered and the filtered product is dried using a rotavapor at reduced pressure. The product obtained is purified by repeated crystallisation in ethyl acetate, the crystals are filtered and left to dry at room temperature and at reduced pressure.

The derivative is characterised by TLC analysis (eluent: CH₂Cl₂/ethyl acetate 90/10 and R_f = 0.77) and by IR spectroscopy.

Yield of the reaction 93%

Example 6

5 Preparation of p-NO₂-phenylester of stearic acid (acylating agent)

23.3 grams of stearic acid is solubilised in 1 litre of CH₂Cl₂, after which 11.4 g (0.082 mol.) of p-NO₂-phenol and 16.9 g (0.082 mol.) of DCC (Dicyclohexylcarbodiimide) are added. The reaction proceeds for 2 hours during which time the solution is boiled and refluxed.

- 10 Subsequently the dicyclohexylurea is filtered and the filtered product is dried using a rotavapor at reduced pressure. The product obtained is purified by repeated crystallisation in absolute ethanol, the crystals are filtered and left to dry at room temperature at reduced pressure.

The derivative is characterised by TLC analysis (eluent: CH₂Cl₂/ethyl acetate 90/10 and R_f = 0.82) and by IR spectroscopy.

Yield of the reaction 87%

Example 7

Preparation of p-NO₂-phenylester of o-acetyl salicylic acid (acylating agent)

- 20 14.7 g of acetylsalicylic acid is solubilised in 1 litre of CH₂Cl₂, after which 11.4 g (0.082 mol.) of p-NO₂-phenol and 16.9 g (0.082 mol.) of DCC (Dicyclohexylcarbodiimide) are added. The reaction proceeds for 2 hours during which time the solution is boiled and refluxed.

- Subsequently, the dicyclohexylurea that forms is filtered and the filtered product is dried using a rotavapor at reduced pressure. The product obtained is purified by repeated crystallisation in absolute ethanol, the crystals are filtered and left to dry at room temperature at reduced pressure.

The derivative is characterised by TLC analysis (eluent: CH₂Cl₂/ethyl acetate 90/10 and R_f=0.82) and by IR spectroscopy.

Yield of the reaction 80%

Example 8**Preparation of partially N-acylated hyaluronic acid (with the benzoic acid derivative)**

One gram (1.6 mmol.) of DHA/TBA (26% deacetylation) is solubilised in 50 ml of DMSO, after which 5 ml of a 10% solution of p-NO₂-phenylester of benzoic acid (prepared according to example 3) in DMSO is added. The reaction proceeds for 24 hours, under agitation at room temperature, after which it is blocked by adding 2.5 ml of a saturated solution of NaCl. This is left to react for 30 minutes and then 100 ml of ethanol is slowly added. The precipitate thus obtained is filtered through a Gooch, washed with ethanol and ethyl ether and lastly dried at room temperature and at reduced pressure.

The derivative is analysed by TLC (after hydrolysis of the amide), colorimetric analysis of the percentage of free NH₂ groups and IR and UV spectroscopy.

Yield of the reaction	85%
% free NH ₂	11%
% N-acylation	15%

Example 9**Preparation of partially N-acylated hyaluronic acid (with the derivative of cinnamic acid)**

One gram (1.6 mmol.) of DHA/TBA (26% deacetylation) is solubilised in 50ml of NMP, after which 10 ml of a 10% solution of p-NO₂-phenylester of cinnamic acid (prepared according to example 4) in NMP is added. The reaction proceeds for 24 hours, under agitation, at room temperature, after which it is blocked by adding 2.5 ml of a solution saturated with NaCl. This is left to react for 30 minutes and lastly 100 ml of ethanol is slowly added. The precipitate thus obtained is filtered through a Gooch crucible, washed with ethanol/water 9:1, ethyl ether and lastly dried at room temperature at reduced pressure.

The derivative is analysed by TLC (after hydrolysis of the amide), colorimetric analysis of the percentage of free NH₂ groups and IR and UV spectroscopic analysis.

	Yield of the reaction	85%
5	% free NH ₂	11%
	% N-acylation	15%

Example 10

Preparation of partially N-acylated hyaluronic acid (with a derivative of dodecanoic acid)

- 10 One gram (1.6 mmol.) of DHA/TBA (26% deacetylation) is solubilised in 50 ml of NMP, after which 3.2 ml of a 10% solution of p-NO₂-phenylester of dodecanoic acid (prepared according to example 5) in NMP is added. The reaction proceeds for 24 hours, under agitation, at room temperature, after which it is blocked by adding 2.5 ml of a saturated solution of NaCl.
- 15 This is left to react for 30 minutes, after which 100 ml of ethanol is gently added. The precipitate obtained is filtered through a Gooch, washed with ethanol and ethyl ether and lastly dried at room temperature at reduced pressure.

The derivative is analysed by TLC (after hydrolysis of the amide),
20 colorimetric analysis of the percentage of free NH₂ groups and IR and UV spectroscopy.

	Yield of the reaction	88%
	% freeNH ₂	10%
	% N-acylation	16%

Example 11

Preparation of partially N-acylated hyaluronic acid (with the derivative of stearic acid)

- One gram (1.6 mmol) of DHA/TBA (26% deacetylation) is solubilised in 50 ml of NMP, after which 6 ml of a 10% solution of p-NO₂-phenylester of
30 stearic acid (prepared according to example 6) in NMP is added. The reaction proceeds for 24 hours under agitation at room temperature after

which it is blocked by adding 2.5 ml of a saturated solution of NaCl. This is left to react for 30 minutes and then 100 ml of ethanol is slowly added. The precipitate thus obtained is filtered through a Gooch filter, washed with ethanol and ethyl ether and lastly left to dry at room temperature and reduced pressure.

The derivative is analysed by TLC (after hydrolysis of the amide), colorimetric analysis of the percentage of free NH₂ groups and IR and UV spectroscopy.

Yield of the reaction	85%
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% free NH ₂	12%
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% N-acylation	14%
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IR spectroscopy (Figure 1): the figure shows the difference between the IR spectrum of the amide and that of hyaluronic acid sodium salt. In the spectrum of the amide, there is an evident peak in the area of 2900 cm⁻¹, due to the stretching of the CH₂ of the stearate.

Example 12

Preparation of partially N-acylated hyaluronic acid (with acetyl salicylic acid derivative)

One gram (1.6 mmol.) of DHA/TBA is solubilised in 50 ml of NMP, after which 3.2 ml of a 10% solution of p-NO₂-phenylester of acetyl salicylic acid (prepared according to example 7) in NMP is added. The reaction proceeds for 24 hours under agitation at room temperature, after which it is blocked by adding 2.5 ml of a saturated solution of NaCl. This is left to react for 30 minutes and lastly 100 ml of ethanol is slowly added. The precipitate thus obtained is filtered through a Gooch crucible, washed with ethanol and ethyl ether and then dried at room temperature and reduced pressure.

The derivative is analysed by TLC (after hydrolysis of the amide), colorimetric analysis of the percentage of free NH₂ groups and IR and UV spectroscopy.

Yield of the reaction	90%
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% free NH₂ 10%

% N-acylation 16%

Example 13

Preparation of benzylamide of hyaluronic acid

5 Two grams (3.2 mmol.) of tetrabutylammonium salt of hyaluronic acid (HA/TBA) is solubilised in 100 ml of DMSO. This solution is supplemented with 3 ml of humid acid resin in DMSO and 784 mg (4.8 mmol.) of 1,1-carbonyldiimidazole. This is left to react under agitation for 12 hours, after which it is filtered through a Gooch crucible to eliminate
10 the resin and the filtered product is supplemented with 1 ml (9.6 mmol) of benzylamine. This is left to react for 48 hours and then 5 ml of a saturated solution of NaCl is added and it is left under agitation for 30 minutes. It is supplemented with 200 ml of acetone and the precipitate thus obtained is filtered and dried at reduced pressure.

15 The dry derivative is characterised by TLC, IR and HPLC analysis.

% of amidation 25%

IR spectroscopy (Figures 2 and 3): the spectrum in figure 2 clearly shows a peak at 1537 cm⁻¹ due to bending in the NH plane (the amide band) and a peak at about 730 cm⁻¹ due to bending of the CH outside the plane
20 of the aromatic ring. Figure 3 shows the difference between the graph relative to the amide and that of the sodium salt of hyaluronic acid.

Example 14

Preparation of benzylamide of hyaluronic acid

Two grams (3.2 mmol.) of tetrabutylammonium salt of hyaluronic acid
25 (HA/TBA) is solubilised in 100 ml of DMSO. The solution is adjusted to pH 3 with HCl 1M and then 784 mg (4.8 mmol.) of 1,1-carbonyldiimidazole is added. This is left to react under agitation for 12 hours, then it is filtered through a Gooch crucible to eliminate the resin and 1 ml (9.6 mmol.) of benzylamine is added to the filtered product. This
30 is left to react for 48 hours, then 5 ml of a saturated solution of NaCl is added and left under agitation for 30 minutes. To this is added 200 ml of

acetone, the precipitate thus obtained is filtered and dried under reduced pressure.

The dry derivative is characterised by TLC, IR and HPLC analysis.

% amidation 15%

5 **Example 15**

Preparation of benzylamide of hyaluronic acid

Two grams (5.2 mmol.) of hyaluronic acid in acid form is solubilised in 100 ml of DMF. To this solution is added 854 mg (5.2 mmol.) of 1,1-carbonyldiimidazole. This is left to react under agitation for 6 hours, after
10 which 1.13 ml (10.4 mmol.) of benzylamine is added. The reaction proceeds for 48 hours, and is then blocked by adding 200 ml of acetone. The precipitate thus obtained is filtered and dried under reduced pressure.

The dry derivative is characterised by TLC, TR and HPLC analysis.

15 % amidation 60%

Example 16

Preparation of benzylamide of hyaluronic acid

Two grams (5.2 mmol.) of hyaluronic acid in acid form is solubilised in 100 ml of DMF. To this solution is added 2 ml of pyridine, 3.68 g (0.026
20 mol.) of p-NO₂-phenol and pyridine chloride until a pH of 7/8 is reached. Lastly, 5.3 (0.026 mol.) of DCC and 2.8 (0.026 mol.) of benzylamine are added. This is left to react under agitation for 16 hours after which the reaction is blocked by adding 200 ml of acetone. The precipitate thus obtained is filtered and dried under reduced pressure.

25 The dry derivative is characterised by TLC, IR and HPLC analysis.

% amidation 5%

Example 17

Preparation of benzylamide of hyaluronic acid

Two grams (3.2 mmol.) of HA/TBA is solubilised in 100 ml of DMSO. The
30 solution is insufflated with gaseous HCl until the reaction mixture reaches a pH of between 4.5 and 5. Subsequently, 518 mg (3.2 mmol.) of

carbonyldiimidazole is added. It is left to react under agitation for one hour at room temperature, after which 0.700 ml (6.4 mmol.) of benzylamine is added. The reaction proceeds for 16-18 hours. After this time, 5 ml of a solution saturated with NaCl is added. It is precipitated by adding 200 ml of acetone and the precipitate thus obtained is filtered and dried under reduced pressure.

The dry derivative is characterised by TLC (after hydrolysis), IR and HPLC analysis.

% amidation 50%

10 **Example 18**

Preparation of the octylamide of hyaluronic acid

Two grams (3.2 mmol.) of HA/TBA is solubilised in 100 ml of DMSO. The solution is insufflated with gaseous HCl till the reaction mixture reaches a pH of between 4.5 and 5. Subsequently, 207 mg (1.28 mmol.) of carbonyldiimidazole is added. It is left to react under agitation for one hour at room temperature, after which 0.417 ml (3.2 mmol.) of octylamine is added. The reaction proceeds for 16-18 hours. At the end of this time, 5 ml of a solution saturated with NaCl is added. It is precipitated by adding 200 ml of acetone and the precipitate obtained is filtered and dried under reduced pressure.

The dry derivative is characterised by TLC (after hydrolysis), IR and HPLC analysis.

% amidation 25%

Example 19

25 **Preparation of the dodecyl amide of hyaluronic acid:**

Two grams (3.2 mmol.) of HA/TBA is solubilised in 100 ml of DMSO. The solution is insufflated with gaseous HCl till the reaction mixture reaches a pH of between 4.5 and 5. Subsequently, 104 mg (0.64 mmol.) of carbonyldiimidazole is added. It is left to react, under agitation, for one hour at room temperature, after which 600 mg (3.2 mmol.) of dodecylamine is added. The reaction proceeds for 16-18 hours. After this

time, 5 ml of a solution saturated with NaCl is added. It is precipitated by adding 200 ml of acetone and the precipitate obtained is filtered and dried under reduced pressure.

The dry derivative is characterised by TLC (after hydrolysis), IR and HPLC analysis.

% amidation 15%

Example 20

Preparation of the hexadecylamide of hyaluronic acid:

Two grams (3.2 mmol.) of HA/TBA is solubilised in 100 ml of DMSO. The solution is insufflated with gaseous HCl till the reaction mixture reaches a pH of between 4.5 and 5. Subsequently, 52 mg (0.32 mmol.) of carbonyldiimidazole is added and left to react under agitation for one hour at room temperature, after which 780 mg (3.2 mmol.) of hexadecylamine is added. The reaction proceeds for 16-18 hours. After this time, 5 ml of a solution saturated with NaCl is added. It is precipitated by adding 200 ml of acetone and the precipitate obtained is filtered and dried under reduced pressure.

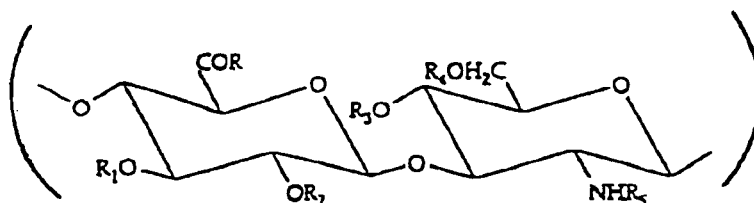
The dry derivative is characterised by TLC (after hydrolysis), IR and HPLC analysis.

20 % amidation 5%

The invention being thus described, it is clear that these methods can be modified in various ways. Such modifications are not to be considered as divergences from the spirit and purpose of the invention and any modification that would appear evident to an expert in the field comes within the scope of the following claims:

CLAIMS

1. An amide of hyaluronic acid or a derivative thereof which comprises at least one repetitive unit of the following general formula:



wherein:

R = NR₆R₇, or alcoholic group of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, OH, O-, alcoholic group of hyaluronic acid, amino group of deacylated hyaluronic acid;

R₁, R₂, R₃, R₄ = H, SO₃⁻, acyl group derived from a carboxylic acid of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, -CO- (CH₂)₂-COOY; Y = negative charge, or H;

R₅ = -CO-CH₃, H, SO₃⁻, acyl group derived from a carboxylic acid of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, acyclic group of hyaluronic acid;

R₆ = is H or a aliphatic, aromatic, arylaliphatic, cycloaliphatic, or heterocyclic group, substituted or unsubstituted;

R₇ = is H or a aliphatic, aromatic, arylaliphatic, cycloaliphatic, or heterocyclic group, substituted or unsubstituted;

wherein at least one of R or R₅ forms an amide group.

2. Amidic, water-soluble compounds obtained by reaction of the carboxylic groups of hyaluronic acid with an amino group of the

aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series for use in ophthalmology and in ophthalmic surgery.

3. Amidic compounds according to claim 1, wherein the hyaluronic acid derivatives are total or partial esters with aliphatic, aromatic, arylaliphatic, cycloaliphatic, heteroaliphatic alcohols.

4. Amidic compounds according to claim 1, wherein the hyaluronic acid derivatives are cross-linked compounds wherein part or all of the carboxy groups of the D-glucuronic residue form inner or inter-molecular esters with the alcoholic functions of the same polysaccharide chain or other chains respectively.

5. Amidic compounds according to claim 1, wherein the hyaluronic acid derivatives are cross-linked compounds wherein a part or all of the carboxy groups of the D-glucuronic residue are made to react with polyalcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic, heterocyclic series, generating cross-linking by means of spacer chains.

6. Amidic compounds according to claim 1, salified with heavy metals.

7. Amidic compounds according to claim 6, wherein the heavy metals are those of the 4th, 5th and 6th group of the table of elements and preferably silver, cobalt, iron, copper, zinc, arsenic, strontium, zirconium, antimony, gold, cesium, tungsten, selenium, platinum, ruthenium, bismuth, tin, titanium and mercury.

8. Amidic compounds according to claims 1-7, salified with pharmacologically active substances.

9. Amidic compounds according to claim 8, wherein the pharmacologically active substances are antibiotics, anti-infective, antimicrobial, antiviral, cytostatic, antitumoral, anti-inflammatory, wound healing agents, anaesthetics, cholinergic or adrenergic agonists and antagonists, antithrombotic, anticoagulant,

6 haemostatic, fibrinolytic, thrombolytic agents, proteins and their
7 fragments, peptides, polynucleotides.

1 10. Amidic compounds and their salts according to claims 1-9, alone or
2 in association with one another and/or with pharmacologically
3 active substances for the preparation of pharmaceutical
4 compositions.

1 11. Amidic compounds and their salts according to claim 10, wherein
2 the pharmacologically active substances are antibiotics, anti-
3 infective, antimicrobial, antiviral, cytostatic, antitumoral, anti-
4 inflammatory, wound healing, anaesthetic agents, cholinergic or
5 adrenergic agonists or antagonists, antithrombotic, anticoagulant,
6 haemostatic, fibrinolytic, thrombolytic agents, proteins and their
7 fragments, peptides, polynucleotides, growth factors, enzymes,
8 vaccines, substances used in the treatment of diseases associated
9 with genetic defects, deforming diseases and hereditary diseases.

1 12. Amidic compounds according to the previous claims, in association
2 with radioactive or non-radioactive substances, used in contrast
3 systems as labels in *in vivo* diagnostics to identify and treat tumoral
4 tissues or damaged tissues.

1 13. Pharmaceutical compositions containing the amidic compounds
2 and their salts according to claims 1-9, alone or in association with
3 one another or with other pharmacologically active substances.

1 14. Biomaterials constituted by amidic compounds and their salts
2 according to claims 1-9, alone or in association with one another or
3 with other natural, semisynthetic, synthetic polymers and,
4 optionally, with biologically active substances.

1 15. Biomaterials according to claim 14, wherein the natural polymers
2 are collagen, co-precipitates of collagen and glycosaminoglycans,
3 cellulose, polysaccharides in the form of gels such as chitin,
4 chitosan, pectin or pectic acid, agar, agarose, xanthane, gellan,

5 alginic acid or alginates, polymannans or polyglycans, starch,
6 natural gums.

1 16. Biomaterials according to claim 14, wherein the semisynthetic
2 polymers are collagen cross-linked with agents such as aldehydes
3 or precursors of the same, dicarboxylic acids or their halogenides,
4 diamines, derivatives of cellulose, hyaluronic acid, chitin or
5 chitosan, gellan, xanthane, pectin or pectic acid, polyglycans,
6 polymannan, agar, agarose, natural gum or glycosaminoglycans.

1 17. Biomaterials according to claim 14, wherein the synthetic polymers
2 are polylactic acid, polyglycolic acid or copolymers of the same or
3 their derivatives, polydioxanes, polyphosphazenes, polysulphonic
4 resins, polyurethanes, PTFE.

1 18. Biomaterials according to claims 14-17, in association with fibrin,
2 and optionally with other biologically active substances for the
3 preparation of surgical glues.

1 19. Biomaterials according to claims 14-17 for the preparation of
2 scaffolds for cell cultures.

1 20. Biomaterials according to claims 14-17 for the preparation of
2 surgical and health-care articles.

1 21. Biomaterials according to claims 14-17, in the form of guide
2 channels, gauzes, threads, gels, hydrogels, tampons, films,
3 membranes, sponges, non-woven fabrics, microspheres,
4 nanospheres and associations of the same.

1 22. Surgical and health-care articles according to claim 20, in the form
2 of guide channels, gauzes, threads, gels, hydrogels, tampons, films,
3 membranes, sponges, non-woven fabrics, microspheres,
4 nanospheres.

1 23. Biomaterials according to claims 14-17 for use in surgery,
2 haemodialysis, cardiology, dermatology, ophthalmology,
3 otorhinolaryngology, dentistry, orthopaedics, gynaecology, urology,

4 in extra-corporeal blood circulation and oxygenation, in cosmetics,
5 in angiology.

1 24. Biomaterials according to claim 23 where surgery should be taken
2 to mean internal or osteoarticular surgery, neurosurgery,
3 anastomotic, viscoelastic, ophthalmic, oncological, plastic,
4 aesthetic, otorhinolaryngological, abdominal-pelvic,
5 urogynaecological or cardiovascular surgery, such as in the
6 preparation of cardiac valves, vascular stents, in the prevention of
7 post-surgical adhesions and hypertrophic scarring.

1 25. Amidic compounds and their salts according to claims 1-9 for the
2 coating of biomedical objects such as bypasses, venous catheters,
3 shunts, catheters, guide channels, probes, cardiac valves, artificial
4 tendons, bone and cardiovascular prostheses, contact lenses, soft
5 tissue prostheses, prostheses of animal origin, blood oxygenators,
6 artificial kidneys, hearts, pancreas and livers, blood bags, syringes,
7 surgical instruments, filtration systems, laboratory instruments,
8 containers for cell cultures and for the regeneration of cells and
9 tissues, supports for peptides, proteins, antibodies.

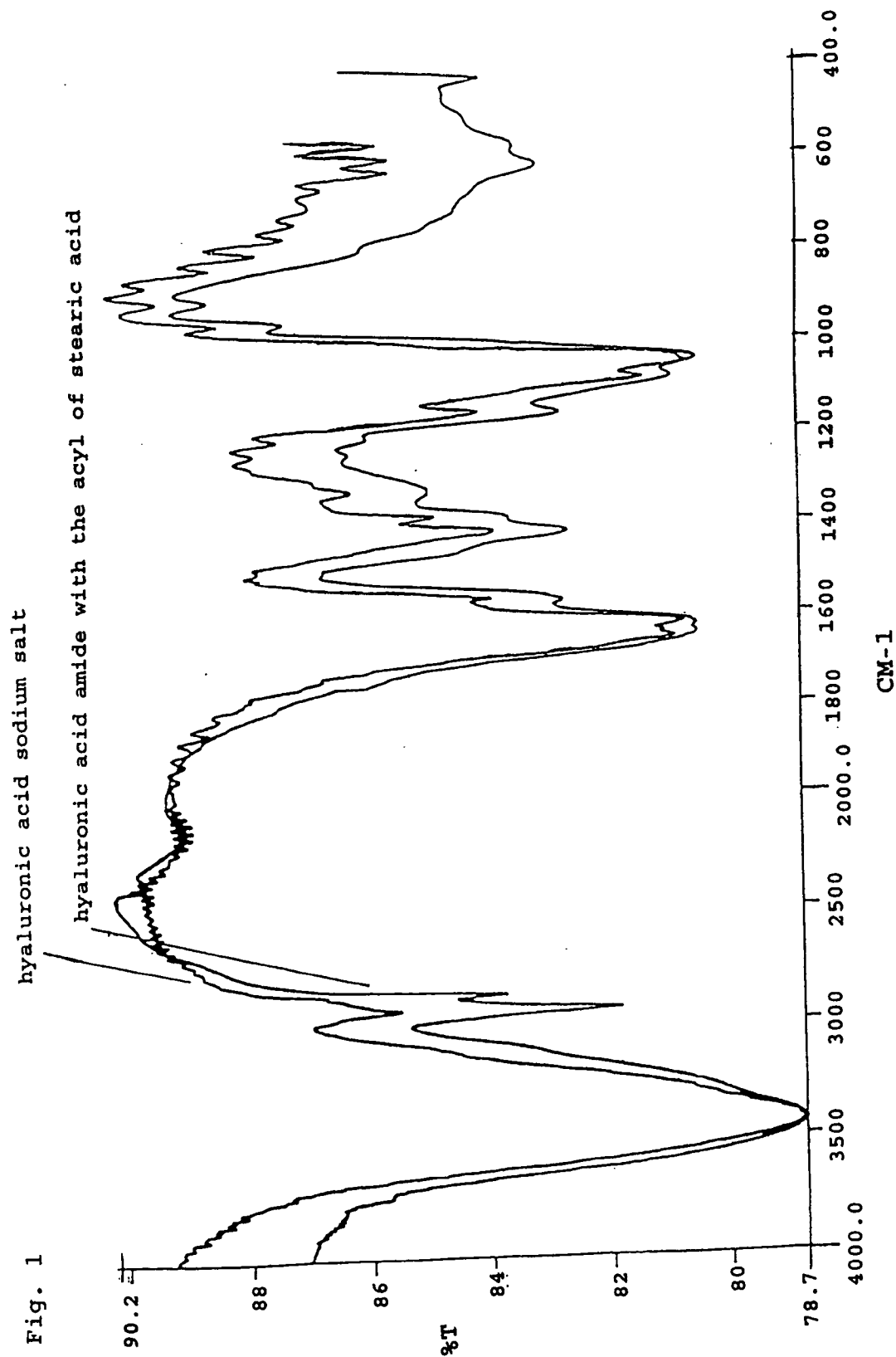
1 26. Use of the amidic compounds according to claims 7-8, in
2 dermatology, ophthalmology, dentistry, stomatology, rheumatology,
3 urology, gynaecology, internal surgery, as food supplements, anti-
4 oxidant, anti-rheumatic, anti-tumoral, anti-inflammatory, analgesic,
5 anti-ulcer agents.

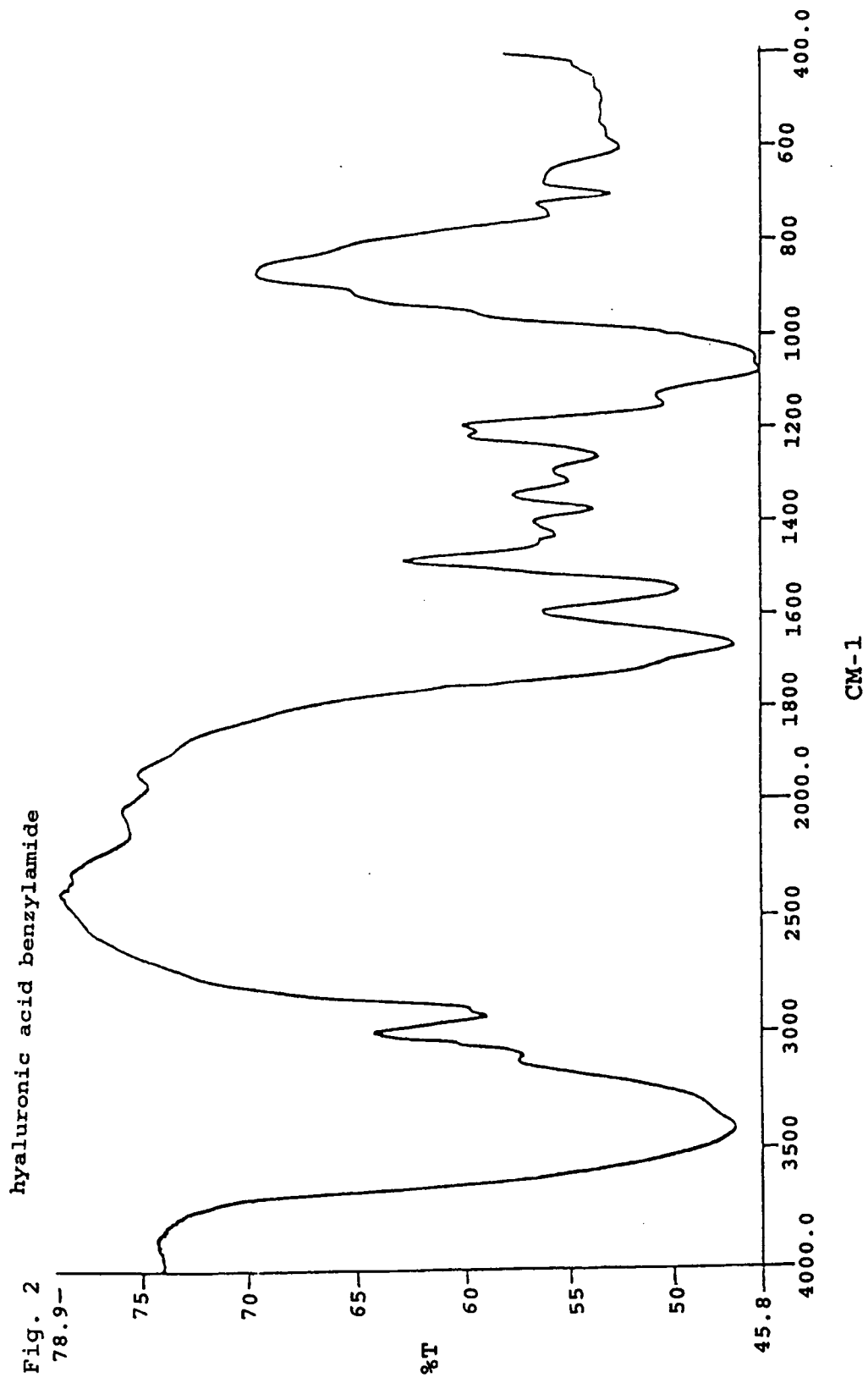
1 27. Use of the amidic compounds according to claims 1-5 for the
2 preparation of salts with pharmacologically active substances.

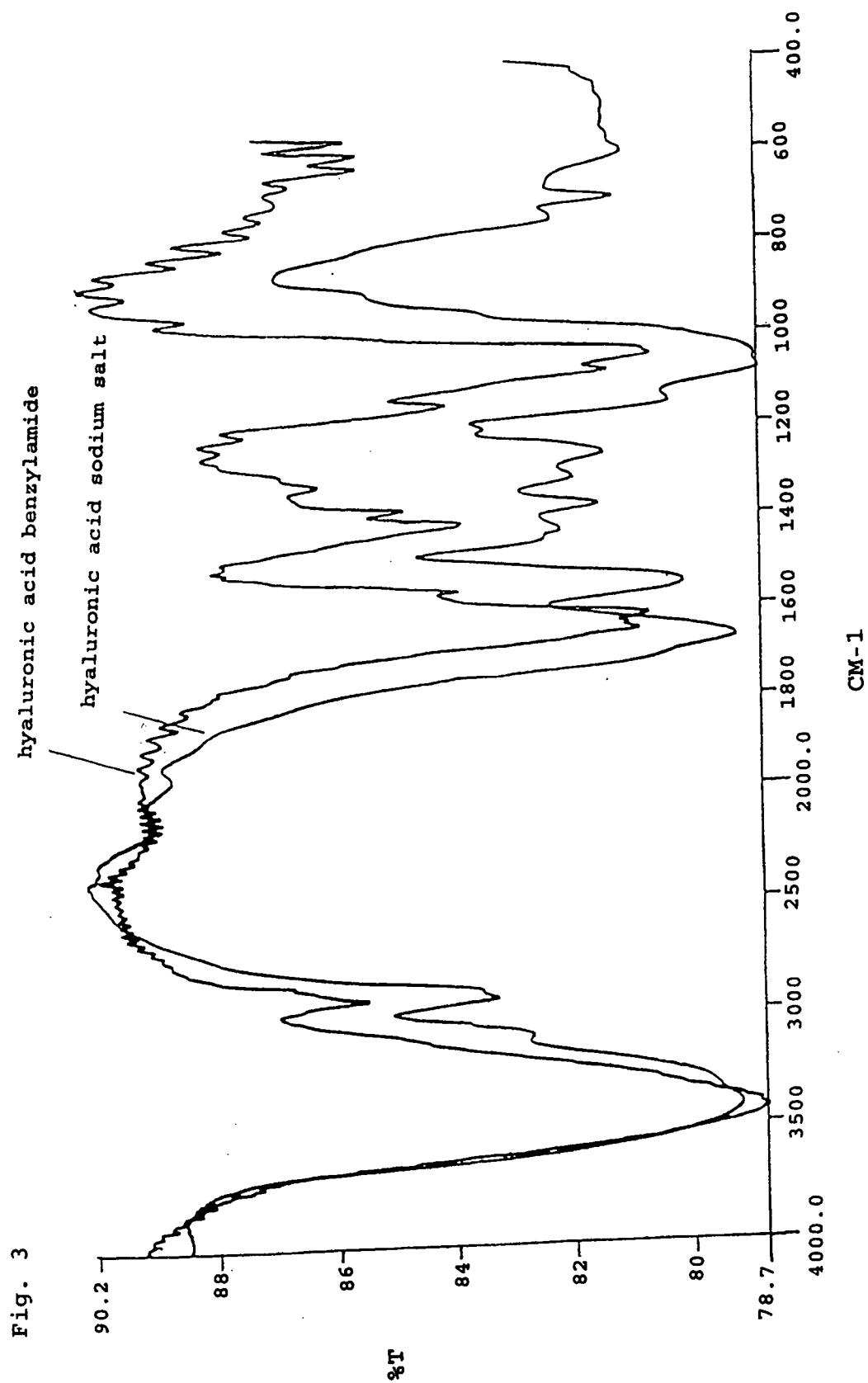
1 28. Use of the amidic compounds and their salts according to claims 1-
2 9 alone or in association with one another and/or with
3 pharmacologically active substances for the preparation of
4 pharmaceutical compositions, biomaterials, surgical and health-
5 care articles, slow release systems and systems for the coating of
6 biomedical objects.

- 1 29. Use of the amidic compounds and their salts according to claims 1-
2 9, in association with radioactive and non-radioactive substances,
3 used in contrast systems as labels in *in vivo* diagnostics for the
4 identification and treatment of tumoral tissues or damaged tissues.
- 1 30. Use of the amidic compounds and their salts according to claim 28,
2 wherein the biomaterials are in the form of guide channels, gauzes,
3 threads, gels, hydrogels, tampons, films, membranes, sponges, non-
4 woven fabrics, microspheres, nanospheres and associations of the
5 same.
- 1 31. Use of the amidic compound according to the previous claims, in
2 surgery, haemodialysis, cardiology, dermatology, ophthalmology,
3 otorhinolaryngology, dentistry, orthopaedics, gynaecology, urology,
4 in extracorporeal blood circulation and oxygenation, in cosmetics
5 and in angiology.
- 1 32. Use of the amide compounds according to claim 31, where surgery
2 means internal, osteo-articular surgery, neurosurgery, anastomotic,
3 viscoelastic, ophthalmic, oncological, plastic aesthetic,
4 otorhinolaryngological, abdominal pelvic, urogynaecological,
5 cardiovascular surgery such as in the preparation of cardiac valves,
6 vascular stents, in the prevention of post-surgical adhesions and in
7 hypertrophic scarring.
- 1 33. Use of biomaterials according to claims 14-17, in association with
2 fibrin, and optionally with other biologically active substances for
3 the preparation of surgical glues.
- 1 34. Use of biomaterials according to claims 14-17 for the preparation of
2 scaffolds for cell cultures.
- 1 35. Process for the preparation of amides on the nitrogen of hyaluronic
2 acid or a deacetylated derivative thereof involving the following
3 steps:
4 a) deacetylation reaction;

- 5 b) preparation of the quaternary ammonium salt of the
6 deacetylated compound;
- 7 c) preparation of the acylating agent in the form of active ester;
- 8 d) N-acylation reaction between the quaternary ammonium salt
9 of hyaluronic acid or a deacetylated derivative thereof and the
10 acylating agent.
- 1 36. Process according to claim 35, wherein the deacetylation reaction is
2 obtained by using hydrazine sulphate/hydrazine.
- 1 37. Process according to claim 35, wherein the quaternary ammonium
2 salt is the tetrabutylammonium salt.
- 1 38. Process according to claim 35, wherein the active ester is the
2 paranitrophenyl ester of aliphatic, aromatic, arylaliphatic,
3 cycloaliphatic, heterocyclic acid, chosen for the formation of the
4 amide.
- 1 39. Process for the preparation of the amides on the carboxyl of
2 hyaluronic acid or a derivative thereof, involving the following steps:
- 3 a) activation of the carboxy groups by reaction of the same, in
4 the acid form or as a quaternary ammonium salt, with an
5 activating agent, in acid solution or on acid resin;
- 6 b) reaction with an amine of the aliphatic, aromatic,
7 arylaliphatic, cycloaliphatic, heterocyclic series.
- 1 40. Process according to claim 39, wherein the activating agent is 1,1-
2 carbonyldiimidazole.







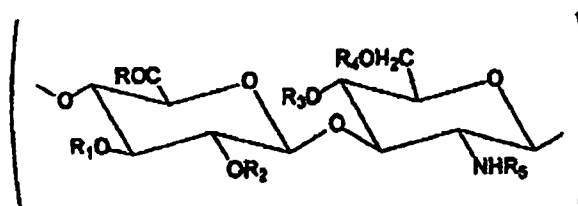
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REVENDICATIONS

1. Composés amidiques hydrosolubles de l'acide hyaluronique ou d'un dérivé de celui-ci comprenant au moins une unité de répétition de formule générale suivante :

5



dans laquelle :

10 R = NR₆R₇, ou un groupe alcoolique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, OH, O-, un groupe alcoolique de l'acide hyaluronique, un groupe amino de l'acide hyaluronique désacylé ;

15 R₁, R₂, R₃, R₄ = H, SO₃⁻, un groupe acyle dérivé d'un acide carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, -CO-(CH₂)₂-COOY ; Y = charge négative, ou H ;

20 R₅ = -CO-CH₃, H, SO₃⁻, un groupe acyle dérivé d'un acide carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, un groupe acylique de l'acide hyaluronique ;

R₆ = H ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

$R_7 = H$ ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

dans laquelle au moins l'un parmi R ou R_5 forme un groupe amide, à condition que lorsque R_6 est H , R_7 n'est pas - $CH_2CO_2CH_3$, et que le composé amidique hydrosoluble ne soit pas combiné avec un ingrédient médicinal par l'intermédiaire d'une liaison amide.

10 2. Composés amidiques hydrosolubles obtenus par réaction des groupes carboxyliques de l'acide hyaluronique avec un groupe amino de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique destinés à être utilisés en ophtalmologie et en chirurgie
15 ophtalmique.

3. Composés amidiques selon la revendication 1, dans lesquels les dérivés de l'acide hyaluronique sont des esters totaux ou partiels avec des alcools aliphatiques, aromatiques, arylaliphatiques, cycloaliphatiques ou
20 hétéroaliphatiques.

4. Composés amidiques selon la revendication 1, dans lesquels les dérivés de l'acide hyaluronique sont des
25 composés réticulés dans lesquels une partie ou tous les groupes carboxy du résidu D-glucuronique forment des esters intra ou intermoléculaires avec les fonctions alcooliques de la même chaîne polysaccharidique ou d'autres chaînes respectivement.

30

5. Composés amidiques selon la revendication 1, dans lesquels les dérivés de l'acide hyaluronique sont des

composés réticulés dans lesquels une partie ou tous les groupes carboxy du résidu D-glucuronique sont tels qu'ils réagissent avec des polyalcools de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou
5 hétérocyclique, générant une réticulation à l'aide de chaînes espaceurs.

6. Composés amidiques selon la revendication 1, transformés en sels avec des métaux lourds.

10

7. Composés amidiques selon la revendication 6, dans lesquels les métaux lourds sont ceux du 4^{ème}, 5^{ème} et 6^{ème} groupe du tableau de classification des éléments et de préférence l'argent, le cobalt, le fer, le cuivre, le
15 zinc, l'arsenic, le strontium, le zirconium, l'antimoine, l'or, le césium, le tungstène, le sélénium, le platine, le ruthénium, le bismuth, l'étain, le titane et le mercure.

20 8. Composés amidiques selon les revendications 1 à 7, transformés en sels avec des substances pharmacologiquement actives.

9. Composés amidiques selon la revendication 8, dans
25 lesquels les substances pharmacologiquement actives sont des antibiotiques, des anti-infectieux, des antimicrobiens, des antiviraux, des cytostatiques, des antitumoraux, des anti-inflammatoires, des agents de cicatrisation des blessures, des anesthésiques, des
30 agonistes et des antagonistes cholinergiques ou adrénergiques, des antithrombiques, des anticoagulants, des hémostatiques, des fibrinolytiques, des

thrombolytiques, des protéines et leurs fragments, des peptides, des polynucléotides.

10. Composés amidiques et leurs sels selon les
5 revendications 1 à 9, seuls ou en association les uns avec les autres et/ou avec des substances pharmacologiquement actives pour la préparation de compositions pharmaceutiques.

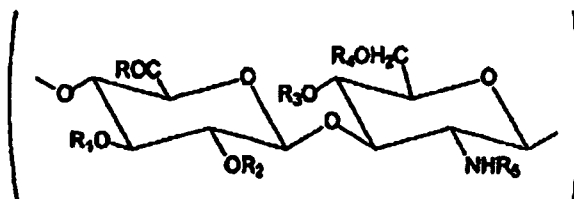
10 11. Composés amidiques et leurs sels selon la revendication 10, dans lesquels les substances pharmacologiquement actives sont des antibiotiques, des anti-infectieux, des antimicrobiens, des antiviraux, des
15 cytostatiques, des antitumoraux, des anti-inflammatoires, des agents de cicatrisation des blessures, des anesthésiques, des agonistes ou des antagonistes cholinergiques ou adrénergiques, des antithrombiques, des anticoagulants, des hémostatiques, des fibrinolytiques, des thrombolytiques, des protéines et leurs fragments,
20 des peptides, des polynucléotides, des facteurs de croissance, des enzymes, des vaccins, des substances utilisées dans le traitement des maladies associées à des défauts génétiques, des maladies déformantes et des maladies héréditaires.

25

12. Composés amidiques selon les revendications précédentes, en association avec des substances radioactives ou non radioactives, utilisés dans des systèmes de contraste en tant que marqueurs pour le
30 diagnostic in vivo afin d'identifier et de traiter des tissus tumoraux ou des tissus endommagés.

13. Compositions pharmaceutiques contenant des composés amidiques hydrosolubles de l'acide hyaluronique ou d'un dérivé de celui-ci comprenant au moins une unité de répétition de formule générale suivante :

5



dans laquelle :

10 R = NR₆R₇, ou un groupe alcoolique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, OH, O-, un groupe alcoolique de l'acide hyaluronique, un groupe amino de l'acide hyaluronique désacylé ;

15 R₁, R₂, R₃, R₄ = H, SO₃⁻, un groupe acyle dérivé d'un acide carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, -CO-(CH₂)₂-COOY ; Y = charge négative, ou H ;

20 R₅ = -CO-CH₃, H, SO₃⁻, un groupe acyle dérivé d'un acide carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, un groupe acylique de l'acide hyaluronique ;

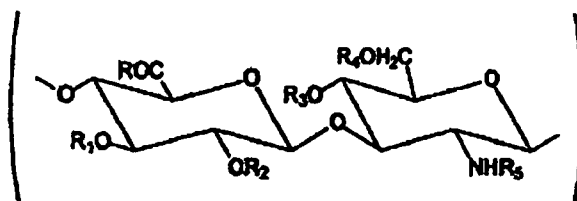
R₆ = H ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

25 R₇ = H ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

dans laquelle au moins un parmi R ou R₅ forme un groupe amide,

seuls ou en association les uns avec les autres ou avec d'autres substances pharmacologiquement actives et en association avec un support pharmaceutiquement acceptable, à condition que le composé amidique hydrosoluble ne soit
 5 pas combiné avec un ingrédient médicinal par l'intermédiaire d'une liaison amide.

14. Biomatériaux à base de composés amidiques hydrosolubles de l'acide hyaluronique ou d'un dérivé de
 10 celui-ci comprenant au moins une unité de répétition de formule générale suivante :



15 dans laquelle :

R = NR_6R_7 , ou un groupe alcoolique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, OH, O-, un groupe alcoolique de l'acide hyaluronique, un groupe amino de
 20 l'acide hyaluronique désacylé ;

$\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4 = \text{H}, \text{SO}_3^-$, un groupe acyle dérivé d'un acide carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, $-\text{CO}-(\text{CH}_2)_2-\text{COOY}$; Y = charge négative, ou H ;

25 $\text{R}_5 = -\text{CO}-\text{CH}_3, \text{H}, \text{SO}_3^-$, un groupe acyle dérivé d'un acide carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, un groupe acylique de l'acide hyaluronique ;

$R_6 = H$ ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

$R_7 = H$ ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

dans laquelle au moins un parmi R ou R_5 forme un groupe amide,

seuls ou en association les uns avec les autres ou avec d'autres polymères naturels, semisynthétiques, synthétiques et, éventuellement, avec des substances biologiquement actives, à condition que le composé amidique hydrosoluble ne soit pas combiné avec un ingrédient médicinal par l'intermédiaire d'une liaison amide.

15. Biomatériaux selon la revendication 14, dans lesquels les polymères naturels sont le collagène, les coprécipités de collagène et de glycosaminoglycanes, la cellulose, les polysaccharides sous forme de gels tels que la chitine, le chitosan, la pectine ou l'acide pectique, l'agar, l'agarose, le xanthane, le gellane, l'acide alginique ou les alginates, les polymannanes ou les polyglycanes, l'amidon, les gommes naturelles.

16. Biomatériaux selon la revendication 14, dans lesquels les polymères semisynthétiques sont le collagène réticulé avec des agents tels que des aldéhydes ou des précurseurs d'aldéhydes, des acides dicarboxyliques ou leurs halogénures, des diamines, des dérivés de cellulose, l'acide hyaluronique, la chitine ou le chitosan, le gellane, le xanthane, la pectine ou l'acide pectique, les

polyglycanes, les polymannanes, l'agar, l'agarose, la gomme naturelle ou les glycosaminoglycanes.

17. Biomatériaux selon la revendication 14, dans lesquels
5 les polymères synthétiques sont l'acide polylactique, l'acide polyglycolique ou les copolymères de l'acide polyglycolique ou leurs dérivés, les polydioxanes, les polyphosphazènes, les résines polysulphoniques, les polyuréthanes, le PTFE.

10

18. Biomatériaux selon les revendications 14 à 17, en association avec la fibrine, et éventuellement avec d'autres substances biologiquement actives pour la préparation de colles chirurgicales.

15

19. Biomatériaux selon les revendications 14 à 17 pour la préparation d'échafaudages pour des cultures cellulaires.

20

20. Biomatériaux selon les revendications 14 à 17 pour la préparation d'articles chirurgicaux et de soins de santé.

25

21. Biomatériaux selon les revendications 14 à 17, sous forme de canaux de guidage, de gazes, de fils, de gels, d'hydrogels, de tampons, de films, de membranes, d'éponges, de tissus non-tissés, de microsphères, de nanosphères et associations de ceux-ci.

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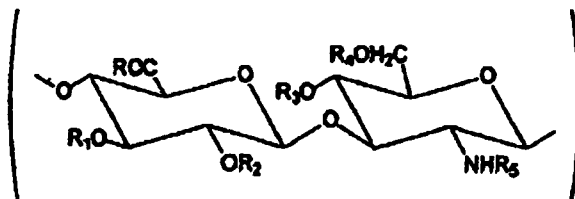
22. Articles chirurgicaux et de soins de santé selon la revendication 20, sous la forme des canaux de guidage, de gazes, de fils, de gels, d'hydrogels, de tampons, de films, de membranes, d'éponges, de tissus non-tissés, de microsphères ou de nanosphères.

23. Biomatériaux selon les revendications 14 à 17 destinés à être utilisés en chirurgie, en hémodialyse, en cardiologie, en dermatologie, en ophtalmologie, en oto-
5 rhino-laryngologie, en dentisterie, en orthopédie, en gynécologie, en urologie, dans le domaine de la circulation sanguine extra corporelle et l'oxygénation, en cosmétique ou en angiologie.
- 10 24. Biomatériaux selon la revendication 23 dans lesquels la chirurgie sous-entend la chirurgie interne ou ostéoarticulaire, la neurochirurgie, la chirurgie anastomotique, viscoélastique, ophtalmique, oncologique, plastique, esthétique, oto-rhino-laryngologique,
15 abdomino-pelvienne, uro-gynécologique ou cardiovasculaire, telle que dans la préparation de valves cardiaques, d'endoprothèses vasculaires, dans la prévention des adhérences postchirurgicales et de la cicatrisation hypertrophique.
- 20 25. Composés amidiques et leurs sels selon les revendications 1 à 9 destinés au revêtement des objets biomédicaux tels que des déviations, des cathéters veineux, des anostomoses « shunts », des cathéters, des
25 canaux de guidage, des sondes, des valves cardiaques, des tendons artificiels, des prothèses osseuses et cardiovasculaires, des lentilles de contact, des prothèses de tissu mou, des prothèses d'origine animale, des oxygénateurs de sang, des reins, des coeurs, des
30 pancréas et des foies artificiels, des sacs sanguins, des seringues, des instruments chirurgicaux, des systèmes de filtration, des instruments de laboratoire, des

réipients pour les cultures cellulaires et pour la régénération des cellules et des tissus, des supports pour les peptides, les protéines, les anticorps.

- 5 26. Utilisation des composés amidiques selon les revendications 7 à 8, en dermatologie, en ophtalmologie, en dentisterie, en stomatologie, en rhumatologie, en urologie, en gynécologie, en chirurgie interne, en tant que suppléments alimentaires, agents antioxydants, anti-
10 rhumatismaux, anti-tumoraux, anti-inflammatoires, analgésiques ou anti-ulcéreux.

27. Utilisation des composés amidiques hydrosolubles de l'acide hyaluronique ou d'un dérivé de celui-ci
15 comprenant au moins une unité de répétition de formule générale suivante :



- 20 dans laquelle :

R = NR₆R₇, ou un groupe alcoolique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, OH, O-, un groupe alcoolique de l'acide hyaluronique, un groupe amino de
25 l'acide hyaluronique désacylé ;

R₁, R₂, R₃, R₄ = H, SO₃⁻, un groupe acyle dérivé d'un acide carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, -CO-(CH₂)₂-COOY ; Y = charge négative, ou H ;

$R_5 = -CO-CH_3$, H, SO_3^- , un groupe acyle dérivé d'un acide carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, un groupe acylique de l'acide hyaluronique ;

5 $R_6 = H$ ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

$R_7 = H$ ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

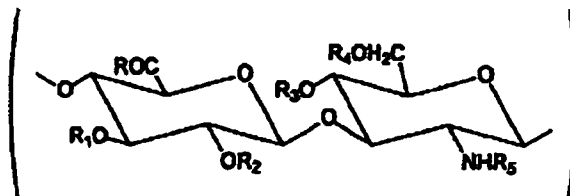
10 dans laquelle au moins l'un parmi R ou R_5 forme un groupe amide,

à condition que le composé amidique hydrosoluble ne soit pas combiné avec un ingrédient médicinal par

15 l'intermédiaire d'une liaison amide,

pour la préparation de sels avec des substances pharmacologiquement actives.

28. Utilisation des composés amidiques hydrosolubles de
20 l'acide hyaluronique ou d'un dérivé de celui-ci comprenant au moins une unité de répétition de formule générale suivante :



25

dans laquelle :

$R = NR_6R_7$, ou un groupe alcoolique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, OH, O-, un groupe

alcoolique de l'acide hyaluronique, un groupe amino de l'acide hyaluronique désacylé ;

$R_1, R_2, R_3, R_4 = H, SO_3^-$, un groupe acyle dérivé d'un acide carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, -CO-(CH₂)₂-COOY ; Y = charge négative, ou H ;

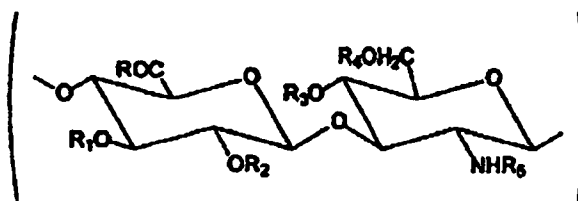
$R_5 = -CO-CH_3, H, SO_3^-$, un groupe acyle dérivé d'un acide carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, un groupe acylique de l'acide hyaluronique ;

$R_6 = H$ ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

$R_7 = H$ ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

dans laquelle au moins l'un parmi R ou R₅ forme un groupe amide, seuls ou en association les uns avec les autres et/ou avec des substances pharmaceutiquement actives pour la préparation de compositions pharmaceutiques, de biomatériaux, d'articles chirurgicaux et de soins de santé, de systèmes à libération lente et de systèmes destinés au revêtement des objets biomédicaux, à condition que le composé amidique hydrosoluble ne soit pas combiné avec un ingrédient médicinal par l'intermédiaire d'une liaison amide.

29. Utilisation des composés amidiques hydrosolubles de l'acide hyaluronique ou d'un dérivé de celui-ci comprenant au moins une unité de répétition de formule générale suivante :



dans laquelle :

R = NR_6R_7 , ou un groupe alcoolique de la série
 5 aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, OH, O-, un groupe alcoolique de l'acide hyaluronique, un groupe amino de l'acide hyaluronique désacylé ;

$\text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4 = \text{H}, \text{SO}_3^-$, un groupe acyle dérivé d'un acide
 10 carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, $-\text{CO}-(\text{CH}_2)_2-\text{COOY}$; Y = charge négative, ou H ;

$\text{R}_5 = -\text{CO}-\text{CH}_3, \text{H}, \text{SO}_3^-$, un groupe acyle dérivé d'un acide
 15 carboxylique de la série aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, un groupe acylique de l'acide hyaluronique ;

$\text{R}_6 = \text{H}$ ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

20 $\text{R}_7 = \text{H}$ ou un groupe aliphatique, aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique, substitué ou non substitué ;

dans laquelle au moins l'un parmi R ou R_5 forme un groupe
 amide, en association avec des substances radioactives et
 25 non radioactives, utilisés dans des systèmes de contraste en tant que marqueurs pour le diagnostic in vivo afin d'identifier et de traiter des tissus tumoraux ou des tissus endommagés, à condition que le composé amidique

hydrosoluble ne soit pas combiné avec un ingrédient médicinal par l'intermédiaire d'une liaison amide.

30. Utilisation des composés amidiques et de leurs sels
5 selon la revendication 28, dans laquelle les biomatériaux sont sous forme de canaux de guidage, de gazes, de fils, de gels, d'hydrogels, de tampons, de films, de membranes, d'éponges, de tissus non tissés, de microsphères, de nanosphères et associations de ceux-ci.

10

31. Utilisation du composé amidique selon les revendications précédentes, en chirurgie, en hémodialyse, en cardiologie, en dermatologie, en ophtalmologie, en oto-rhino-laryngologie, en dentisterie, en orthopédie, en
15 gynécologie, en urologie, dans le domaine de la circulation sanguine extra corporelle et l'oxygénation, en cosmétique ou en angiologie.

32. Utilisation des composés amidiques selon la
20 revendication 31, où la chirurgie sous-entend la chirurgie interne ou ostéoarticulaire, la neurochirurgie, la chirurgie anastomotique, viscoélastique, ophtalmique, oncologique, plastique, esthétique, oto-rhino-laryngologique, abdomino-pelvienne, uro-gynécologique,
25 cardiovasculaire, telle que dans la préparation de valves cardiaques, d'endoprothèses vasculaires, dans la prévention d'adhésions postchirurgicales et de la cicatrisation hypertrophique.

30 33. Utilisation des biomatériaux selon les revendications 14 à 17, en association avec la fibrine, et

éventuellement avec d'autres substances biologiquement actives pour la préparation de colles chirurgicales.

34. Utilisation des biomatériaux selon les revendications
5 14 à 17 pour la préparation d'échafaudages pour des cultures cellulaires.

35. Procédé de préparation d'amides sur l'azote de l'acide hyaluronique ou d'un dérivé désacétylé de celui-
10 ci mettant en jeu les étapes suivantes :

- a) réaction de désacétylation ;
- b) préparation du sel d'ammonium quaternaire du composé désacétylé ;
- c) préparation de l'agent d'acylation sous forme d'un
15 ester actif ;
- d) réaction de N-acylation entre le sel d'ammonium quaternaire de l'acide hyaluronique ou un dérivé désacétylé de celui-ci et l'agent d'acylation.

20 36. Procédé selon la revendication 35, dans lequel la réaction de désacétylation est obtenue en utilisant un mélange sulfate d'hydrazine/hydrazine.

37. Procédé selon la revendication 35, dans lequel le sel
25 d'ammonium quaternaire est le sel de tétrabutylammonium.

38. Procédé selon la revendication 35, dans lequel l'ester actif est l'ester paranitrophénylique d'un acide aliphatique, aromatique, arylaliphatique,
30 cycloaliphatique ou hétérocyclique, choisi pour la formation de l'amide.

39. Procédé de préparation des composés amidiques hydrosolubles selon la revendication 1 ayant les amides sur le carboxyle de l'acide hyaluronique ou d'un dérivé de celui-ci, mettant en jeu les étapes suivantes :

- 5 a) activation des groupes carboxy par réaction de ceux-ci, sous la forme acide ou d'un sel d'ammonium quaternaire, avec un agent d'activation, dans une solution acide ou sur une résine acide ;
- b) réaction avec une amine de la série aliphatique,
- 10 aromatique, arylaliphatique, cycloaliphatique ou hétérocyclique.

40. Procédé selon la revendication 39, dans lequel l'agent d'activation est le 1,1-carbonyldiimidazole.