

DEXTRAN FUNCTIONALIZED BY HYDROPHOBIC AMINO ACIDS

The present invention relates to novel biocompatible polymers based on dextran.

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These polymers can be used especially for the administration of active ingredient(s) (AI) to humans or to animals for therapeutic and/or prophylactic purposes.

10 The present invention relates to novel amphiphilic dextran derivatives functionalized by at least one hydrophobic alpha-amino acid. These novel dextran derivatives have good biocompatibility, and their hydrophobicity can readily be modulated without altering their
15 biocompatibility.

Among the amphiphilic dextrans, the carboxymethyl dextrans from Biodex described in US patent 6,646,120 are modified by benzylamine. That hydrophobic group does not belong to
20 the family of the alpha-amino acids.

Dellacherie et al. have also described amphiphilic dextrans (Durand, A. et al., *Biomacromolecules* **2006**, 7, 958-964) (Durand, Alain et al., *Colloid Polym. Sci.* **2006**,
25 284, 536-545) obtained by reaction of the hydroxyl functional groups of the dextran with epoxides (phenyl glycidyl ether, 1,2-epoxyoctane or 1,2-epoxydodecane). The described amphiphilic polymers are therefore not functionalized by amino acid derivatives.

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In US patent 5,750,678, Bauer et al. describe dextrans functionalized by C10 to C14 fatty acids. The resulting polymers are amphiphilic but are not modified by hydrophobic amino acids.

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A recent review of dextran-based functional polymers (Heinze, Thomas et al., *Adv Polym Sci* **2006**, 205, 199-291) does not take into account dextran functionalized by a hydrophobic amino acid.

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Accordingly, the invention relates to a dextran functionalized by at least one hydrophobic alpha-amino acid radical, designated AA, said alpha-amino acid being grafted or bonded to the dextran by a bonding arm R and a functional group F,

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- R being a chain containing from 1 to 18 carbon atoms, optionally branched and/or unsaturated and containing one or more heteroatoms, such as O, N or/and S, and having at least one acid functional group,

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- F being an ester, a thioester, an amide, a carbonate, a carbamate, an ether, a thioether or an amine,

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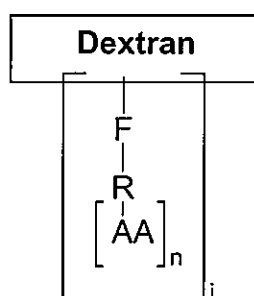
- AA being a hydrophobic amino acid radical, L or D, product of the coupling between the amine of the amino acid and an acid carried by the group R.

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A hydrophobic amino acid radical is understood as being the product of coupling between the amine of the amino

acid and an acid carried by the group R, said dextran being amphiphilic at neutral pH.

According to the invention, the functionalized dextran can correspond to the following general formulae:



Formula I

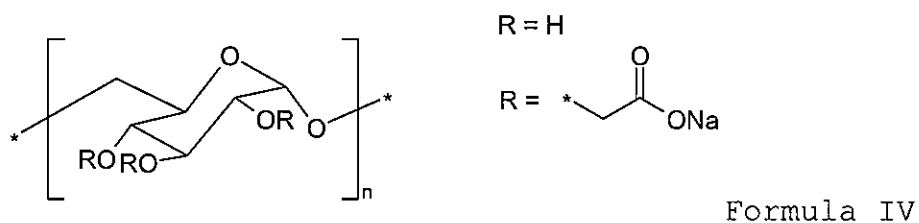
- R being a chain containing from 1 to 18 carbon atoms, optionally branched and/or unsaturated and containing one or more heteroatoms, such as O, N or/and S, and having at least one acid functional group,
 - F being an ester, a thioester, an amide, a carbonate, a carbamate, an ether, a thioether or an amine,
 - AA being a hydrophobic amino acid radical, L or D, product of the coupling between the amine of the amino acid and an acid carried by the group R,
- i represents the molar fraction of substituent F-R-[AA]_n per glycoside unit and is from 0.1 to 2,
- n represents the molar fraction of R groups substituted by AA and is from 0.05 to 1.

When R is not substituted by AA, the acid(s) of the group R are alkaline cation carboxylates, preferably such as Na, K,

5 said dextran being amphiphilic at neutral pH.

In an embodiment, F is an ester, a carbonate, a carbamate or an ether.

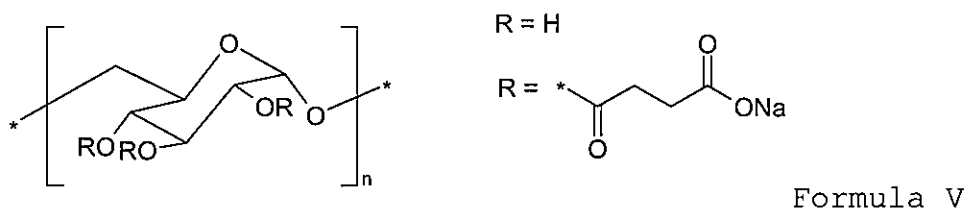
10 In an embodiment, the polysaccharide according to the invention is a carboxymethyl dextran (DMC) of formula IV



15 or the corresponding acid.

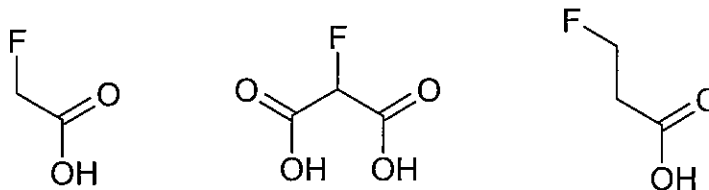
In an embodiment, the polysaccharide according to the invention is a dextran monosuccinic ester or succinic acid dextran (DSA) of formula V

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or the corresponding acid.

In an embodiment, the polysaccharide according to the invention is characterized in that the group R is selected from the following groups:

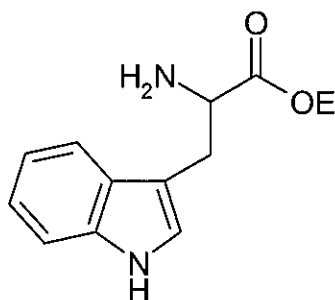


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or their alkaline cation salts.

In an embodiment, the dextran according to the invention is characterized in that the hydrophobic amino acid is selected from tryptophan derivatives, such as tryptophan, tryptophanol, tryptophanamide, 2-indole ethylamine and their alkaline cation salts.

In an embodiment, the dextran according to the invention is characterized in that the tryptophan derivatives are selected from the tryptophan esters of formula II

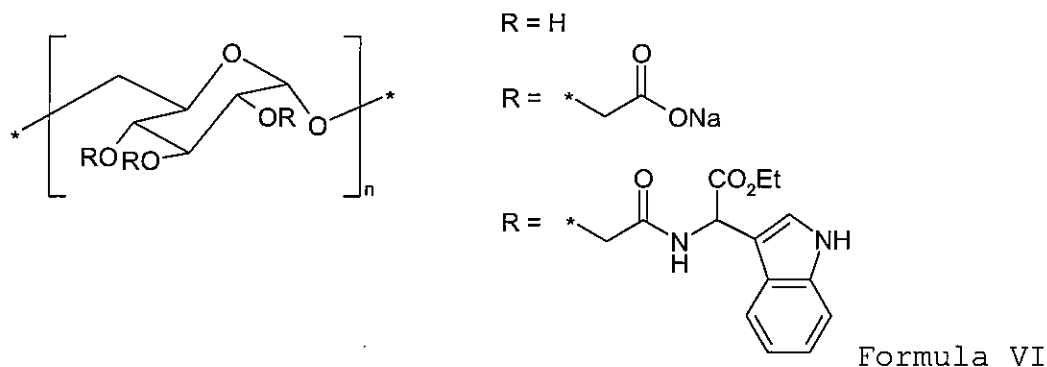


Formula II

E being a group which can be:

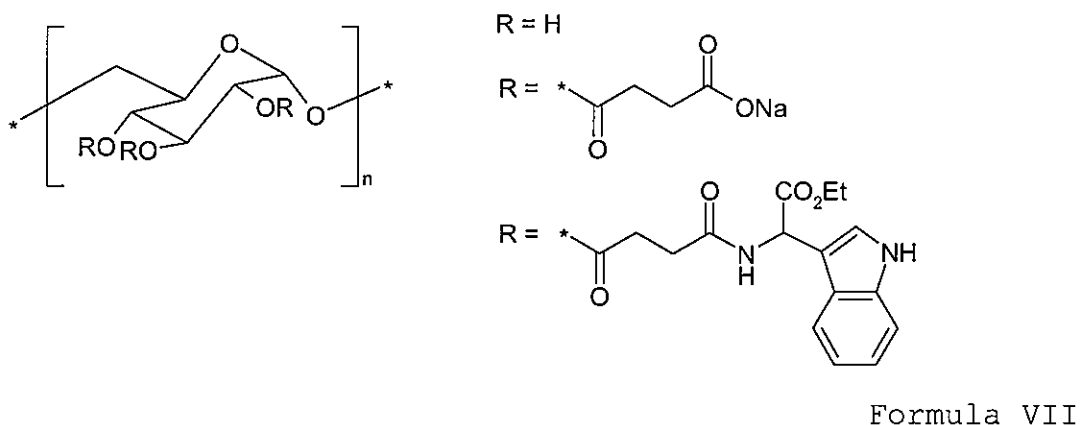
- 20
- a linear or branched C_1 - to C_8 -alkyl,
 - a linear or branched C_6 - to C_{20} -alkylaryl or -arylalkyl.

In an embodiment, the dextran according to the invention is a carboxymethyl dextran modified by the ethyl ester of tryptophan, of formula VI:



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In an embodiment, the dextran according to the invention is a dextran monosuccinic ester or succinic acid dextran (DSA) modified by the ethyl ester of tryptophan, of formula VII:

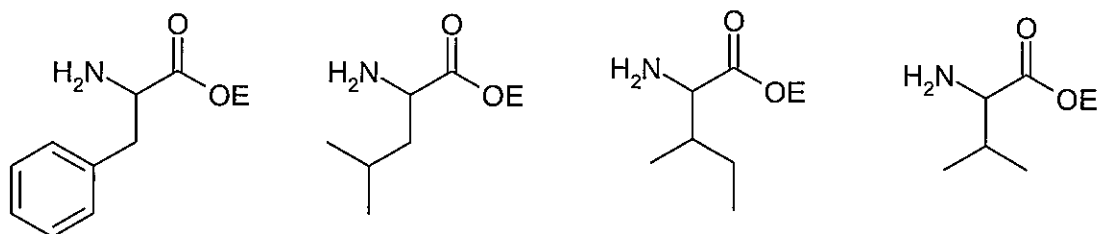


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In an embodiment, the dextran according to the invention is characterized in that the hydrophobic amino acid is selected from phenylalanine, leucine, isoleucine and valine and their alcohol, amide or decarboxylated derivatives.

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In an embodiment, the dextran according to the invention is characterized in that the derivatives of phenylalanine, leucine, isoleucine and valine are selected from the esters of those amino acids of formula III



Formulae III

E being as defined hereinbefore.

10 The dextran can have a degree of polymerization m of from 10 to 10,000.

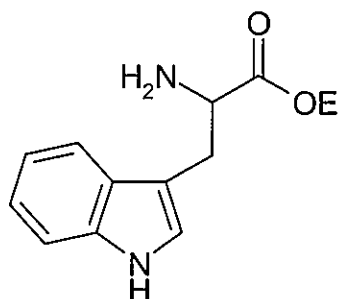
In an embodiment, it has a degree of polymerization m of from 10 to 1000.

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In another embodiment, it has a degree of polymerization m of from 10 to 500.

20 The dextrans according to the invention are obtained by grafting an ester of the amino acid in question onto the dextran modified by a group R.

In an embodiment, an ester of formula II

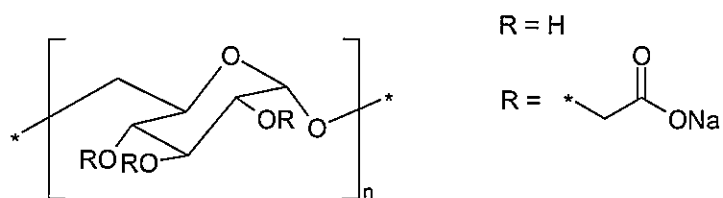


Formula II

E being a group which can be:

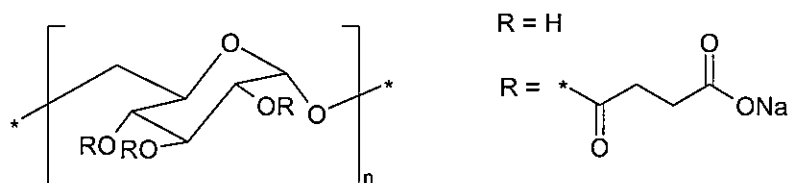
- a linear or branched C₁- to C₈-alkyl,
- 5 • a linear or branched C₆- to C₂₀-alkylaryl or -arylalkyl

is grafted onto a dextran (DMC) of formula IV



Formula IV

- 10 In another embodiment, an ester of formula II as defined above is grafted onto a dextran (DSA) of formula V



Formula V

- 15 The invention relates also to a pharmaceutical composition comprising one of the dextrans according to the invention as described hereinbefore, and at least one active ingredient.

Active ingredient is understood as being a product in the form of a single chemical entity or in the form of a combination having physiological activity. Said active ingredient can be exogenous, that is to say it is supplied
5 by the composition according to the invention. It can also be endogenous, for example the growth factors which will be secreted in a wound during the first phase of scarring and which it will be possible to retain on said wound by means of the composition according to the invention.

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The invention relates also to a pharmaceutical composition according to the invention as described hereinbefore, characterized in that it is administrable by the oral, nasal, vaginal, buccal route.

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The invention relates also to a pharmaceutical composition according to the invention as described hereinbefore, characterized in that it is obtained by drying and/or lyophilization.

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The invention relates also to a pharmaceutical composition according to the invention as described hereinbefore, characterized in that it is administrable in the form of a stent, a film or a coating of implantable biomaterials, in
25 the form of an implant.

The invention relates also to a pharmaceutical composition according to the invention as described hereinbefore, characterized in that the active ingredient is selected
30 from the group constituted by proteins, glycoproteins, peptides and non-peptide therapeutic molecules.

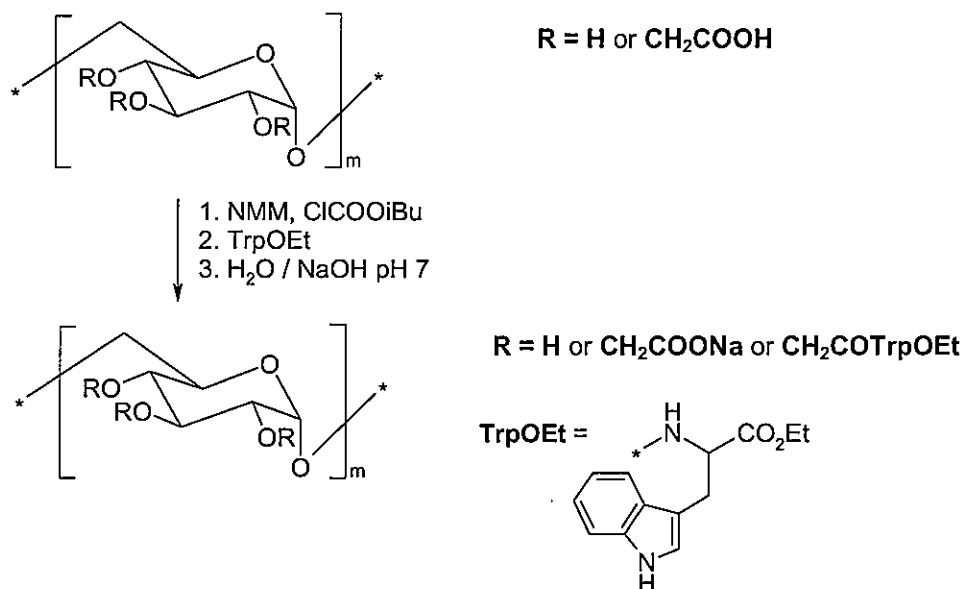
The possible pharmaceutical compositions are either in liquid form (nanoparticles or microparticles suspended in water or in mixtures of solvents) or in the form of a powder, an implant or a film.

In the case of local and systemic release, the possible modes of administration are by the intravenous, subcutaneous, intradermal, intramuscular, oral, nasal, vaginal, ocular, buccal route, etc.

The invention relates also to the use of the functionalized dextrans according to the invention in the preparation of pharmaceutical compositions as described hereinbefore.

Example 1: Synthesis of a carboxymethyl dextran modified by the ethyl ester of tryptophan

The acid functional groups ($i = 1.0$) of a carboxymethyl dextran having an average DP of 250 (10 g) are activated in the presence of N-methylmorpholine (4.7 g) and isobutyl chloroformate (6.4 g) in DMF (180 ml). The ethyl ester hydrochloride of tryptophan (5.4 g, Bachem) neutralized by TEA (2.0 g) in DMF (54 ml) is then grafted onto the activated polymer at 4°C for 45 minutes. After hydrolysis of the remaining activated acids (94 ml of water), the polymer is diluted in water (720 ml) and the pH is fixed at 7 by addition of sodium hydroxide solution. The polymer is then purified by ultrafiltration. The resulting polymer has the following structure:



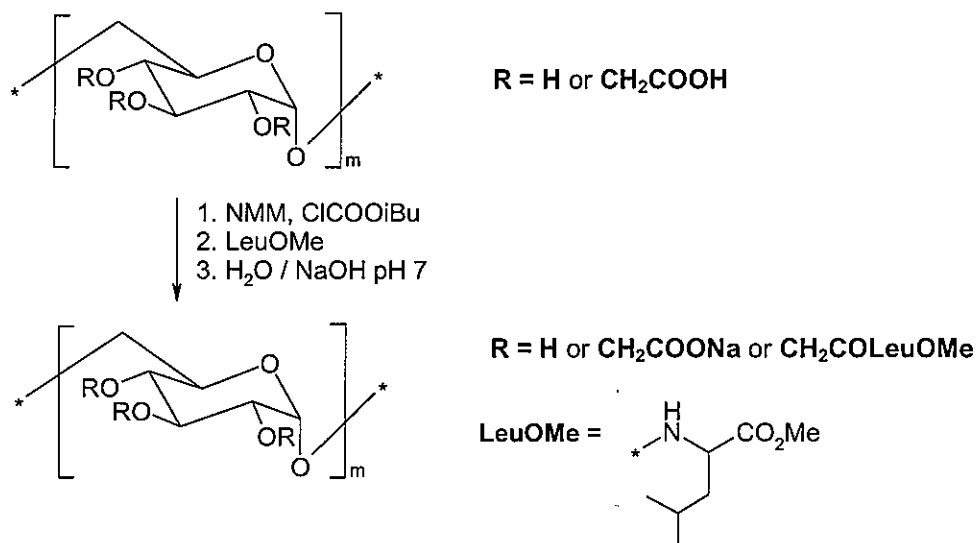
The molar fraction of acids modified by the ethyl ester of tryptophan is 0.45 according to 1H -NMR in $D_2O/NaOD$ ($n = 0.45$). The molar fraction of unmodified acids and acids modified by a glycoside unit is 1.0 ($i = 1.0$).

Example 2: Synthesis of a carboxymethyl dextran modified by the methyl ester of leucine

10 The acid functional groups ($i = 1.0$) of a carboxymethyl dextran having an average DP of 250 (10 g) are activated in the presence of N-methylmorpholine (4.7 g) and isobutyl chloroformate (6.4 g) in DMF (180 ml). The methyl ester hydrochloride of leucine (3.7 g, Bachem) neutralized by

15 TEA (2.0 g) in DMF (54 ml) is then grafted onto the activated polymer at $4^\circ C$ for 45 minutes. After hydrolysis of the remaining activated acids (94 ml of water), the polymer is diluted in water (720 ml) and the pH is fixed at 7 by addition of sodium hydroxide solution. The polymer

is then purified by ultrafiltration. The resulting polymer has the following structure:

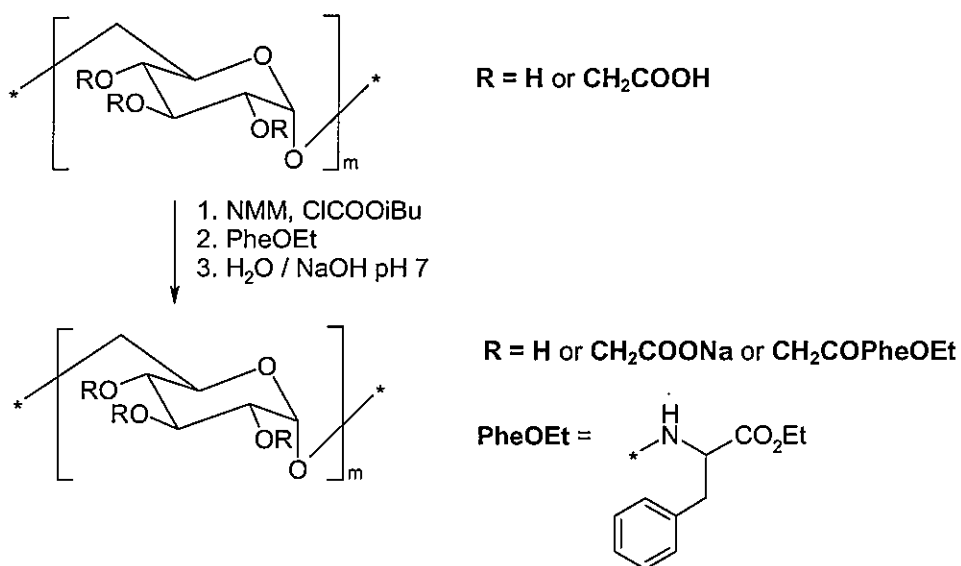


The molar fraction of acids modified by the methyl ester of leucine is 0.30 according to $^1\text{H-NMR}$ in $\text{D}_2\text{O}/\text{NaOD}$ ($n = 0.30$). The molar fraction of unmodified acids and acids modified by a glycoside unit is 1.0 ($i = 1.0$).

Example 3: Synthesis of a carboxymethyl dextran modified by the ethyl ester of phenylalanine

The acid functional groups ($i = 1.0$) of a carboxymethyl dextran having an average DP of 250 (10 g) are activated in the presence of N-methylmorpholine (4.7 g) and isobutyl chloroformate (6.4 g) in DMF (180 ml). The ethyl ester hydrochloride of phenylalanine (4.6 g, Bachem) neutralized by TEA (2.0 g) in DMF (54 ml) is then grafted onto the activated polymer at 4°C for 45 minutes. After hydrolysis of the remaining activated acids (94 ml of water), the polymer is diluted in water (720 ml) and the pH is fixed at 7 by addition of sodium hydroxide solution. The polymer

is then purified by ultrafiltration. The resulting polymer has the following structure:



The molar fraction of acids modified by the ethyl ester of
 5 phenylalanine is 0.45 according to ¹H-NMR in D₂O/NaOD (n =
 0.45). The molar fraction of unmodified acids and acids
 modified by a glycoside unit is 1.0 (i = 1.0).

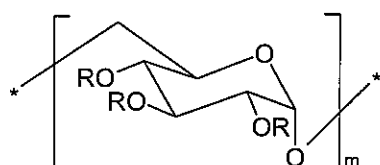
Example 4: Synthesis of a carboxymethyl dextran modified
 10 by the sodium salt of tryptophan

The polymer obtained in Example 1 is dissolved in water
 (30 mg/ml) and the pH is fixed at 12.5 by addition of 1N
 sodium hydroxide solution. After stirring overnight at
 15 ambient temperature, the product is purified by ultra-
 filtration.

The molar fraction of acids modified by the sodium salt of
 tryptophan is 0.45 according to ¹H-NMR in D₂O (n = 0.45).
 20 The molar fraction of unmodified acids and acids modified
 by a glycoside unit is 1.0 (i = 1.0).

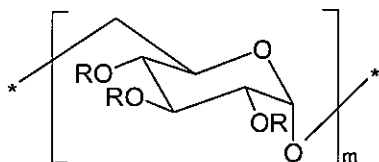
Example 5: Synthesis of a succinic acid dextran modified by the ethyl ester of tryptophan

- 5 The dextran having an average DP of 250, D40, (10 g, Amersham Biosciences) is dissolved in DMSO (25 ml) at 40°C. To that solution there are added succinic anhydride in solution in DMF (6.2 g in 25 ml) and N-methylmorpholine, NMM, diluted in DMF (6.2 g in 25 ml). After 1 hour's
10 reaction, the reaction mixture is diluted in water (400 ml) and the polymer is purified by ultrafiltration. The molar fraction of succinic ester formed per glycoside unit is 1.0 according to $^1\text{H-NMR}$ in $\text{D}_2\text{O}/\text{NaOD}$ ($i = 1.0$).
- 15 DSA in aqueous solution (350 g of a solution at 28 mg/ml) is acidified on ion exchange resin (300 ml of moist resin, Purolite, C100H). The resulting solution is frozen and then lyophilized.
- 20 Acidified DSA (8 g) is dissolved in DMF (115 ml) at ambient temperature. The solution is cooled to 0°C, and ethyl chloroformate (3.3 g) and then NMM (3.1 g) are added thereto. The ethyl ester hydrochloride of tryptophan (3.7 g, Bachem) neutralized by TEA (1.4 g) in DMF (37 ml)
25 is then added to the reaction mixture at 4°C, and the mixture is stirred for 45 minutes. After hydrolysis of the remaining activated acids, the polymer is diluted in water (530 ml) and the pH is fixed at 7 by addition of sodium hydroxide solution. The polymer is then purified by
30 ultrafiltration. The resulting polymer has the following structure:

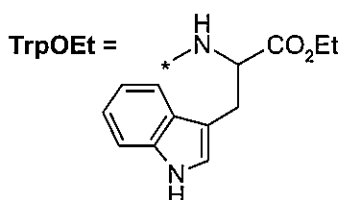


R = H or COCH₂CH₂COOH

1. NMM, ClCOOEt
2. TrpOEt
3. H₂O / NaOH pH 7



R = H or COCH₂CH₂COONa or COCH₂CH₂COTrpOEt



The molar fraction of acids modified by the ethyl ester of tryptophan is 0.45 according to ¹H-NMR in D₂O/NaOD (n = 0.45). The molar fraction of unmodified acids and acids modified by a glycoside unit is 1.0 (i = 1.0).

Example 6: Synthesis of a succinic acid dextran modified by the ethyl ester of tryptophan

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The dextran having an average DP of 250, D40, (20 g, Amersham Biosciences) is dissolved in DMSO (50 ml) at 40°C. To that solution there are added succinic anhydride in solution in DMF (24.7 g in 50 ml) and N-methylmorpholine, NMM, diluted in DMF (25.0 g in 50 ml). After 3 hours' reaction, the reaction mixture is diluted in water (800 ml) and the polymer is purified by ultrafiltration. The molar fraction of succinic ester formed per glycoside unit is 1.8 according to ¹H-NMR in D₂O/NaOD (i = 1.8).

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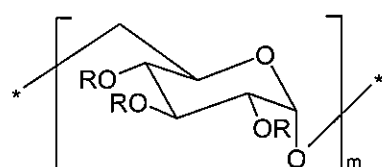
DSA in aqueous solution (720 g of a solution at 29.5 mg/ml) is acidified on ion exchange resin (300 ml of moist resin, Purolite, C100H). The resulting solution is frozen and then lyophilized.

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Acidified DSA (22.3 g) is dissolved in DMF (542 ml) at ambient temperature. The solution is cooled to 0°C, and ethyl chloroformate (13.4 g) and then NMM (12.5 g) are added thereto. The ethyl ester hydrochloride of tryptophan (7.5 g, Bachem) neutralized by TEA (2.8 g) in DMF (75 ml) is then added to the reaction mixture at 4°C, and the mixture is stirred for 45 minutes. After hydrolysis of the remaining activated acids, the polymer is diluted in water (530 ml) and the pH is fixed at 7 by addition of sodium hydroxide solution. The polymer is then purified by ultrafiltration. The resulting polymer has the following structure:

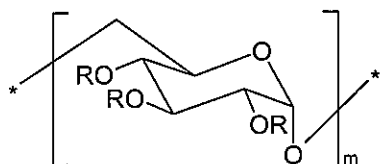
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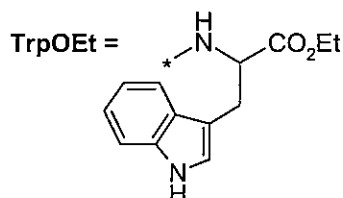


$R = H \text{ or } COCH_2CH_2COOH$

1. NMM, ClCOOEt
2. TrpOEt
3. H₂O / NaOH pH 7



$R = H \text{ or } COCH_2CH_2COONa \text{ or } COCH_2CH_2COTrpOEt$



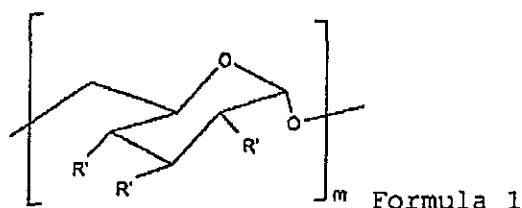
The molar fraction of acids modified by the ethyl ester of tryptophan is 0.25 according to ¹H-NMR in D₂O/NaOD (n =

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0.25). The molar fraction of unmodified acids and acids modified by a glycoside unit is 1.8 ($i = 1.8$).

CLAIMS

- 1 Functionalized dextran, characterized in that it corresponds to the following general formula:



wherein:

- each R' independently represents -OH or -F-R-[AA]_n, a molar fraction of substituent -F-R-[AA]_n per glycoside unit being from 0.1 to 0.2,
- m is the degree of polymerization,
- R represents a chain containing from 1 to 18 carbon atoms and having at least one acid functional group prior to attachment to AA, the chain optionally being branched and/or unsaturated and comprising one or more heteroatoms,
- F represents an ester, a thioester, an amide, a carbonate, a carbamate, an ether, a thioether or an amine,
- AA represents a hydrophobic amino acid radical, L or D, derived from a coupling between an amine of an amino acid and the at least one acid functional group of R, the amino acid, prior to attachment to group R, being selected from the group consisting of tryptophan, phenylalanine, leucine, isoleucine and valine, and alcohol, amide or decarboxylated derivatives thereof, and alkaline cation salts thereof,
- n represents the molar fraction of R groups substituted by AA and is from 0.05 to 1,

- when R is not substituted by AA, the acid(s) of the group R are alkaline cation carboxylates, and
 - the functionalized dextran is amphiphilic at neutral pH.
- 2 The functionalized dextran according to claim 1, wherein the group F is an ester, a carbonate, a carbamate or an ether.
 - 3 The functionalized dextran according to claim 1, wherein prior to attachment of the AA moiety,

F is an ether,

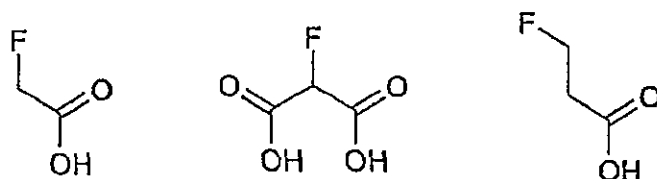
R is $-\text{CH}_2-\text{COONa}$ or the corresponding acid, and

m is from 10 to 10,000.
 - 4 The functionalized dextran according to claim 1, wherein, prior to attachment of the AA moiety,

F is an ester,

R is $-\text{CH}_2-\text{CH}_2-\text{COONa}$ or the corresponding acid, and

m is from 10 to 10,000.
 - 5 The functionalized dextran according to claim 1, wherein, prior to attachment of the moiety, $-\text{F}-\text{R}$ is selected from the group consisting of:



and alkaline cation salts thereof.

- 6 The functionalized dextran according to any one of the preceding claims, wherein the hydrophobic amino acid is a tryptophan or alkaline cation salts thereof.
- 7 The functionalized dextran according to any one of claims 1 to 5, wherein the hydrophobic amino acid AA is selected from the group consisting of phenylalanine, leucine, isoleucine and valine and alcohol, amide or decarboxylated derivatives thereof.
- 8 The functionalized dextran according to any one of the preceding claims, wherein a degree of polymerization of the functionalized dextran is from 10 to 10,000.
- 9 A pharmaceutical composition, comprising the functionalized dextran according to any one of the preceding claims and at least one active ingredient.
- 10 The functionalized dextran according to claim 1, wherein the hydrophobic amino acid AA is a sodium salt of tryptophan.
- 11 The functionalized dextran according to claim 1, wherein, prior to attachment to the AA moiety,

F is an ether,

R is $-\text{CH}_2-\text{COONa}$ or the corresponding acid, and

m is from 10 to 10,000.
- 12 A carboxymethyl dextran modified by a sodium salt of tryptophan.
- 13 The functionalized dextran according to claim 1, wherein:
 - prior to attachment of the AA moiety, F is an ether, and R is $-\text{CH}_2-\text{COONa}$ or alkaline salt thereof, and

- 21 -

- the AA moiety represents an alkaline cation salt of phenylalanine.