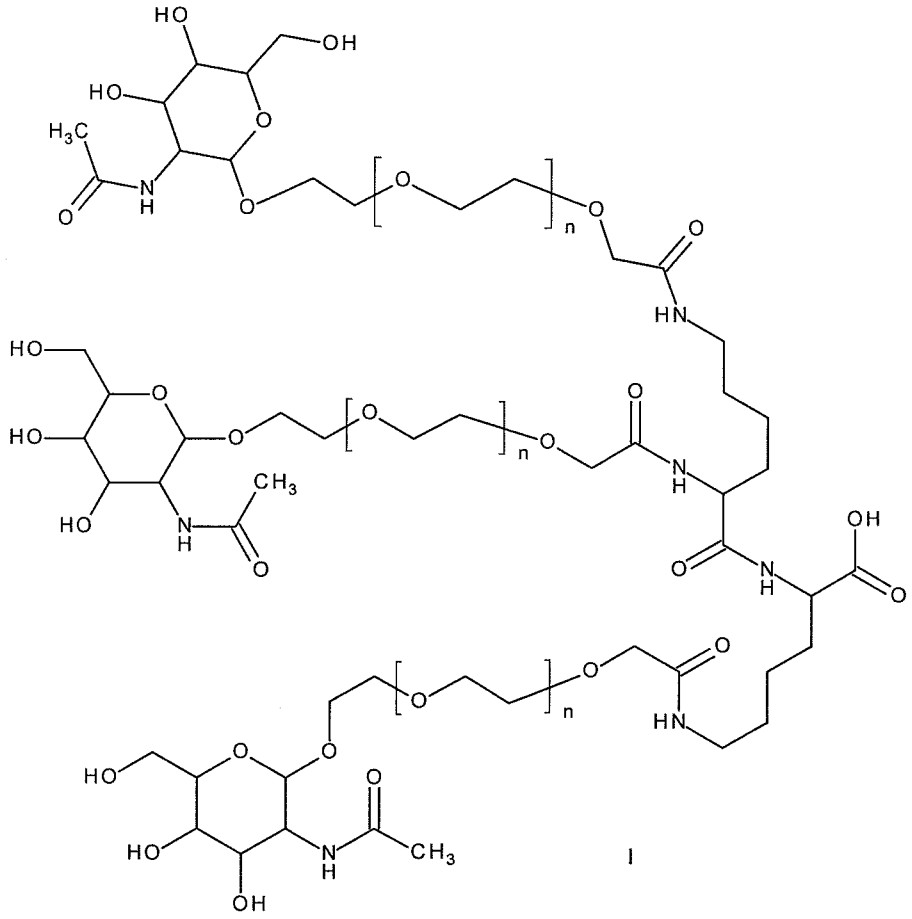
PROCESS FOR THE PREPARATION OF FALNAC ACID DERIVATIVES

Summary

The disclosure relates to a new process for the preparation of GalNAc derivatives of the formula I



I

wherein n is an integer between 0 and 10 and its salts, corresponding enantiomers and/ or optical isomers thereof.

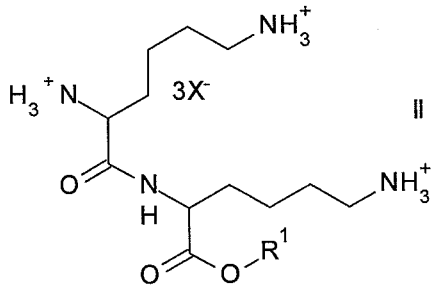
GalNAc derivatives of formula I are usually the targeting moiety of conjugates comprising the GalNAc moiety and certain oligonucleotides. The GalNAc moiety due to its affinity to the asialoglycoprotein receptor which is located on the liver cell enables functional delivery of oligonucleotide conjugates to the liver cell. Such GalNAc cluster antisense conjugates have the potential to act as pharmacokinetic modulators and are e.g. described in the PCT Publication WO 2012/083046 or in the US Patent Application Publication US 2011/0207799. While these publications also disclose processes for the preparation of the GalNAc derivatives it was found that these processes do not meet the standard for a technical scale synthesis.

Object of the disclosure therefore is to provide an improved method for the preparation of the GalNAc derivatives of formula I which meets the requirements of an industrial scale process.

Further object of the disclosure is the use of the GalNAc acid derivative of formula I for the preparation of therapeutically valuable GalNAc oligonucleotide conjugates and of a process for the preparation of such conjugates.

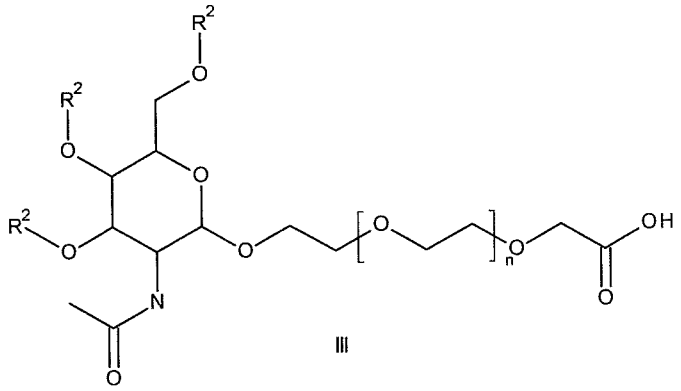
The object could be achieved with the process of the present disclosure which comprises

a) the coupling of a triamine salt of formula II

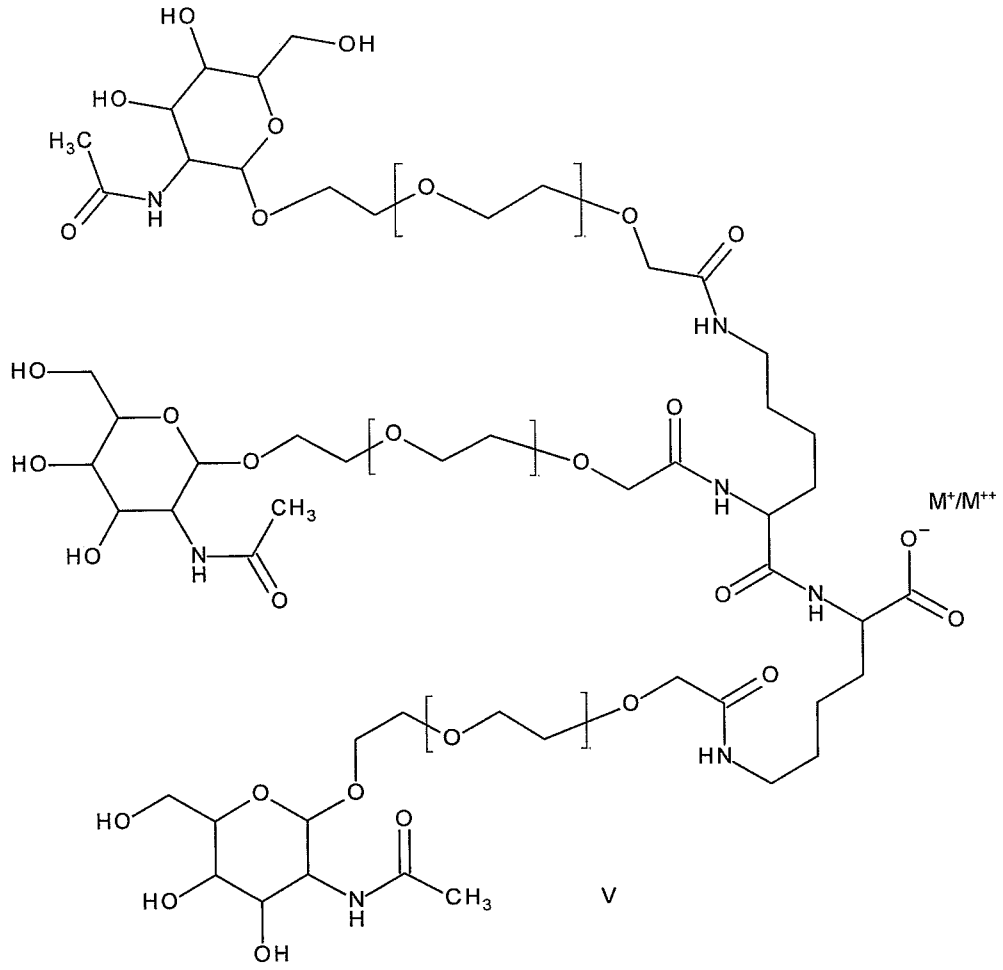


II

wherein R^1 is an ester protecting group and X is an anion of an acid with a tetrahydropyran acid of formula III



III



V

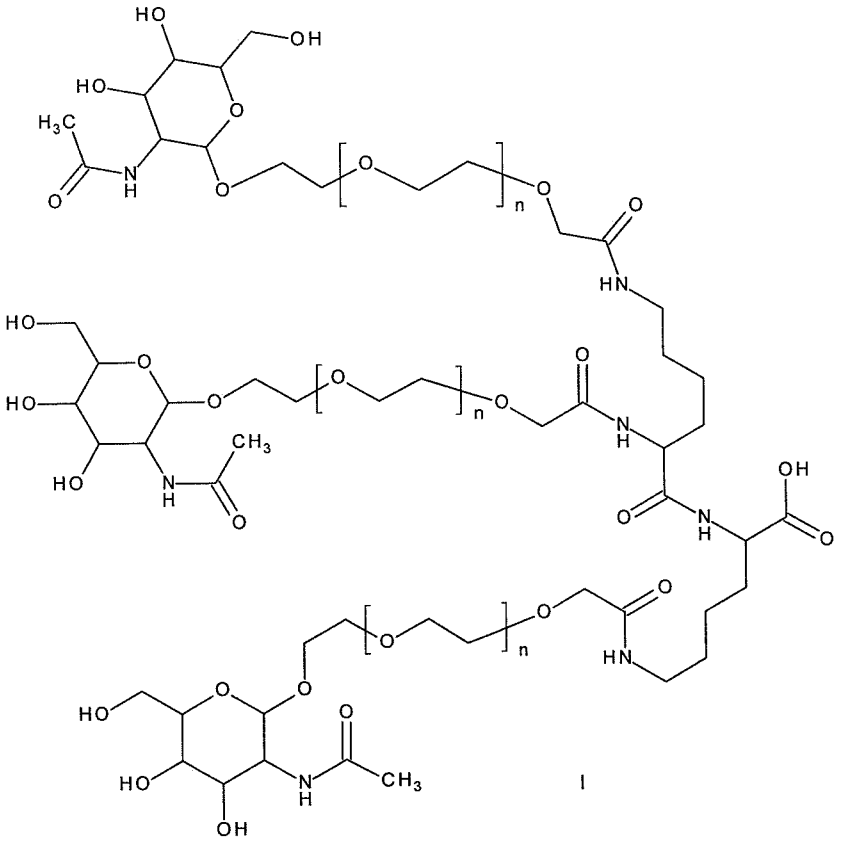
c)

wherein n is as above and M is a metal kation;

and

c) optionally the transformation of the GalNAc acid salt of formula V into the GalNAc acid derivative of formula I.

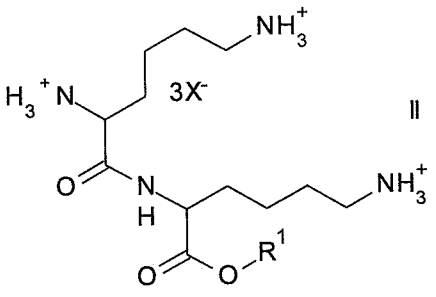
In one aspect, the disclosure relates to process for the preparation of GalNAc acid derivatives of the formula I



I

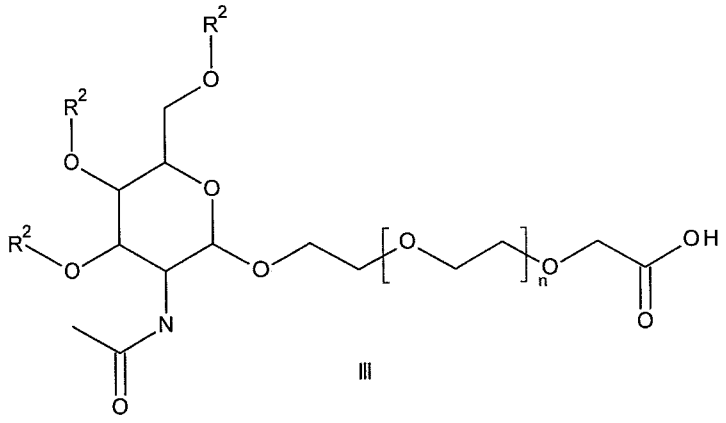
wherein n is an integer between 0 and 10 and its salts, corresponding enantiomers and/ or optical isomers thereof, comprising

a) the coupling of a triamine salt of formula II



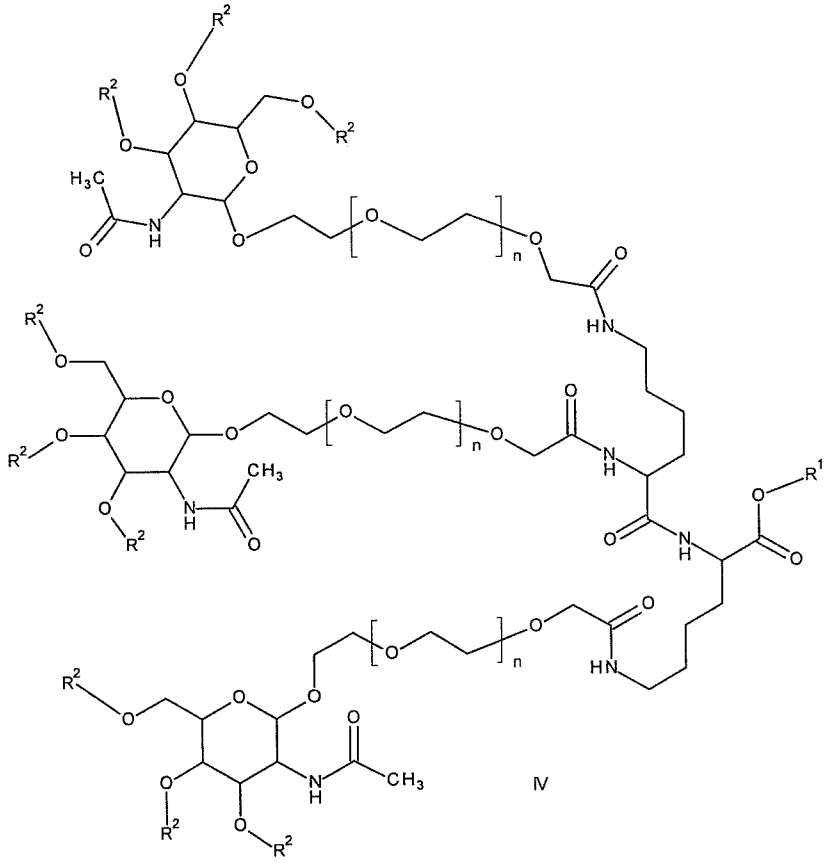
II

wherein R^1 is an ester protecting group and X is an anion of an acid with a tetrahydropyran acid of formula III



III

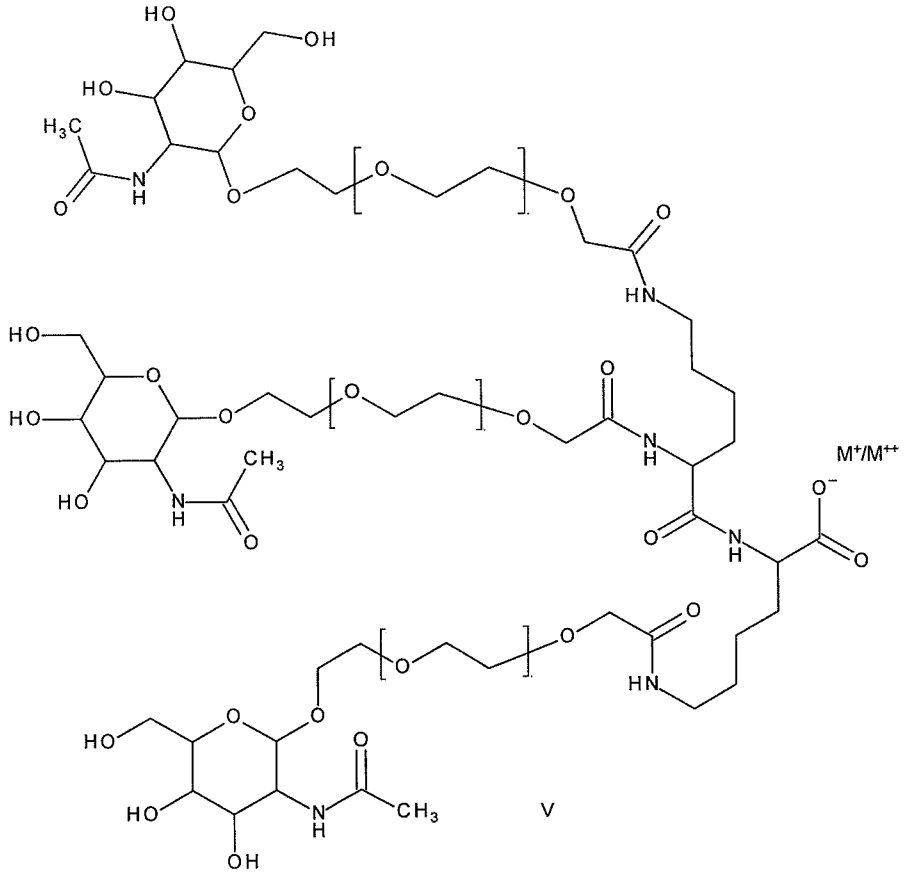
wherein R^2 is a hydroxy protecting group and n is as above in the presence of a peptide coupling agent, an amine base and an organic solvent to form the GalNAc ester of formula IV



IV

wherein R^1 and R^2 and n are as above;

b) the removal of the ester protecting group R^1 and of the hydroxy protecting groups R^2 in the presence of a mineral base to form the GalNAc acid salt of formula V



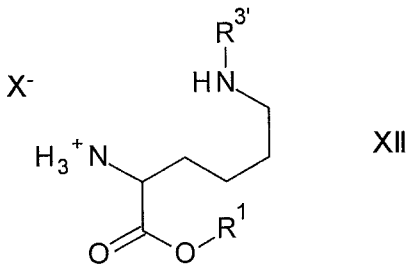
V

wherein n is as above and M is a metal kation;

and

c) optionally to transform GalNAc acid salt of formula V into the GalNAc acid derivative of formula I.

In another aspect, the disclosure relates to amine salt of formula XII



XII

wherein R^1 is benzyl, $R^{3'}$ is Boc and X is the anion of methanesulfonic acid.

In another aspect, the disclosure relates to process for the preparation of GalNAc oligonucleotide conjugates comprising the steps

a3) preparing the GalNAc acid derivative of formula I or the GalNAc acid salt of formula V according to any one of claims 1 to 27 and

b3) conjugating the GalNAc acid derivative of formula I or the GalNAc acid salt of formula V under peptide coupling conditions with an oligonucleotide.

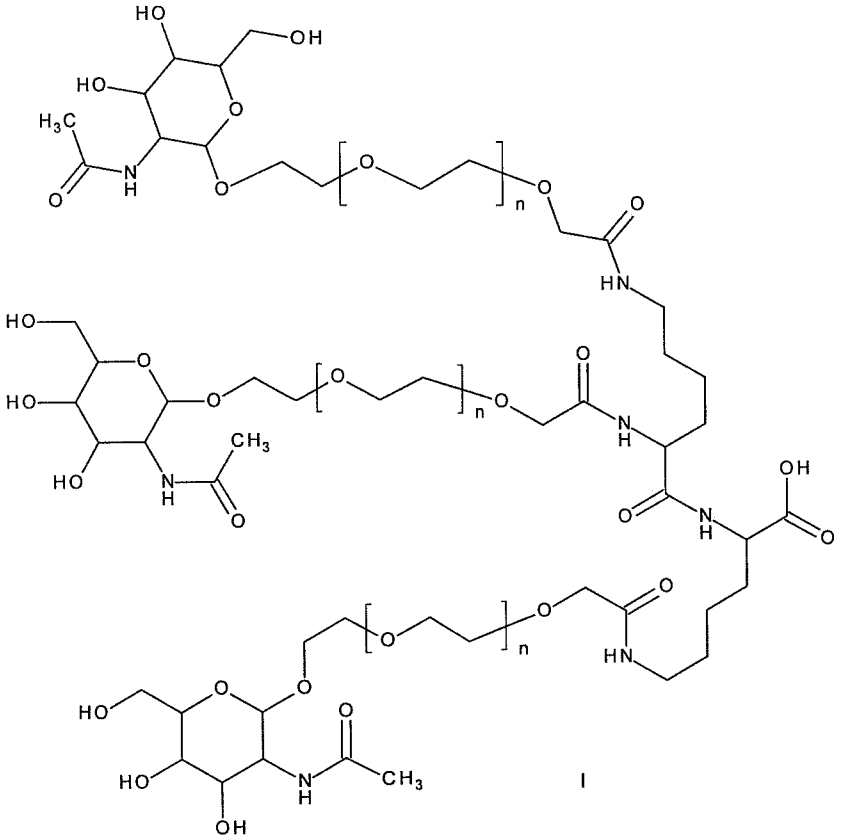
The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

Whenever a chiral carbon is present in a chemical structure, it is intended that all stereoisomers associated with that chiral carbon are encompassed by the structure as pure stereoisomers as well as mixtures thereof.

The term "C_{1-7} alkyl" denotes a monovalent linear or branched saturated hydrocarbon group of 1 to 7 carbon atoms, and in more particular embodiments 1 to 4 carbon atoms. Examples of alkyl include methyl, ethyl, propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, or tert-butyl.

Claims:

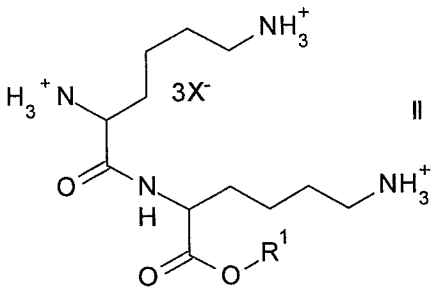
1. Process for the preparation of GalNAc acid derivatives of the formula I



I

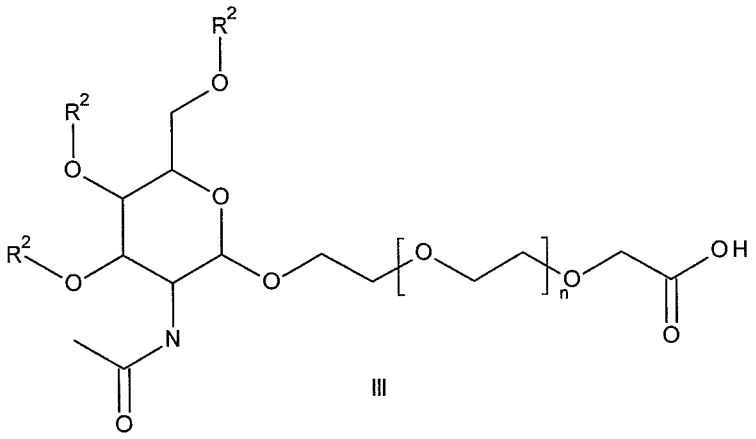
wherein n is an integer between 0 and 10 and its salts, corresponding enantiomers and/ or optical isomers thereof, comprising

a) the coupling of a triamine salt of formula II



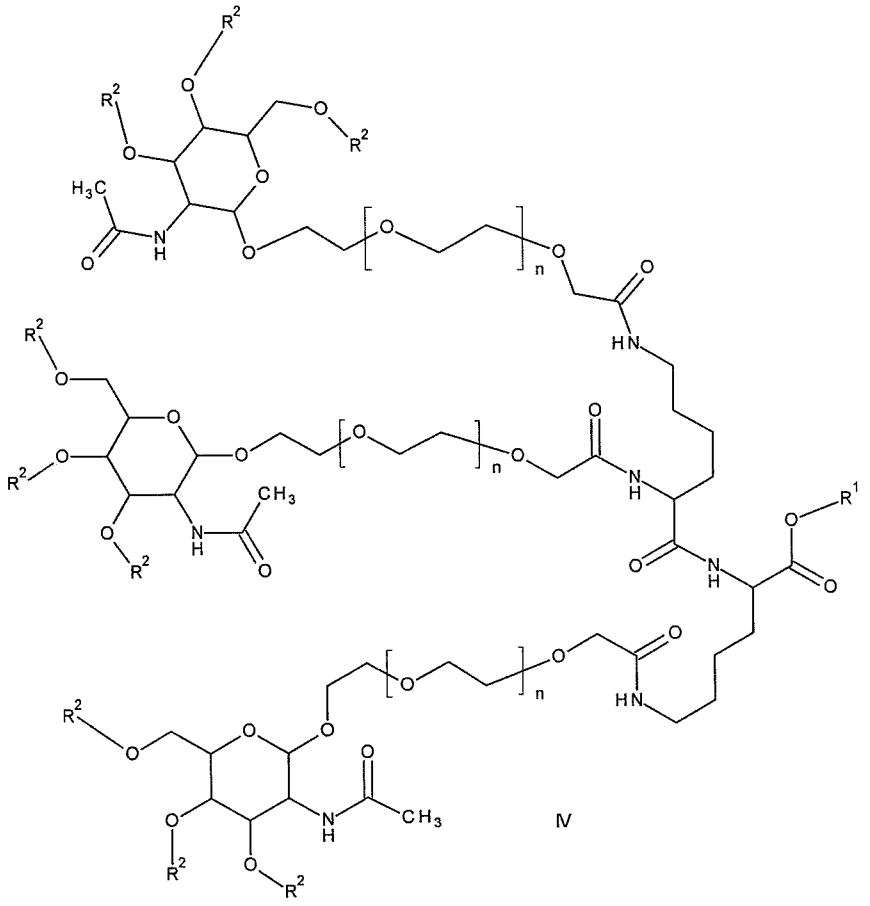
II

wherein R^1 is an ester protecting group and X is an anion of an acid with a tetrahydropyran acid of formula III



III

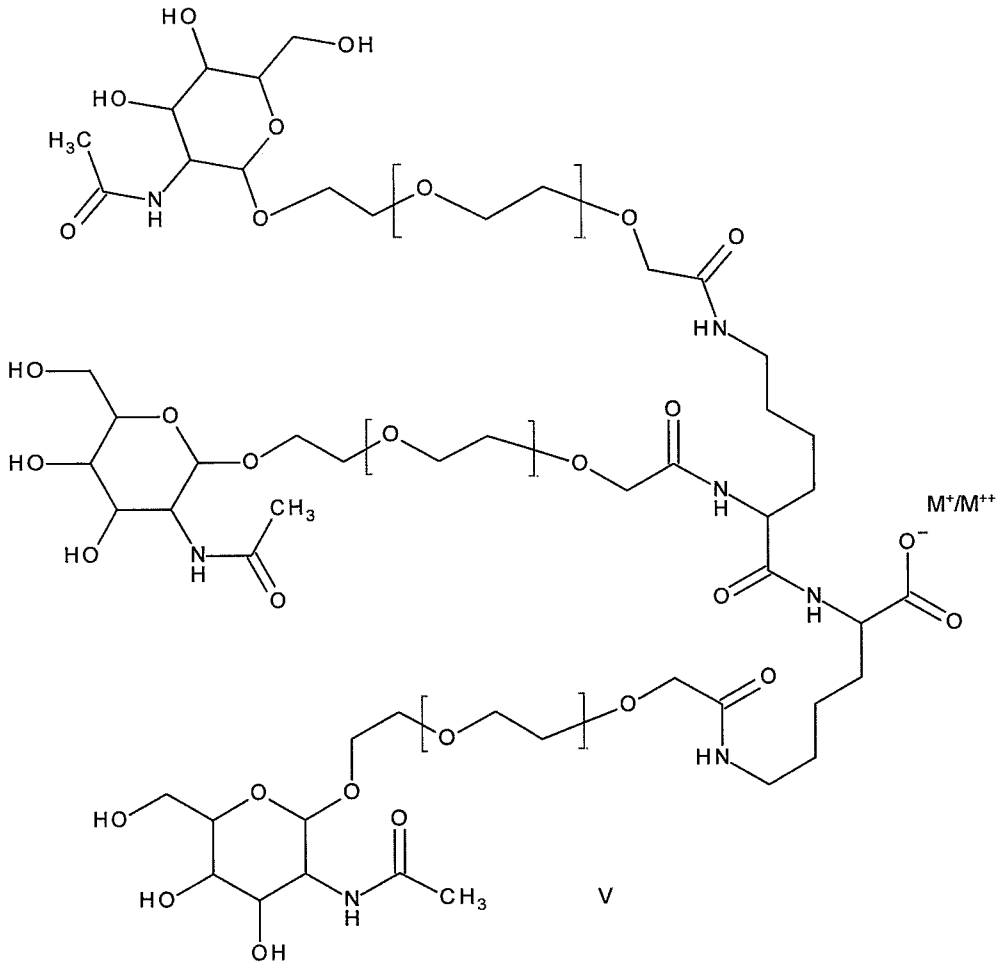
wherein R^2 is a hydroxy protecting group and n is as above in the presence of a peptide coupling agent, an amine base and an organic solvent to form the GalNAc ester of formula IV



IV

wherein R^1 and R^2 and n are as above;

b) the removal of the ester protecting group R^1 and of the hydroxy protecting groups R^2 in the presence of a mineral base to form the GalNAc acid salt of formula V



wherein n is as above and M is a metal kation;

and

c) optionally to transform GalNAc acid salt of formula V into the GalNAc acid derivative of formula I.

2. Process of claim 1, wherein n is an integer between 0 and 5 and the ester protecting group R^1 is C_{1-7}-alkyl or phenyl-C_{1-7}-alkyl, wherein the phenyl group is optionally substituted with

halogen or C_{1-7}-alkyl, the hydroxy protecting group R^2 is acetyl and X is selected from the anion of a sulfonic acid.

3. Process of any one of claims 1 or 2, wherein the peptide coupling agent is n-propylphosphonic acid anhydride and the amine base is a tertiary amine.

4. Process of any one of claim 1 to 3, wherein the coupling in step a) takes place in an organic solvent is a polar aprotic solvent at a reaction temperature from 20°C to 70°C.

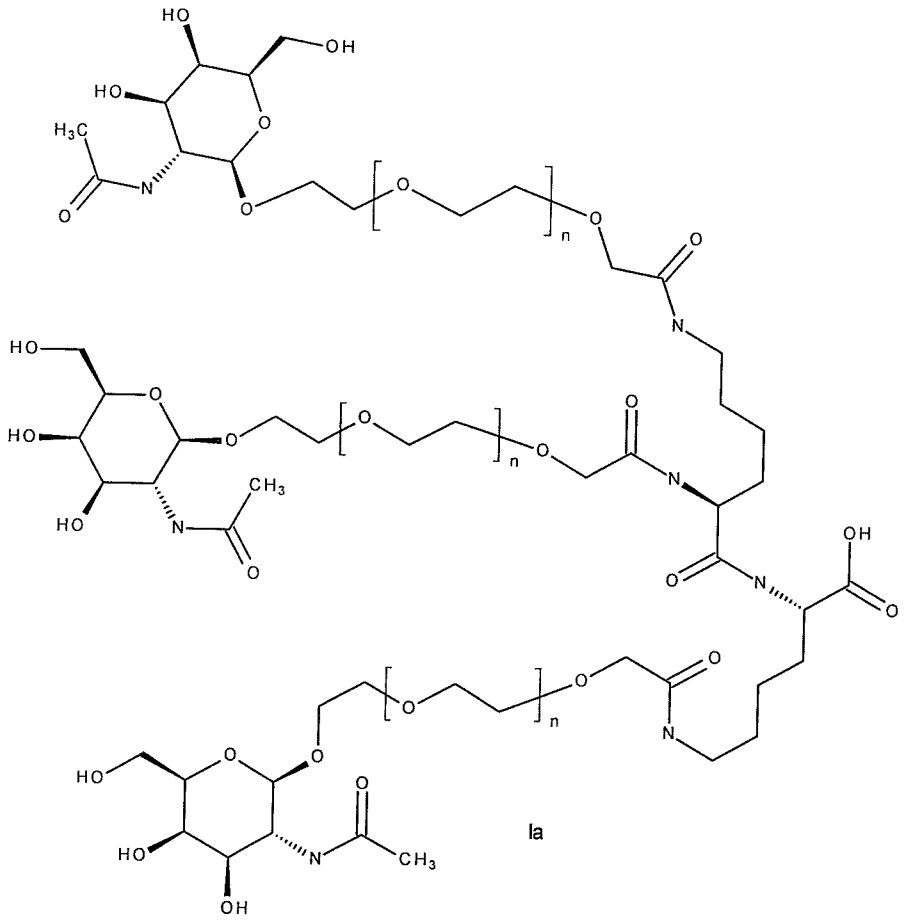
5. Process of any one of claims 1 to 4, wherein the mineral base for the removal of the ester protecting group R^1 in step b) is an alkali hydroxide.

6. Process of any one of claims 1 to 5, wherein the step a) and b) are combined and performed in one step without isolating the GalNAc ester of formula V.

7. Process of any one of claims 1 to 6, wherein the optional transformation of the GalNAc acid salt of formula V into the GalNAc acid derivative of formula I is performed via kation exchange or via treatment with an acid.

8. Process of any one of claims 1 to 7, wherein the GalNAc acid derivative of formula I is an enantiomer of the formula Ia

AND Enantiomer

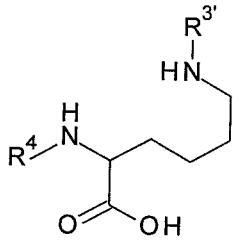


Ia

wherein n is as above or a salt, a corresponding enantiomer and/ or optical isomer thereof.

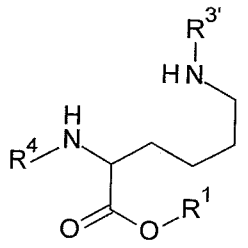
9. Process of claim 1 wherein the process for producing the triamine of formula II comprises the steps

a1) transforming the carboxylic acid of formula X



X

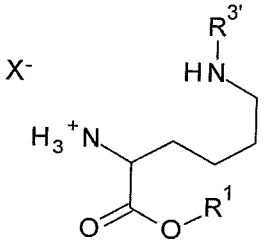
wherein $R^{3'}$ and R^4 are different and independent of each other are amino protecting groups into an ester of formula XI



XI

wherein R^1 is an ester protecting group and $R^{3'}$ and R^4 are as above;

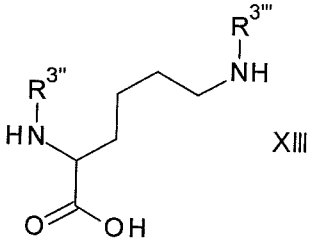
b1) removing the amino protecting group R^4 and subsequent forming an amine salt of formula XII



XII

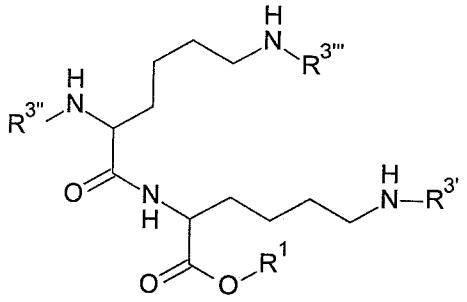
wherein $R^1$and $R^{3'}$ are as above and X^- is an acid anion;

c1) coupling the amine salt of formula XII with a hexanoic acid derivative of formula XIII



XIII

wherein $R^{3''}$ and $R^{3'''}$ are amino protecting groups to form the protected triamine of formula XIV



XIV

wherein $R^{3'}$, $R^{3''}$, $R^{3'''}$ and R^1 are as above;

d1) converting the protected triamine of formula XIV with an acid into the triamine salt of formula II.

10. Process of claim 9, wherein $R^{3'}$, $R^{3''}$ and $R^{3'''}$ are the same and protecting groups which are cleavable under acidic conditions and R^4 is a protecting group which is cleavable under basic conditions or by way of hydrogenolysis.

11. Process of claim 10, wherein $R^{3'}$, $R^{3''}$ and $R^{3'''}$ are Boc and R^4 is FMOC.

12. Process of any one of claims 9 to 11, wherein the transformation in step a1) takes place with benzyl alcohol in the presence of an activating agent, an amine catalyst and an aprotic organic solvent at a reaction temperature of 20°C to 50°C.

13. Process of any one of claims 9 to 11, wherein the amino protecting group R^4 is FMOC and its removal in step b1) is performed with a secondary aliphatic amine in a polar aprotic solvent at a reaction temperature of 20°C to 50°C.

14. Process of any one of claims 9 to 11, wherein the subsequent formation of the amine salt of formula XII in step b1) is effected with a sulfonic acid.

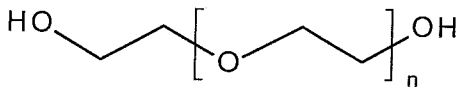
15. Process of any one of claims 9 to 11, wherein the coupling in step c1) is performed with n-propylphosphonic acid anhydride as coupling agent in the presence of a tertiary amine and a polar aprotic solvent at a reaction temperature from 20°C to 50°C.

16. Process of any one of claims 9 to 11, wherein in step d1) the triamine salt of formula II is formed with a sulfonic acid in a polar aprotic solvent at a reaction temperature of 20°C to 80°C.

17. Process of claim 16, wherein a polar aprotic solvent is selected which prevents the triamine salt of formula II to crystallize.

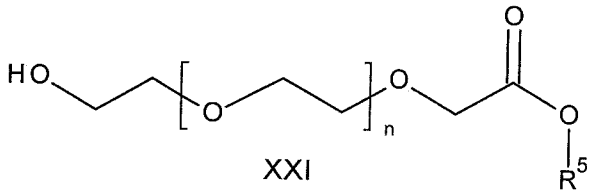
18. Process of claim 1, wherein the process for producing the tetrahydropyran acid of formula III comprises

a2) the transformation of the diol of formula XX



XX

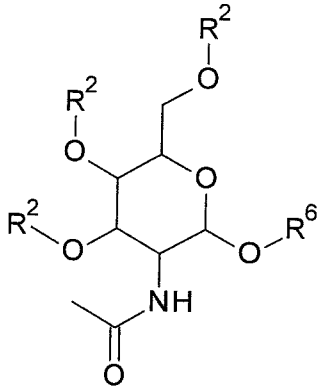
wherein n is as above into the alcohol ester of formula XXI



XXI

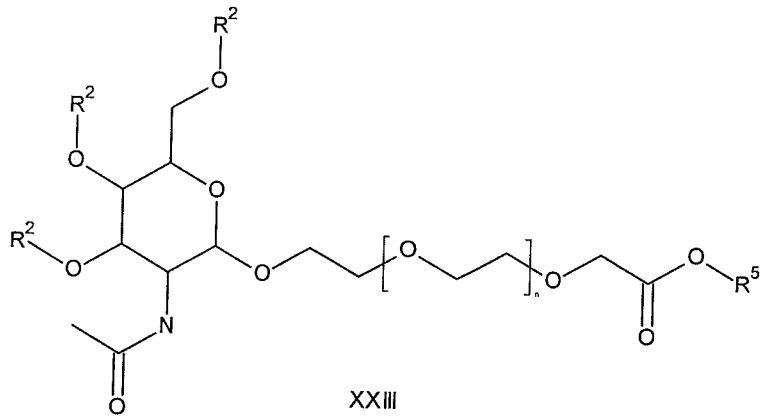
wherein n is as above and R^5 is an ester protecting group;

b2) the coupling of the alcohol ester of formula XXI with a tetrahydropyran derivative of formula XXII



XXII

wherein R^2 and R^6 independent of each other are hydroxy protecting groups to form a tetrahydropyran ester of formula XXIII



XXIII

wherein n, R^2 and R^5 are as above ;

c2) the removal of the ester group to form the tetrahydropyran acid of formula III.

19. Process of claim 18, wherein the hydroxy protecting group R^2 is acetyl, the ester protecting group R^5 is benzyl and the hydroxy protecting group R^6 is acetyl.

20. Process of claims 18 or 19, wherein in a first step of step a2) the diol of formula XX is deprotonated with an alkali metal alcoholate in the presence of a polar protic or polar aprotic solvent at a reaction temperature from 50°C to 120°C.

21. Process of claims 18 or 19, wherein in a second step of step a2) an acetic acid moiety is introduced with a halogen acetic acid or with a salt thereof in the presence of a polar protic or polar aprotic solvent at a reaction temperature from 50°C to 120°C.

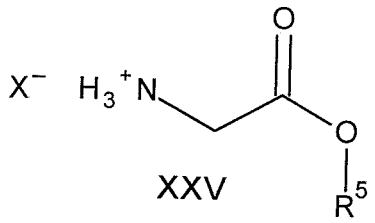
22. Process of claims 18 or 19, wherein in a third step of step a2) the alcohol ester of formula XXI wherein R^5 is benzyl is formed with a benzyl halogenide or a benzyl sulfonylester in a polar aprotic solvent at a reaction temperature from 20°C to 120°C.

23. Process of claim 18 or 19, wherein in step b2) the alcohol ester of formula XXI is coupled with the tetrahydropyran derivative of formula XXII in the presence of a halogenated sulfonic acid in the presence of a polar aprotic solvent at a reaction temperature from 0°C to 140°C.

24. Process of claim18 or 19, wherein in step c2) the benzylester group is removed by a catalytic hydrogenation with hydrogen in the presence of a hydrogenation catalyst.

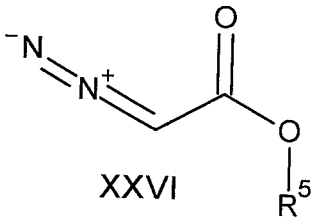
25. Process of claim 18, wherein the formation of the alcohol ester of formula XXI comprises

a3) the diazotization of an 2-amino acetate of formula XXV



XXV

wherein R^5 is as above and X is a halogen atom with a nitrite salt to form the 2-diazo compound of formula XXVI



XXVI

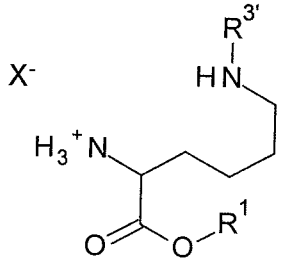
wherein R^5 is as above; and

b3) the transformation of the 2-diazo compound of formula XXVI with the diol of formula XX.

26. Process of claim 25, wherein the diazotization in step a3) is performed with an alkali nitrite in the presence of a solvent mixture of water and a non-polar aprotic solvent at a reaction temperature of -10°C to 10°C.

27. Process of claim 25 or 26, wherein the transformation of the 2-diazo compound with the diol of formula XX in step b3) is performed in the presence of a Lewis acid and a non-polar aprotic solvent at a reaction temperature of -10°C to 10°C.

28. Amine salt of formula XII



XII

wherein R^1 is benzyl, $R^{3'}$ is Boc and X is the anion of methanesulfonic acid.

29. Process for the preparation of GalNAc oligonucleotide conjugates comprising the steps

a3) preparing the GalNAc acid derivative of formula I or the GalNAc acid salt of formula V according to any one of claims 1 to 27 and

b3) conjugating the GalNAc acid derivative of formula I or the GalNAc acid salt of formula V under peptide coupling conditions with an oligonucleotide.

30. Process of claim 29, wherein the GalNAc acid salt of formula V is used.