CLAIMS

1. A method for preparing a sample in which a substance A containing a hydrazide group is bonded to a sugar chain and/or a sugar derivative via hydrazone formation between the hydrazide group of the substance A and the reducing end of the sugar chain and/or the sugar derivative.

2. The method for preparing a sample as set forth in claim 1, in which said substance A comprises a moiety containing chromophore or fluorophore.

3. The method for preparing a sample as set forth in claim 1, in which said substance A is a substance selected from the following substances or a salt thereof,

(substance A): 5-Dimethylaminonaphthalene-1-sulfonyl hydrazine (Dansylhydrazine); 2-hydrazinopyridine; 9-fluorenylmethyl carbazate (Fmoc hydrazine); benzylhydrazine; 4, 4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionoc acid, hydrazide; 2-(6,8-difluoro-7-hydroxy-4-methylcoumarin)acetohydrazide; 7-diethylaminocoumarin-3-carboxylic acid, hydrazide (DCCH); phenylhydrazine; 1-Naphthaleneacethydrazide; 2-hydrazinobenzoic acid; biotin hydrazide; and phenylacetic hydrazide.

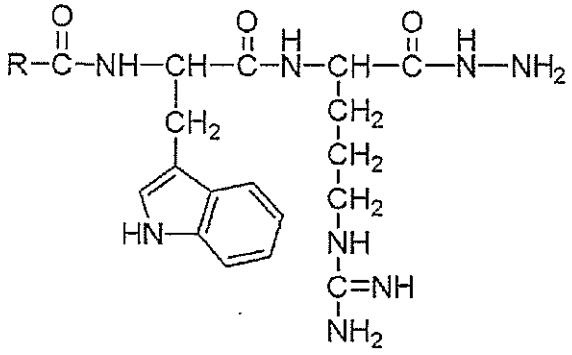
4. The method for preparing a sample as set forth in claim 1, in which said substance A contains a moiety consisting of at least one of an arginine residue, a tryptophan residue, a phenylalanine residue, a tyrosine residue, a cysteine residue

and a derivative thereof.

5. The method for preparing a sample as set forth in claim 4, in which said substance A is a compound having a structure of the following formula (1),

[Chemical Formula 1]

(Formula 1)

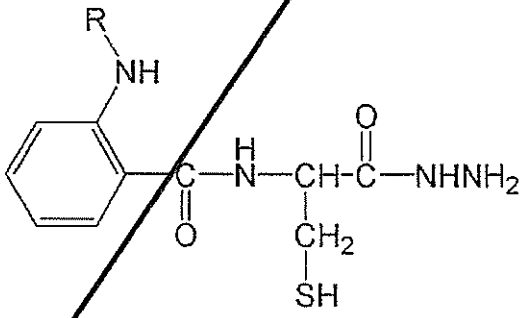


wherein, in the formula, R represents $-CH_3$ or $-CD_3$.

6. The method for preparing a sample as set forth in claim 1, in which said substance A has a structure of the following formula (2),

[Chemical Formula 2]

(Formula 2)

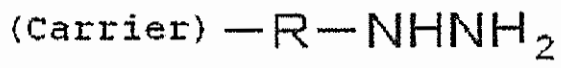


wherein, in the formula, R represents any of H, $-COCH_3$ or $-COCD_3$.

7. The method for preparing a sample as set forth in claim 1, in which said substance A is represented by the following formula (3),

[Chemical Formula 3]

(Formula 3)

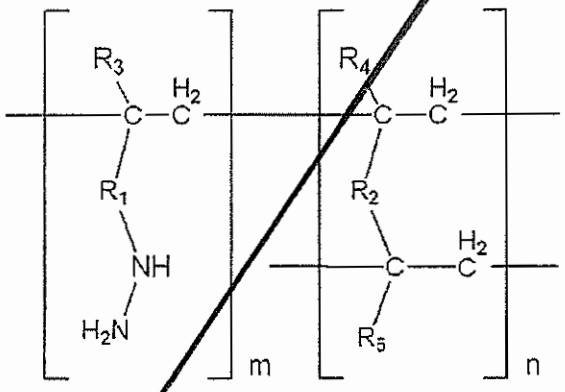


wherein, in the formula, the carrier represents a polymer matrix; and R represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-.

8. The method for preparing a sample as set forth in claim 7, in which said substance A has a crosslinked polymer structure represented by the following formula (4),

[Chemical Formula 4]

(Formula 4)

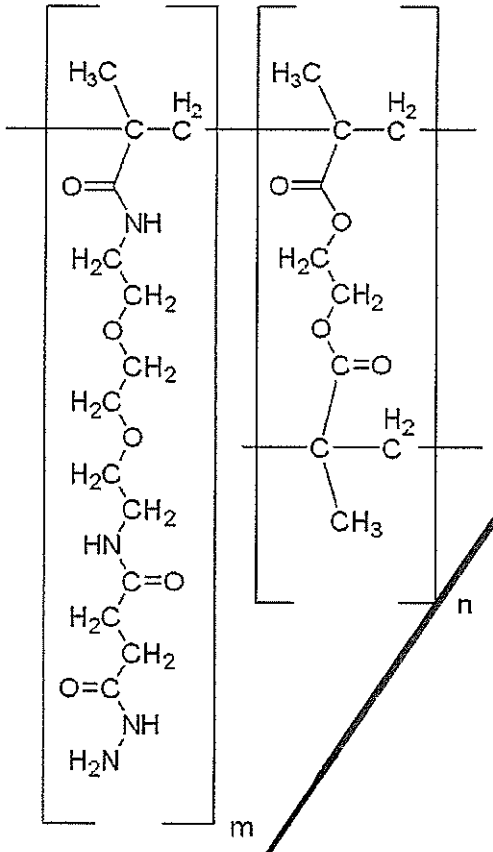


wherein, in the formula, R_1 and R_2 represent a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-; R_3, R_4 and R_5 represent H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms; and m and n represent the number of monomer units.

9. The method for preparing a sample as set forth in claim 7, in which said substance A has a crosslinked polymer structure represented by the following formula (5),

[Chemical Formula 5]

(Formula 5)



wherein, in the formula, m and n represent the number of monomer units.

10. The method for preparing a sample as set forth in any one of claims 7 to 9, in which said substance A is a polymer particle having an average particle diameter of equal to or more than 0.1 and equal to or less than 500 μm.

11. The method for preparing a sample as set forth in any one

of claims 7 to 10, in which said substance A is a polymer particle having a hydrazide group of a dry weight of not less than 100 nmol per 1 mg.

12. The method for preparing a sample as set forth in any one of claims 7 to 10, in which said substance A is a polymer particle having a hydrazide group of a dry weight of not less than 0.5 μmol per 1 mg.

13. The method for preparing a sample as set forth in any one of claims 7 to 12, in which said substance A is stable at the pH of 3 to 8.

14. The method for preparing a sample as set forth in any one of claims 7 to 12, in which said substance A is stable under pressure of at least not more than 1 MPa.

15. The method for preparing a sample as set forth in claim 7, in which the carrier in said formula (3) is a substance to be directly bonded to a solid phase substrate or a surface of the solid phase substrate.

16. A method for preparing a sample which involves the sugar chain capture step comprising binding a substance A to a sugar chain and/or a sugar derivative according to the method for preparing a sample as set forth in any one of claims 1 to 15, and

the sugar chain release step comprising the action of a substance B containing an aminooxy group or a hydrazide group

on a complex of the substance A and the sugar chain and/or the sugar derivative, captured in the sugar chain capture step, and binding said sugar chain and/or said sugar derivative while cut off from said substance A to said substance B in accordance with the hydrazone-oxime exchange reaction or hydrazone-hydrazone exchange reaction occurred between said complex and said substance B.

17. The method for preparing a sample as set forth in claim 16, in which said substance B comprises a moiety containing chromophore or fluorophore.

18. The method for preparing a sample as set forth in claim 16, in which said substance B is a substance selected from the group consisting of the following substances containing a hydrazide group or the following substances containing an aminooxy group or a salt,

(substances containing a hydrazide group) 5-Dimethylaminonaphthalene-1-sulfonyl hydrazine (Dansylhydrazine); 2-hydrazinopyridine; 9-fluorenylmethyl carbazate (Fmoc hydrazine); benzylhydrazine; 4, 4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionoc acid, hydrazide; 2-(6,8-difluoro-7-hydroxy-4-methylcoumarin)acetohydrazide; 7-diethylaminocoumarin-3-carboxylic acid, hydrazide (DCCH); phenylhydrazine; 1-Naphthaleneacethydrazide; 2-hydrazinobenzoic acid; and phenylacetic hydrazide; or

(substances containing an aminooxy group) O-benzylhydroxylamine; O-phenylhydroxylamine; O-(2,3,4,5,6-

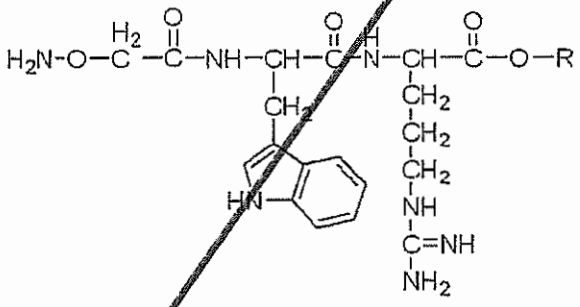
pentafluorobenzyl)hydroxylamine; O-(4-nitrobenzyl)hydroxylamine; 2-aminooxypyridine; 2-aminooxymethylpyridine; 4-[(aminooxyacetyl)amino]benzoic acid methyl ester; 4-[(aminooxyacetyl)amino]benzoic acid ethyl ester; and 4-[(aminooxyacetyl)amino]benzoic acid n-butyl ester.

19. The method for preparing a sample as set forth in claim 16, in which said substance B comprises a moiety consisting of at least one of an arginine residue, a tryptophan residue, a phenylalanine residue, a tyrosine residue, a cysteine residue and a derivative thereof.

20. The method for preparing a sample as set forth in claim 16, in which said substance B has a structure represented by the following formula (7),

[Chemical Formula 6]

(Formula 7)



wherein, in the structural formula, R represents $-CH_3$ or $-CD_3$.

21. The method for preparing a sample as set forth in claim 16, in which said substance B is a solid phase carrier.

22. The method for preparing a sample as set forth in claim 21, in which said substance B is a solid phase carrier containing an aminooxy group.

23. The method for preparing a sample as set forth in claim 22, in which said substance B is represented by the following formula (12),

[Chemical Formula 7]

(Formula 12)

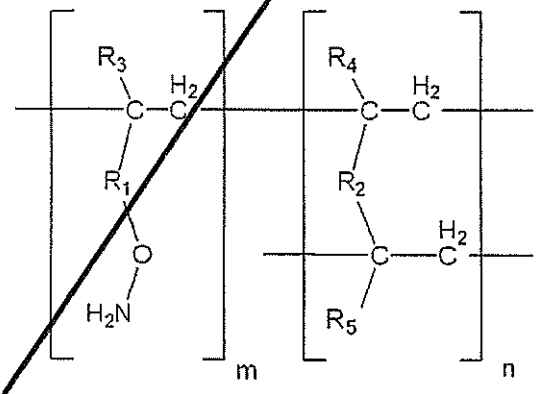


wherein, in the formula, the carrier represents a polymer matrix; and R represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-.

24. The method for preparing a sample as set forth in claim 23, in which said substance B is a polymer particle having a structure represented by the following formula (8),

[Chemical Formula 8]

(Formula 8)



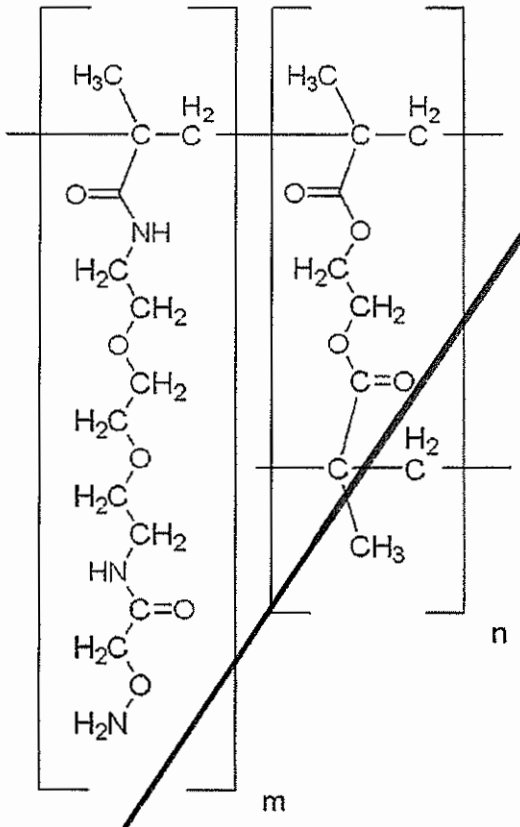
wherein, in the formula, R_1 and R_2 represent a hydrocarbon

chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-; R_3, R_4 and R_5 represent H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms; and m and n represent the number of monomer units.

25. The method for preparing a sample as set forth in claim 23, in which the substance B is a polymer particle having a structure represented by the following formula (9),

[Chemical Formula 9]

(Formula 9)



wherein, in the formula, m and n represent the number of monomer units.

26. The method for preparing a sample as set forth in claim 21, in which said solid phase carrier is a substance to be

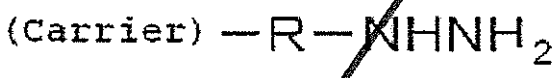
directly bonded to a solid phase substrate or a surface of the solid phase substrate.

27. The method for preparing a sample as set forth in any one of claims 16 to 25, in which the hydrazone-oxime exchange reaction or the hydrazone-hydrazone exchange reaction is carried out at least one or more times after the completion of said sugar chain release step.

28. A method for preparing a sample in which a hydrazone bond is dissociated and a sugar chain and/or a sugar derivative is released by treating a substance A having a structure represented by the following formula (3), (4) or (5) under acidic conditions to which the sugar chain and/or the sugar derivative is bonded,

[Chemical Formula 10]

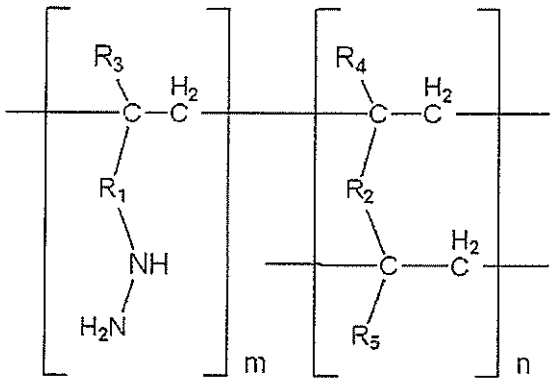
(Formula 3)



wherein, in the formula, the carrier represents a polymer matrix; and R represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-,

[Chemical Formula 11]

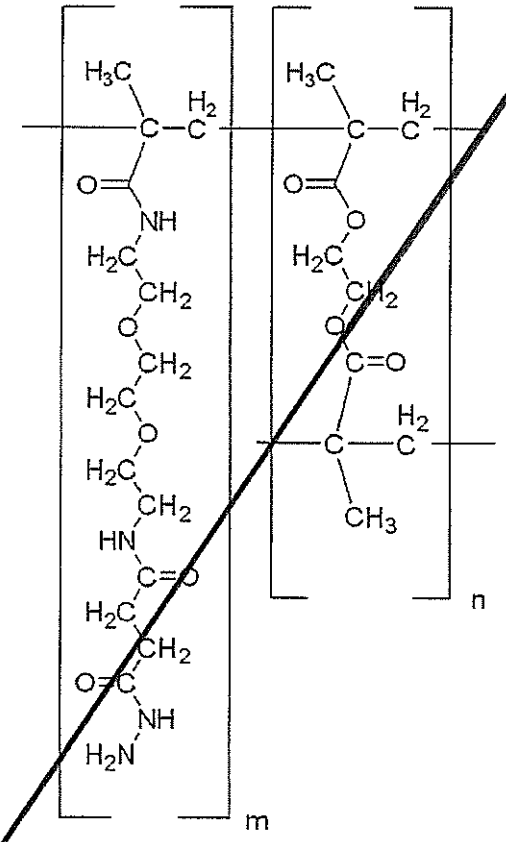
(Formula 4)



wherein, in the formula, R_1 and R_2 represent a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-; R_3, R_4 and R_5 represent H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms; and m and n represent the number of monomer units,

[Chemical Formula 12]

(Formula 5)



wherein, in the formula, m and n represent the number of

monomer units.

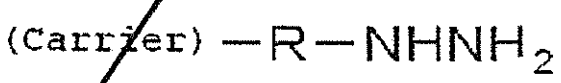
29. The method for preparing a sample as set forth in claim 28, in which the treatment under acidic conditions is carried out using a trifluoroacetic acid solution of 0.01 to 10 volume percentage.

30. The method for preparing a sample as set forth in claim 29, in which the treatment under acidic conditions is carried out using a trifluoroacetic acid solution of 0.01 to 1 volume percentage at 25 to 80 degree centigrade for 5 to 60 minutes.

31. A method for preparing a substance A represented by the following formula (3), in which a polymer particle containing carboxylic acid ester is obtained by polymerizing a raw material containing a carboxylic acid ester monomer having a polymerizable group in the presence of a crosslinking agent, and then the polymer particle containing carboxylic acid ester is treated with a hydrazine solution of a concentration of not less than 10 volume percentage,

[Chemical Formula 13]

(Formula 3)



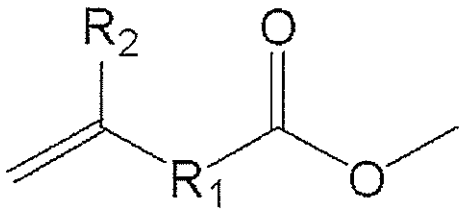
wherein, in the formula, the carrier represents a polymer matrix; and R represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-.

32. The method for preparing a substance A as set forth in claim 31, in which said carboxylic acid ester monomer is carboxylic acid methyl ester.

33. A monomer having a structure represented by the following formula (10),

[Chemical Formula 14]

(Formula 10)



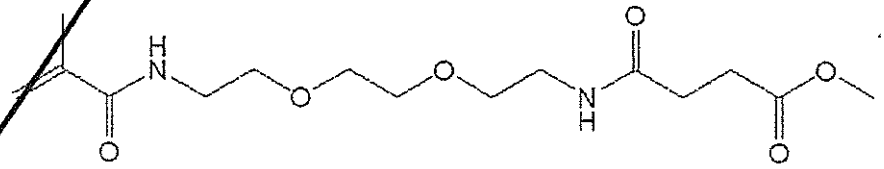
wherein, in the formula, R_1 represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-; and R_2 represents H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms.

34. A polymer obtained by polymerizing the monomer as set forth in claim 33.

35. A monomer having a structure represented by the following formula (11),

[Chemical Formula 15]

(Formula 11)

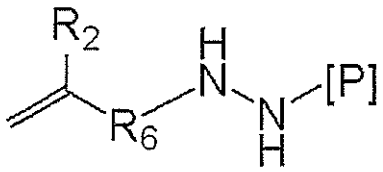


36. A polymer obtained by polymerizing the monomer as set forth in claim 35.

37. A monomer having a structure represented by the following formula (13),

[Chemical Formula 16]

(Formula 13)



wherein, in the formula, R_2 represents H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms; R_6 represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-; and [P] represents a protective group.

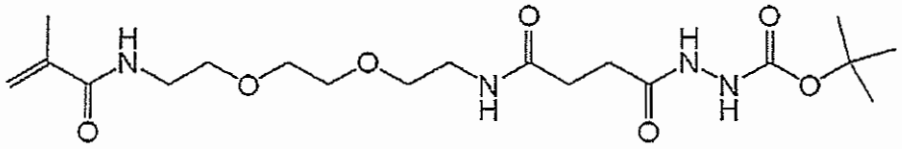
38. A polymer obtained by polymerizing the monomer as set forth in claim 37.

39. The polymer as set forth in claim 38, obtained by subjecting said protective group [P] of the formula (13) to deprotection by the acid treatment.

40. A monomer having a structure represented by the following formula (14),

[Chemical Formula 17]

(Formula 14)



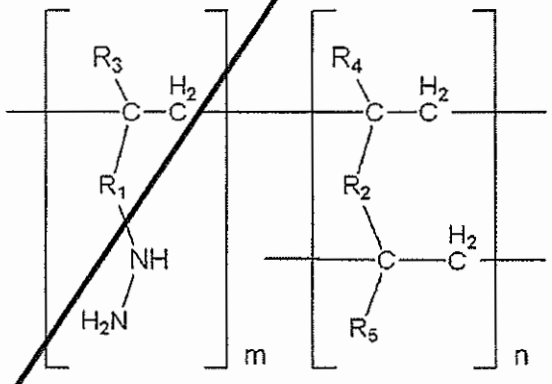
41. A polymer obtained by polymerizing the monomer as set forth in claim 40.

42. A polymer obtained by subjecting a t-butoxycarbonyl group of the polymer as set forth in claim 41 to deprotection by the acid treatment.

43. A method for preparing a substance A represented by the following formula (4), in which a polymer particle containing carboxylic acid ester is obtained by polymerizing a monomer having a structure of the following formula (10) in the presence of a crosslinking agent, and then the polymer particle containing carboxylic acid ester is treated with a hydrazine solution of a concentration of not less than 10 volume percentage,

[Chemical Formula 18]

(Formula 4)

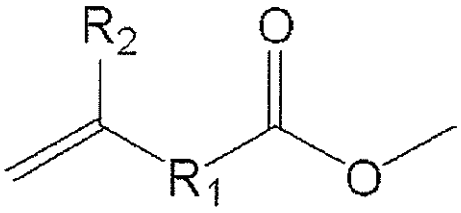


wherein, in the formula, R_1 and R_2 represent a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted

with -O-, -S-, -NH-, -CO- or -CONH-; R_3, R_4 and R_5 represent H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms; and m and n represent the number of monomer units,

[Chemical Formula 19]

(Formula 10)

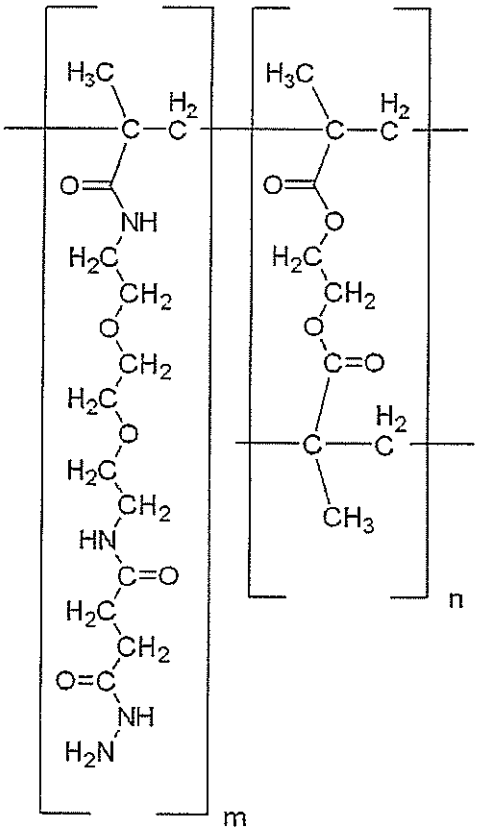


wherein, in the formula, R_1 represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-; and R_2 represents H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms.

44. A method for preparing a substance A represented by the following formula (5), in which a polymer particle containing carboxylic acid ester is obtained by polymerizing a monomer having a structure of the following formula (11) in the presence of a crosslinking agent, and then the polymer particle containing carboxylic acid ester is treated with a hydrazine solution of a concentration of not less than 10 volume percentage,

[Chemical Formula 20]

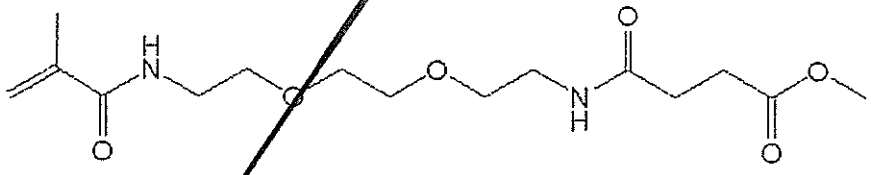
(Formula 5)



wherein, in the formula, m and n represent the number of monomer units,

[Chemical Formula 21]

(Formula 11)



45. A method of applying a solid phase carrier onto which a sugar chain and/or a sugar derivative obtained by subjecting a mixture or a specific fraction from the mixture to the isolation and purification is immobilized as a carrier for collecting a substance having a bonding property or affinity to the sugar chain and/or the sugar derivative.

46. A method for preparing a sugar chain microarray, in which a sugar chain and/or a sugar derivative is immobilized onto a surface of a solid phase substrate composed of a solid phase carrier comprising a substance B having a structure represented by the following formula (3) or (12) according to the following steps (1) to (4),

(step 1) a step of purifying and/or isolating the sugar chain and/or the sugar derivative by a specific separation means by binding the sugar chain and/or the sugar derivative to a compound containing a soluble hydrazide group,

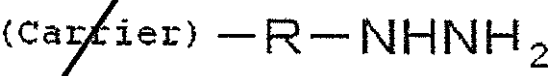
(step 2) a step of dispensing drops of a solution of the compound obtained in the step (1) in a row onto said solid phase substrate,

(step 3) a step of proceeding a reaction of exchanging a sugar chain-substance A bond with a solid phase substrate-sugar chain bond by incubating the solid phase substrate after the completion of dispensing drops under prescribed conditions, and immobilizing the sugar chain and/or the sugar derivative onto the solid phase substrate, and

(step 4) a step of washing and removing the unreacted substance on the solid phase substrate,

[Chemical Formula 22]

(Formula 3)

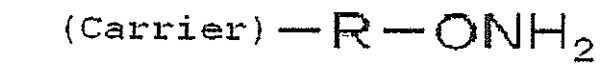


wherein, in the formula, the carrier represents a polymer matrix; and R represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -

CO- or -CONH-,

[Chemical Formula 23]

(Formula 12)



wherein, in the formula, the carrier represents a polymer matrix; and R represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-.

47. A sugar chain microarray produced by the method as set forth in claim 46.

48. A system for searching a sugar-binding substance for singularly binding to or adsorbing the immobilized sugar chain and/or the immobilized sugar derivative by bringing a solution containing a specimen into contact with a surface of the sugar chain mircoarray as set forth in claim 47, incubating and washing under prescribed conditions, and then detecting the sugar-binding substance collected at the sugar chain and/or the sugar derivative of a drop-dispensed portion on the solid phase substrate.

49. A system for evaluating the recognition specificity of a binding protein by bringing a solution containing a specimen into contact with a surface of the sugar chain mircoarray as set forth in claim 47, collecting a sugar binding protein in said specimen at the sugar chain and/or the sugar derivative of a drop-dispersed region on the solid phase substrate, and

quantitatively analyzing the collected amount by a prescribed quantifying means.

50. A method for preparing sugar chain affinity beads, in which a sugar chain and/or a sugar derivative is immobilized onto a surface of the polymer composed of a solid phase carrier comprising a substance B having a structure represented by the following formula (3) or (12),

[Chemical Formula 24]

(Formula 3)



wherein, in the formula, the carrier represents a polymer matrix; and R represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-,

[Chemical Formula 25]

(Formula 12)



wherein, in the formula, the carrier represents a polymer matrix; and R represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-.

51. The method for preparing sugar chain affinity beads as set forth in claim 50, in which the sugar chain and/or the sugar derivative is immobilized onto a surface of said polymer particle according to the following steps (1) to (3),

(step 1) a step of purifying and/or isolating the sugar chain and/or the sugar derivative by a specific separation means by binding the sugar chain and/or the sugar derivative to a compound having a soluble hydrazide group,

(step 2) a step of immobilizing the sugar chain and/or the sugar derivative onto the polymer particle by bringing the compound obtained in the step (1) into contact with said polymer particle and incubating under prescribed conditions for exchanging a sugar chain-hydrazide group-containing compound bond with a sugar chain-polymer particle bond, and

(step 3) a step of washing and removing the unreacted substance on the polymer particle.

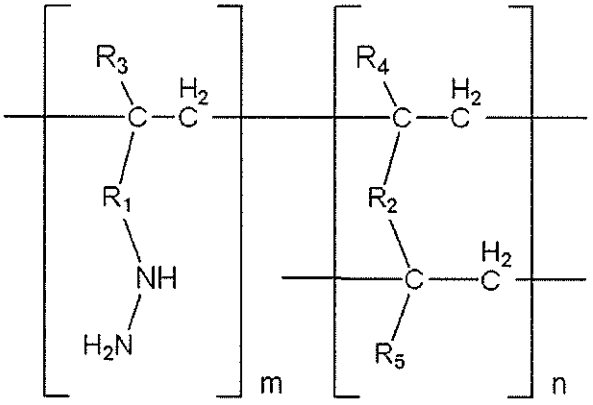
52. Sugar chain affinity beads prepared by the method as set forth in claim 50 or 51.

53. A system for bringing a solution containing a specimen into contact with the sugar chain affinity beads as set forth in claim 52, incubating under prescribed conditions and washing, and then isolating the captured sugar-binding substance.

CLAIMS

1. A method for preparing a sample in which a substance A containing a hydrazine group and having a crosslinked polymer structure represented by the following formula (4) is bonded to a sugar chain and/or a sugar derivative via hydrazone formation between the hydrazine group of the substance A and the reducing end of the sugar chain and/or the sugar derivative,

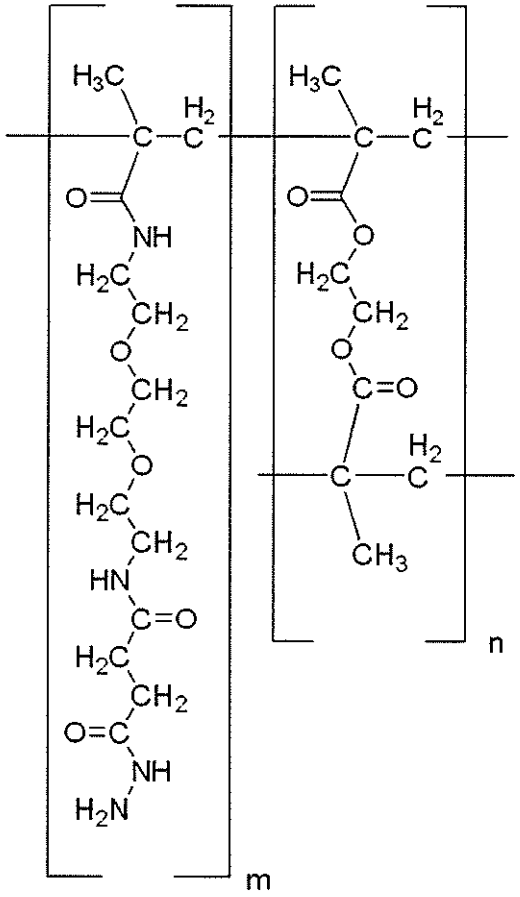
(Formula 4)



wherein, in the formula, R_1 and R_2 represent a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-; R_3, R_4 and R_5 represent H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms; and m and n represent the number of monomer units.

2. The method for preparing a sample as set forth in claim 1, in which said substance A has a crosslinked polymer structure represented by the following formula (5),

(Formula 5)



wherein, in the formula, m and n represent the number of monomer units.

3. The method for preparing a sample as set forth in claims 1 or 2, in which said substance A is a polymer particle having an average particle diameter of equal to or more than 0.1 and equal to or less than 500 μm.

4. The method for preparing a sample as set forth in any one of claims 1 to 3, in which said substance A is a polymer particle having a hydrazide group of a dry weight of not less than 100 nmol per 1 mg.

5. The method for preparing a sample as set forth in any one of claims 1 to 4, in which said substance A is stable at the pH of 3 to 8.

6. The method for preparing a sample as set forth in any one of claims 1 to 4, in which said substance A is stable under pressure of at least not more than 1 MPa.

7. A method for preparing a sample which involves the sugar chain capture step comprising binding a substance A to a sugar chain and/or a sugar derivative according to the method for preparing a sample as set forth in any one of claims 1 to 6, and

the sugar chain release step comprising the action of a substance B containing an aminooxy group or a hydrazine group on a complex of the substance A and the sugar chain and/or the sugar derivative, captured in the sugar chain capture step, and binding said sugar chain and/or said sugar derivative while cut off from said substance A to said substance B in accordance with the hydrazone-oxime exchange reaction or hydrazone-hydrazone exchange reaction occurred between said complex and said substance B.

8. The method for preparing a sample as set forth in claim 7, in which said substance B comprises a moiety containing chromophore or fluorophore.

9. The method for preparing a sample as set forth in claim 7,

in which said substance B is a substance selected from the group consisting of the following substances containing a hydrazine group or the following substances containing an aminooxy group or a salt,

(substances containing a hydrazine group) 5-Dimethylaminonaphthalene-1-sulfonyl hydrazine (Dansylhydrazine); 2-hydrazinopyridine; 9-fluorenylmethyl carbazate (Fmoc hydrazine); benzylhydrazine; 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionoc acid, hydrazide; 2-(6,8-difluoro-7-hydroxy-4-methylcoumarin)acetohydrazide; 7-diethylaminocoumarin-3-carboxylic acid, hydrazide (DCCH); phenylhydrazine; 1-Naphthaleneacethydrazide; 2-hydrazinobenzoic acid; and phenylacetic hydrazide; or

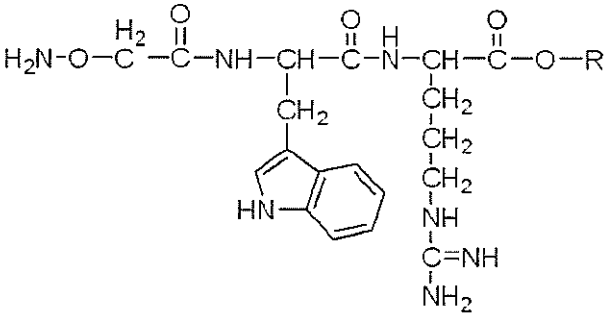
(substances containing an aminooxy group) O-benzylhydroxylamine; O-phenylhydroxylamine; O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine; O-(4-nitrobenzyl)hydroxylamine; 2-aminooxypyridine; 2-aminooxymethylpyridine; 4-[(aminooxyacetyl)amino]benzoic acid methyl ester; 4-[(aminooxyacetyl)amino]benzoic acid ethyl ester; and 4-[(aminooxyacetyl)amino]benzoic acid n-butyl ester.

10. The method for preparing a sample as set forth in claim 7, in which said substance B comprises a moiety consisting of at least one of an arginine residue, a tryptophan residue, a phenylalanine residue, a tyrosine residue, a cysteine residue

and a derivative thereof.

11. The method for preparing a sample as set forth in claim 7, in which said substance B has a structure represented by the following formula (7),

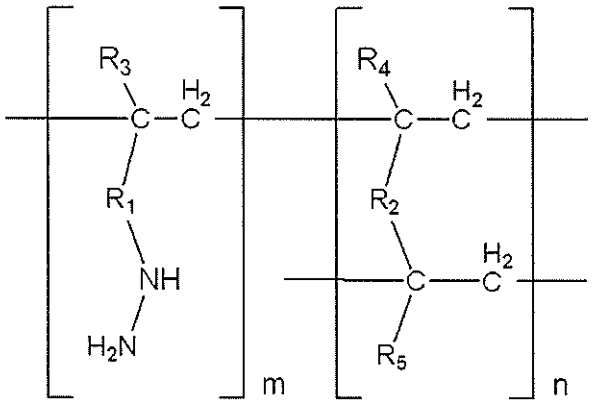
(Formula 7)



wherein, in the structural formula, R represents $-CH_3$ or $-CD_3$.

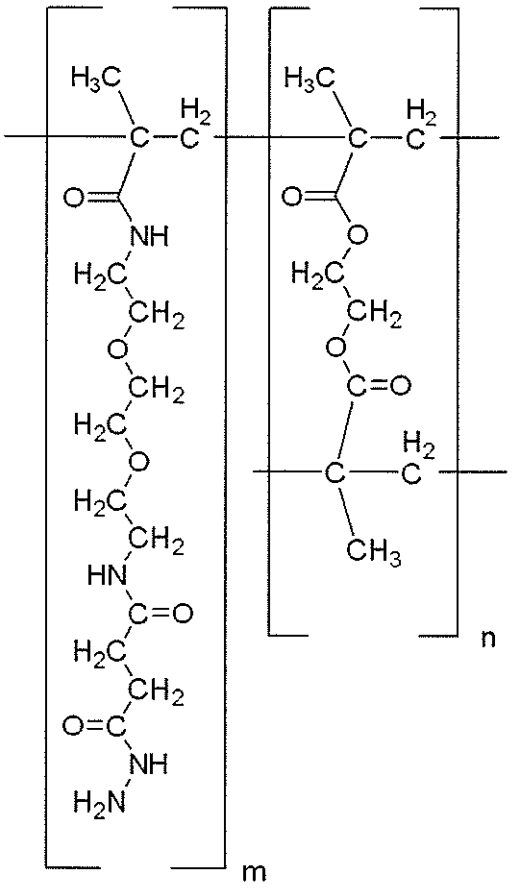
12. A method for preparing a sample in which a hydrazone bond is dissociated and a sugar chain and/or a sugar derivative is released by treating a substance A having a structure represented by the following formula (4) or (5) under acidic conditions to which the sugar chain and/or the sugar derivative is bonded,

(Formula 4)



wherein, in the formula, R_1 and R_2 represent a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-; R_3, R_4 and R_5 represent H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms; and m and n represent the number of monomer units,

(Formula 5)



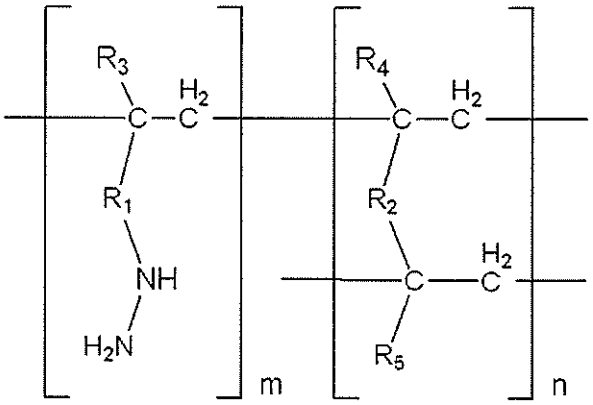
wherein, in the formula, m and n represent the number of monomer units.

13. An analytical sample prepared by the method for preparing a sample as set forth in any one of claims 1 to 12.

14. A method for preparing a substance A represented by the

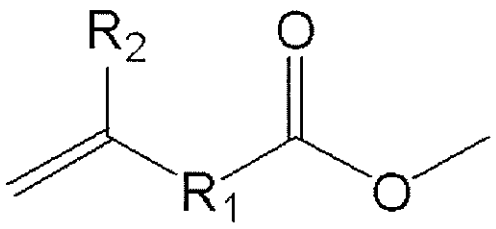
following formula (4), in which a polymer particle containing carboxylic acid ester is obtained by polymerizing a monomer having a structure of the following formula (10) in the presence of a crosslinking agent, and then the polymer particle containing carboxylic acid ester is treated with a hydrazine solution of a concentration of not less than 10 volume percentage,

(Formula 4)



wherein, in the formula, R_1 and R_2 represent a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-, -S-, -NH-, -CO- or -CONH-; R_3, R_4 and R_5 represent H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms; and m and n represent the number of monomer units,

(Formula 10)

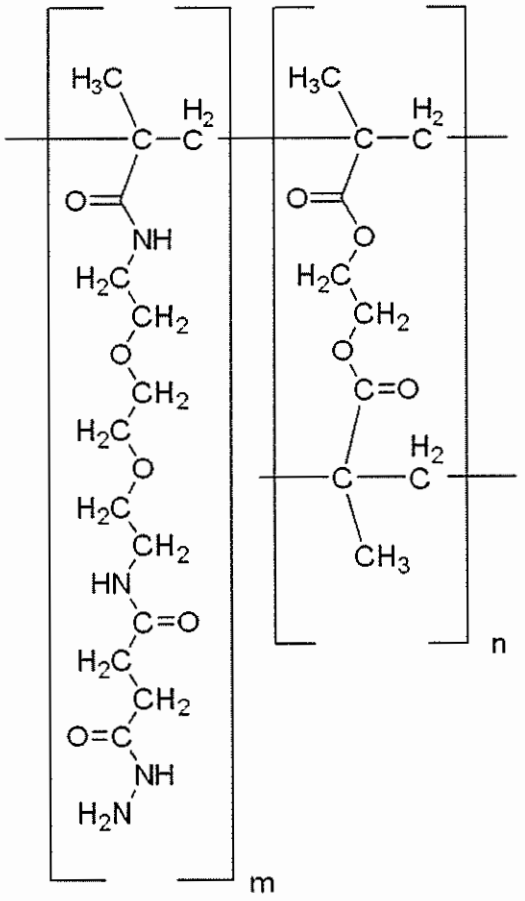


wherein, in the formula, R_1 represents a hydrocarbon chain having 1 to 20 carbon atoms which may be interrupted with -O-,

-S-, -NH-, -CO- or -CONH-; and R_2 represents H, CH_3 or a hydrocarbon chain having 2 to 5 carbon atoms.

15. A method for preparing a substance A represented by the following formula (5), in which a polymer particle containing carboxylic acid ester is obtained by polymerizing a monomer having a structure of the following formula (11) in the presence of a crosslinking agent, and then the polymer particle containing carboxylic acid ester is treated with a hydrazine solution of a concentration of not less than 10 volume percentage,

(Formula 5)



wherein, in the formula, m and n represent the number of monomer units.

(Formula 11)

