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(54) **CUCURBITURIL DERIVATIVE-BONDED SOLID SUBSTRATE AND BIOCHIP USING THE SAME**
CUCURBITURILDERIVAT-GEBUNDENES FESTSTOFFSUBSTRAT UND BIOCHIP UNTER
VERWENDUNG DAVON
SUBSTRAT SOLIDE LIE AU DERIVE DE CUCURBITURILE ET PUCE BIOLOGIQUE UTILISANT CE
SUBSTRAT

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| <ul style="list-style-type: none"> • LESAICHERRE MARIE-LAURE ET AL: "Intein-mediated biotinylation of proteins and its application in a protein microarray" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 124, no. 30, 31 July 2002 (2002-07-31), pages 8768-8769, XP002475329 ISSN: 0002-7863 • JON SANG YONG ET AL: "Facile synthesis of cucurbit[n]uril derivatives via direct functionalization: expanding utilization of cucurbit[n]uril." JOURNAL OF THE AMERICAN CHEMICAL SOCIETY 27 AUG 2003, vol. 125, no. 34, 27 August 2003 (2003-08-27), pages 10186-10187, XP002475330 ISSN: 0002-7863 | <ul style="list-style-type: none"> • ANGEW. CHEM. INT. ED. vol. 29, no. 23, 2000, pages 4257 - 4260, XP008064000 • BIOCONJU. CHEM. vol. 13, no. 6, 2002, pages 1181 - 1185, XP001171923 • CHEM. SOC. REV. vol. 31, no. 2, 2002, pages 96 - 107, XP008064008 • CHEMISTRY vol. 8, no. 2, 2002, pages 498 - 508, XP008064007 |
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Description

TECHNICAL FIELD

[0001] The present invention relates to a cucurbituril derivative-bonded solid substrate, and more particularly, to a solid substrate covalently bonded with a cucurbituril derivative which can immobilize a biomaterial by a non-covalent interaction, and its applications.

BACKGROUND ART

[0002] After the human genome sequence was drafted in 2000, gene expression could be understood at mRNA level. Thereafter, developments of personalized medicines or diagnostic reagents based on Individual genome information have been anticipated. Therefore, there has arisen a need to rapidly trace the expression levels of a large number of genes. In this regard, there was developed a DNA chip capable of simultaneously performing assays of a thousand to ten thousand genes. However, a gene assay alone cannot provide information about proteins which are not only gene products but also biomaterials essential for biological activity. Therefore, there has been suggested a protein chip, a corresponding concept of a DNA chip, which can perform simultaneous assays of a large number of proteins.

[0003] Reference will now be made to FIGS. 1 and 2 of the accompanying diagrammatic drawings, in which FIG. 1 illustrates a protein chip; FIG. 2 illustrates degeneration of a protein; and FIG. 3 illustrates a cucurbituril derivative-covalent bonded solid substrate .

[0004] The concept of the protein chip is based on the protein microarrays which contain chemically or biochemically treated surfaces for specific interaction with proteins of interest. As illustrated in FIG. 1, in which 1 is a functional group for linkage with a solid substrate, 2 is a molecular body, 3 is a functional group for linkage with a biomaterial, and 4 is the solid substrate, a protein chip can be made as follows. First, a thin film is formed on a solid substrate 4 using compounds with functional groups 1 for linkages with the solid substrate 4 and another functional groups 3 for linkages with biomaterials such as proteins. Then, biomaterials such as proteins can be immobilized on the solid substrate 4 via chemical or physical interactions between biomaterial and the terminal functional group 3. FIG. 1 illustrates the solid substrate 4 having a thin film made of compounds with the functional groups 1 for linkages with the solid substrate 4 and the functional groups 3 for linkages with biomaterials such as proteins.

[0005] Hitherto, many researchers have used covalent bonds between the functional groups 3 of FIG. 1 and proteins to immobilize the proteins on a solid substrate. When covalent bonds between the functional groups 3 of FIG. 1 and proteins are formed, the proteins can be immobilized on the surface of a solid substrate.

[0006] However, it is well-known that their specificities or activities toward substrates are seriously affected by the immobilization method, because the specificity and activity are strongly related to their specific three-dimensional structures and orientation of their active sites, referring to the following FIG. 2, in which 1 is a solid substrate, 5 is a linkage layer, 6 is a protein with a damaged active site, and 7 is a protein with a preserved active site. Therefore, the three-dimensional structures of proteins may be damaged when the proteins are covalently bonded to a solid substrate, thereby causing degeneration of the proteins, like a protein 6 of FIG. 2. This is because the function of proteins is dependent on their specific three-dimensional structures formed by chains of amino acids constituting the proteins. To maintain the function of a protein chip, like a protein 7 of FIG. 2, an active site must not be bonded to the linkage layer 5 to preserve the functionality of the active site.

[0007] To solve this problem, many methods have been developed for immobilizing proteins to a surface of a solid substrate via non-covalent bonds.

[0008] By way of an example, a study about the attachment of proteins to a solid substrate by a coordination bond was reported. Paborsky et al. suggested a coordination linkage between proteins fused with histidine, which is an amino acid known to well bind with Ni, Cu, etc., and a surface of a solid substrate to which Ni is attached by nitrilotriacetic acid (NTA) [Paborsky, L. R.; Dunn, K. E.; Gibbs, C. S.; and Dougherty, J. P., Anal. Biochem. 1996, 234, pp. 60-65].

[0009] Frey et al. reported the attachment of an intermediate, such as polylysine, capable of ionically binding with proteins, to a solid substrate, to immobilize the proteins on the solid substrate [Frey, Brian L.; Jordan, Claire E.; Kornguth, Steven; and Corn, Robert M., Anal. Chem. 1995, 67, 4452-4457].

[0010] Recently, Tae-Sun Kim et al. reported a hydrogen bond between proteins and a solid substrate having crown ether derivatives, paying attention to a hydrogen bond between ammonium groups abundantly present at non-active sites of proteins and crown ether groups (Korean Patent Application Nos. 10-1999-0061074 and 10-2000-0038491).

[0011] However, the bond strength of most non-covalent bonds is much less than that of covalent bonds. Therefore, proteins having non-covalent bonds with a solid substrate may be detached from the solid substrate when contact with chemical materials used in immunoassay. In this regard, many attempts have been made to immobilize proteins on a solid substrate via stronger non-covalent bonds.

[0012] Recently, Yao and co-workers reported a solid substrate for a protein chip in which avidin, a type of protein, is

immobilized on the solid substrate via a covalent bond [Lesaichere, M.-L.; Lue, R. Y. P.; Chen, G. Y. J.; Zhu, Q.; and Yao, S. Q. J., Am. Chem. Soc. 2002, 124, 8768]. Avidin is known to bind with four small molecules of biotin by a coupling constant of about 10^{15} M^{-1} , which is the strongest non-covalent bond among currently known non-covalent bonds [Wilchek, M.; Bayer, E. A. Avidin-Biotin Technology. In Methods in Enzymology 1990, 184]. According to the report by Yao et al., probe proteins are fused with biotin and then are immobilized on a solid substrate treated with avidin. Reportedly, the probe proteins are not detached from the solid substrate even under a very severe environment. However, this method has an economical limitation of avidin being costly, even though there is an advantage in that a coupling constant of avidin-biotin interaction is very large.

[0013] Therefore, a cost effective method for immobilizing proteins to a solid substrate using a non-covalent bond with strong interaction is required.

[0014] WO 2004/072151 (Article 54(3) EPC document) discloses a cucurbituril-bonded silica gel useful for removal of air pollutants or water contaminants, and separation and purification of biological, organic, inorganic, or ionic substances.

[0015] WO 2003/004500 discloses cucurbituril derivatives of specific formulations, having enhanced solubility in common solvents.

DETAILED DESCRIPTION OF THE INVENTION

Technical Goal of the Invention

[0016] The present invention provides a cucurbituril derivative-bonded solid substrate in which a cucurbituril derivative is covalently bonded to a modified solid substrate.

[0017] The present invention also provides a protein chip using the cucurbituril derivative-bonded solid substrate.

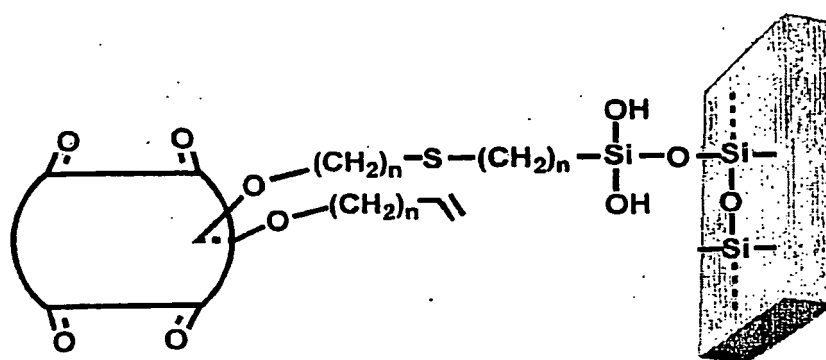
[0018] The present invention also provides a gene chip using the cucurbituril derivative-bonded solid substrate.

[0019] The present invention also provides a sensor for biomaterial assay using the cucurbituril derivative-bonded solid substrate.

Disclosure of the Invention

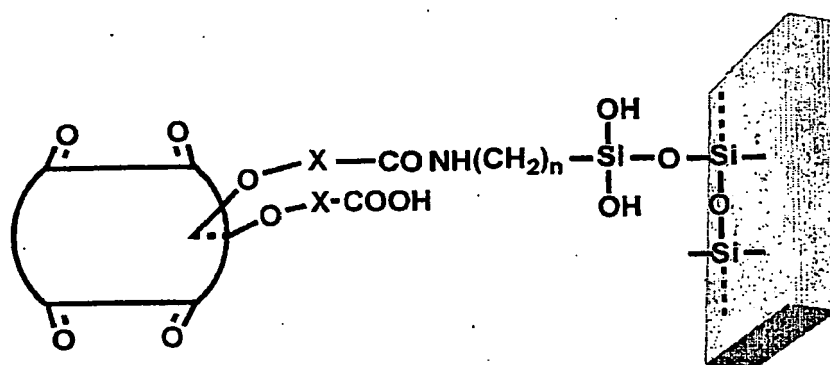
[0020] According to an aspect of the present invention, there is provided a cucurbituril derivative-bonded solid substrate wherein the substrate is a glass, a silicon wafer, an indium tin oxide (ITO) glass, an aluminum oxide substrate, or a titanium dioxide substrate and is selected from the group consisting of substrates represented by Formulae 3 through 6:

<Formula 3>



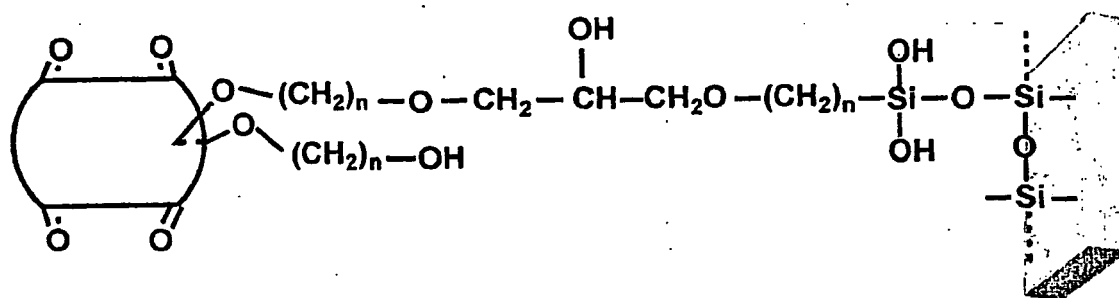
wherein each n is independently an integer of 1 to 20;

<Formula 4>



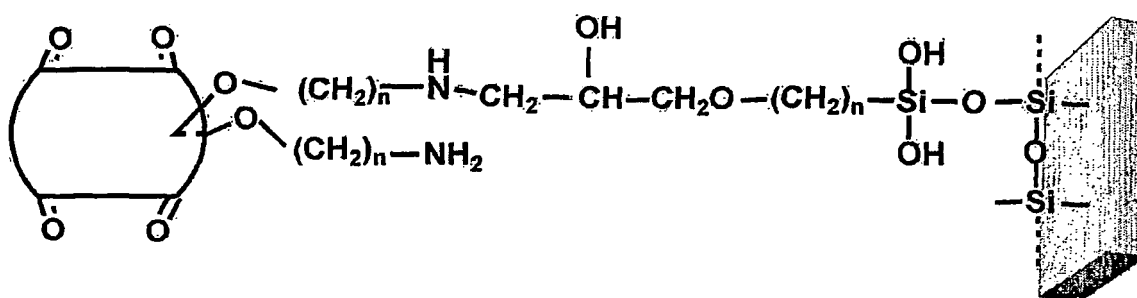
wherein n is an integer of 1 to 20 and X is a dialkylsulfide group with a substituted or unsubstituted alkyl moiety of C₁-C₂₀ or a substituted or unsubstituted alkyl group of C₁-C₂₀;

<Formula 5>



wherein n is an integer of 1 to 20; and

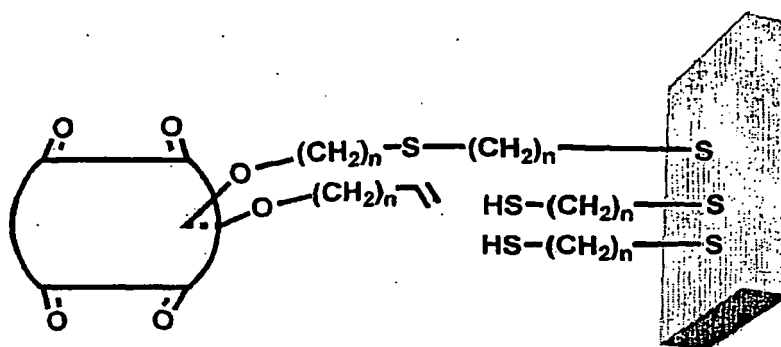
<Formula 6>



wherein n is an integer of 1 to 20.

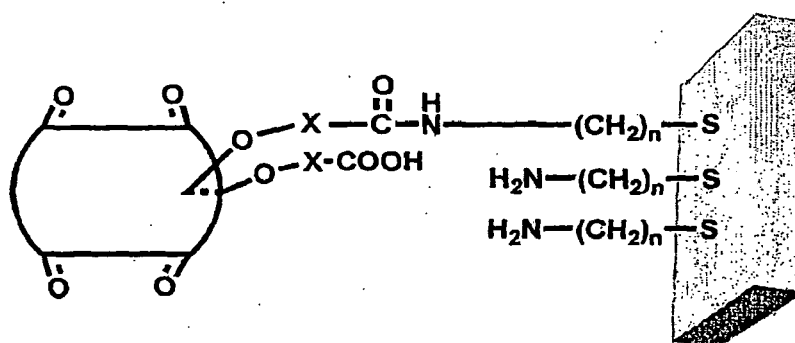
[0021] According to another aspect of the present invention, a cucurbituril derivative-bonded solid substrate is selected from the group consisting of substrates represented by Formulae 8 through 11:

<Formula 8>



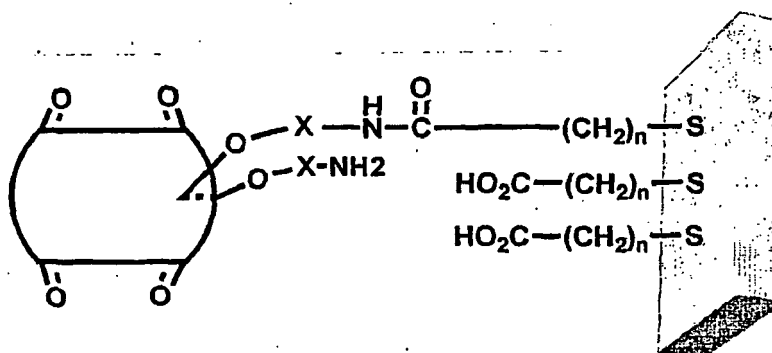
wherein each n is independently an integer of 1 to 20;

<Formula 9>



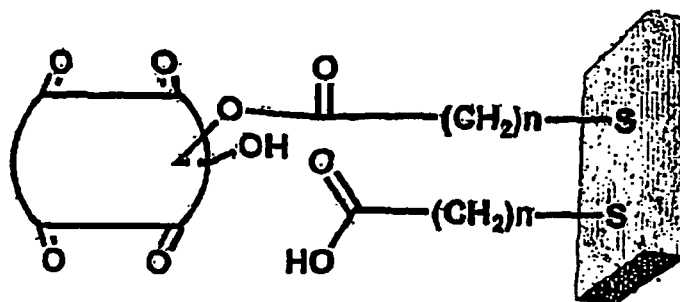
wherein each n is independently an integer of 1 to 20 and X is a dialkylsulfide group with a substituted or unsubstituted alkyl moiety of C_1-C_{20} or a substituted or unsubstituted alkyl group of C_1-C_{20} ;

<Formula 10>



wherein each n is independently an integer of 1 to 20 and X is a dialkylsulfide group with a substituted or unsubstituted alkyl moiety of C_1-C_{20} or a substituted or unsubstituted alkyl group of C_1-C_{20} ; and

<Formula 11>



wherein each n is independently an integer of 1 to 20.

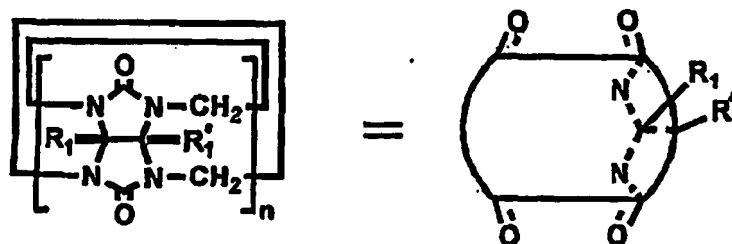
[0022] The solid substrate may be a substrate made of gold, silver, platinum, or copper.

[0023] The invention extends to a protein chip, a gene chip and a sensor for biomaterial assay, all including a cucurbituril derivative-bonded solid substrate according to any of the preceding aspects of the invention.

[0024] Hereinafter, examples of the present invention will be described.

[0025] A cucurbituril derivative represented by Formula 1 below has a functional group suitable for a covalent linkage with a solid substrate:

<Formula 1>

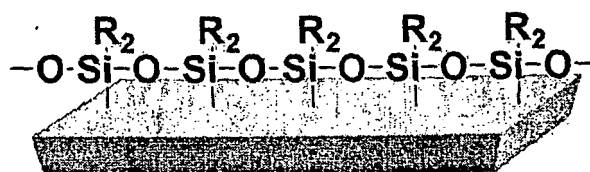


wherein n is an integer of 4 to 20, and R_1 and R_1' are each independently an alkenyloxy group with an unsaturated bond end and a substituted or unsubstituted alkyl moiety of C_1-C_{20} , a carboxyalkylsulfinyloxy group with a substituted or unsubstituted alkyl moiety of C_1-C_{20} , a carboxyalkyloxy group with a substituted or unsubstituted alkyl moiety of C_2-C_8 , an aminoalkyloxy group with a substituted or unsubstituted alkyl moiety of C_2-C_8 , or a hydroxyalkyloxy group with a substituted or unsubstituted alkyl moiety of C_2-C_8 .

[0026] The solid substrate may be a glass, a silicon wafer, an indium tin oxide (ITO) glass, an aluminum oxide substrate, or a titanium dioxide substrate. Examples of hydroxycucurbituril and its mother cucurbituril used as a synthetic material for the compound of Formula 1 above are disclosed together with their structural formulae and synthetic methods in Korean Patent Application Nos. 02-68362, 02-318, 01-57573, 01-39756, and 00-33028.

[0027] The cucurbituril derivative of Formula 1 is covalently bonded to a codified solid substrate with various end functional groups to form a desired solid substrate. For this, a modified solid substrate of Formula 2 below may be used:

<Formula 2>



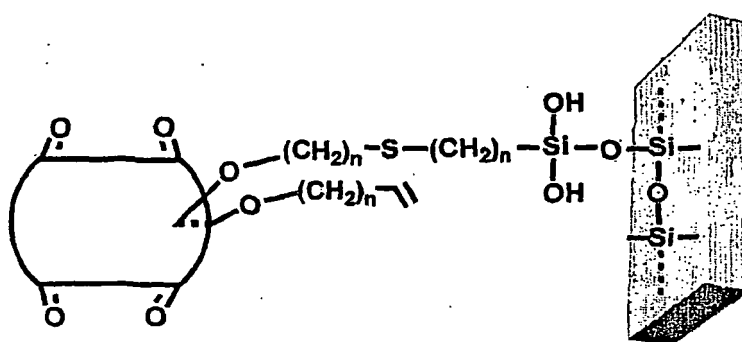
wherein R_2 is an alkyl group of C_1 - C_{10} with an end functional group selected from thiol, amine, epoxy, isocyan, and isothiocyan.

[0028] For example, the modified substrate of Formula 2 may be prepared by reacting a silane having an end functional group, such as thiol, amino, and epoxy, with a metal oxide substrate containing a -OH surface group by washing.

[0029] A cucurbituril-bonded solid substrate can be prepared by covalently bonding the cucurbituril derivative of Formula 1 with the modified solid substrate of Formula 2. That is, the cucurbituril derivative of Formula 1 is covalently bonded to the modified solid substrate of Formula 2 by reacting end functional groups of the cucurbituril derivative, such as a carboxyl group, an amine group, a hydroxyl group, or an allyl group, with end functional groups of the modified solid substrate, such as an amine group, an epoxy group, or a thiol group.

[0030] Examples of the solid substrate thus prepared are presented in the following Formulae 3 through 6 and their preparation methods will now be described.

<Formula 3>



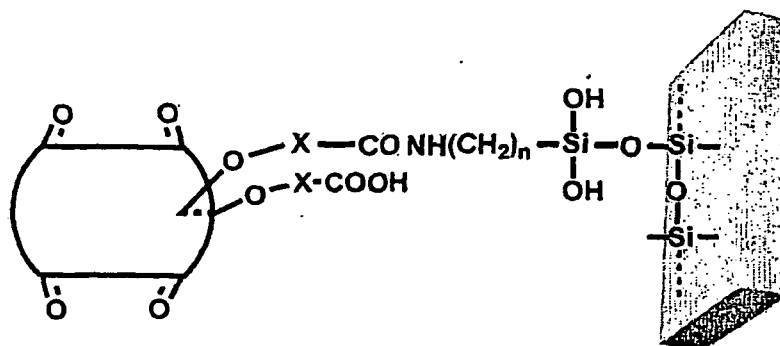
wherein each n is independently an integer of 1 to 20.

[0031] A substrate of Formula 3 may be obtained by sulfido-bond formation between a cucurbituril derivative and a solid substrate, in detail, by radical reaction between a thiol-modified metal oxide substrate and alkenyloxycucurbituril.

[0032] In more detail, the preparation of the substrate of formula 3 by radical reaction includes, but is not limited to, the steps of:

- (a) dissolving alkenyloxycucurbituril in an organic solvent such as chloroform and methanol;
- (b) adding a catalytic amount of AIBN (2,2-azobisisobutyronitrile) to the reaction mixture and then placing the resultant reaction mixture in a crystal tube;
- (c) adding a thiol-modified metal oxide substrate to the reaction mixture;
- (d) removing residual oxygen by the supply of nitrogen or argon to the reaction mixture;
- (e) exposing the reaction mixture to ultraviolet light for several days, for example, 3 days; and
- (f) washing the resultant solution with excess organic solvent followed by filtration to obtain a metal oxide substrate linked with cucurbituril by a sulfido-bond.

[0033] The exposure to ultraviolet light in step (e) may be substituted by heating at 80 to 120°C.



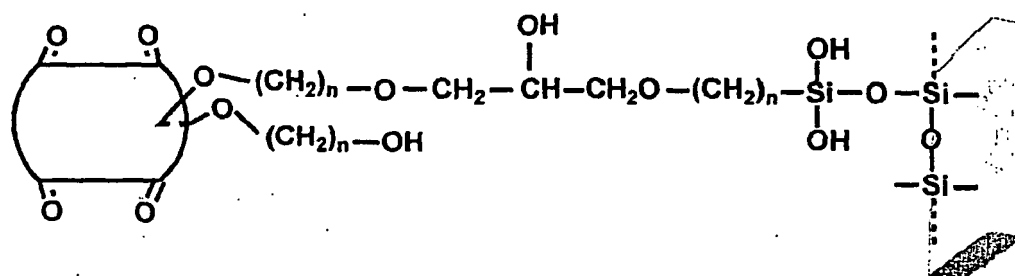
[0034] The substrate of Formula 4 may be obtained by amide bond formation between a cucurbituril derivative and a solid substrate, in detail, by amide bond formation between a carboxyl-ended cucurbituril derivative and an amino-modified metal oxide substrate.

(a) adding 1-ethyl-3-(3-dimethylaminopropyl)carboimidehydrochloride and N-hydroxysuccinimide or N,N-dimethylacetamide to a solution of carboxyl-ended cucurbituril derivative in distilled dimethylformamide;

(b) adding an amino-modified solid substrate to the reaction mixture followed by stirring at room temperature for 12 hours or more;

(c) washing the resultant metal oxide substrate with water and an organic solvent followed by drying to prepare a metal oxide substrate linked with cucurbituril by an amide bond.

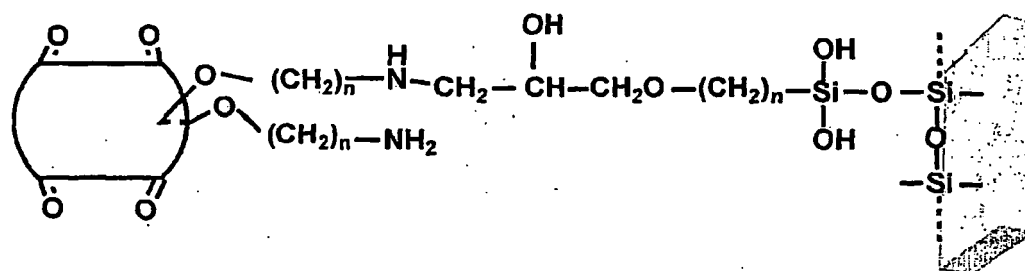
<Formula 5>



[0036] the substrate of Formula 5 may be obtained by ether bond formation between a cucurbituril derivative and a solid substrate, in detail, by a nucleophilic substitution reaction between a hydroxyl-ended cucurbituril derivative and an epoxy-modified metal oxide substrate.

- (a) adding hydroxyalkyloxycucurbituril with an end hydroxyl group to a dimethylformamide solvent;
- (b) gradually adding an epoxy-modified metal oxide substrate and a catalytic amount of boron trichloride to the reaction mixture;
- (c) stirring the reaction mixture at room temperature for 1 to 24 hours followed by further stirring at 85°C for 1 to 24 hours; and
- (d) washing the resultant metal oxide substrate with water and an organic solvent followed by drying to prepare a metal oxide substrate linked with cucurbituril by an ether bond.

<Formula 6>



wherein n is an integer of 1 to 20.

[0038] The substrate of Formula 6 may be obtained by amino bond formation between a cucurbituril derivative and a solid substrate, in detail by a nucleophilic substitution reaction between an amino-ended cucurbituril derivative and an epoxy-modified metal oxide substrate.

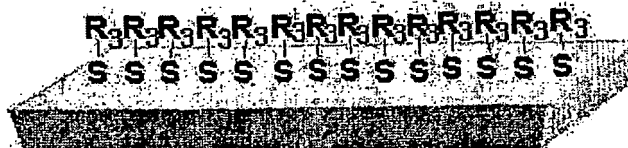
[0039] The preparation of the substrate of Formula 6 by the nucleophilic substitution reaction includes the steps of:

- (a) dissolving aminoalkyloxycucurbituril with an end amino group in a phosphate buffer (pH 7 to 10);
- (b) adding an epoxy-modified metal oxide substrate to the reaction mixture;
- (c) stirring the reaction mixture at room temperature for 1 to 24 hours; and
- (d) washing the resultant metal oxide substrate with water and an organic

solvent followed by drying to prepare a metal oxide substrate linked with cucurbituril by an amino bond.

[0040] A cucurbituril derivative-bonded solid substrate may also be prepared by covalently bonding the cucurbituril derivative of Formula 1 to a solid substrate of Formula 7 below:

<Formula 7>



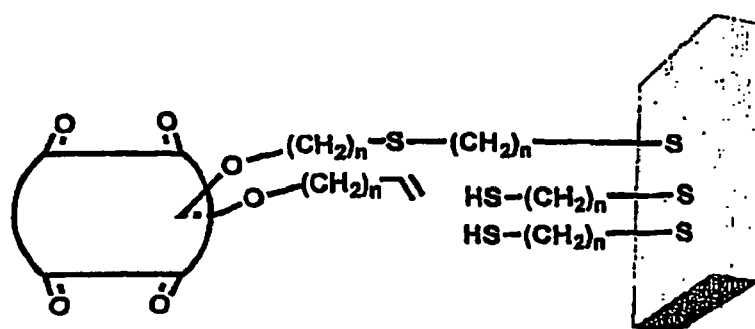
wherein R_3 is an alkyl group of C_1 - C_{10} with an end functional group selected from thiol, amine, epoxy, isocyan, and isothiocyan.

[0041] For example, the modified substrate of Formula 7 may be prepared by reacting a thiol compound having an end functional group, such as thiol, amino, and carboxyl group, with a metal oxide substrate containing a -OH surface group by washing.

[0042] A covalent bond between the cucurbituril derivative of Formula 1 and the modified solid substrate of Formula 7 enables the formation of another cucurbituril derivative-bonded solid substrate. That is, the cucurbituril derivative of Formula 1 is covalently bonded to the modified solid substrate of Formula 7 by reacting end functional groups of the cucurbituril derivative, such as carboxyl group, an amino group, or a thiol group, with end functional groups of the modified solid substrate, such as an amino group, a carboxyl group, or a thiol group.

[0043] Examples of the solid substrate thus prepared are presented in the following Formulae 8 through 11 and their preparation methods will now be described.

<Formula 8>



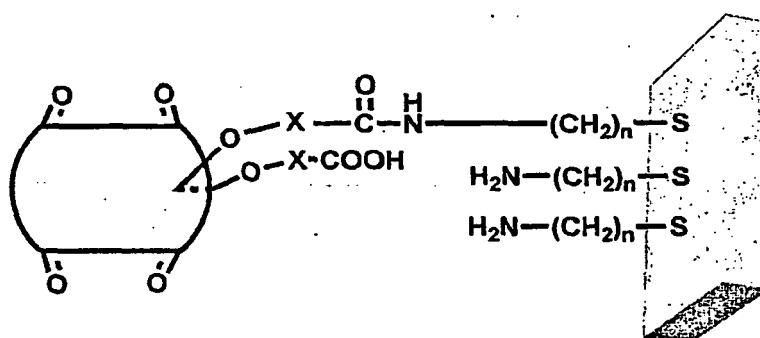
wherein each n is independently an integer of 1 to 20.

[0044] A substrate of Formula 8 may be obtained by sulfide-bond formation between a cucurbituril derivative and a metal substrate, in detail, by radical reaction between a thiol-modified metal substrate and alkenyloxycucurbituril.

[0045] In more detail, the preparation of the substrate of Formula 8 by radical reaction includes, but is not limited to, the steps of:

- (a) dissolving alkenyloxycucurbituril in an organic solvent such as chloroform and methanol;
- (b) adding a catalytic amount of AIBN (2,2-azobisisobutyronitrile) to the reaction mixture and then placing the resultant reaction mixture in a crystal tube;
- (c) adding a thiol-modified metal substrate to the reaction mixture;
- (d) removing residual oxygen by the supply of nitrogen or argon to the reaction mixture;
- (e) exposing the reaction mixture to ultraviolet light for several days, for example, 3 days; and
- (f) washing the resultant solution with an excess organic solvent followed by filtration to obtain a metal oxide substrate linked with cucurbituril by a sulfide-bond.

<Formula 9>



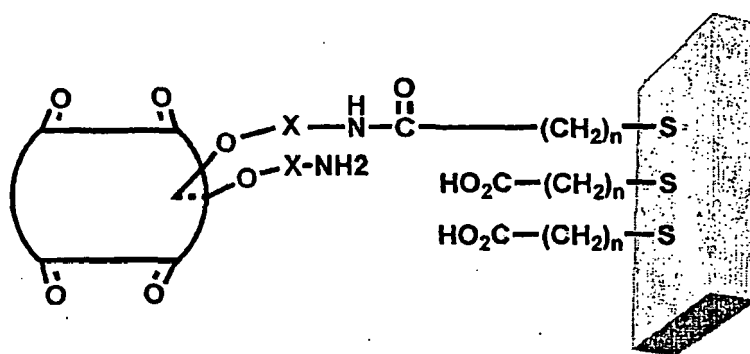
wherein each n is independently an integer of 1 to 20 and X is a dialkylsulfide group with a substituted or unsubstituted alkyl moiety of C_1 - C_{20} or a substituted or unsubstituted alkyl group of C_1 - C_{20} .

[0046] The substrate of Formula 9 may be obtained by amide bond formation between a cucurbituril derivative and a metal substrate, in detail, by amide bond formation between a carboxyl-ended cucurbituril derivative and an amino-modified metal substrate.

[0047] In more detail, the preparation of the substrate of Formula 9 includes, but is not limited to, the steps of:

- (a) adding 1-ethyl-3-(3-dimethylaminopropyl)carboimidehydrochloride and N-hydroxysuccinimide or N,N-dimethylacetamide to a solution of carboxyl-ended cucurbituril in distilled dimethylformamide;
- (b) adding an amino-modified metal substrate to the reaction mixture followed by stirring at room temperature for 12 hours or more; and
- (c) washing the resultant metal substrate with water and an organic solvent followed by drying to prepare a metal substrate linked with cucurbituril by an amide bond.

<Formula 10>



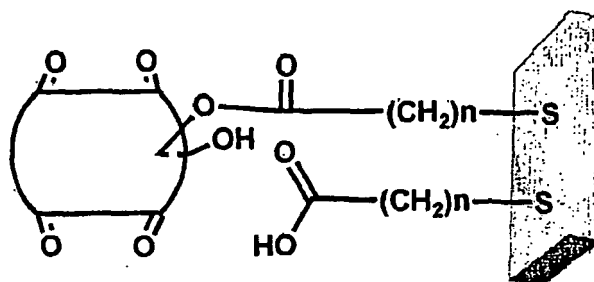
wherein each n is independently an integer of 1 to 20 and X is a alkylsulfidoalkyl group with a substituted or unsubstituted alkyl moiety of C_1 - C_{20} or a substituted or unsubstituted alkyl group of C_1 - C_{20} ,

[0048] The substrate of Formula 10 may be prepared by amide bond formation between a cucurbituril derivative and a metal oxide substrate, in detail, by amide bond formation between a carboxyl-ended cucurbituril derivative and an amino-modified metal oxide substrate.

[0049] In more detail, the preparation of the substrate of Formula 10 includes, but is not limited to, the steps of:

- (a) dissolving 1-ethyl-3-(3-dimethylaminopropyl)carboimidehydrochloride and N-hydroxysuccinimide or N,N-dimethylacetamide in distilled dimethylformamide and adding a carboxyl-modified metal substrate to the reaction mixture;
- (b) adding an amino-ended cucurbituril derivative to the reaction mixture followed by stirring at room temperature for 12 hours or more; and
- (c) washing the resultant metal substrate with water and an organic solvent followed by drying to prepare a metal substrate linked with cucurbituril by an amide bond.

<Formula 11>



wherein each n is independently an integer of 1 to 20.

[0050] The preparation of the substrate of Formula 11 includes the steps of:

- (a) dissolving methylmorpholine and ethylchloroformate to distilled dimethylformamide and adding a carboxyl-modified metal substrate followed by stirring for several minutes;
- (b) washing the resultant metal substrate with an organic solvent followed by drying to obtain a metal substrate containing carboxylic anhydride;
- (c) dissolving hydroxyl-ended cucurbituril and methylmorpholine to distilled dimethylformamide and adding the resultant metal substrate to the reaction mixture; and
- (d) washing the resultant metal substrate with water and an organic solvent followed by drying to prepare a metal substrate linked with cucurbituril by an ester bond.

[0051] Preferably, a cucurbituril derivative-bonded solid substrate according to the present invention is further subjected to drying and purification after being sufficiently washed with water and an organic solvent to remove residual impurities.

[0052] The present invention also provides a protein chip including the cucurbituril derivative-bonded solid substrate. Cucurbituril has carbonyl groups on the entrance of its cavity, and thus, can retain various ionic compounds such as organic cations and high polarity compounds by charge-polarity interactions, polarity-polarity interactions, or hydrogen bonds. In particular, cucurbituril is bonded to diaminoalkane salt by a coupling constant of about 10^6 M^{-1} , which is smaller than that of avidin-biotin interaction but is larger than that of a common non-covalent bond such as a coordination bond or a hydrogen bond. Therefore, a protein chip including the cucurbituril derivative-bonded solid substrate has advantages such as low manufacturing costs, a strong coupling constant, and immobilization of proteins on a solid substrate without damage to active sites of the proteins.

[0053] In addition, the cucurbituril derivative-bonded solid substrate of the present invention is covalently bonded to genes or biomaterials, and thus, can be used in preparation of a gene chip and a sensor for biomaterial assay.

Effect of the Invention

[0054] As apparent from the above description, a cucurbituril derivative-bonded solid substrate according to the present invention enables immobilization of proteins on a surface of the solid substrate via a non-covalent bond with a very strong coupling constant. Based on this property of the solid substrate, a protein chip with no damage to active sites of proteins can be prepared in a cost-effective manner.

[0055] FIG. 3 is a diagram that illustrates a cucurbituril derivative-covalent bonded solid substrate.

BEST MODE FOR CARRYING OUT THE INVENTION

[0056] Hereinafter, the present invention will be described more specifically by Example. However, the following Examples are provided only for illustrations and thus the present invention is not limited to or by them.

Example 1: Preparation of cucurbituril derivative-bonded solid substrate

[0057] A glass substrate was washed with a piranha solution (a 3:1 mixture of sulfuric acid and hydrogen peroxide) to introduce a hydroxyl group to a surface of the glass substrate, sufficiently dried under a reduced pressure, and added in a 20 ml vial under a nitrogen atmosphere. Then, a 10 mM solution of (3-mercaptopropyl)triethoxysilane in toluene was added thereto and incubated at room temperature to perform silanization. After the silanization was completed, the glass substrate was washed with toluene and heated at 120°C under a reduced pressure for one hour. The glass substrate was placed in a crystal tube, and a solution of 10 mg allyloxycucurbit[6]uril of Formula 1 where R_1 is an allyloxy group in a 1:1 mixed solvent of chloroform and methanol was added thereto. The reaction mixture underwent oxygen removal by the supply of nitrogen in the crystal tube and then exposed to ultraviolet light with a wavelength of 300 nm for 38 hours. After the reaction terminated, the resultant glass substrate was sequentially washed with dimethylsulfoxide, dimethylformamide, chloroform, methanol, and acetone, and dried under a reduced pressure.

Example 2: Preparation of cucurbituril derivative-bonded solid substrate

[0058] A glass substrate was washed with a piranha solution to introduce a hydroxyl group to a surface of the glass substrate, sufficiently dried under a reduced pressure, and added in a 20 ml vial under a nitrogen atmosphere. Then, a 10 mM solution of (3-aminopropyl)triethoxysilane in toluene was added thereto and incubated at room temperature to perform silanization. After the silanization was completed, the glass substrate was washed with toluene and heated at 120°C under a reduced pressure for one hour. 10 mg of carboxymethylsulfinylpropyloxycucurbit[6]uril of Formula 1 where R_1 is a carboxymethylsulfinylpropyloxy group was dissolved in 10 mL of dimethylformamide, and 150 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) and 3 mg of N-hydroxysuccinimide were then added thereto. The amino-modified glass substrate was placed in the resultant solution and stirred at room temperature for 12 hours. After the reaction terminated, the resultant glass substrate was sequentially washed with dimethylformamide, methanol, water, and acetone, and dried under a reduced pressure.

Example 3: Preparation of cucurbituril derivative-bonded solid substrate

[0059] A glass substrate was washed with a piranha solution to introduce a hydroxyl group to a surface of the glass substrate, sufficiently dried under a reduced pressure, and added in a 20 ml vial under a nitrogen atmosphere. Then, a 10 mM solution of (3-aminopropyl)triethoxysilane in toluene was added thereto and incubated at room temperature to perform silanization. After the silanization was completed, the glass substrate was washed with toluene and heated at 120°C under a reduced pressure for one hour. The resultant amino-modified glass substrate was immersed in a solution of 100 mg of succinic anhydride in dimethylformamide and stirred at room temperature for 12 hours. After the reaction

terminated, the glass substrate was sequentially washed with dimethylformamide, water, methanol, and acetone, and dried under a reduced pressure. 10 mg of aminocucurbit[6]uril of Formula 1 where R_1 is an amino group was dissolved in 10 mL of dimethylformamide, and 150 mg of EDAC and 3 mg of N-hydroxysuccinimide were then added thereto. Then, the glass substrate was placed in the resultant solution and stirred at room temperature for 12 hours. After the reaction terminated, the resultant glass substrate was sequentially washed with dimethylformamide, methanol, water, and acetone, and dried under a reduced pressure.

Example 4: Preparation of cucurbituril derivative-bonded solid substrate

[0060] A glass substrate was washed with a piranha solution to introduce a hydroxyl group to a surface of the glass substrate, sufficiently dried under a reduced pressure, and added in a 20 ml vial under a nitrogen atmosphere. Then, a 10 mM solution of (3-glycidioxypropyl)triethoxysilane in toluene was added thereto and incubated at room temperature to perform silanization. After the silanization was completed, the glass substrate was washed with toluene and heated at 120°C under a reduced pressure for one hour. 10 mg of 2-hydroxyethyloxycucurbit[6]uril of Formula 1 where R_1 is a 2-hydroxyethyloxy group and the resultant glycidioxy-modified glass substrate were placed in 10 mL of dimethylformamide. Then, a catalytic amount of boron trifluoride (BF_3) and diethylether (Et_2O) were added thereto and stirred at room temperature for two hours, followed by further stirring at 85°C for 12 hours. After the reaction terminated, the resultant glass substrate was sequentially washed with dimethylformamide, chloroform, methanol, water, and acetone, and dried under a reduced pressure.

Example 5: Preparation of cucurbituril derivative-bonded solid substrate

[0061] A glass substrate was washed with a piranha solution to introduce a hydroxyl group to a surface of the glass substrate, sufficiently dried under a reduced pressure, and added in a 20 ml vial under a nitrogen atmosphere. Then, a 10 mM solution of (3-glycidioxypropyl)triethoxysilane in toluene was added thereto and incubated at room temperature to perform silanization. After the silanization was completed, the glass substrate was washed with toluene and heated at 120°C under a reduced pressure for one hour. 10 mg of 2-aminoethyloxycucurbit[6]uril of Formula 1 where R_1 is a 2-aminoethyloxy group and the resultant glycidioxy-modified glass substrate were placed in a phosphate buffer (pH 8.8) and stirred for 12 hours. After the reaction terminated, the resultant glass substrate was immersed in 10 mL of 0.2N HCl solution, stirred for 30 minutes, sequentially washed with water, acetone, and methanol, and dried under a reduced pressure.

Examples 6: Preparation of cucurbituril derivative-bonded solid substrate

[0062] A gold-deposited silicon wafer was washed with a piranha solution, sufficiently dried under a reduced pressure, and added in a 20 ml vial under a nitrogen atmosphere. Then, a 1 mM solution of 1,8-octanedithiol in ethanol was added thereto and incubated at room temperature to obtain a thiol-modified gold substrate. The thiol-modified gold substrate (in Formula 2, R_2 is a propylthiol group) was placed in a test tube and a solution of allyloxycucurbit[6]uril of Formula 1 where R_1 is an allyloxy group in 5 mL of a 1:1 mixed solvent of chloroform and methanol was added thereto. The reaction mixture underwent oxygen removal by the supply of nitrogen in the test tube and then exposed to ultraviolet light with a wavelength of 300 nm for 36 hours. After the reaction terminated, the resultant gold substrate was sequentially washed with dimethylsulfoxide, dimethylformamide, chloroform, methanol, and acetone, and dried under a reduced pressure.

Example 7: Preparation of cucurbituril derivative-bonded solid substrate

[0063] A gold-deposited silicon wafer was washed with a piranha solution, sufficiently dried under a reduced pressure, and added in a 20 ml vial under a nitrogen atmosphere. Then, a 1 mM solution of 2-aminoethanediol in ethanol was added thereto and incubated at room temperature to obtain an amino-modified gold substrate. 10 mg of carboxymethylsulfanylpropyloxycucurbit[6]uril of Formula 1 wherein R_1 is a carboxymethylsulfanylpropyloxy group was dissolved in 10 mL of dimethylformamide, and 150 mg of EDAC and 3 mg of N-hydroxysuccinimide were added thereto. The amino-modified gold substrate was added to the resultant solution and stirred at room temperature for 12 hours. After the reaction terminated, the gold substrate was sequentially washed with dimethylformamide, methanol, water, and acetone, and dried under a reduced pressure.

Example 8: Preparation of cucurbituril derivative-bonded solid substrate

[0064] A gold-deposited silicon wafer was washed with a piranha solution, sufficiently dried under a reduced pressure, and added in a 20 ml vial under a nitrogen atmosphere. Then, a 1 mM solution of 11-mercaptoundecanoic acid in ethanol

was added thereto and incubated at room temperature to obtain a carboxyl-modified gold substrate. The carboxyl-modified gold substrate was immersed in a solution of 100 mg of succinic anhydride in dimethylformamide and stirred at room temperature for 12 hours. 10 mg of aminocucurbit[6]uril of Formula 1 wherein R_1 is an amino group was dissolved in 10 mL of dimethylformamide, and 150 mg of EDAC and 3 mg of N-hydroxysuccinimide were added thereto. The carboxyl-modified gold substrate was added to the resultant solution and stirred at room temperature for 12 hours. After the reaction terminated, the resultant gold substrate was sequentially washed with dimethylformamide, methanol, water, and acetone, and dried under a reduced pressure.

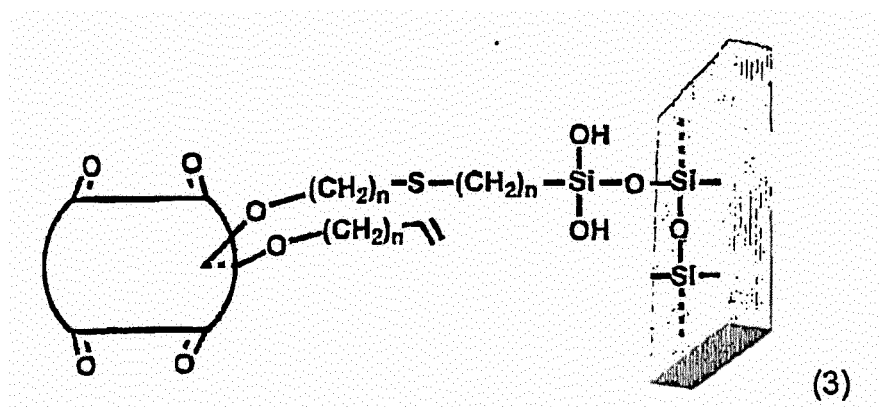
Example 9: Preparation of cucurbituril derivative-bonded solid substrate

[0065] A gold-deposited silicon wafer was washed with a piranha solution, sufficiently dried under a reduced pressure, and added in a 20 ml vial under a nitrogen atmosphere. Then, a 1 mM solution of 11-mercaptopundecanoic acid in ethanol was added thereto and incubated at room temperature to obtain a carboxyl-modified gold substrate. The gold substrate was immersed in 10 mL of anhydrous dimethylformamide under a nitrogen atmosphere, and 100 μl of N-methylmorpholine and 100 μl of ethylchloroformate were sequentially added thereto, followed by stirring for 24 hours. After the reaction terminated, the gold substrate was several times washed with diethylether and dried under a reduced pressure. 10 mL of anhydrous dimethylformamide was added to the gold substrate and 10 mL of 2-hydroxyethyloxycucurbit[6]uril of Formula 1 wherein R_1 is a 2-hydroxyethyloxy group under a nitrogen atmosphere and stirred for 24 hours. After the reaction terminated, the gold substrate was sequentially washed with dimethylformamide, water, methanol, and acetone, and dried under a reduced pressure.

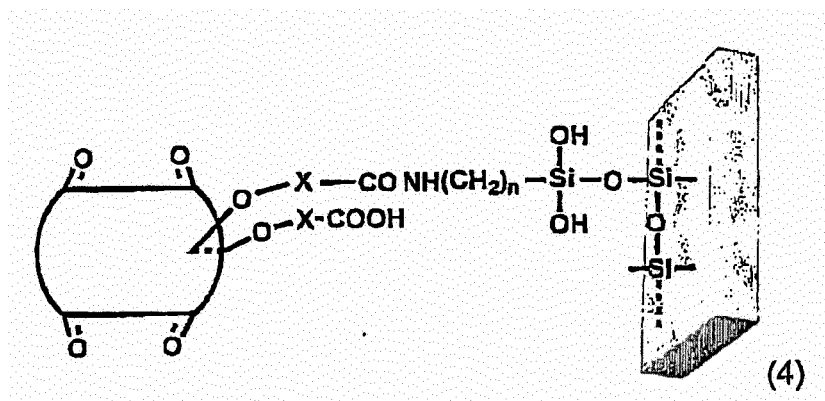
[0066] While the above Examples has been particularly shown and described in terms of only specific bonds between cucurbituril and a solid substrate, it will be understood by those of ordinary skill in the art that synthesis of a solid substrate linked with cucurbituril is possible by various types of bonds.

Claims

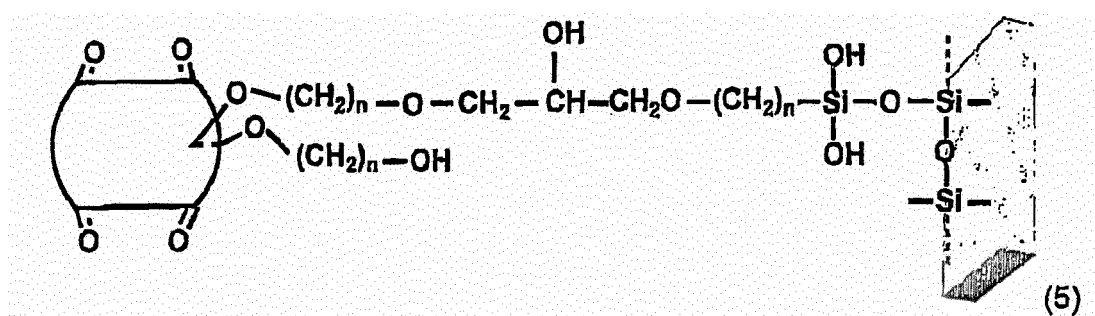
1. A cucurbituril derivative-bonded solid substrate wherein the substrate is a glass, a silicon wafer, an indium tin oxide (ITO) glass, an aluminum oxide substrate, or a titanium dioxide substrate and is selected from the group consisting of substrates represented by Formulae 3 through 6:



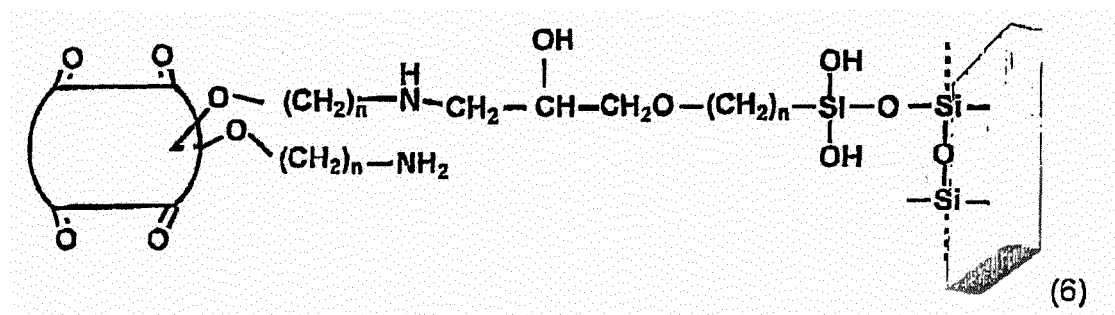
wherein each n is independently an integer of 1 to 20;



15 wherein n is an integer of 1 to 20 and X is a dialkylsulfide group with a substituted or unsubstituted alkyl moiety of C₁-C₂₀ or a substituted or unsubstituted alkyl group of C₁-C₂₀;

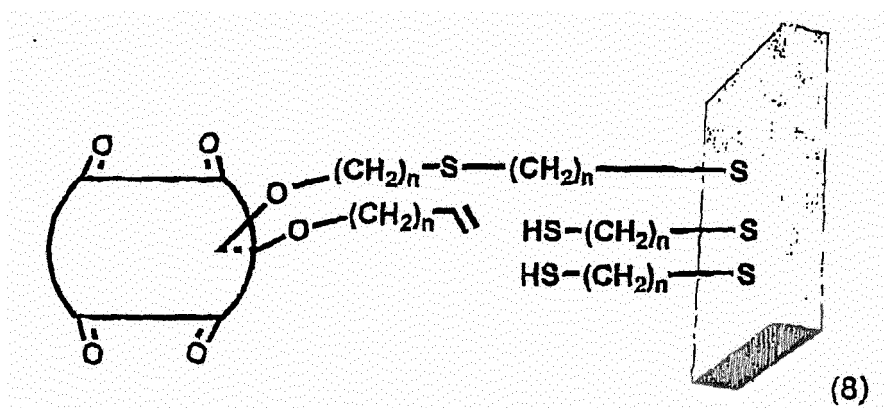


30 wherein n is an integer of 1 to 20; and

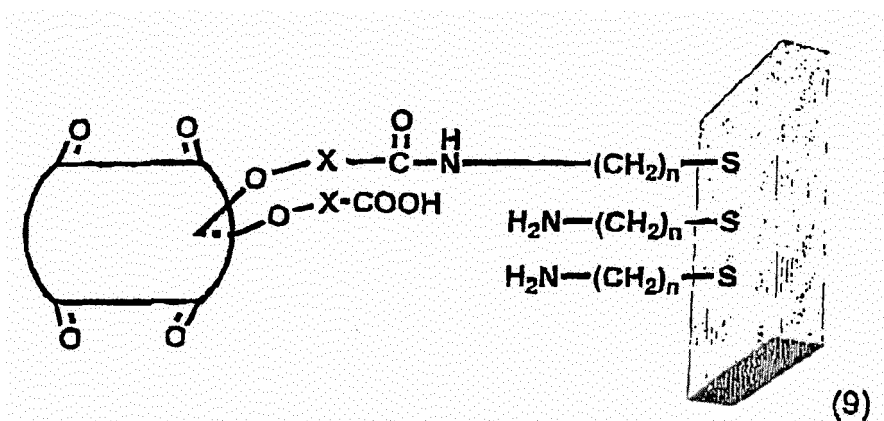


45 wherein n is an integer of 1 to 20.

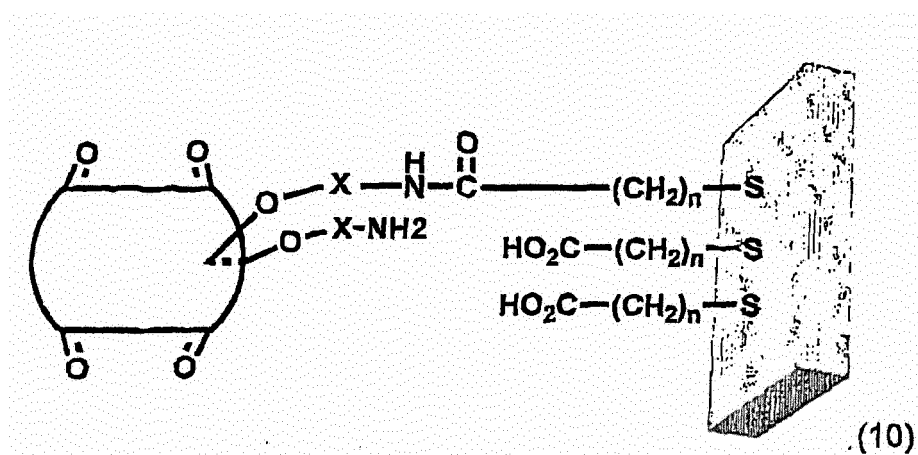
- 50
- 55
2. A cucurbituril derivative-bonded solid substrate selected from the group consisting of substrates represented by Formulae 8 through 11:



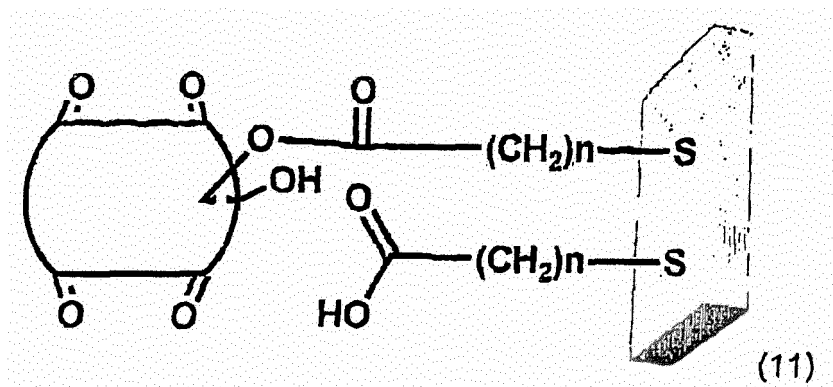
wherein each n is independently an integer of 1 to 20;



wherein each n is independently an integer of 1 to 20 and X is a dialkylsulfide group with a substituted or unsubstituted alkyl moiety of $\text{C}_1\text{-C}_{20}$ or a substituted or unsubstituted alkyl group of $\text{C}_1\text{-C}_{20}$;



wherein each n is independently an integer of 1 to 20 and X is a dialkylsulfide group with a substituted or unsubstituted alkyl moiety of $\text{C}_1\text{-C}_{20}$ or a substituted or unsubstituted alkyl group of $\text{C}_1\text{-C}_{20}$; and

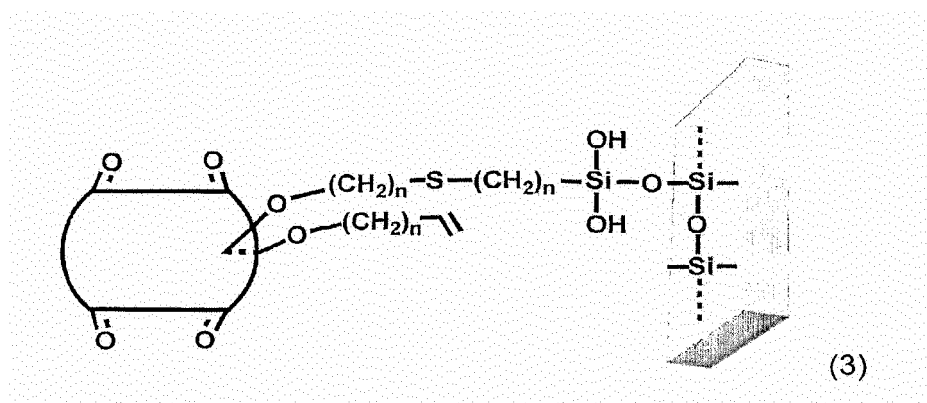


wherein each n is independently an integer of 1 to 20.

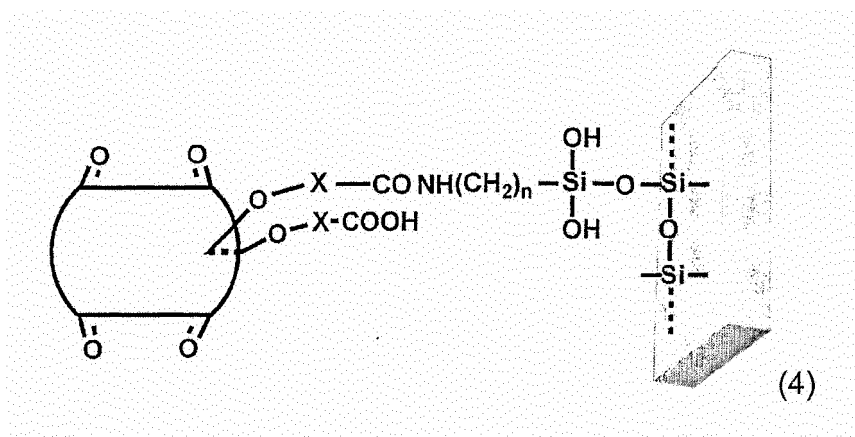
3. The cucurbituril derivative-bonded solid substrate of claim 2, wherein the solid substrate is a substrate made of gold, silver, platinum, or copper.
4. A protein chip comprising the cucurbituril derivative-bonded solid substrate of any one of claims 1 through 3.
5. A gene chip comprising the cucurbituril derivative-bonded solid substrate of any one of claims 1 through 3.
6. A sensor for biomaterial assay comprising the cucurbituril derivative-bonded solid substrate of any one of claims 1 through 3.

Revendications

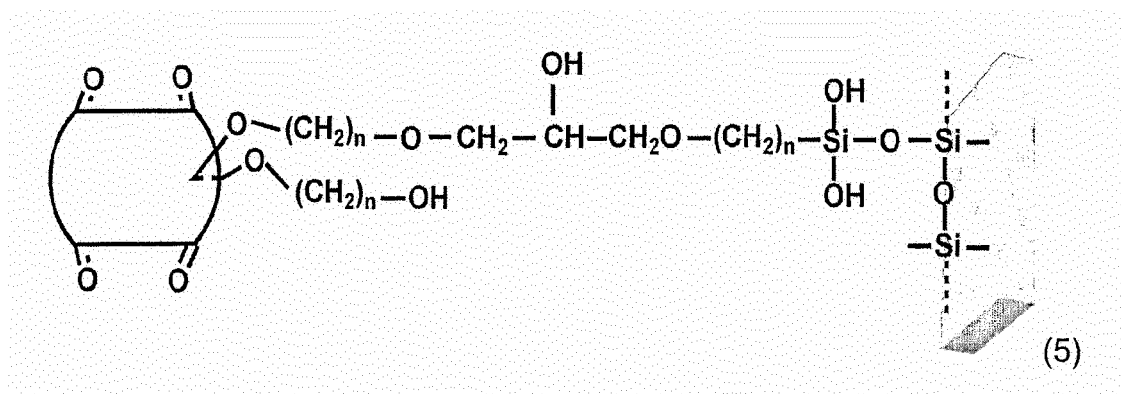
1. Substrat solide lié à un dérivé de cucurbiturile, lequel substrat est un verre, une plaque de silicium, un verre en indium/oxyde d'étain (ITO), un substrat en oxyde d'aluminium ou un substrat en dioxyde de titane et est choisi dans le groupe constitué par les substrats représentés par les Formules 3 à 6 :



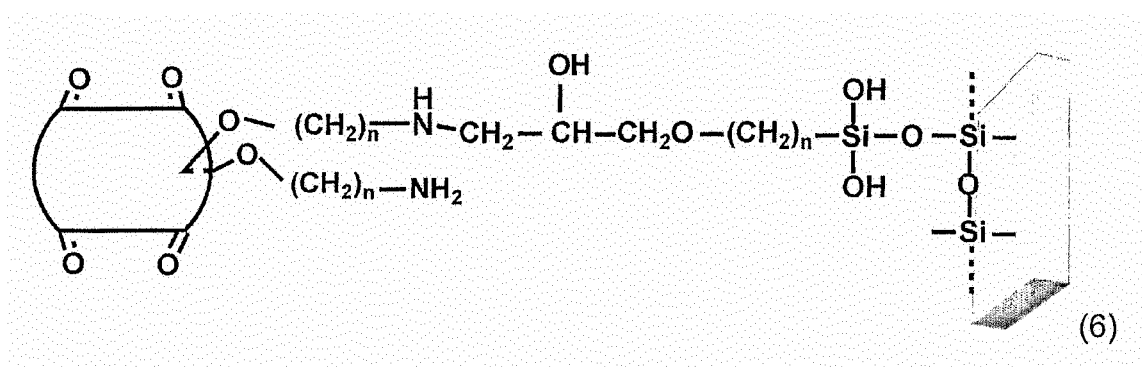
dans laquelle chaque n est indépendamment un nombre entier de 1 à 20 ;



dans laquelle n est un nombre entier de 1 à 20 et X est un groupe dialkylsulfure ayant un fragment alkyle en C₁-C₂₀ substitué ou non substitué ou un groupe alkyle en C₁-C₂₀ substitué ou non substitué ;

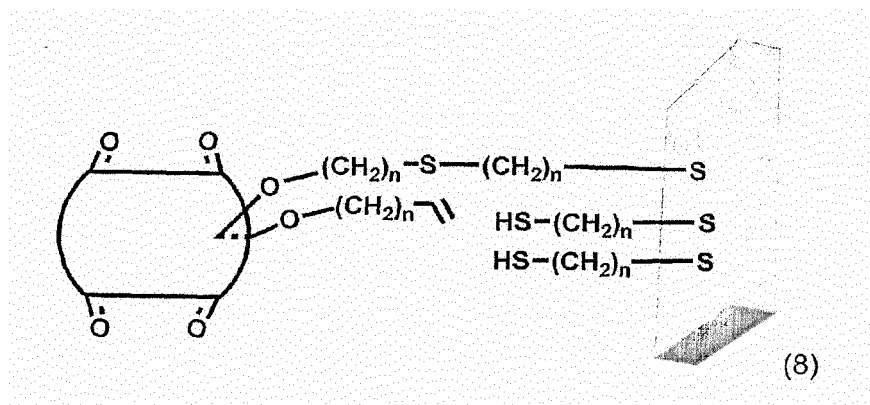


dans laquelle n est un nombre entier de 1 à 20 ; et

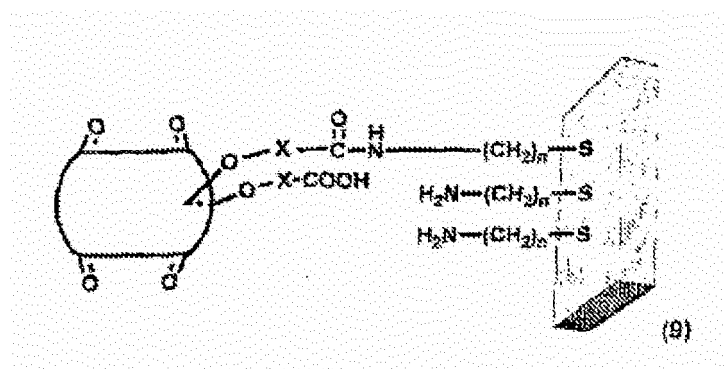


dans laquelle n est un nombre entier de 1 à 20.

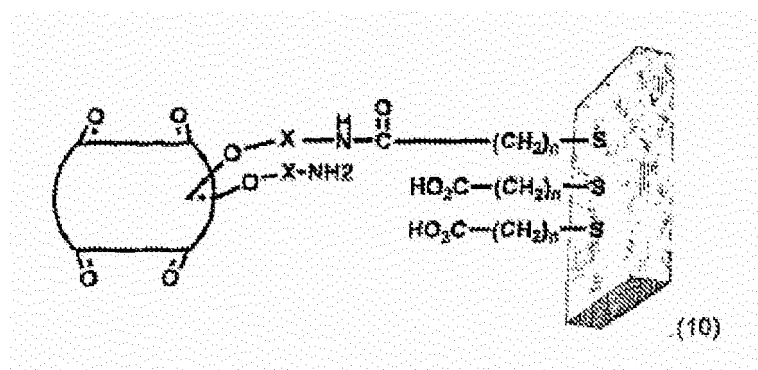
2. Substrat solide lié à un dérivé de cucurbiturile choisi dans le groupe constitué par des substrats représentés par les Formules 8 à 11 :



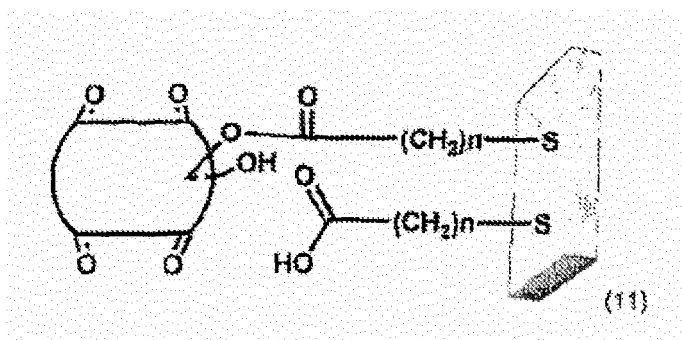
dans laquelle chaque n est indépendamment un nombre entier de 1 à 20 ;



dans laquelle chaque n est indépendamment un nombre entier de 1 à 20 et X est un groupe dialkylsulfure ayant un fragment alkyle substitué ou non substitué de C₁ à C₂₀ ou un groupe alkyle substitué ou non substitué de C₁ à C₂₀ ;



dans laquelle chaque n est indépendamment un nombre entier de 1 à 20 et X est un groupe dialkylsulfure ayant un fragment alkyle substitué ou non substitué de C₁ à C₂₀ ou un groupe alkyle substitué ou non substitué de C₁ à C₂₀ ;

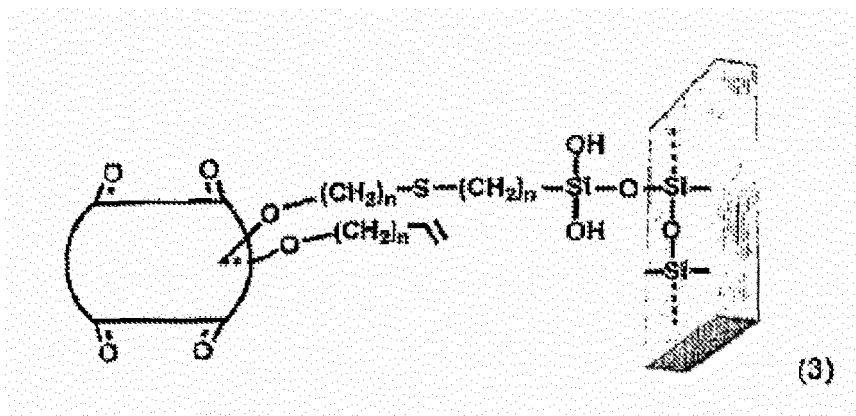


dans laquelle chaque n est indépendamment un nombre entier de 1 à 20.

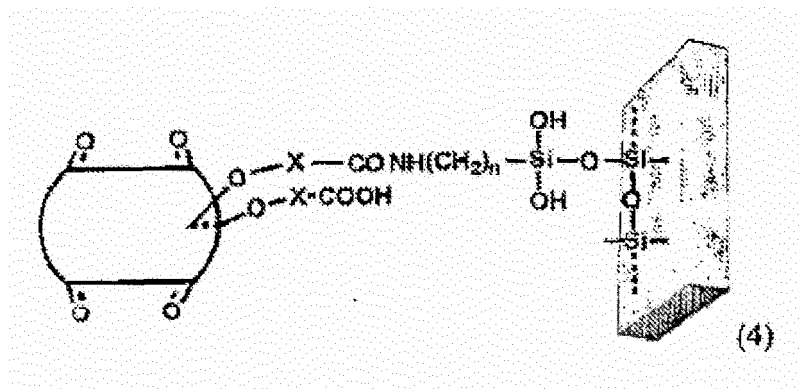
3. Substrat solide lié à un dérivé de cucurbiturile de la revendication 2, dans lequel le substrat solide est un substrat en or, en argent, en platine ou en cuivre.
4. Puce à protéines comprenant le substrat solide lié à un dérivé de cucurbiturile de l'une quelconque des revendications 1 à 3.
5. Puce à ADN comprenant le substrat solide lié à un dérivé de cucurbiturile de l'une quelconque des revendications 1 à 3.
6. Capteur pour essai de biomatériaux comprenant le substrat solide lié à un dérivé de cucurbiturile de l'une quelconque des revendications 1 à 3.

Patentansprüche

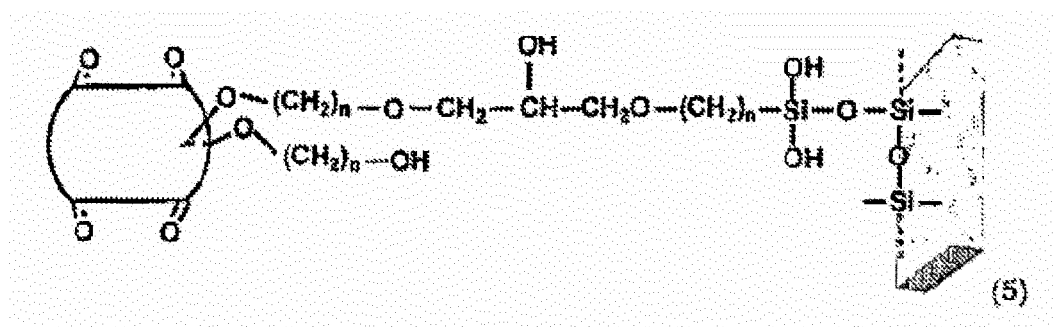
1. An Cucurbiturilderivat gebundenes festes Substrat, wobei das Substrat ein Glas, ein Siliciumwafer, ein Indium-Zinn-Oxid- (ITO-) Glas, ein Aluminiumoxidsubstrat oder ein Titandioxidsubstrat ist und aus der Gruppe ausgewählt ist bestehend aus Substraten, die durch die Formeln 3 bis 6 dargestellt sind:



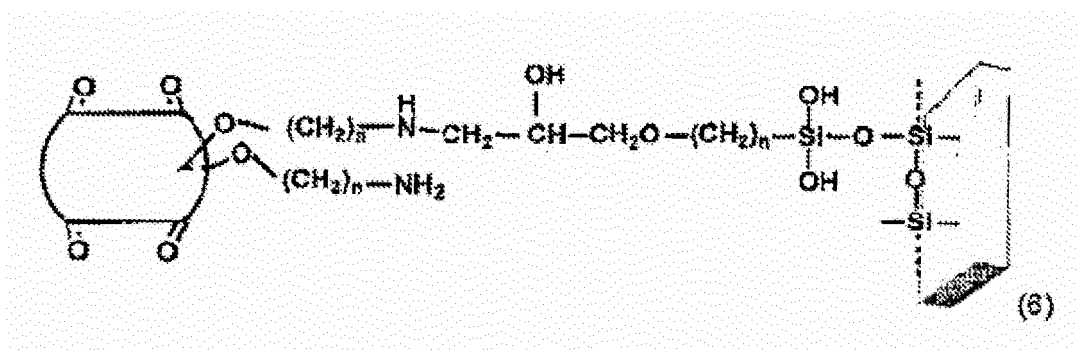
wobei jedes n eine ganze Zahl von 1 bis 20 ist;



wobei n eine ganze Zahl von 1 bis 20 ist und X eine Dialkylsulfidgruppe mit einem substituierten oder unsubstituierten Alkylanteil von C_1 - C_{20} oder einer substituierten oder unsubstituierten Alkyl-Gruppe von C_1 - C_{20} ist ;

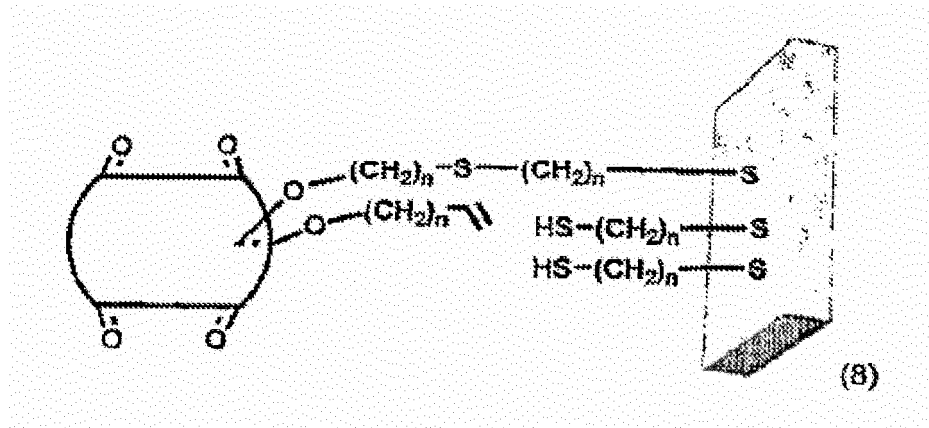


wobei n eine ganze Zahl von 1 bis 20 ist; und

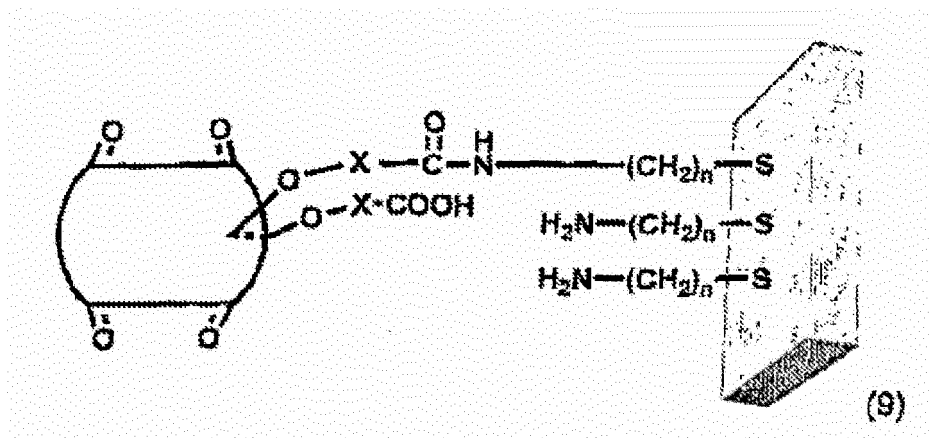


wobei n eine ganze Zahl von 1 bis 20 ist.

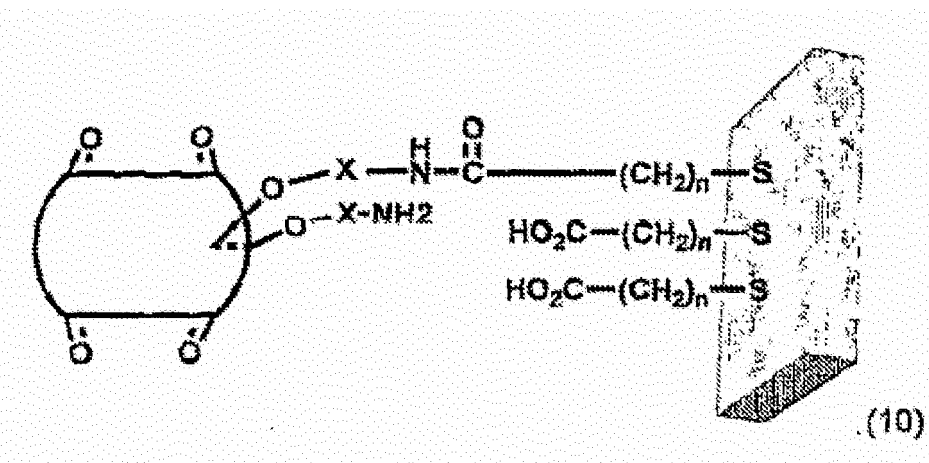
2. An Cucurbiturilderivat gebundenes festes Substrat ausgewählt aus der Gruppe bestehend aus Substraten, die durch die Formeln 8 bis 11 dargestellt sind:



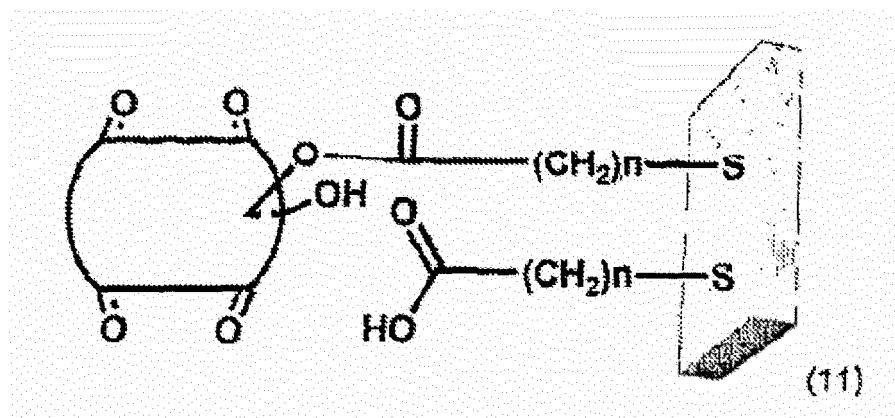
wobei n unabhängig eine ganze Zahl von 1 bis 20 ist;



wobei n unabhängig eine ganze Zahl von 1 bis 20 ist und X eine Dialkylsulfidgruppe mit einem substituierten oder unsubstituierten Alkylanteil von C_1 - C_{20} oder einer substituierten oder unsubstituierten Alkyl-Gruppe von C_1 - C_{20} ist;



wobei n unabhängig eine ganze Zahl von 1 bis 20 ist und X eine Dialkylsulfidgruppe mit einem substituierten oder unsubstituierten Alkylanteil von C_1 - C_{20} oder einer substituierten oder unsubstituierten Alkyl-Gruppe von C_1 - C_{20} ist;



wobei n unabhängig eine ganze Zahl von 1 bis 20 ist.

3. An Cucurbiturilderivat gebundenes festes Substrat nach Anspruch 2, wobei das feste Substrat ein Substrat ist, das aus Gold, Silber, Platin oder Kupfer hergestellt ist.
4. Protein-Chip umfassend das an Cucurbiturilderivat gebundene feste Substrat nach einem der Ansprüche 1 bis 3.
5. Gen-Chip umfassend das an Cucurbiturilderivat gebundene feste Substrat nach einem der Ansprüche 1 bis 3.
6. Sensor für Biomaterial-Assay umfassend das an Cucurbiturilderivat gebundene feste Substrat nach einem der Ansprüche 1 bis 3.

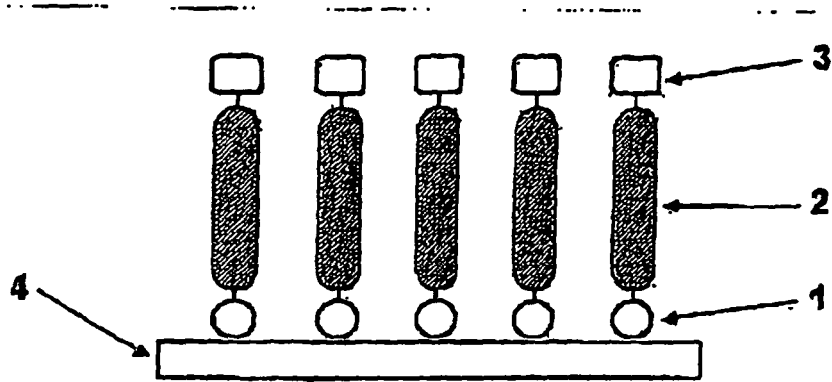


FIG. 1

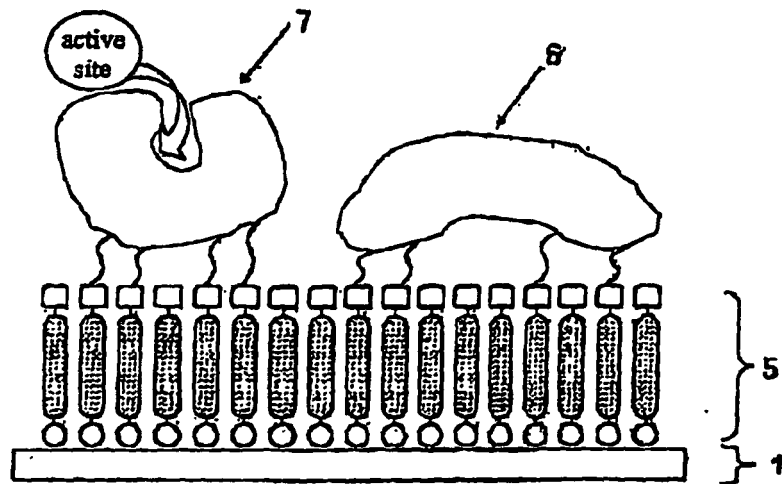


FIG. 2

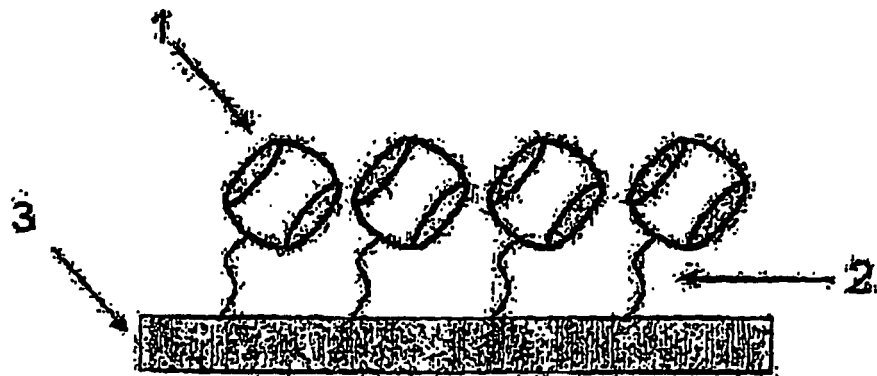


FIG. 3

REFERENCES CITED IN THE DESCRIPTION

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