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(54) DETECTION OF GLUCOSE IN SOLUTIONS ALSO CONTAINING AN ALPHA-HYDROXY ACID OR A BETA-DIKETONE

(76) Inventors: **George Y. Daniloff**, N. Potomac, MD (US); **Aristotle G. Kalivretenos**, Columbia, MD (US); **Alexandre V. Nikolaitchik**, Damascus, MD (US)

Correspondence Address:
ROTHWELL, FIGG, ERNST & MANBECK, P.C.
1425 K STREET, N.W.
SUITE 800
WASHINGTON, DC 20005 (US)

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(57) **ABSTRACT**

Compositions and methods for determining the presence or concentration of glucose in a sample which may also contain an alpha-hydroxy acid or a beta-diketone. The method uses a compound having at least two recognition elements for glucose, oriented such that the interaction between the compound and glucose is more stable than the interaction between the compound and the alpha-hydroxy acid or beta-diketone, such that the presence of the alpha-hydroxy acid or the beta-diketone does not substantially interfere with said determination.

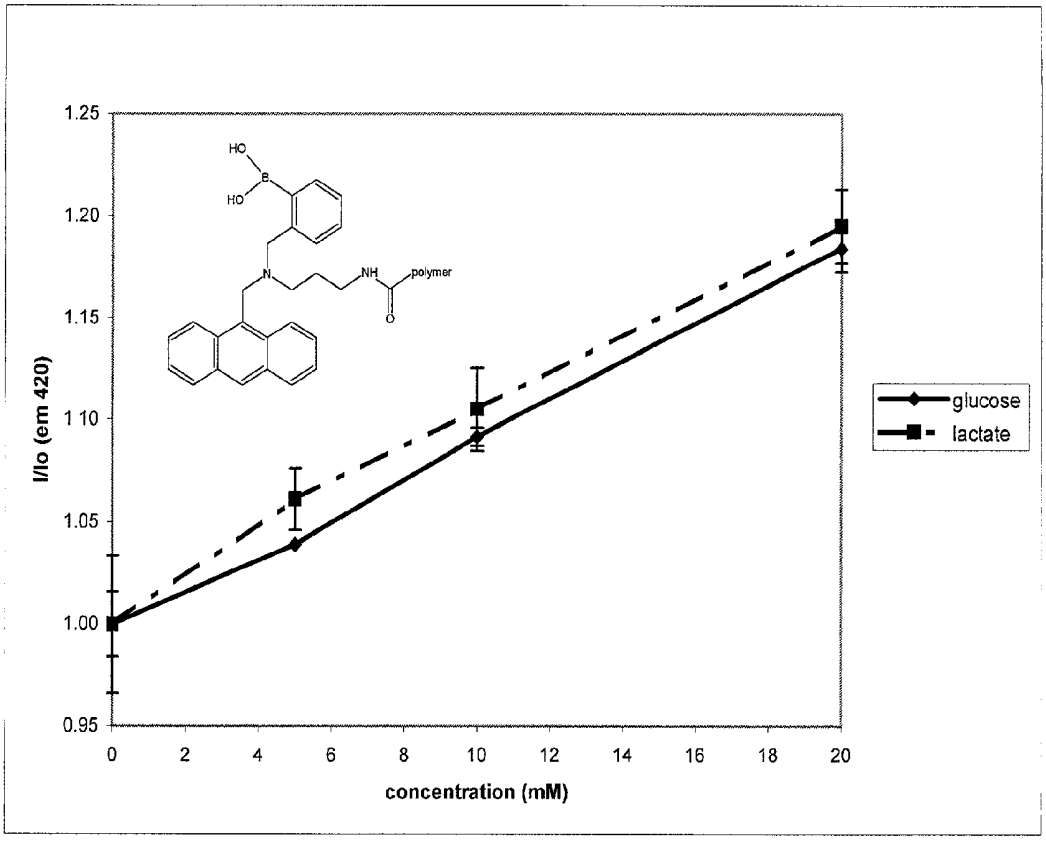

1.25
1.20
1.15
1 10
1.05
1.00
0.95
I/Io (em 420)
0
2
4
6
8
10
12
14
16
18
20
concentration (mM)
glucose
lactate
HO
B
HO
N
NH
polymer
O

FIGURE 1

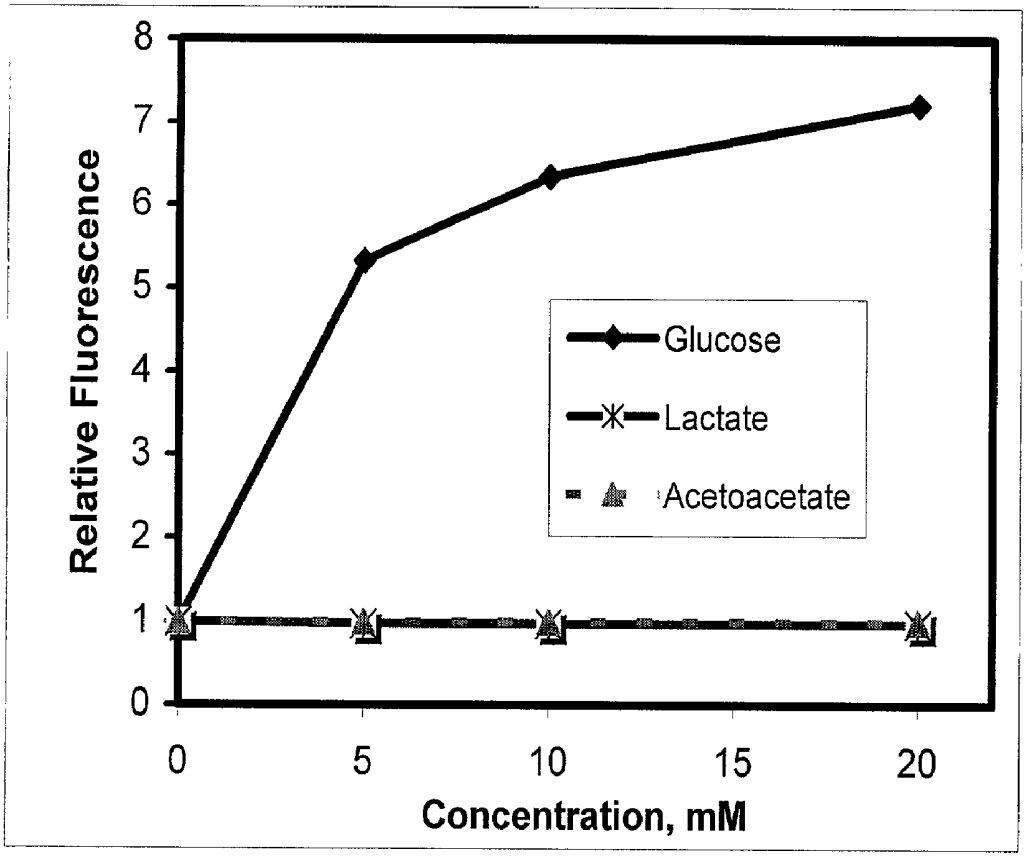

Relative Fluorescence
8
7
6
5
4
3
2
1
0
0
5
10
15
20
Concentration, mM
Glucose
Lactate
Acetoacetate

FIGURE 2

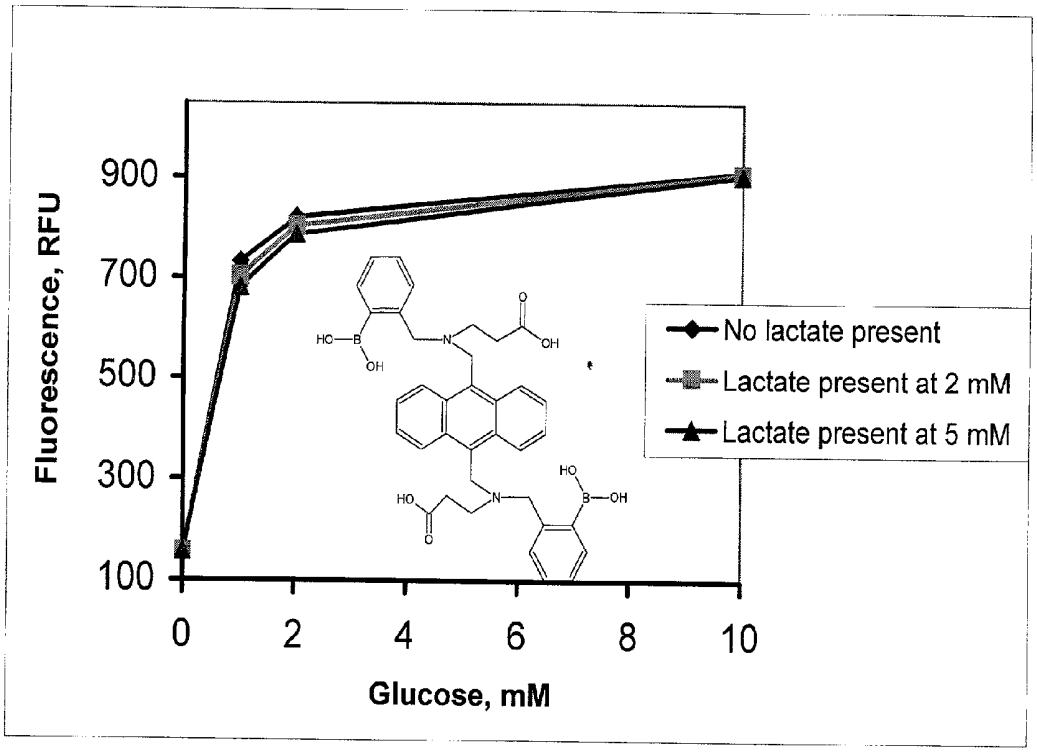

Fluorescence, RFU
900
700
500
300
100
0
2
4
6
8
10
Glucose, mM
No lactate present
Lactate present at 2 mM
Lactate present at 5 mM

FIGURE 3

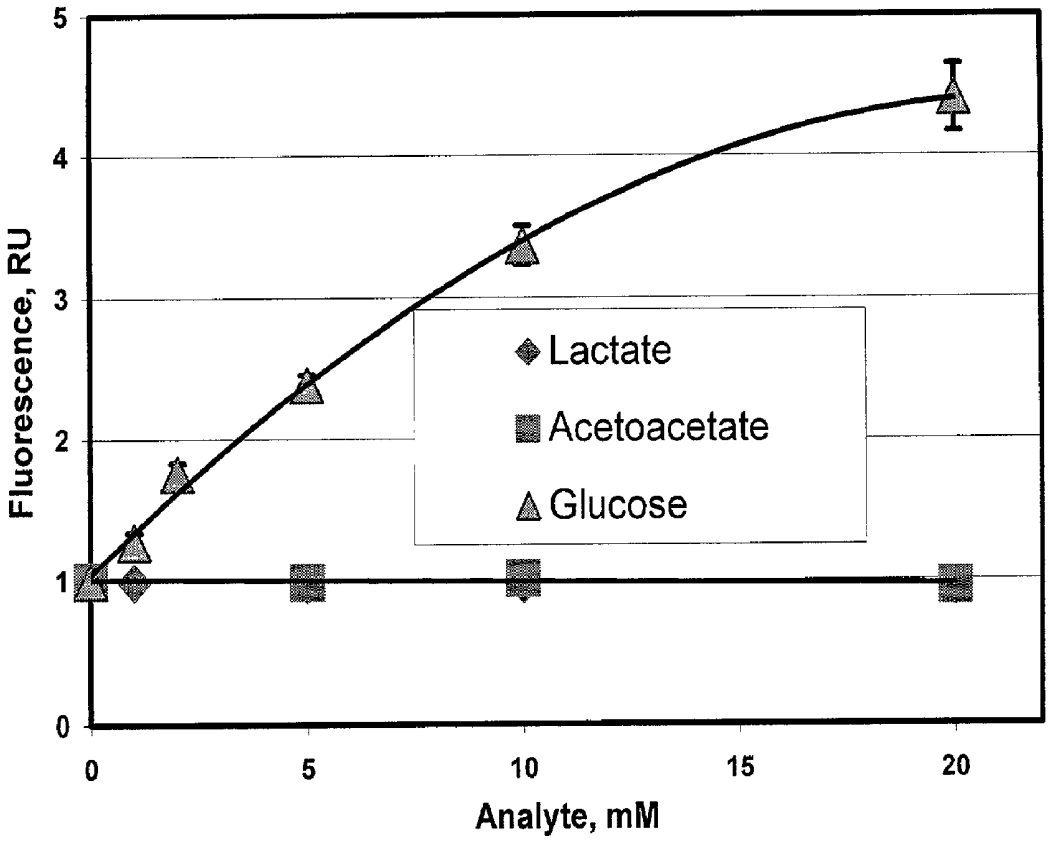

Fluorescence, RU
Analyte, mM
Lactate
Acetoacetate
Glucose
0
1
2
3
4
5
0
5
10
15
20

FIGURE 4

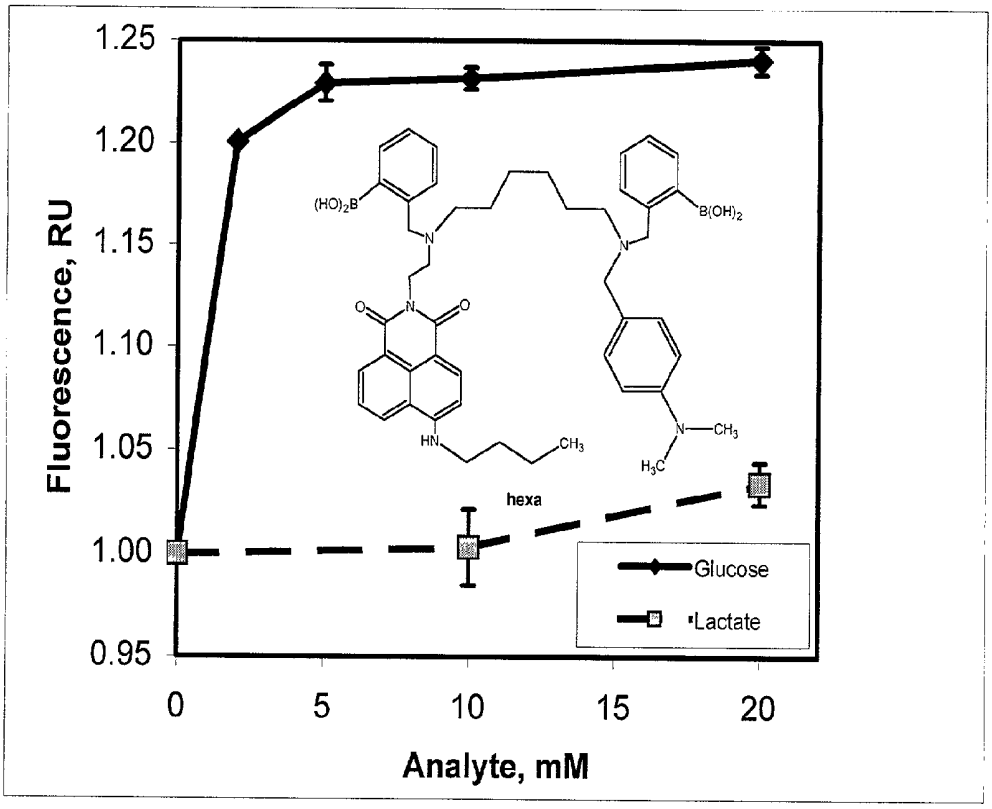

1.25
1.20
1.15
1.10
1.05
1.00
0.95
Fluorescence, RU
0
5
10
15
20
Analyte, mM
hexa
Glucose
Lactate
(HO)2B
B(OH)2
N
O
HN
CH3
H3C

FIGURE 5

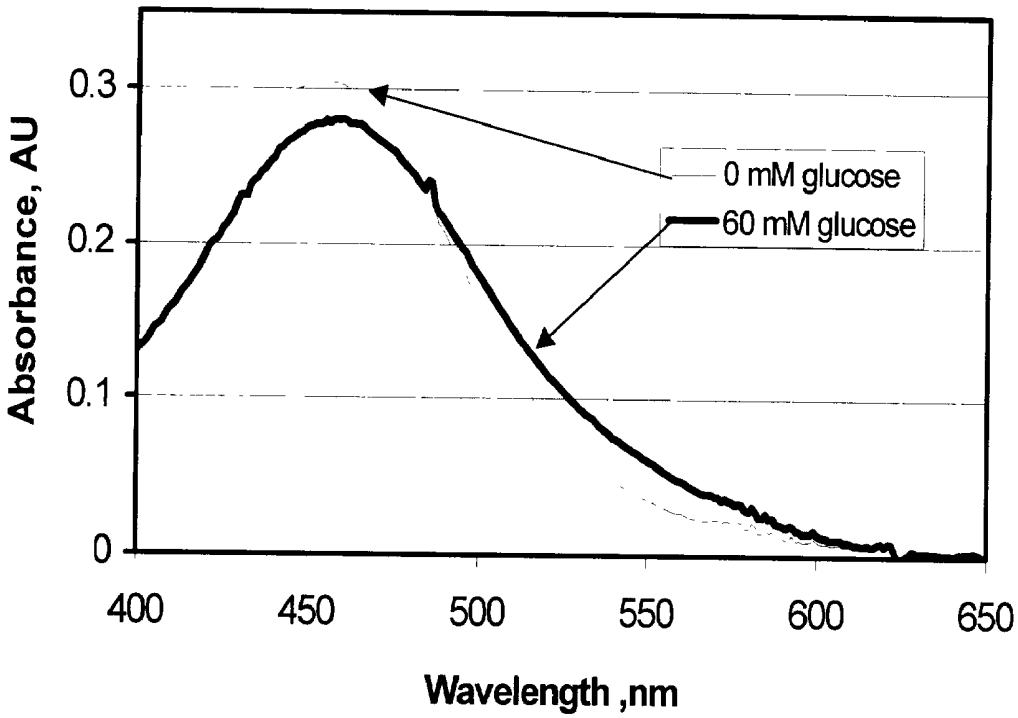

0.3
0.2
0.1
0
Absorbance, AU
400
450
500
550
600
650
Wavelength ,nm
0 mM glucose
60 mM glucose

FIGURE 6

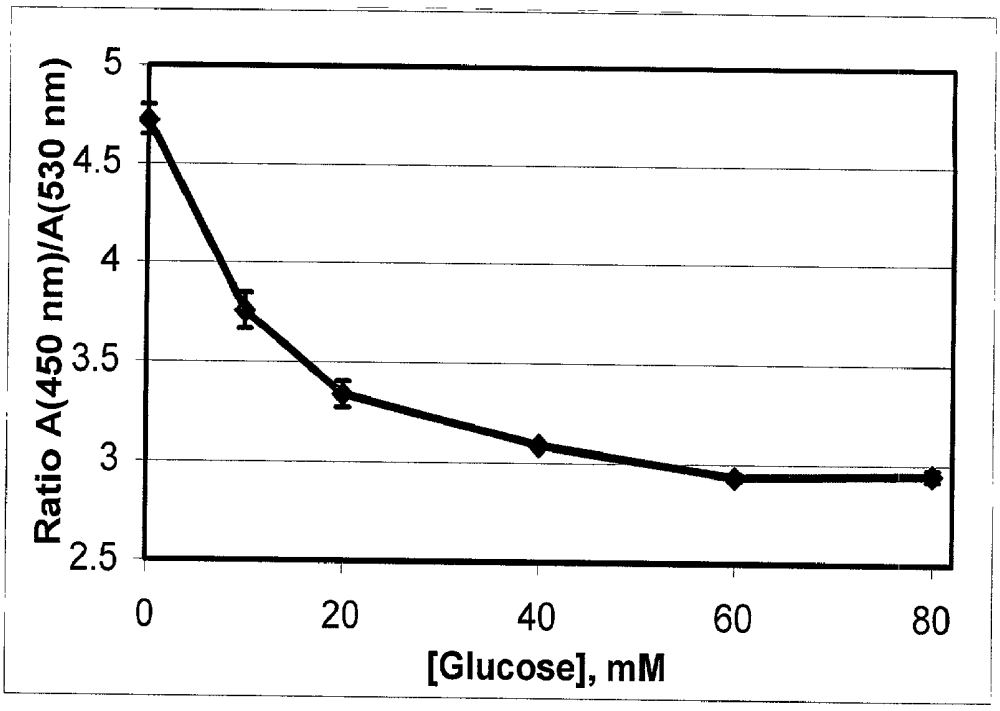


FIGURE 7

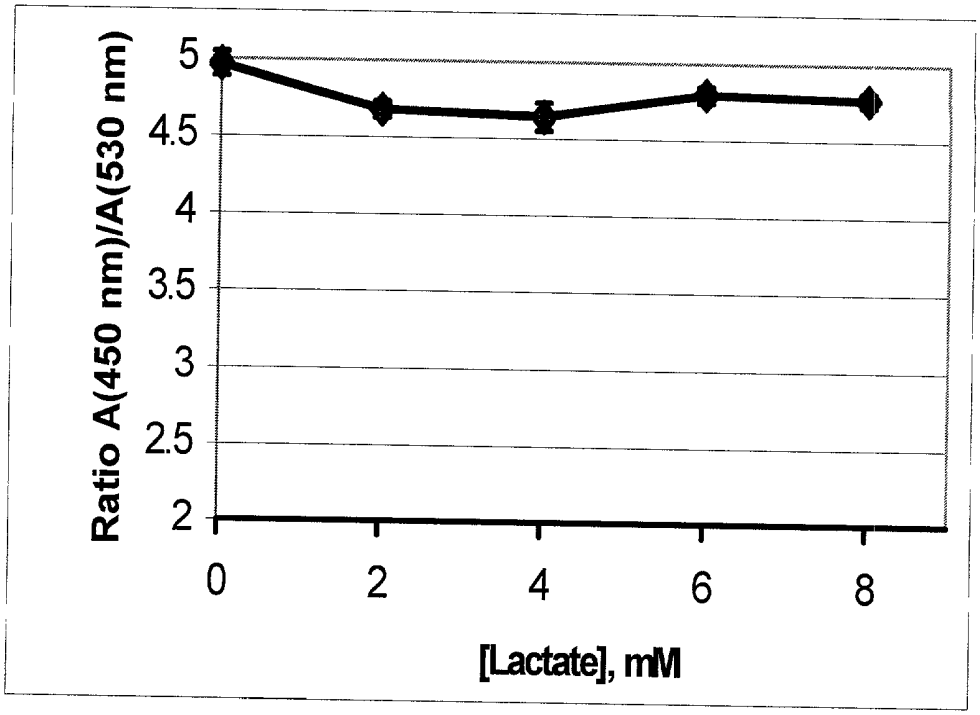


FIGURE 8

DETECTION OF GLUCOSE IN SOLUTIONS ALSO CONTAINING AN ALPHA-HYDROXY ACID OR A BETA-DIKETONE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

BACKGROUND OF THE INVENTION

[0003] 1. Field of the Invention

[0004] The present invention relates to the detection of glucose in samples which may also contain potential interfering compounds, such as a-hydroxy acids or β-diketones.

[0005] 2. Description of the Related Art

[0006] The complexation of carbohydrates, including glucose, with phenylboronic acid has been known for a long time and the reversibility of that interaction has served as a basis for the chromatographic separation of sugars. Specifically, in 1959, Lorand and Edwards reported association constants for aqueous associations of phenylboronic acid with many saturated polyols; binding interactions ranged from very weak (e.g., ethylene glycol, K_d=360 mM) to moderately strong (e.g., glucose, K_d=9.1 mM). See J. Yoon, et al., *Bioorganic and Medicinal Chemistry* 1(4):267-71 (1993). The binding mechanism is believed to occur through bonding of adjacent hydroxyl groups on glucose to hydroxyl groups on a boronate moiety.

[0007] U.S. Pat. No. 5,503,770 (James, et al.) describes a fluorescent boronic acid-containing compound that emits fluorescence of a high intensity upon binding to saccharides, including glucose. The fluorescent compound has a molecular structure comprising a fluorophore, at least one phenylboronic acid moiety and at least one amine-providing nitrogen atom where the nitrogen atom is disposed in the vicinity of the phenylboronic acid moiety so as to interact intramolecularly with the boronic acid. Such interaction thereby causes the compound to emit fluorescence upon saccharide binding. See also T. James, et al., *J. Am. Chem. Soc.* 117(35):8982-87 (1995).

[0008] Additionally, fluorescent sensors using an anthrylboronic acid-containing compound for detecting blood glucose are known in the art. For example, J. Yoon, et al., *J. Am. Chem. Soc.* 114:5874-5875 (1992) describe that anthrylboronic acid can be used as a fluorescent chemosensor for signaling carbohydrate binding, including binding of glucose and fructose.

[0009] Unfortunately, compounds which interact with glucose in the manner described above also have a tendency to interact with other compounds having hydroxyl groups, thus reducing the specificity of a glucose assay, especially when assaying physiological samples which may contain interfering amounts of lactate, acetoacetate, etc. For example, some diabetic patients also develop lactic acidosis, in which blood lactate levels are greater than 5 mmol/liter. Thus, there remains a great need for glucose assays which are relatively insensitive to potentially interfering hydroxyl compounds, such as lactate.

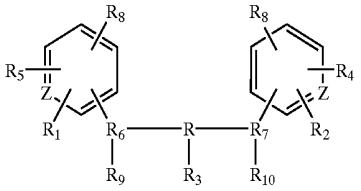
BRIEF SUMMARY OF THE INVENTION

[0010] In one aspect, the present invention is directed to a method for detecting the presence or concentration of glucose in a sample which may also contain an α-hydroxy acid or a β-diketone, which comprises:

[0011] a) exposing the sample to a compound having at least two recognition elements for glucose, oriented such that the interaction between the compound and glucose is more stable than the interaction between the compound and the α-hydroxy acid or β-diketone, said compound also containing a detectable moiety having a detectable quality that changes in a concentration-dependent manner when said compound is exposed to glucose in said sample; and

[0012] b) measuring any change in said detectable quality to thereby determine the presence or concentration of glucose in said sample, wherein the presence of the α-hydroxy acid or the β-diketone does not substantially interfere with said determination.

[0013] In another aspect, the present invention is directed to a compound having the following structure



[0014] wherein:

[0015] R_1 and R_2 are the same or different and are selected from the following: i) hydrogen; ii) a substituent to modify the pKa and hydrolytic stability of the R_8 moiety, iii) a detectable moiety, or iv) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0016] R_3 is hydrogen or a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0017] R_4 and R_5 are the same or different and are selected from the following: i) hydrogen, ii) a substituent to modify the pKa and hydrolytic stability of the R_8 moiety, iii) a detectable moiety, or iv) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0018] each Z is independently carbon or nitrogen;

[0019] R_6 and R_7 are the same or different and are i) linking groups having from zero to ten contiguous or branched carbon and/or heteroatoms, or ii) a linking group capable of attachment to a solid support or a

polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0020] R is selected from the following: i) an aliphatic and/or aromatic spacer containing from 1 to 10 contiguous atoms selected from the group consisting of carbon, oxygen, nitrogen, sulfur and phosphorus, ii) a detectable moiety, or iii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0021] each R_8 is the same or different and is a moiety capable of interaction with the vicinal diol groups present in glucose; and

[0022] R_9 and R_{10} are the same or different, and are i) hydrogen, ii) a detectable moiety, or iii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0023] with the proviso that the indicator compound contains at least one detectable moiety associated therewith, either directly or as part of the solid support or polymeric matrix.

[0024] In another aspect, the present invention is directed to a detection system which comprises a compound described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] **FIG. 1** illustrates the normalized fluorescence emission (I/Io@420 nm) of an indicator as described in Example 1.

[0026] **FIG. 2** illustrates the normalized fluorescence emission (I/Io@428 nm) of an indicator as described in Example 2.

[0027] **FIG. 3** illustrates the normalized fluorescence emission (I/Io@428 nm) of an indicator as described in Example 3.

[0028] **FIG. 4** illustrates the normalized fluorescence emission (I/Io@427 nm) of an indicator as described in Example 4.

[0029] **FIG. 5** illustrates the normalized fluorescence emission (I/Io@540 nm) of an indicator as described in Example 5.

[0030] **FIG. 6** illustrates the absorbance spectra of an indicator as described in Example 6.

[0031] FIGS. **7-8** illustrate the ratio of the absorbance (450 nm/530 nm)of an indicator as described in Example 6.

DETAILED DESCRIPTION OF THE INVENTION

[0032] In one aspect, the present invention provides a way to detect the presence or concentration of glucose in a sample which may also contain interfering compounds, such as α-hydroxy acids or β-diketones. Such potentially interfering compounds include lactate, acetoacetate, β-hydroxy butyric acid, etc.

[0033] The present invention is carried out using an indicator compound which is capable of recognizing glucose in a sample, but which is less likely to recognize interfering compounds in the sample. The indicator compound has at least two recognition elements for glucose, oriented such that the interaction between the indicator compound and glucose is more stable than the interaction between the indicator compound and the interfering compounds.

[0034] Suitable recognition elements include moieties which are capable of a preferably reversible interaction with glucose, especially with the diol groups present in glucose. Several such recognition elements are known, and preferably include boronic acid, boronate ion, arsenious acid, arsenite ion, telluric acid, tellurate ion, germanic acid, germanate ion, etc. Most preferred are recognition elements containing boron.

[0035] The recognition elements are preferably spaced on the indicator compound a suitable distance from each other so as to allow at least two of the recognition elements to interact with a glucose molecule, resulting in increased specificity. In general, the recognition elements may have a spacer of up to about 30 atoms between them. Preferably, the recognition elements are oriented such that they are capable of being about 6 Å apart when interacting with glucose.

[0036] The indicator compounds of the present invention have a detectable quality that changes in a concentration-dependent manner when the compound is exposed to a sample containing glucose. Many such qualities are known and may be used in the present invention. For example, the indicator compound may include a luminescent (fluorescent or phosphorescent) or chemiluminescent moiety, an absorbance based moiety, etc. The indicator compound may include an energy donor moiety and an energy acceptor moiety, each spaced such that there is a detectable change when the indicator compound interacts with glucose. The indicator compound may include a fluorophore and a quencher, configured such that the fluorophore is quenched by the quencher when glucose is absent. In that situation, when glucose is present, the indicator undergoes a conformational change which causes the quencher to move sufficiently distant from the fluorophore so that fluorescence is emitted. Conversely, the fluorophore and quencher may be configured such that in the absence of glucose, they are sufficiently separated and the fluorophore emits fluorescence; upon interaction with glucose, the fluorophore and quencher are moved in sufficient proximity to cause quenching. The conformational change concept is described in more detail in our co-pending application Serial No. filed concurrently herewith, entitled "Detection of Analytes", incorporated herein by reference.

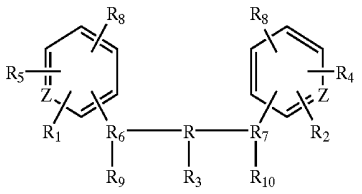
[0037] Other detectable moieties include those whose fluorescence is affected by glucose interaction via photoinduced electron transfer or inductive effects. These include the lanthanide chelates disclosed in copending U.S. application Ser. No. 09/265,979 filed Mar. 11, 1999 (and published as PCT International Application WO 99/46600 on Sep. 16, 1999), incorporated herein by reference; polyaromatic hydrocarbons and their derivatives; coumarins; BoDiPy; dansyl; catechols; etc. Another class of moieties include those whose absorbance spectrum changes upon interaction of the indicator compound with glucose, including Alizarin Red, etc. Another class of moieties include those whose fluorescence is modulated by proximity effects, e.g., energy donor/acceptor pairs such as dansyl/dabsyl, etc.

[0038] Preferably, the detectable quality is a detectable spectral change, such as changes in absorptive characteristics (e.g., absorbtivity and/or spectral shift), in fluorescent decay time (determined by time domain or frequency domain measurement), fluorescent intensity, fluorescent anisotropy or polarization; a spectral shift of the emission spectrum; a change in time-resolved anisotropy decay (determined by time domain or frequency domain measurement), etc.

[0039] The indicator compounds of the present invention, if soluble, may be used directly in solution if so desired. On the other hand, if the desired application so requires, the indicator compounds may be immobilized (such as by mechanical entrapment or covalent or ionic attachment) onto or within an insoluble surface or matrix such as glass, plastic, polymeric materials, etc. When the indicator compound is entrapped within, for example, another polymer, the entrapping material preferably should be sufficiently permeable to glucose to allow suitable interaction between glucose and the indicator compound.

[0040] If the indicator compounds are sparingly soluble or insoluble in water, yet detection in an aqueous medium is desired, the indicator compound may be co-polymerized with a hydrophilic monomer to form a hydrophilic macromolecule as described in co-pending U.S. application Ser. No. 09/632,624, filed Aug. 4, 2000, the contents of which are incorporated herein by reference.

[0041] Preferred indicator compounds have the following structure:



[0042] wherein:

[0043] R_1 and R_2 are the same or different and are selected from the following: i) hydrogen; ii) a substituent to modify the pKa and hydrolytic stability of the R_8 moiety, iii) a detectable moiety, or iv) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0044] R_3 is hydrogen or a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0045] R_4 and R_5 are the same or different and are selected from the following: i) hydrogen, ii) a substituent to modify the pKa and hydrolytic stability of the R_8 moiety, iii) a detectable moiety, or iv) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0046] each Z is independently carbon or nitrogen;

[0047] R_6 and R_7 are the same or different and are i) linking groups having from zero to ten contiguous or branched carbon and/or heteroatoms, or ii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0048] R is selected from the following: i) an aliphatic and/or aromatic spacer containing from 1 to 10 contiguous atoms selected from the group consisting of carbon, oxygen, nitrogen, sulfur and phosphorus, ii) a detectable moiety, or iii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0049] each R_8 is the same or different and is a moiety capable of interaction with the vicinal diol groups present in glucose; and

[0050] R_9 and R_{10} are the same or different, and are i) hydrogen, ii) a detectable moiety, or iii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

[0051] with the proviso that the indicator compound contains at least one detectable moiety associated therewith, either directly or as part of the solid support or polymeric matrix.

[0052] Suitable groups for modifying the pKa and hydrolytic stability of the R_8 moieties would be readily apparent to one of ordinary skill, and include groups such as halogen; nitro; amino; halogen substituted alkyl; optionally substituted carboxyl; acyl; keto; nitrile; amide; ester; alkoxy; etc. Suitable linking groups include alkyl; aryl; acyl; polyamide; polyether; all optionally substituted, and combinations thereof.

[0053] It will be understood that when any of the substituents is a detectable moiety, that could also include suitable linking groups which link the detectable moiety to the rest of the indicator compound. Suitable linking groups include those listed above in the prior paragraph. Suitable detectable moieties include those defined above.

[0054] R_8 is preferably selected from the group consisting of boronic acid, boronate ion, arsenious acid, arsenite ion, telluric acid, tellurate ion, germanic acid, germanate ion, and combinations thereof.

[0055] It will also be understood from the above definition that the present compounds and detection systems may be in polymeric form. Thus, an integral compound (containing recognition elements and detectable moiety) could be linked to an existing polymer, or the integral compound in monomeric form could be polymerized or co-polymerized with another suitable monomer to form a polymer. Alternatively, two separate monomeric components (e.g., one containing the recognition elements, and one containing a detectable moiety) could be co-polymerized so that the resulting polymer contains all necessary elements of the system (see Example 6).

[0056] Many uses exist for the indicator compounds of the present invention, including uses as indicators in the fields of energy, medicine and agriculture. For example, the indicator compounds can be used to detect sub-levels or supra-

levels of glucose in physiological buffers or fluids, such as blood, plasma, serum, interstitial fluid, cerebrospinal fluid, urine, saliva, intraocular fluid, lymph, tears, or sweat, thus providing valuable information for diagnosing or monitoring such diseases as diabetes and adrenal insufficiency.

[0057] Medical/pharmaceutical production of glucose for human therapeutic application requires monitoring and control.

[0058] Uses for the present invention in agriculture include detecting levels of glucose in soybeans and other agricultural products. Glucose must be carefully monitored in critical harvest decisions for such high value products as wine grapes. As glucose is the most expensive carbon source and feedstock in fermentation processes, glucose monitoring for optimum reactor feed rate control is important in power alcohol production. Reactor mixing and control of glucose concentration also is critical to quality control during production of soft drinks and fermented beverages, which consumes the largest amounts of glucose and fermentable (vicinal diol) sugars internationally.

[0059] When the indicator compounds incorporate fluorescent indicator substituents, various detection techniques also are known in the art. For example, the compounds of the invention can be used in fluorescent sensing devices (e.g., U.S. Pat. No. 5,517,313) or can be bound to polymeric material such as test paper for visual inspection. This latter technique would permit, for example, glucose measurement in a manner analogous to determining pH with a strip of litmus paper. The compounds described herein may also be utilized as simple reagents with standard benchtop analytical instrumentation such as spectrofluorometers or clinical analyzers as made by Shimadzu, Hitachi, Jasco, Beckman and others. These molecules would also provide analyte specific chemical/optical signal transduction for fiber optic-based sensors and analytical fluorometers as made by Ocean Optics (Dunedin, Fla.), or Oriel Optics.

[0060] U.S. Pat. No. 5,517,313, the disclosure of which is incorporated herein by reference, describes a fluorescence sensing device in which the compounds of the present invention can be used to determine the presence or concentration of glucose in a liquid medium. The sensing device comprises a layered array of a fluorescent indicator molecule-containing matrix (hereafter "fluorescent matrix"), a high-pass filter and a photodetector. In this device, a light source, preferably a light-emitting diode ("LED"), is located at least partially within the indicator material, or in a waveguide upon which the indicator matrix is disposed, such that incident light from the light source causes the indicator molecules to fluoresce. The high-pass filter allows emitted light to reach the photodetector, while filtering out scattered incident light from the light source. The fluorescence of the indicator molecules employed in the device described in U.S. Pat. No. 5,517,313 is modulated, e.g., attenuated or enhanced, by the local presence of glucose.

[0061] In the sensor described in U.S. Pat. No. 5,517,313, the material which contains the indicator molecule is permeable to the analyte. Thus, the analyte can diffuse into the material from the surrounding test medium, thereby affecting the fluorescence emitted by the indicator compounds. The light source, indicator compound-containing material, high-pass filter and photodetector are configured such that at least a portion of the fluorescence emitted by the indicator compounds impacts the photodetector, generating an electrical signal which is indicative of the concentration of glucose in the surrounding medium.

[0062] In accordance with other possible embodiments for using the indicator compounds of the present invention, sensing devices also are described in U.S. Pat. Nos. 5,910,661, 5,917,605 and 5,894,351, all incorporated herein by reference.

[0063] The compounds of the present invention can also be used in an implantable device, for example to continuously monitor blood glucose levels in vivo. Suitable devices are described in, for example, co-pending U.S. patent application Ser. No. 09/383,148 filed Aug. 26, 1999, as well as U.S. Pat. Nos. 5,833,603, 6,002,954 and 6,011,984, all incorporated herein by reference.

[0064] The compounds of the present invention can be prepared by persons skilled in the art without an undue amount of experimentation using readily known reaction mechanisms and reagents, for example including reaction mechanisms which are consistent with the general procedures described below.

EXAMPLE 1

[0065] Water Soluble Copolymer of Anthracene Derivative and MAPTAC

[0066] I. Synthesis of Mono-Boronate-Anthracene Indicator Co-Polymerized in Water-Soluble Polymer:

[0067] A. 9-[3-(methacrylamido)propylamino]methylanthracene

[0068] To a suspension of N-(3-aminopropyl)methacrylamide hydrochloride salt (11.82 g, 66.0 mmole, 3.0 equiv.) and DBMP (10 mg as inhibitor) in 250 mL $CHCl_3$ at 0° C. was added dropwise DIEA (18.5 g, 25.0 mL, 144 mmole, 6.5 equiv.) over a 20 min period. The mixture was allowed to warm to 25° C. and then recooled to 0° C. To the cooled mixture was added dropwise a solution of 9-chloromethylanthracene (5.0 g, 22 mmole) in $CHCl_3$ (100 mL) over a 1 h period. The mixture was subsequently stirred at 25° C. for 1 h, 50° C. for 12 h and then 70° C. for 2 hours. At this time, the mixture was washed with 4×60 mL portions of water, and the combined aqueous layers were extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, decanted and concentrated in vacuo. The crude material was purified by silica gel chromatography (flash silica gel, 2-5% CH_3OH/CH_2Cl_2) to yield 2.44 g (33%) of a solid product.

[0069] TLC: Merck silica gel 60 plates, Rf 0.39 with 90/10 CH_2Cl_2/CH_3OH, see with UV (254/366), ninhydrin stain.

[0070] B. 9-[N-[2-(5,5-dimethylborinan-2-yl)benzyl]-N-[3-(methacrylamido)propylamino]methylanthracene.

[0071] To a solution of 9-[3-(methacrylamido)propylamino]-methylanthracene (2.44 g, 7.34 mmole) and DBMP (10 mg as inhibitor) in 200 mL $CHCl_3$ at 0° C. was added DIEA (2.85 g, 3.84 mL, 22.0 mmole, 3.0 equiv.) in portions over a 10 min period, followed by the dropwise addition of a solution of (2-bromomethylphenyl)boronic acid neopentyl ester (2.49 g, 8.81 mmole, 1.2 equiv.) over a 30 min period. The mixture was subsequently stirred at 25° C. for 20 hours. At this time, the mixture was washed with water, and the

combined aqueous layers were extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, decanted and concentrated in vacuo. The crude material was purified by silica gel chromatography (flash silica gel, 2-5% CH_3OH/CH_2Cl_2) to yield 2.50 g (76%) of a lightly yellow crystalline solid.

[0072] Mp: 72-73° C.

[0073] TLC: Merck silica gel 60 plates, Rf 0.36 with 90/10 CH_2Cl_2/CH_3OH, see with UV (254/366), ninhydrin stain.

[0074] C. Water Soluble Copolymer of 9-[N-[2-(5,5-dimethyl-borinan-2-yl)benzyl]-N-[3-(methacrylamido)propylamino]-methylanthracene and MAPTAC (1:20 molar ratio).

[0075] To a solution of 9-[N-[2-(5,5-dimethylborinan-2-yl)-benzyl]-N-[3-(methacrylamido)propylamino]methylanthracene (0.0490 g, 0.105 mmole) and [3-(methacrylamido)propyl]-trimethylammonium chloride (MAPTAC, 50 wt % aqueous solution, 0.48 g, 0.90 mL, 2.1 mmole, 20 equiv.) in 1.5 mL ethylene glycol was added 4,4'-azobis(cyanovaleric acid) (0.008 g, 0.03 mmole, 1.4 mole % of total monomer). The solution was purged with argon gas for 5 min and then heated to 60° C. in the dark for 18 hours. At this time, the viscous solution was cooled to 25° C., diluted with 5 mL water and dialyzed through a cellulose acetate membrane (MWCO 3500) against 3×4 L of water. The dialyzed material was concentrated to dryness to yield 0.339 g (68%) of a yellow glassy solid.

[0076] II. Modulation of Fluorescence with Glucose and Lactate

[0077] The modulation of the fluorescence of the copolymer (which contains a single recognition element) prepared in this example by glucose and lactate was determined. **FIG. 1** shows the normalized fluorescence emission (I/Io@420 nm) of 0.5 mg/mL solutions of the copolymer (1:20 molar ratio) in PBS containing a) 0-20 mM glucose; b) 0-20 mM lactate. Spectra were recorded using a Shimadzu RF-5301 spectrafluorometer with excitation@365 nm; excitation slits at 1.5 nm; emission slits at 5 nm; ambient temperature. Error bars are standard deviation with duplicate values for each data point. The fluorescence of the copolymer was affected by the presence of glucose and lactate.

EXAMPLE 2

[0078] Modulation of Bis-Boronate-Indicator Covalently Attached to Water-Soluble Polymer by Glucose and Potential Physiological Interferences.

[0079] I. Synthesis of Single-Methacrylate Monomer of Bis-Boronate-Anthracene Indicator

[0080] A. 9,10-bis[[2-(2-hydroxyethoxy)ethylamino]methyl]-anthracene.

[0081] To a solution of 2-(2-aminoethoxy)ethanol (31.4 g, 30.0 mL, 299 mmole, 20.9 equiv.) in 40 mL $CHCl_3$ at 23° C. was added 9,10-bis(chloromethyl)anthracene (3.94 g, 14.3 mmole). The solution was stirred in the dark for 67 hours. At this time, added 100 mL CH_2Cl_2 and washed with 1×50 mL and 2×100 mL portions of $NaHCO_3$ (saturated aqueous solution). The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated to yield 4.67 g (79%) of a yellow powder. Product (~85% pure by RP-HPLC) was carried on as is.

[0082] HPLC conditions: HP 1100 HPLC chromatograph, Vydac 201TP 10×250 mm column, 0.100 mL injection, 2 mL/min, 370 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100%B 2 min, retention time 15.6 min.

[0083] B. 9,10-bis[N-[2-(5,5-dimethylborinan-2-yl)benzyl]-N-[2-(2-hydroxyethoxy)ethylamino]methyl]anthracene.

[0084] A solution of 9,10-bis[[2-(2-hydroxyethoxy)-ethylamino]methyl]anthracene (4.02 g, 9.75 mmole), DIEA (12.6 g, 17.0 mL, 97.5 mmole, 10.0 equiv.) and (2-bromomethylphenyl)boronic acid neopentyl ester (13.7 g, 48 mmole, 4.9 equiv.) in 125 mL $CHCl_3$ at 23° C. was stirred in the dark for 46 hours. At this time, the reaction mixture was concentrated initially by rotary evaporation, then using a vacuum pump to remove the DIEA. The residue was purified by alumina column chromatography (150 g activated neutral alumina, 0-3% CH_3OH/CH_2Cl_2) to yield 5.67 g (70%) of a viscous oil which solidified upon standing. Product (~85 % pure by RP-HPLC) was carried on as is.

[0085] TLC: Merck basic alumina plates, Rf 0.33 with 95/5 CH_2Cl_2/CH_3OH, see with UV (254/366)

[0086] HPLC conditions: HP 1100 HPLC chromatograph, Vydac 201TP 10×250 mm column, 0.100 mL injection, 2 mL/min, 370 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 18.8 min.

[0087] C. 9-[N-[2-(5,5-dimethylborinan-2-yl)benzyl]-N-[2-(2-methacroyloxyethoxy)ethylamino]methyl]-10-[N-[2-(5,5-dimethylborinan-2-yl)benzyl]-N-[2-(2-hydroxyethoxy-)ethylamino]-methyl]anthracene.

[0088] A solution of 9,10-bis[N-[2-(5,5-dimethyl-borinan-2-yl)benzyl]-N-[2-(2-hydroxyethoxy)ethylamino]-methyl] anthracene (0.298 g, 0.359 mmole), methacrylic acid (0.304 g, 0.300 mL, 3.53 mmole, 9.84 equiv.), DCC (0.965 g, 4.68 mmole, 13.0 equiv.) and N,N-dimethyl-aminopyridine (0.020 g, 0.16 mmole, 0.46 equiv.) in 15 mL CH_2Cl_2 at 23° C. was stirred in the dark for 4 hours. At this time, the reaction mixture was filtered and concentrated by rotary evaporation. The residue was purified by alumina column chromatography (50 g activated neutral alumina, 0-4% CH_3OH/CH_2Cl_2) to yield 0.150 g (47%) of a yellow solid.

[0089] FAB MS: Calc'd for $C_{52}H_{66}B_2N_2O_9$ $[M]^+$885; Found $[M+1]^+$886.

[0090] TLC: Merck basic alumina plates, Rf 0.45 with 95/5 CH_2Cl_2/CH_3OH, see with UV (254/366).

[0091] HPLC: HP 1100 HPLC chromatograph, Vydac 201TP 10×250 mm column, 0.100 mL injection, 2 mL/min, 370 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 21 min.

[0092] D. Water Soluble Copolymer of 9-[N-[2-(5,5-dimethyl-borinan -2-yl)benzyl]-N-[2-(2-methacroyloxyethoxy) ethyl-amino]methyl]-10-[N-[2-(5,5-dimethylborinan-2-yl benzyl]-N-[2-(2-hydroxyethoxy)ethylamino]-methyl]anthracene and TMAMA (1:50 molar ratio).

[0093] To a solution of [2-(methacryloxy)ethyl]trimethylammonium chloride (TMAMA, 70 wt % aqueous solution, 0.344 g monomer, 1.66 mmole, 50 equiv.) in 0.600 mL water was added a solution of 9-[N-[2-(5,5-dimethylborinan-2-yl)-benzyl]-N-[2-(2-methacroyloxyethoxy)ethylamino]methyl]-10-[N-[2-(5,5-dimethylborinan-2-yl)benzyl]-N-[2-(2-hydroxyethoxy)ethylamino]methyl]anthracene (0.0024 g, 0.0033 mmole) in 3.00 mL MeOH. To this mixture was added 4,4'-azobis(4-cyanovaleric acid) (0.0075 g, 0.027 mmole, 1.6 mole % of total monomer). The solution was filtered through a 0.45μ membrane filter, was purged with nitrogen gas and then heated in the dark at 55° C. for 16 hours. At this time, the viscous solution was cooled to 25° C. and concentrated in vacuo. The residue was diluted with 20 mL water and filtered through a 0.2μ membrane filter. The polymer solution was dialyzed through a cellulose acetate membrane (MWCO 3500) against 2×4 L of water. From the dialysis was obtained 38.5 mL of polymer solution. Concentration of a portion of this solution to dryness indicated 0.0075 g polymer per 1.0 mL solution. Overall 0.289 g (77%) yield of polymer.

[0094] II. Modulation of Fluorescence with Glucose, Lactate and Acetoacetate

[0095] The modulation of the fluorescence of the copolymer (which contains two recognition elements) prepared in this example by glucose, lactate and acetoacetate was determined. **FIG. 2** shows the normalized fluorescence emission (I/Io@428 nm) of a 1.5 mg/mL solution of anthracene bis boronate-TMAMA (1:50 mole ratio) copolymer in PBS containing a) 0-20 mM glucose; b) 0-20 mM lactate; c) 0-20 mM lithium acetoacetate. Spectra were recorded using a Shimadzu RF-5301 spectrafluorometer with excitation@365 nm; excitation slits at 1.5 nm; emission slits at 1.5 nm; ambient temperature. The fluorescence of the copolymer was affected by the presence of glucose, but not by the presence of lactate or acetoacetate.

EXAMPLE 3

[0096] Effect of Lactate in Solution on the Dose Response Effect of Glucose on the Fluorescence of Bis-Boronate-Anthracene Indicator

[0097] A. 9,10-bis[[2-(tert-butoxycarbonyl)ethylamino]methyl]-anthracene.

[0098] A solution of β-alanine tert-butyl ester hydrochloride (3.06 g, 16.8 mmole, 5.09 equiv.), DIEA (4.27 g, 5.75 mL, 33.0 mmole, 10.00 equiv.) and 9,10-bis(chloromethyl)anthracene (0.910 g, 3.31 mmole) in 75 mL $CHCl_3$ at 23° C. was stirred in the dark for 93 hours. The solution was filtered and washed with 1×40 mL and 2×60 mL portions of $NaHCO_3$ (saturated aqueous solution) The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated to yield a crude yellow solid. The residue was purified by silica gel column chromatography (30 g gravity grade gel, 0-3% CH_3OH/CH_2Cl_2) to yield 1.06 g (65%) of a viscous yellow-orange oil. Product was carried on as is.

[0099] TLC: Merck silica gel 60 plates, Rf 0.33 with 95/5 CH_2Cl_2/CH_3OH, see with UV (254/366).

[0100] B. 9,10-bis[N-[2-(5,5-dimethylborinan-2-yl)benzyl]-N-[2-(tert-butoxycarbonyl)ethylamino]methyl]anthracene.

[0101] A solution of 9,10-bis[[2-(tert-butoxycarbonyl)-ethylamino]methyl]anthracene (1.60 g, 3.25 mmole), DIEA (4.45 g, 6.00 mL, 34.4 mmole, 10.6 equiv.) and (2-bromomethylphenyl)boronic acid neopentyl ester (4.80 g, 17.0 mmole, 5.22 equiv.) in 30 mL $CHCl_3$ at 23° C. was stirred in the dark for 4.5 days. At this time, 45 mL $CHCl_3$ were added to the mixture and the solution was washed with 2×25 mL portions of $NaHCO_3$ (saturated aqueous solution). The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated to yield a crude reddish oil. The residue was purified by alumina column chromatography (100 g activated neutral alumina, 0-3% CH_3OH/CH_2Cl_2) to yield ~3.5 g of an orange solid. The product was dissolved, followed by the formation of a white precipitate (DIEA-HBr salt). The solution was filtered and the filtrate concentrated to yield 2.72 g (93%) of an orange solid. Product (>80% pure by RP-HPLC) was carried on as is.

[0102] TLC: Merck basic alumina plates, Rf 0.66 with 95/5 CH_2Cl_2/CH_3OH, see with UV (254/366).

[0103] HPLC conditions: HP 1100 HPLC chromatograph, Vydac 201TP 10×250 mm column, 0.100 mL injection, 2 mL/min, 370 nm detection, A=water (0.1% HFBA) and B=MECN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 23.9 min.

[0104] C. 9,10-bis[N-(2-boronobenzyl)-N-(3-(propanoyl)amino]-methyl)-anthracene.

[0105] A solution of 9,10-bis[N-[2-(5,5-dimethylborinan-2-yl)benzyl]-N-[2-(tert-butoxycarbonyl)ethylamino]methyl]an thracene (0.556 g, 0.620 mmole) in 5 mL 20% TFA/CH_2Cl_2 at 23° C. was stirred in the dark for 25 hours. At this time, the reaction mixture was concentrated under a stream of N_2 gas. The residue was triturated with 3×10 mL portions of ether. The residual solid was dried in vacuo to yield 0.351 g (87%) of a fluffy yellow powder.

[0106] FAB MS: Glycerol matrix; Calcd for $C_{40}H_{46}B_2N_2O_{10}$ (bis glycerol adduct) $[M]^+$760; Found $[M]^+$ 760.

[0107] HPLC: HP 1100 HPLC chromatograph, Waters 5×100 mm NovaPak HR C18 column, 0.025 mL injection, 0.75 mL/min, 1.5 mL injection loop, 360 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 16.7 min.

[0108] D. Modulation of Fluorescence with Glucose and Lactate

[0109] The modulation of the fluorescence of the indicator compound (which contains two recognition elements) prepared in this example by glucose and lactate was determined. **FIG. 3** shows the fluorescence (at 428 nm) of 75 μM solutions of bis carboxylate bis-boronate-anthracene indicator in PBS containing a) 0-10 mM glucose, 0 mM lactate; b) 0-10 mM glucose, 2 mM lactate; c) 0-10 mM glucose, 5 mM lactate. Spectra were recorded using a Shimadzu RF-5301 spectrafluorometer with excitation@365 nm; excitation slits at 1.5 nm; emission slits at 1.5 nm; ambient temperature. All points measured in triplicate, with ±1 SD error bars included. The presence of lactate did not substantially affect the fluorescence modulation of the indicator by glucose.

EXAMPLE 4

[0110] Selectivity of Bis-Boronate Glucose Indicator for Glucose vs. Lactate and Acetoacetate when Indicator Covalently Immobilized in the Hydrogel

[0111] Preparation of N,N-dimethylacrylamide Hydrogel with Glucose Indicator:

[0112] A solution of N,N-dimethylacrylamide (40% wt.) and N,N'-methylenebisacrylamide (0.8% wt.) in ethylene glycol was prepared. 9,10-bis[N-[2-(5,5-dimethylborinan-2-yl)-benzyl]-N-[3-(methacrylamido)propylamino]methylanthracene (17.8 mg, 2×10^{-5} mole) and 40 μL of aqueous ammonium persulfate (5% wt) were combined with 1 mL of ethylene glycol monomer solution. The resulting solution was placed in a glove box purged with nitrogen. An aqueous solution of N,N,N',N'-tetramethylethylenediamine (80 μL, 5% wt.) was added to the monomer formulation to accelerate polymerization. The resulting formulation was poured in a mold constructed from microscope slides and 100 micron stainless steel spacer. After being kept for 8 hours in nitrogen atmosphere the mold was placed in phosphate buffered saline (PBS) (10 mM PBS, pH=7.4), the microscope slides were separated, and the hydrogel was removed. The hydrogel was washed with 100 mL of PBS containing 1 mM lauryl sulfate sodium salt and 1 mM EDTA sodium salt for 3 days, the solution being changed every day, followed by washing with DMF/PBS (10/90 by vol., 3×100 mL), and finally with PBS (pH=7.4, 3×100 mL). The resulting hydrogel polymer was stored in PBS (10 mM PBS, pH=7.4) containing 0.2% wt. sodium azide and 1 mM EDTA sodium salt.

[0113] Modulation of Fluorescence with Glucose, Lactate and Acetoacetate

[0114] The modulation of the fluorescence of the indicator compound (which contains two recognition elements) prepared in this example by glucose, lactate and acetoacetate was determined. **FIG. 4** shows the normalized fluorescence emission (I/Io@427 nm) of a hydrogel containing the glucose recognition molecule of this example in 10 mM PBS, pH 7.4 containing 0.2% NaN_3 and 1 mM EDTA containing various amounts of sodium-L-lactate, lithium acetoacetate or a-D-glucose. Data were recorded using a Shimadzu RF-5301 spectrofluorometer with excitation@365 nm (slit 3 nm) and emission at 427 nm (slit=3 nm) at low sensitivity at 37° C. using a temperature controlled sample holder. The cuvettes containing 3 mL of the desired solution were equilibrated at 37° C. for 15 minutes before measurement. Each hydrogel sample was measured in four independent samples. Error bars are standard deviation with quadruplicate values for each data point. The hydrogels containing a glucose recognition molecule were prepared as previously described. The hydrogels were mounted on glass slides and covered with polyester mesh in PMMA cuvettes at 450 to the incident light. Solutions of 1, 5, 10 and 20 mM sodium L-lactate [Aldrich], 5, 10 and 20 mM lithium acetoacetate [Aldrich], and 1, 2, 4, 5, 10, and 20 mM α-D-glucose were prepared in 10 mM PBS, pH 7.4 containing 0.2% NaN_3 and 1 mM EDTA. The fluorescence of the copolymer was affected by the presence of glucose, but not by the presence of lactate or acetoacetate.

EXAMPLE 5

[0115] Glucose Selectivity vs. Lactate Using Bis-Boronate Recognition and Proximity Quenching Signal Generation

[0116] A. N-(2,2-diethoxyethyl)-4-bromo-1,8-naphthalimide.

[0117] A suspension of 4-bromo-1,8-naphthalic anhydride (10.0 g, 36.1 mmol) and aminoacetaldehyde diethyl acetal (4.81 g, 5.26 mL, 36.1 mmol, 1 equiv.) in 45 mL EtOH was stirred at 45° C. for 3 days. At this time, the resulting suspension was filtered, washing with EtOH and the residue was dried to yield 13.3 g (94%) of a light brown solid product.

[0118] TLC: Merck silica gel 60 plates plates, Rf 0.17 with 98/2 CH_2Cl_2/CH_3OH, see with UV (254/366).

[0119] HPLC: HP 1100 HPLC chromatograph, Waters 5×100 mm NovaPak HR C18 column, 0.050 mL injection, 0.75 mL/min, 1.5 mL injection loop, 360 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 24.2 min.

[0120] B. N-(2,2-diethoxyethyl)-4-butylamino-1,8-naphthalimide.

[0121] A solution of N-(2,2-diethoxyethyl)-4-bromo-1,8-naphthalimide (0.797 g, 2.03 mmol) and n-butylamine (1.48 g, 2.00 mL, 20.2 mmol, 9.96 equiv.) in 8 mL NMP was heated at 45° C. for 66 hours. At this time, the resulting suspension was allowed to cool to 25° C., followed by filtration. The residue was dissolved with 50 mL ether and extracted 3×50 mL water. The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated to yield a crude yellow powder. The crude material was purified by silica gel chromatography (25 g gravity grade gel, 0-1% CH_3OH/CH_2Cl_2) to yield 0.639 g (82%) of a yellow powder.

[0122] TLC: Merck silica gel 60 plates, Rf 0.71 with 95/5 CH_2Cl_2/CH_3OH, see with UV (254/366).

[0123] HPLC: HP 1100 HPLC chromatograph, Waters 5×100 mm NovaPak HR C18 column, 0.050 mL injection, 0.75 mL/min, 1.5 mL injection loop, 450 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 23.5 min.

[0124] C. N-(2-oxoethyl)-4-butylamino-1,8-naphthalimide.

[0125] A solution of N-(2,2-diethoxyethyl)-4-butylamino-1,8-naphthalimide (0.622 g, 1.62 mmol) and p-toluenesulfonic acid mono hydrate (0.010 g, 0.053 mmol, 0.032 equiv.) in 25 mL acetone was stirred at 25° C. for 18 hours. At this time, the solution was concentrated and the residue purified by silica gel chromatography (25 g gravity grade gel, 0-1% CH_3OH/CH_2Cl_2) to yield 0.470 g (94%) of an orange solid.

[0126] TLC: Merck silica gel 60 plates, Rf 0.61 with 95/5 CH_2Cl_2/CH_3OH, see with UV (254/366).

[0127] ^1H NMR (400 MHz, $CDCl_3$); δ1.03 (t, 3H, J=7.3 Hz), 1.53 (m, 2H), 1.78 (m, 2H), 3.38 (t, 2H, J=7.2 Hz), 5.02 (s, 2H), 6.64 (d, 1H, J=8.6 Hz), 7.52 (dd, 1H, J=7.4, 8.3 Hz), 8.08 (dd, 1H, J=1 Hz, 8.5 Hz), 8.38 (d, 1H, J=8.3 Hz), 8.46 (dd, 1 H, J=1.0, 7.3 Hz), 9.75 (s, 1H)

[0128] HPLC: HP 1100 HPLC chromatograph, Waters 5×100 mm NovaPak HR C18 column, 0.050 mL injection, 0.75 mL/min, 1.5 mL injection loop, 450 nm detection,

A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 19.6 min.

[0129] D. N-(4-dimethylaminobenzyl)-1,6-diaminohexane.

[0130] A suspension of 4-dimethylaminobenzaldehyde (1.00 g, 6.70 mmol), Na_2SO_4 (6.70 g, 47.2 mmol, 7.04 equiv.) and 1,6-diaminohexane (3.89 g, 33.5 mmol, 5.00 equiv.) in 20 mL anhydrous EtOH was stirred in the dark at 25° C. under an atmosphere of nitrogen gas for 18 hours. At this time, the solution was filtered and $NaBH_4$ (1.73 g, 45.8 mmol, 6.84 equiv.) was added to the filtrate. The suspension was stirred at 25° C. for 5 hours. At this time, the reaction mixture was concentrated and the residue dissolved in 50 mL water and extracted 3×50 mL ether. The combined organic extracts were washed 2×50 mL water. The combined aqueous extracts were extracted 2×50 mL ether. The combined organic extracts were dried over Na_2SO_4, filtered and concentrated to yield 1.35 g (81%) of a viscous oil.

[0131] TLC: Merck silica gel 60 plates plates, Rf 0.58 with 80/15/5 $CH_2Cl_2/CH_3OH/iPrNH_2$, see with ninhydrin stain, UV (254/366).

[0132] HPLC: HP 1100 HPLC chromatograph, Waters 5×100 mm NovaPak HR C18 column, 0.050 mL injection, 0.75 mL/min, 1.5 mL injection loop, 280 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 13.3 min.

[0133] E. N-2-[5-(N-4-dimethylaminobenzyl)aminohexyl]amino-ethyl) -4-butylamino-1,8-naphthalimide.

[0134] To a suspension of N-(2-oxoethyl)-4-butylamino-1,8-naphthalimide (0.346 g, 1.11 mmol) in 25 mL anhydrous MeOH was added a solution of N-(4-dimethylamino-benzyl) -1,6-diaminohexane (0.554 g, 2.22 mmol, 2.00 equiv.) and acetic acid (0.067 g, 1.1 mmol, 1.0 equiv.) in 20 mL anhydrous MeOH. To this mixture was added a solution of $NaCNBH_3$ (0.070 g, 1.1 mmol, 1.0 equiv.) in 5 mL anhydrous MeOH. The reaction mixture was stirred at 25° C. for 15 hours. At this time, the MeOH was removed by rotary evaporation and the reidue was dissolved in 30 mL water. The solution was adjusted to pH 2 with 1 N HCl and then stirred for 1 hour at 25° C. At this time, the solution was adjusted to pH 12 with 1 N NaOH and subsequently extracted 3×50 mL CH_2Cl_2. The combined organic extracts were washed 3×50 mL water, dried over anhydrous Na_2SO_4, filtered and concentrated to yield a crude brown oil. The crude material was purified by silica gel chromatography (35 g flash grade gel, 0-50% CH_3OH/CH_2Cl_2, then 45/50/5 $CH_3OH/CH_2Cl_2/iPrNH_2$) to yield 0.190 g (32%) of diamine product.

[0135] FAB MS: Calc'd for $C_{33}H_{45}N_5O_2$ $[M]^+$544; Found $[M]^+$ 544.

[0136] TLC: Merck silica gel 60 plates, Rf 0.42 with 80/20 CH_2Cl_2/CH_3OH, see with ninhydrin stain and UV (254/366).

[0137] HPLC: HP 1100 HPLC chromatograph, Waters 5×100 mm NovaPak HR C18 column, 0.050 mL injection, 0.75 mL/min, 1.5 mL injection loop, 450 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 17.6 min.

[0138] F. N-2-[5-(N-4-dimethylaminobenzyl)-5-[2-(5,5-dimethylborinan-2-yl)benzyl]aminohexyl]-[2-(5,5-dimethyl-borinan-2-yl)benzyl]aminoethyl-4-butylamino-1,8-naphthalimide.

[0139] To a solution of N-2-[5-(N-4-dimethylaminobenzyl)-aminohexyl]aminoethyl)-4-butylamino-1,8-naphthalimide (0.150 g, 0.276 mmole) and DIEA (0.355 g, 0.478 mL, 2.81 mmole, 10.0 equiv.) in 5 mL $CHCl_3$ was added a solution of (2-bromomethylphenyl)boronic acid neopentyl ester (0.390 g, 1.38 mmole, 5.00 equiv.) in 2 mL $CHCl_3$. The solution was subsequently stirred at 25° C. for 27 hours. At this time, the mixture was concentrated and the residue was purified by alumina column chromatography (100 g activated neutral alumina, 0-5% CH_3OH/CH_2Cl_2) to yield 0.024 g (19%) of a viscous brown oil.

[0140] FAB MS (glycerol matrix): Calc'd for $C_{53}H_{67}B_2N_5O_8$ $[M]^+$924 (bis glycerol adduct in place of bis neopentyl ester of boronic acids); Found $[M]^+$924.

[0141] TLC: Merck neutral alumina plates, Rf 0.62 with 80/20 CH_2Cl_2/CH_3OH, see with UV (254/366).

[0142] HPLC: HP 1100 HPLC chromatograph, Waters 5×100 mm NovaPak HR C18 column, 0.050 mL injection, 0.75 mL/min, 1.5 mL injection loop, 450 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 20.7 min.

[0143] G. N-2-[5-(N-4-dimethylaminobenzyl)-5-[2-(borono)benzyl]aminohexyl]-[2-(borono)benzyl]amino-ethyl -4-butylamino-1,8-naphthalimide (nBuF-hexa-Q bis-boronate).

[0144] The free bis boronic acid product used in glucose studies results from dissolution of N-2-[5-(N-4-dimethylaminobenzyl) -5-[2-(5,5-dimethylborinan-2-yl)benzyl] amino-hexyl]-[2-(5,5-dimethylborinan-2-yl)benzyl]amino-ethyl-4-butylamino-1,8-naphthalimide in the MeOH/PBS buffer system.

[0145] H. Modulation of Fluorescence with Glucose and Lactate.

[0146] The modulation of the fluorescence of the indicator compound (which contains two recognition elements) prepared in this sample by glucose and lactate was determined. **FIG. 5** shows the normalized fluorescence emission (I/Io@535 nm) of 0.015 mM solutions of the indicator compund in 70/30 MeOH/PBS containing a) 0-20 mM glucose; b) 0-20 mM lactate. Spectra were recorded using a Shimadzu RF-5301 spectrafluorometer with excitation@450 nm; excitation slits at 1.5 nm; emission slits at 1.5 nm; ambient temperature. Error bars are standard deviation with triplicate values for each data point. The fluorescence of the indicator was affected by the presence of glucose, but not substantially affected by the presence of lactate.

EXAMPLE 6

[0147] Effect of Glucose or Lactate on Acrylamide Gel Containing N-[3-(methacrylamido)propyl]-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonamide (Alizarin Red S Monomer) and α,α'-bis[N-[2-(5,5-dimethylborinan-2-yl-

)benzyl]-N-[3-(methacrylamido)propylamino]-1,4-xylene (Bis Boronic Acid Monomer):

[0148] A. 3,4-Dihydroxy-9,10-dioxo-2-anthracenesulfonyl Chloride:

[0149] 3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid sodium salt (1.4 g, 3.9 mmoles) was combined with 30 mL of chlorosulfonic acid and heated to 90° C. for 5 hours, after which the solution was cooled to 0° C. and poured into 100 g of ice. After the ice melted the solution was extracted with CH_2Cl_2 (3×100 mL), methylene chloride extracts were combined, dried with Na_2SO_4 and evaporated to produce 0.87 g of solid (Yield 66%).

[0150] B. N-[3-(methacrylamido)propyl]-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonamide:

[0151] 3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonyl chloride (96 mg, 0.28 mmoles) and N-(3-aminopropyl) methacrylamide hydrochloride (108 mg, 0.6 mmoles) were combined with 20 mL of CH_2Cl_2. To this suspension Et_3N (303 mg, 3 mmoles) was added. The mixture was stirred at room temperature for 24 hours, filtered, and solvent was evaporated. The resulting solid was subjected to column chomatography on SiO_2 (10 g) with CH_2Cl_2/MeOH (90/10) as an eluent. The product was obtained as a red solid (80 mg, 64% yield).

[0152] FAB MS: Calculated for $C_{21}H_{20}N_2O_7S$ M^+445; Found M^+445.

[0153] HPLC: HP 1100 HPLC chromatograph, Waters 5×100 mm NovaPak HR C18 column, 0.100 mL injection, 0.75 mL/min, 2 mL injection loop, 370 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 17.67 min.

[0154] C. α,α'-bis[3-(methacrylamido)propylamino]-1,4-xylene.

[0155] A solution of N-(3-aminopropyl)methacrylamide hydrochloride salt (3.00 g, 16.8 mmole, 2.21 equiv.), DIEA (6.5 g, 8.8 mL, 50 mmole, 6.6 equiv.), terephthaldicarboxaldehyde (1.02 g, 7.60 mmole) and Na_2SO_4 (10.7 g, 75.3 mmole, 9.91 equiv.) in 75 mL anhydrous MeOH was stirred in the dark at 25° C. for 18 hours. At this time, more Na_2SO_4 (10.7 g, 75.3 mmole, 9.91 equiv.) was added and stirring continued for 6 hours longer. At this time, the solution was filtered and $NaBH_4$ (1.73 g, 45.7 mmole, 6.01 equiv.) was added to the filtrate in portions and subsequently stirred at 25° C. for 21 hours. The suspension was filtered through Celite and the filtrate was concentrated. The residue was dissolved in 100 mL CH_2Cl_2 and washed 1×25 mL saturated aqueous $NaHCO_3$. The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated to yield a viscous oil. The product was carried on as is.

[0156] HPLC: HP 1100 HPLC chromatograph, Vydac 201TP 10×250 mm column, 0.100 mL injection, 2.00 mL/min, 260 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 15.8 min.

[0157] D. α,α'-bis[N-[2-(5,5-dimethylborinan-2-yl)benzyl]-N-[3-(methacrylamido)propylamino]-1,4-xylene.

[0158] A solution of α,α'-bis[3-(methacrylamido)-propylamino]-1,4-xylene (2.94 g, 7.61 mmole), DIEA (2.97 g, 4.00 mL, 23.0 mmoles, 3.02 equiv.), (2-bromomethyl-phenyl)boronic acid neopentyl ester (6.50 g, 23.0 mmole, 3.02 equiv.) and BHT (5 mg as inhibitor) in 75 mL CH_2Cl_2 at 25° C. was stirred in the dark for 28 hours. At this time, the mixture was washed 1×25 mL saturated aqueous $NaHCO_3$. The organic extract was dried over anhydrous Na_2SO_4, filtered and concentrated. To the residue was added 200 mL ether and the suspension was stirred for 18 hours. The suspension was filtered and the residue dissolved in CH_2Cl_2, filtered and the filtrate concentrated. To the solid residue was added 150 mL ether and the suspension was stirred for 18 hours. At this time, the suspension was filtered yielding 1.98 g (33%) of a fluffy pink powder.

[0159] FAB MS: Calc'd for $C_{46}H_{64}B_2N_4O_6$ $[M]^+$790; Found $[M+1]^+$791.

[0160] HPLC: HP 1100 HPLC chromatograph, Waters 5×100 mm NovaPak HR C18 column, 0.050 mL injection, 0.75 mL/min, 280 nm detection, A=water (0.1% HFBA) and B=MeCN (0.1% HFBA), gradient 10% B 2 min, 10-80% B over 18 min, 80-100% B over 2 min, 100% B 2 min, retention time 13.4 min.

[0161] E. Preparation of Acrylamide Gel Containing N-[3-(methacrylamido)propyl]-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonamide (Alizarin Red S Monomer) and α,α'-bis[N-[2-(5,5-dimethylborinan-2-yl)benzyl]-N-[3-(methacrylamido)propylamino]-1,4-xylene:

[0162] Ethylene glycol solution containing 30% wt. acrylamide and 0.8% wt. N,N'-methylenebisacrylamide was prepared. N-[3-(methacrylamido)propyl]-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonamide (1.5 mg, 3.38×10-6 mole) and α,α'-bis[N-[2-(5,5-dimethyl-borinan -2-yl)benzyl]-N-[3-(methacrylamido)propylamino]-1, 4-xylene (28 mg, 3.54×10^{-5} mole) were combined with 800 μL of ethylene glycol monomer solution and 40 μL of 5% wt. aqueous ammonium persulfate. This formulation was placed in a glove box purged with nitrogen along with a mold constructed from glass microscope slides and 100 micron stainless steel spacer. An aqueous solution of N,N,N',N'-tetramethylethylenediamine (40 μL, 5% wt.) was added to the monomer solution to accelerate polymerization and the final formulation was poured into a glass mold. The mold was left under nitrogen atmosphere for 16 hours, after which it was immersed in PBS (pH=7.4) and the glass slides were separated to afford a hydrogel polymer in a form of a thin film. The resulting hydrogel thin film was washed with 100 mL of phosphate buffered saline containing 1 mM lauryl sulfate sodium salt for 3 days, the solution being changed every day, followed by washing with MeOH/PBS (20/80 by vol., 3×100 mL), and finally with PBS (pH=7.4, 3×100 mL). Hydrogel polymer was stored in PBS (10 mM PBS, pH=7.4) containing 0.2% wt. sodium azide and 1 mM EDTA sodium salt.

[0163] F. Modulation of Absorbance with Glucose and Lactate

[0164] The modulation of the absorbance of the indicator hydrogel (which contains two recognition elements) prepared in this example by glucose and lactate was deter-

mined. The acrylamide gel was mounted in PMMA cell in the same way as described in Example 4. Phosphate buffered saline (PBS), pH=7.4 containing desired amount of glucose or sodium lactate was heated to 37° C. in a water bath and placed in the PMMA cell containing the gel after which the PMMA cell was allowed to equilibrate for 15 min at 37° C. Absorbance measurement for each glucose or lactate concentration was conducted in triplicate. For each measurement, absorbance at 650 nm was used as a blank, A(650 nm) was subtracted from all values of A(450 nm) and A(530 nm).

[0165] **FIG. 6** shows the absorbance spectra for acrylamide gel (30%) containing 4 mM Alizarin Red S monomer and 44 mM bis boronic acid monomer with and without glucose. **FIG. 7** shows the effect of glucose on absorbance of acrylamide gel (30%) containing 4 mM Alizarin Red S monomer and 44 mM bis boronic acid monomer. **FIG. 8** shows the effect of sodium lactate on absorbance of acrylamide gel (30%) containing 4 mM Alizarin Red S monomer and 44 mM bis boronic acid monomer. The absorbance of the indicator was affected by the presence of glucose, but not substantially affected by the presence of lactate.

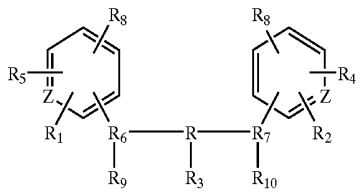
What is claimed is:

1. A method for detecting the presence or concentration of glucose in a sample which may also contain an alpha-hydroxy acid or a beta-diketone, which comprises:

a) exposing the sample to a compound having at least two recognition elements for glucose, oriented such that the interaction between the compound and glucose is more stable than the interaction between the compound and the alpha-hydroxy acid or beta-diketone, said compound also containing a detectable moiety having a detectable quality that changes in a concentration-dependent manner when said compound is exposed to glucose in said sample; and

b) measuring any change in said detectable quality to thereby determine the presence or concentration of glucose in said sample, wherein the presence of the alpha-hydroxy acid or the beta-diketone does not substantially interfere with said determination.

2. The method of claim 1, wherein the compound has the following structure:



wherein:

R_1 and R_2 are the same or different and are selected from the following: i) hydrogen; ii) a substituent to modify the pKa and hydrolytic stability of the R_8 moiety, iii) a detectable moiety, or iv) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

R_3 is hydrogen or a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

R_4 and R_5 are the same or different and are selected from the following: i) hydrogen, ii) a substituent to modify the pKa and hydrolytic stability of the R_8 moiety, iii) a detectable moiety, or iv) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

each Z is independently carbon or nitrogen;

R_6 and R_7 are the same or different and are i) linking groups having from zero to ten contiguous or branched carbon and/or heteroatoms, or ii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

R is selected from the following: i) an aliphatic and/or aromatic spacer containing from 1 to 10 contiguous atoms selected from the group consisting of carbon, oxygen, nitrogen, sulfur and phosphorus, ii) a detectable moiety, or iii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

each R_8 is the same or different and is a moiety capable of interaction with the vicinal diol groups present in glucose; and

R_9 and R_{10} are the same or different, and are i) hydrogen, ii) a detectable moiety, or iii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

with the proviso that the indicator compound contains at least one detectable moiety associated therewith.

3. The method of claim 2, wherein R_8 is selected from the group consisting of boronic acid, boronate ion, arsenious acid, arsenite ion, telluric acid, tellurate ion, germanic acid, germanate ion, and combinations thereof.

4. The method of claim 3, wherein each R_8 is a boronic acid group.

5. The method of claim 2, wherein the compound comprises at least two detectable moieties that are capable of energy transport from one to the other, and wherein said energy transport is modulated by the presence of glucose in the sample.

6. The method of claim 2, wherein at least one of R, R_1, R_2, R_4, R_5, R_9 or R_{10} comprises a fluorophore moiety and further wherein at least one of those groups comprises a quenching moiety, and wherein said fluorophore is either quenched or dequenched when said compound interacts with glucose in the sample.

7. The method of claim 2, wherein the compound comprises a fluorophore, and the fluorescence of said fluorophore is modulated by the interaction of said compound with glucose.

8. The method of claim 1, wherein the sample is a physiological fluid.

9. The method of claim 8, wherein the physiological fluid is selected from the group consisting of blood, plasma,

serum, interstitial fluid, cerebrospinal fluid, urine, saliva, intraocular fluid, lymph, tears, sweat, and physiological buffers.

10. The method of claim 1, wherein the compound is exposed to the sample in solution.

11. The method of claim 1, wherein the compound is immobilized on or within a solid support.

12. The method of claim 11, wherein the solid support is a polymeric matrix.

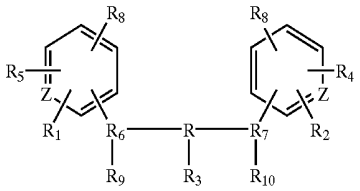
13. The method of claim 1, wherein the compound is associated with an implantable device, and wherein step a) takes place in vivo.

14. The method of claim 2, wherein R is an anthracene residue; R_1, R_2, R_3, R_4 and R_5 are hydrogen; R_6 and R_7 are dimethylamine residues; each R_8 is a boronic acid group; R_9 and R_{10} are aliphatic carboxylic acid residues; and each Z is carbon.

15. The method of claim 14, wherein R_9 and R_{10} are propionic acid residues.

16. The method of claim 2, wherein R is a hexamethylene residue; R_1, R_2, R_3, R_4 and R_5 are hydrogen; R_6 and R_7 are dimethylamine residues; each R_8 is a boronic acid group; R_9 is a naphthalimide residue; R_{10} is a dimethylaminobenzyl residue; and each Z is carbon.

17. A compound having the following structure



wherein:

R_1 and R_2 are the same or different and are selected from the following: i) hydrogen; ii) a substituent to modify the pKa and hydrolytic stability of the R_8 moiety, iii) a detectable moiety, or iv) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

R_3 is hydrogen or a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

R_4 and R_5 are the same or different and are selected from the following: i) hydrogen, ii) a substituent to modify the pKa and hydrolytic stability of the R_8 moiety, iii) a detectable moiety, or iv) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

each Z is independently carbon or nitrogen;

R_6 and R_7 are the same or different and are i) linking groups having from zero to ten contiguous or branched carbon and/or heteroatoms, or ii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

R is selected from the following: i) an aliphatic and/or aromatic spacer containing from 1 to 10 contiguous atoms selected from the group consisting of carbon, oxygen, nitrogen, sulfur and phosphorus, ii) a detectable moiety, or iii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

each R_8 is the same or different and is a moiety capable of interaction with the vicinal diol groups present in glucose; and

R_9 and R_{10} are the same or different, and are i) hydrogen, ii) a detectable moiety, or iii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

with the proviso that the indicator compound contains at least one detectable moiety associated therewith.

18. The compound of claim 17, wherein R_8 is selected from the group consisting of boronic acid, boronate ion, arsenious acid, arsenite ion, telluric acid, tellurate ion, germanic acid, germanate ion, and combinations thereof.

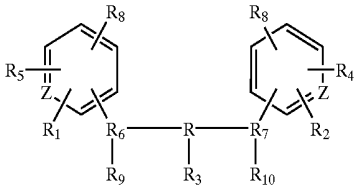
19. The compound of claim 18, wherein each R_8 is a boronic acid group.

20. The compound of claim 17, wherein the compound comprises a fluorophore, and the fluorescence of said fluorophore is modulated by the interaction of said compound with glucose.

21. The compound of claim 17, wherein R is an anthracene residue; R_1, R_2, R_3, R_4 and R_5 are hydrogen; R_6 and R_7 are dimethylamine residues; each R_8 is a boronic acid group; R_9 and R_{10} are aliphatic carboxylic acid residues; and each Z is carbon.

22. The compound of claim 21, wherein R_9 and R_{10} are propionic acid residues.

23. A detection system for detecting the presence or concentration of glucose in a sample which may also contain an alpha-hydroxy acid or a beta-diketone, which comprises a compound having the following structure



wherein:

R_1 and R_2 are the same or different and are selected from the following: i) hydrogen; ii) a substituent to modify the pKa and hydrolytic stability of the R_8 moiety, iii) a detectable moiety, or iv) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

R_3 is hydrogen or a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

R_4 and R_5 are the same or different and are selected from the following: i) hydrogen, ii) a substituent to modify the pKa and hydrolytic stability of the R_8 moiety, iii) a detectable moiety, or iv) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

each Z is independently carbon or nitrogen;

R_6 and R_7 are the same or different and are i) linking groups having from zero to ten contiguous or branched carbon and/or heteroatoms, or ii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

R is selected from the following: i) an aliphatic and/or aromatic spacer containing from 1 to 10 contiguous atoms selected from the group consisting of carbon, oxygen, nitrogen, sulfur and phosphorus, ii) a detectable moiety, or iii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

each R_8 is the same or different and is a moiety capable of interaction with the vicinal diol groups present in glucose; and

R_9 and R_{10} are the same or different, and are i) hydrogen, ii) a detectable moiety, or iii) a linking group capable of attachment to a solid support or a polymeric matrix, said support or matrix optionally containing a detectable moiety;

with the proviso that the indicator compound contains at least one detectable moiety associated therewith.

24. The detection system of claim 23, wherein R_8 is selected from the group consisting of boronic acid, boronate ion, arsenious acid, arsenite ion, telluric acid, tellurate ion, germanic acid, germanate ion, and combinations thereof.

25. The detection system of claim 24, wherein each R_8 is a boronic acid group.

26. The detection system of claim 23, wherein the compound comprises a fluorophore, and the fluorescence of said fluorophore is modulated by the interaction of said compound with glucose.

27. The detection system of claim 23, wherein R is an anthracene residue; R_1, R_2, R_3, R_4 and R_5 are hydrogen; R_6 and R_7 are dimethylamine residues; each R_8 is a boronic acid group; R_9 and R_{10} are aliphatic carboxylic acid residues; and each Z is carbon.

28. The detection system of claim 27, wherein R_9 and R_{10} are propionic acid residues.

* * * * *

专利名称(译)	检测也含有α-羟基酸或β-二酮的溶液中的葡萄糖		
公开(公告)号	US20020090734A1	公开(公告)日	2002-07-11
申请号	US09/754217	申请日	2001-01-05
[标]申请(专利权)人(译)	达尼洛夫GEORGEÿ KALIVRETENOS ARISTOTLE摹 NIKOLAITCHIK ALEXANDRE V		
申请(专利权)人(译)	达尼洛夫GEORGE Y. KALIVRETENOS ARISTOTLE G. NIKOLAITCHIK ALEXANDRE V.		
当前申请(专利权)人(译)	达尼洛夫GEORGE Y. KALIVRETENOS ARISTOTLE G. NIKOLAITCHIK ALEXANDRE V.		
[标]发明人	DANILOFF GEORGE Y KALIVRETENOS ARISTOTLE G NIKOLAITCHIK ALEXANDRE V		
发明人	DANILOFF, GEORGE Y. KALIVRETENOS, ARISTOTLE G. NIKOLAITCHIK, ALEXANDRE V.		
IPC分类号	C07F5/02 G01N33/533 G01N33/542 G01N33/58 G01N33/66 C12Q1/54 G01N33/00		
CPC分类号	C07F5/025 G01N33/533 G01N33/542 G01N33/582 G01N33/66 Y10T436/144444		
外部链接	Espacenet USPTO		

摘要(译)

用于确定样品中葡萄糖的存在或浓度的组合物和方法，其还可含有α-羟基酸或β-二酮。该方法使用具有至少两个葡萄糖识别元素的化合物，其取向使得化合物与葡萄糖之间的相互作用比化合物与α-羟基酸或β-二酮之间的相互作用更稳定，使得存在α-羟基酸或β-二酮基本上不干扰所述测定。