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(21) International Application Number: PCT/US82/00725 (22) International Filing Date: 26 May 1982 (26.05.82) (71) Applicant: THE CHILDREN'S HOSPITAL MEDICAL CENTER [US/US]; 300 Longwood Avenue, Boston, MA 02115 (US). (72) Inventors: GALLOP, Paul, M. ; 118 Walnut Hill Road, Chestnut Hill, MA (US). PAZ, Mercedes ; 221 Kent Street, Brookline, MA (US). (74) Agent: HILLMAN, Robert, E.; Fish & Richardson, 28 State Street, Boston, MA 02109 (US). (81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE, DE (European patent), FR (European patent), GB, GB (European patent), JP, LU (European patent), NL (European patent), SE (European patent).		Published <i>With international search report.</i>
(54) Title: REPORTER COMPOUNDS (57) Abstract Compounds having one of formulae I or II. In formula I, Rp₁ is a group which includes a reporter group comprising a fluorophore, a chromophore, an organometallic group, a therapeutic agent, an antigen, or an isotopically-labelled group, each of X and Y, independently, is N, O, or S, Cg₁ is a carrier group which includes a solubilizing group which is substantially ionized in water, and r is a receptor group which, with X and Y, forms complexes with boronic acids. In formula II, Rp₂ is a group which includes a reporter group comprising a fluorophore, a chromophore, an organometallic group, a therapeutic agent, an antigen, or an isotopically-labelled group, each of X and Y, independently, is N, O, or S, Cg₂ is a carrier group which includes a solubilizing group which is substantially ionized in water, and r is a receptor group which, with X and Y, forms complexes with boronic acids. <pre> graph TD REACTANTS --> PEROXIDE SAMPLE[SAMPLE CONTAINING UNKNOWN] --> PEROXIDE PEROXIDE --> FLUOROPHORE[FLUOROPHORE IN SOLUTION] PEROXIDE --> SUPPORT[INSOLUBLE SUPPORT] FLUOROPHORE --> SUPPORT FLUOROPHORE --> SEPARATE[SEPARATE] SEPARATE --> INSOLUBLE[INSOLUBLE SUPPORT] SEPARATE --> FLUOROPHORE2[FLUOROPHORE IN SOLUTION] FLUOROPHORE2 --> MEASURE[MEASURE WITH FLUOROMETER] </pre>		

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REPORTER COMPOUNDSBackground of the Invention

This application is a continuation-in-part of Gallop et al. "Fluorescent Boronic Acid-Containing Dyes", S.N. 132,908, filed March 24, 1980.

This invention relates to reporter molecules such as dyes and drugs.

It is often desirable that such molecules be capable of being transferred from one phase to another; e.g., from an aqueous to a non-aqueous phase, or across biological membranes.

It is well-known that certain molecules can be rendered water soluble by the introduction into the molecule of a strongly basic or acidic group such as carboxylic acid or sulfonic acid, which confers water solubility by virtue of being ionized in aqueous solution at certain pH values.

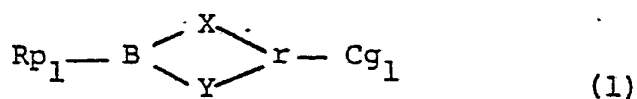
Summary of the Invention

In one aspect, the invention features a new class of reagents which we have named the Boronate-Dependent Phase Transfer (BorAdept) compounds. The new compounds allow groups which can 1) report on conditions within living cells and tissues or 2) modify metabolic parameters within living tissues to be presented to and taken up by cells under non-toxic conditions.

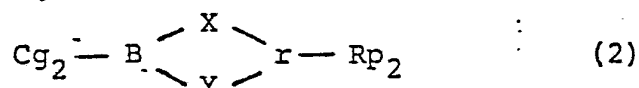
The new compounds have one of two general formulae:



- 2 -



or



In both formulae (1) and (2), each X and Y, independently, is N, O, or S, and r represents a receptor group which, with X and Y, forms adducts or complexes with boronic acids. Cg₁ and Cg₂ are carrier groups which each include a solubilizing group which is substantially ionizable in water, and Rp₁ and Rp₂ are groups which each include a reporter group which is a fluorophore, a chromophore, an organometallic group, a therapeutic agent, an antigen, or an isotopically-labelled group.

The new compounds, because of their water-solubility at or near physiological pH, provide reporter groups with greatly enhanced versatility. Further, the ability of formula (1) and (2) compounds to exist in pH-dependent equilibria with their dissociated forms renders them useful for a broad range of applications. Many of these compounds have the additional advantage provided by the boronic acid-derived portion of the molecule which, when dissociated from the remainder of the molecule, can exhibit the



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selective affinity which boronic acids have for organic compounds having reactive hydroxyl, amino, and thiol groups, the aforesaid equilibria also allow some reporter groups to enter environments
5 where their properties are advantageously altered. For example, some of the fluorescent reporter groups, under certain conditions, can be brought into contact with hydrophobic pockets in cells or organic phases where they exhibit enhanced or
10 quenched fluorescence.

Other aspects of the invention feature applications of the new compounds such as the staining of living cells, proteins, and fabrics, and various assay methods.

15 Among other aspects, the invention also features new boronic acid containing compounds, which are useful both by themselves, and as starting materials for BorAdept compounds.

Other advantages and features of the
20 invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

We turn now to a description of the
25 preferred embodiments of the invention, after first briefly describing the drawings.

Drawings

Figure 1 is a flow chart diagramming an assay method of the invention.

30 Figure 2 is a perspective view of apparatus for carrying out one of said methods.

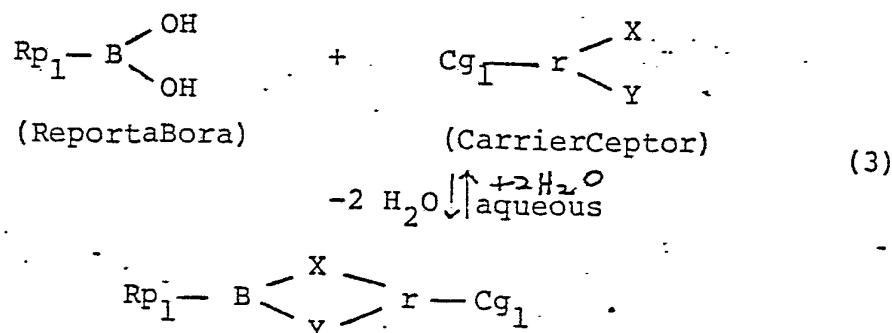


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BorAdept Compounds

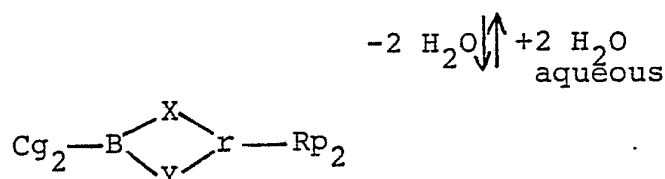
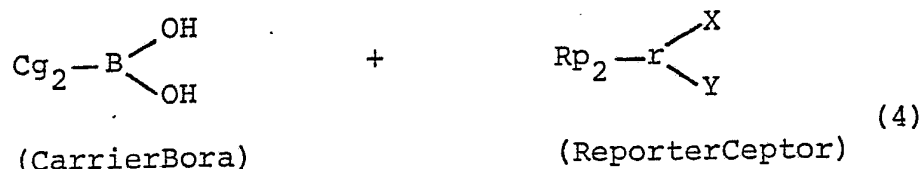
The compounds represented in formula (1) and (2) each is a reaction product between a compound which includes a boronic acid, and a compound which includes a receptor group (r) which, with X and Y, forms adducts or complexes with boronic acids. In both cases, one of the compounds also includes a group (Rp₁ or Rp₂) which includes a reporter group comprising a fluorophore, a chromophore, an organometallic group, a therapeutic agent, an antigen, or an isotopically-labelled group. The other compound includes a carrier group (Cg₁ or Cg₂) which includes a strongly basic or acidic solubilizing group which is substantially ionizable in water. Rp₁ is bonded to boronic acid in formula (1), while Rp₂ is bonded to the receptor in formula (2). Cg₁ is bonded to the receptor in formula (1), while Cg₂ is bonded to boronic acid in formula (2).

The compounds of formula (1) are generally made by the reaction



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The compounds of formula (2) are generally made by the reaction



An important feature of reactions (3) and (4) is that, in aqueous solution, both are reversible; i.e., the BorAdept reagents are in dynamic equilibria with their starting materials. The extent to which, under a given set of circumstances, an equilibrium is pushed in either direction in part determines a compound's usefulness for a given application. The extent to which the equilibrium is pushed toward the formation of the water-soluble formula (1) or (2) compound, at the expense of the starting materials (i.e., push to the right) is dependent on several factors, including the pKa and Lewis acid complexing strength of the boronic acid, the complexing potential of $\text{HX} \begin{array}{l} \nearrow \\ \searrow \end{array} \text{r} \begin{array}{l} \nearrow \text{Y} \\ \searrow \end{array}$ and the local net charge and hydrophobicity of Rp_1 or Rp_2 .

The compounds of formula (1) and (2) can, if desired, contain more than one reporter group, and in such case the properties of the additional

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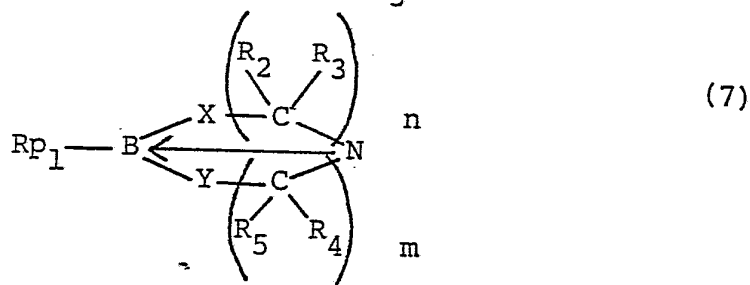
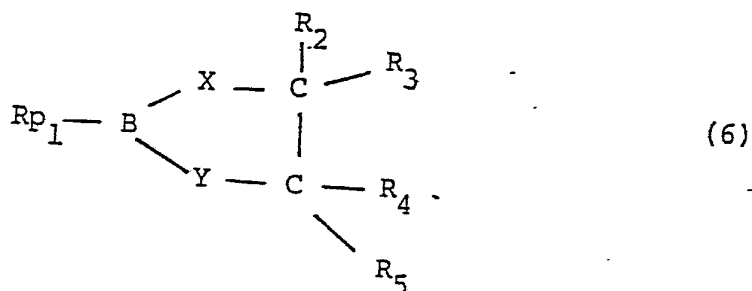
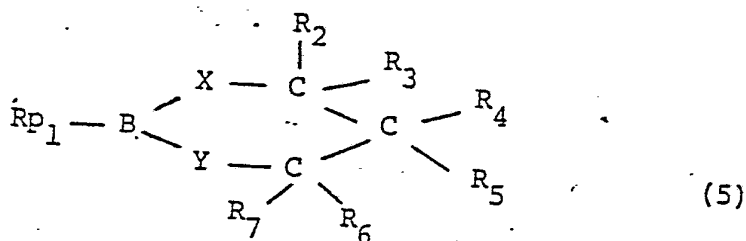
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group or groups can influence the dynamic equilibrium of the compound.

Turning now to a more detailed description of the compounds of formula (1), the CarrierCeptor

5 portion of the molecule, $\begin{array}{c} \text{HX} \\ \text{HY} \end{array} \text{r} - \text{Cg}_1$ most

frequently is of one of three general configurations, so that the formula (1) compound has one of the following general formulae:



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In formulae (5), (6), and (7), each $R_2 - R_7$, independently, is hydrogen, aryl, alkyl, heteroalkyl, hetroaryl, aralkyl, or a solubilizing group which is substantially ionized in water, provided that the compound contains at least one such solubilizing group. Each of n and m , independently, is 0, 2, or 3, provided that both cannot be 0. When n or m is 2 or 3, each $R_2 - R_5$ can be different in successive repeating units. For example, where n is 2, the R_2 attached to the first carbon atom can be hydrogen, while the R_2 attached to the second carbon atom is a solubilizing group.

In the structure of formula (7), known as a transannular complex, the arrow from N to B indicates the attraction between the electron-deficient boron atom and the electron-rich nitrogen atom.

In formulae (5), (6), and (7), each of groups $R_2 - R_7$ preferably contains between 0 and about 12 carbon atoms. The solubilizing group is preferably a sulfonic acid, a carboxylic acid, quaternary ammonium, a guanidinium group, or a phosphate group.

The first step in making a formula (1) compound is to make the desired ReportaBora. This can be done by reacting a desired organic boronic acid with the desired Rp_1 . Alternatively, the desired Rp_1 precursor can undergo hydroboration with catechol borane, or can participate in a Grignard reaction with a boronate ester to make a ReportaBora. If desired, the reporter group can first be incorporated into a compound having a



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desired property, e.g., good electron withdrawing power for enhanced activation of the boronate.

Table I shows the structures of some ReportaBora compounds which provide the Rp_1 -B portion of the compounds of formula (1). Each of these can react with any CarrierCeptor, as defined above, to produce a formula (1) compound. Table I also indicates the utility of the reporter group of each listed ReportaBora. This utility, along with other factors, including choice of CarrierCeptor, allows the choice of starting materials which will react to produce a formula (1) compound possessing the properties required for a given application.

Table II shows the structures and some properties of some preferred CarrierCeptor

compounds which provide $\begin{array}{c} \text{HX} \\ \text{HY} \end{array} \text{r} - \text{Cg}_1$ of

formula (1) compounds. These CarrierCeptors can be reacted with any ReportaBora, such as those listed in Table I, to make a compound of the general formula (1).

Some of the CarrierCeptors listed in Table II are biologically compatible buffers which have been described in publications from the laboratory of Good, as summarized in Ferguson et al. (1980) *Analyt. Biochem.* 104, 300; this paper itself also describes suitable buffers. Only the buffers described in the aforementioned literature which

contain the $\begin{array}{c} \text{HX} \\ \text{HY} \end{array} \text{r} - \text{Cg}_1$ group, as defined herein,

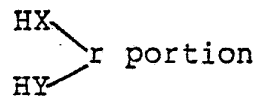
are suitable for making the compounds of formula (1). Table III shows the structures of some



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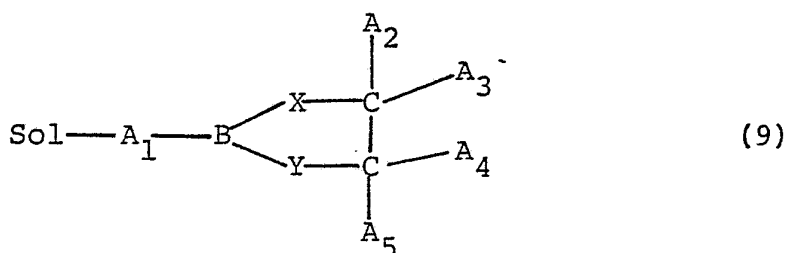
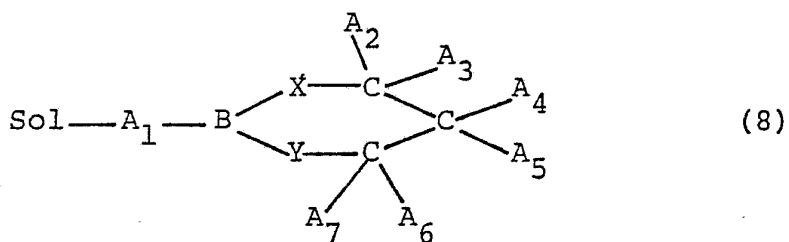
preferred formula (1) compounds made with suitable buffers.

Turning now to a more detailed description of the compounds of formula (2), the



5 of the molecule, as in the case of formula (1) compounds, typically has one of three general configurations, Cg_2 typically includes both a solubilizing group and an organic group linking the

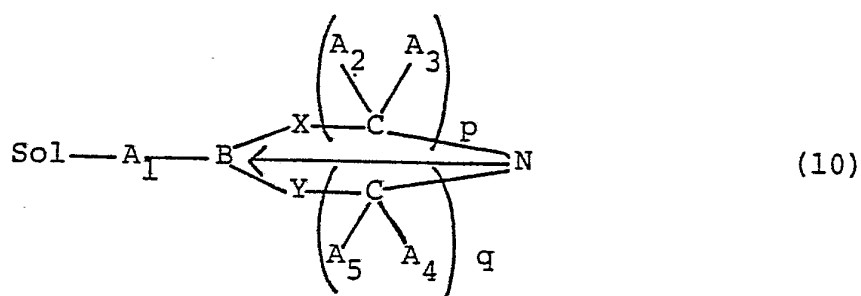
10 solubilizing group to the boron atom. Formula (2) compounds thus typically have one of the following general formulae:



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In formulae (8), (9), and (10), Sol is the
 aforementioned strongly basic or acidic
 solubilizing group and A_1 is alkyl, aryl,
 5 heteroaryl, heteroalkyl, or aralkyl; most
 preferably A_1 contains between 3 and about 12
 carbon atoms. Each $\text{A}_2 - \text{A}_7$, independently, is
 hydrogen, aryl, alkyl, heteroalkyl, heteroaryl,
 aralkyl, or Rp_2 , the group containing a reporter
 10 group, provided that the compound contains at least
 one Rp_2 . Each of $\text{A}_2 - \text{A}_7$, except Rp_2 ,
 preferably contains between 0 and about 12 carbon
 atoms.

Each of p and q , independently, is 0, 2,
 15 or 3, provided that both cannot be 0. When p or q
 is 2 or 3, each $\text{A}_2 - \text{A}_4$ can be different in
 successive repeating units. For example, where p
 is 2, the A_2 attached to the first carbon atom
 can be hydrogen, while the A_2 attached to the
 20 second carbon atom can be Rp_2 .

The first step in making a formula (2)
 compound is to make the desired ReporterCeptor by
 reacting the desired Rp_2 with the desired

$$\begin{array}{c} \text{HX} \\ \text{HY} \end{array} \text{R} \cdot$$

 Rp_2 can consist entirely of the desired reporter
 25 group, or the reporter group can be incorporated

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into an Rp_2 having a desired property; e.g., good

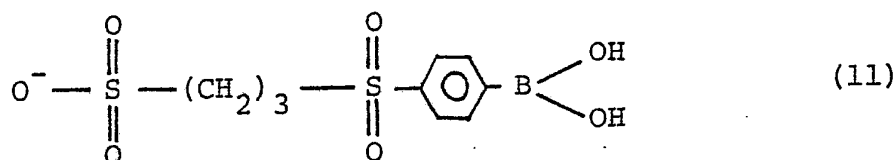
reactivity with $\begin{array}{c} \text{HX} \\ \diagdown \\ \text{r} \\ \diagup \\ \text{HY} \end{array}$. The $\begin{array}{c} \text{HX} \\ \diagdown \\ \text{r} \\ \diagup \\ \text{HY} \end{array}$ can be any

compound which forms adducts or complexes with boronic acids. For example, any of the

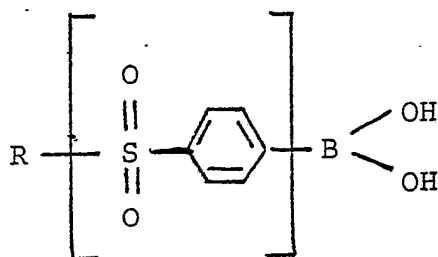
5 CarrierCeptors of Table II, with or without their

solubilizing groups, are suitable $\begin{array}{c} \text{HX} \\ \diagdown \\ \text{r} \\ \diagup \\ \text{HY} \end{array}$ compounds.

A large number of CarrierBoras can be made and reacted with a ReporterCeptor to produce a formula (2). However, there is little advantage
10 (putting aside considerations such as cost) in having a wide variety of CarrierBoras to choose from, and one or a few can be reacted with a wide variety of ReporterCeptors. An example of one such versatile CarrierBora is the compound shown below
15 which, although not commercially available, can be made using conventional methods.



Compound (11) is particularly useful because its boronate group is highly reactive as a result of
20 the strong electron withdrawing action of the sulfonephenyl group



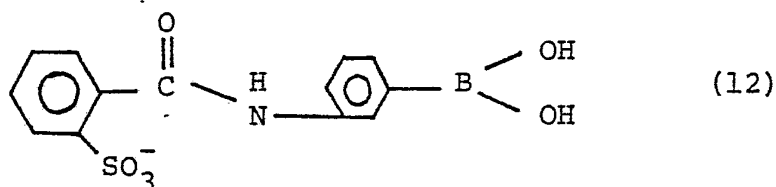
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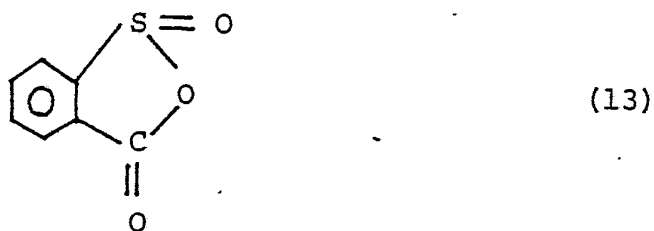
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Other examples of CarrierBoras are m-carboxyphenyl boronic acid, p-sulfophenylboronic acid, m-guanidinophenyl boronic acid, and p-trimethylammoniumphenyl boronic acid. The first
 5 two are negatively charged, while the latter two are positively charged.

If it is desired to bond a reporter group not only to the boronic acid part of the CarrierBora, but to the linking group as well, it
 10 is necessary to provide a reactive site on the A₁ portion of the CarrierBora. An example of a CarrierBora having such a site (NH) is



Compound 12 is made by reacting m-aminophenyl
 15 boronic acid, in aqueous ethanol at pH 8.0, with



which is available commercially.

A formula (2) compound is typically made by dissolving the desired ReporterCeptor in an
 20 organic, water-miscible solvent, e.g., dimethyl acetamide, acetone, or dimethyl formamide, and then adding an excess of the desired CarrierBora in

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water or an appropriate aqueous buffer. Some preferred formula (2) compounds are listed in Table IV.

Both formula (1) and formula (2) compounds can, if desired, contain more than one reporter group. A group, Rp_3 including a second reporter group can be attached at any suitable reactive site on formula (1) or (2) compounds, but will most often be attached at a site on Rp_1 or Rp_2 so that the ReportaBora or ReporterCeptor, dissociated from the carrier part of the molecule, bears both reporter groups. Compound 3 of Table III, an example of such a compound, bears two staining reporter groups. Depending on the application, it can also be advantageous to include, in one compound, any of a number of combinations of two or more reporter groups, e.g., a therapeutic agent together with an isotopically-labelled group, or a fluorophore together with an antigen.

20 Staining of Living Cells, Proteins, and Other Materials

The compounds of both formulae (1) and (2) in which the reporter group is a chromophore, an antigen, an organometallic group, an isotopically-labelled group, or a fluorophore are useful for staining living cells, proteins, and other materials such as fabrics and paper products.

The cell-staining formula (1) compounds (hereinafter referred to as "stains") behave differently from those of formula (2), and will be discussed separately.

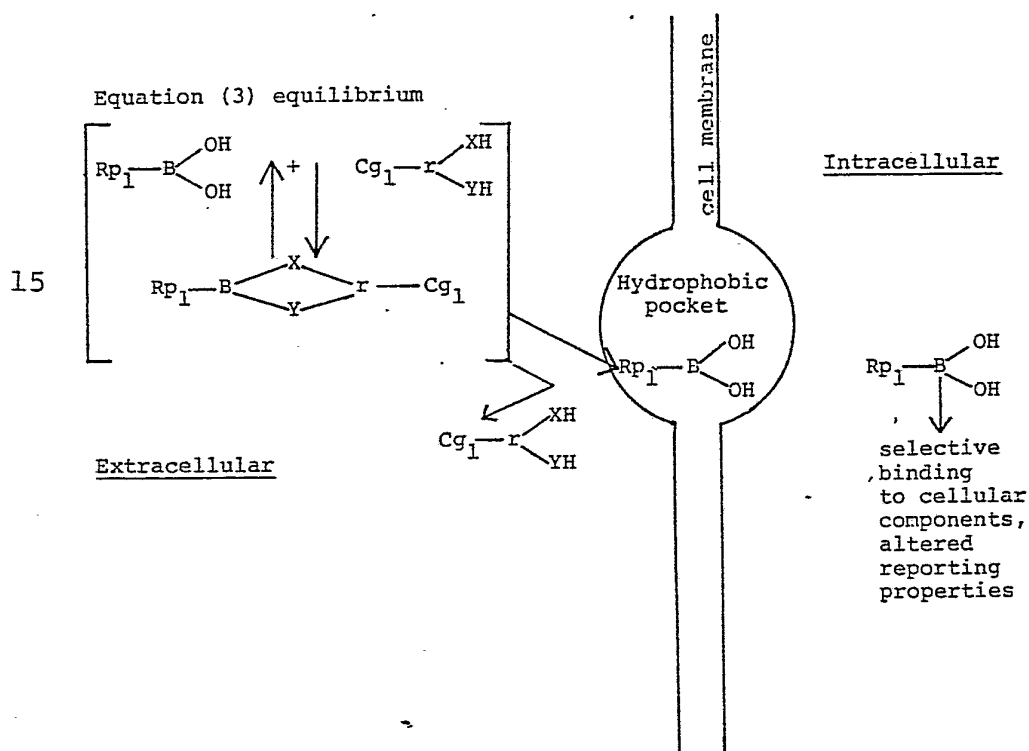
As shown in equation (3), formula (1) compounds, in aqueous solution at pH values around



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physiological pH, exist in dynamic equilibria with their starting materials. The equilibrium permits the presentation of the ReportaBora to cells in media compatible with living cells, and uptake by cell membranes without toxicity.

The diagram below illustrates the mechanism of uptake of ReportaBora by cells. The scheme is general, and is not strictly applicable to all formula (1) compounds. For example, a formula (1) compound having a positively-charged solubilizing group such as quaternary ammonium is more likely to enter the cell intact rather than operating as shown below. Also, many ReportaBoras do not exhibit altered reporting properties inside cells.



- 15 -

As shown in the diagram, the equation (3) equilibrium permits hydrophobic pockets of the cell membrane to selectively extract ReportaBora, which then enters the cell. Once inside the cell, the ReportaBora, carried by lipophilic components of the cell, is capable of selectively staining cellular components. The boronic acid portion of the molecule binds selectively to certain biological groups within cells which contain appropriately distributed hydroxyl, amino, and thiol groups; binding is particularly favored when such groups are in non-aqueous regions of the cell. Certain complex lipids, mucopolysaccharides, liposaccharides, glycoproteins, and proteins contain such reactive groups. Other compounds, e.g., certain conjugated proteins, also contain such reactive groups in appropriate complexing positions resulting from their sequences, confirmations, and configurations.

The selectivity of formula (1) compounds makes them useful as vital cellular stains in the diagnosis of certain disease states, including certain genetic disorders, particularly those which cause mucopolysaccharidosis, liposaccharidosis, or an increase in either the number or size of cellular lysosomal components. For example, Hurler's Syndrome, Hunter's Syndrome, and β -glucuronidase deficiency can be diagnosed by culturing a patient's cells, staining them with a fluorescent formula (1) stain, e.g., compound 1 of Table III, and then examining them using a fluorescent microscope. The vital staining



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patterns and the rate of destaining of certain cellular particulates can provide valuable diagnostic information. The compounds are also useful for gaging the viability and motility of human and animal sperm.

The selectivity of formula (1) compounds also makes them useful in comparing staining patterns of various cells in different cell cycle stages. The stains can also be useful for staining catecholamine-rich regions of cells; for example, the compounds stain epinephrine, norepinephrine, dopamine, di-hydroxyphenylalanine, and 3, 4-hydroxyphenylacetic acid, compounds which are abundant in nervous and adrenal tissue and are thus useful for the diagnosis of a variety of clinical conditions.

Staining of cells can be carried out using any most conventional vital staining techniques, but is preferably carried out according to either of the following two preferred processes.

According to one preferred process, cells to be stained, e.g., fibroblasts, are grown on a coverslip, the culture medium is poured off, the cells are washed with physiological buffer several times, and the coverslip is covered for 5 minutes with a formula (1) compound dissolved in physiological buffer. The coverslip is then washed, turned over onto a slide, and examined using a light or fluorescent microscope, or processed further, e.g., tested for the presence of immune complexes, radioactive label, or heavy metals.



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According to another preferred staining method, ReportaBora is added directly to the cell culture medium in which CarrierCeptor is already present as the buffer. After the desired degree of staining is complete, the medium is poured off and the cells are examined.

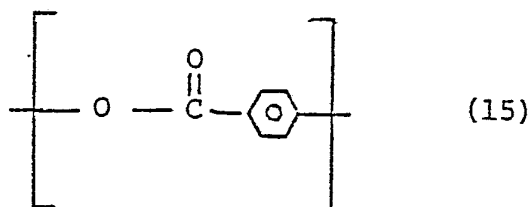
An additional step may be added to either preferred staining method to obtain additional information, such as the relative dynamic stability of the bonds between the stain and various cellular components. After staining, cells are transferred to fresh culture medium and monitored to determine the relative rates at which the stain leaches out of various cellular components. When the formula (1) compound contains an organometallic electron opaque reporter group, following vital staining, the cells are fixed and prepared for examination with an electron microscope.

Although any formula (1) compounds can be used for vital staining, some are more useful than others for certain applications. One factor affecting choice of dye is the strength of the bond between the ReportaBora and the CarrierCeptor; very tight complexes, such as those formed with strong CarrierCeptors such as TAPSO, and with certain ReportaBoras containing strong Lewis acid boronic acids, tend to make it more difficult for cell membranes to extract appreciable amounts of the ReportaBora. For this reason, it is sometimes preferable, for vital staining, to use a formula (1) stain made with a weaker complexing CarrierCeptor such as TES or MOPSO (Table II).



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Because Carrier Ceptors which form weak complexes with boronic acids also tend to be less reactive with boronic acids, when it is desired to make a formula (1) compound using a weak CarrierCeptor such as MOPSO, it is often desirable to employ a more reactive ReportaBora; i.e., one in which the boron atom is more electron deficient. Such a reactive ReportaBora is one in which Rp contains a linking group, proximate to the boron atom, which has strong electron withdrawing power. Referring to Table I, FBIII contains the linking group



which, because of its relatively strong electron withdrawing action, renders FBIII able to complex to a considerable degree with weakly complexing CarrierCeptors such as MOPSO. In contrast ReportaBoras, e.g., FluoraBora I, containing groups such as the sulfamido linkage, which do not exert strong electron withdrawing action, complex to a lesser extent with weakly complexing CarrierCeptors, and formula (1) compounds wherein the ReportaBora is FluoraBora I are thus preferably made with a strong CarrierCeptor such as TAPSO.

Another factor which can influence the choice of formula (1) fluorescent stain for a particular application is whether the fluorophore is enhanced or quenched (inhibited) in hydrophobic



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environments such as the hydrophobic protein pockets which the ReportaBora is likely to seek within a cell. Still another consideration is whether the fluorophore exhibits a shift in
5 emission wavelength in such an environment; both enhancement and emission shift can advantageously increase detectability of dyes within cells.

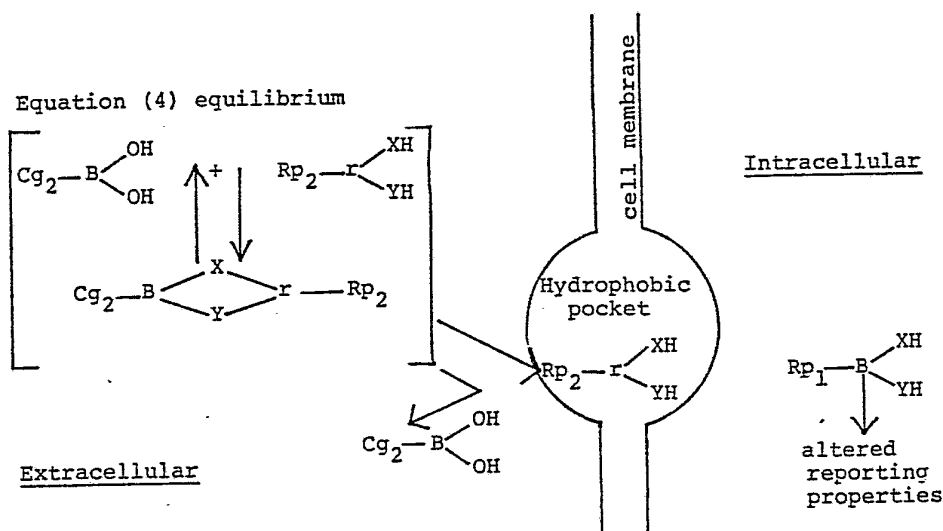
As mentioned above, in the case of formula (1) stains in which the solubilizing group is a
10 positively charged group such as the guanidinium group (e.g., compound 7 of Table III), rather than just the ReportaBora entering the cell, the entire formula (1) compound may enter. After such a stain has entered a cell, hydrophobic regions of the cell
15 compete with the CarrierCeptor portion for the ReportaBora portion, establishing local intracellular equation (3)-type equilibria. In the case of such a stain in which the reporter group is a fluorophore which is enhanced in hydrophobic
20 environments, varying degrees of enhancement in different parts of a cell are correlated with varying degrees of hydrophobicity in those regions; this data can provide additional information about the properties of the cell.

25 Formula (2) dyes can also be used for the vital staining of cells. The diagram below illustrates the mechanism of uptake of ReporterCeptor by cells. As with formula (1) compounds, there are exceptions to this general
30 scheme.



Formula (2) dyes can also be used for the vital staining of cells. The diagram below illustrates the mechanism of uptake of ReporterCeptor by cells. As with formula (1) compounds, there are exceptions to this general scheme.

5



Cells are contacted with an aqueous solution of the stain, which is in dynamic equilibrium with its respective CarrierBora and ReporterCeptor. The cell selectively extracts ReporterCeptor molecules, which enter and stain the cells, and can exhibit altered reporting properties such as fluorescence enhancement or wavelength shift in hydrophobic regions of the cell. Unlike the reporter component of formula (1) stains, those of formula (2) stains are not boronic acids, and do not form such complexes with particular cellular components; their specificity depends instead on the nature of the receptor portion of the molecule.

Formula (2) stains in which the
20 solubilizing group is a positively charged group;
e.g., quaternary ammonium, can also be used for

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vital cell staining. As with formula (1) stains containing such groups, the entire compound may enter the cell and establish local hydrophobicity-dependent equilibria within the cell.

5 A further refinement to vital cell staining using formula (1) and (2) compounds is to employ a stain having more than one staining reporter group. For example, a formula (1) stain

can have the structure $Rp_3 - Rp_1 - B \begin{array}{c} \diagup x \\ \diagdown y \end{array} r - Cg_1$

10 where Rp_1 and Rp_3 each is a group which includes a fluorophore, chromophore, an antigen, an isotopically-labelled group, or an organometallic compound. Compound 12 of Table I, complexed with TAPSO (Table II) is an example of such a compound,
 15 bearing a fluorophore which provides cellular information at the level of the fluorescent microscope, and an electron-opaque organometallic group which provides information on the electron microscope level; the fluorescence pattern of a
 20 stained cell indicates to the electron microscopist that that cell took up stain, indicates which portions of the cell were stained at the level of resolution of the light microscope, and, after preparation for electron microscopy, allows a
 25 comparison of staining patterns within the same cell at the light and ultrastructural levels.

The formula (1) stains can also be used to permanently stain proteins. The preferred method for carrying out such staining is to react the
 30 protein to be stained with the dye under mild conditions; e.g., at room temperature or lower, at



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pH 4 to 10, in the absence of an organic solvent. Excess ReportaBora can then be removed by carrying out dialysis against the CarrierCeptor used to make the stain. For example, to stain serum albumin, compound 9 (Table III) is first made by dissolving highly water-insoluble ChromaBora II (CBII) (Table I) in a very small volume of dimethyl acetamide and then in an excess of TAPSO at pH 9.0. Serum albumin is then added and the mixture then dialyzed against TAPSO to remove excess CBII. Before the dialysis, CBII enters the hydrophobic pockets of serum albumin, leaving its CarrierCeptor, TAPSO, in the aqueous phase. Dialysis against TAPSO removes excess CBII as the formula (1) complex, but is unable to remove CBII from the hydrophobic pockets of the serum albumin, which is thus permanently stained.

Formula (1) and (2) compounds can also be used to stain or brighten a variety of other materials such as fabrics and paper products. Staining is carried out by simply contacting the material to be stained with an aqueous solution of the desired formula (1) or (2) compound (generally a colored or a fluorescent compound) until the desired degree of staining has been achieved.

Brightening of fabrics is carried out using a fluorescent formula (1) or (2) compound, and can often be performed in a conventional clothes washing machine, in soapy or clean water. Many fabrics can be brightened using the new compounds, including cellulose-containing fabrics, such as cotton and linen, and nitrogen-containing



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fabrics such as wool and silk, and various synthetics. When brightening a cellulose-containing fabric, it is preferable to use formula (1) compounds, because the FluoraBora of the equation (3) equilibrium is attracted to the hydroxyl groups of the fabric.

When brightening a nitrogen-containing compound such as wool, formula (1) dyes are also preferred, and the brightening process may include an additional step. Following the step of contacting the fabric with the fluorescent formula (1) compound, the fabric is contacted with an oxidizing agent such as peroxide, which breaks the carbon-boron bond of the formula (1) compound, releasing $Rp_1 - OH$, which is then better locked into the hydrophobic pockets in the fabric. Even greater brightening can be obtained by employing a formula (1) compound in which the fluorescent part of the molecule exhibits enhanced fluorescence in hydrophobic environments.

Peroxide and Enzyme Assays

A colored or fluorescent formula (1) compound can be used to measure, in aqueous solution, an unknown amount of a compound which participates in an enzymatic procedure ("procedure", as used herein, includes one or a series of reactions) in which an enzyme acts on a substrate, or a chemical procedure which results in the production of peroxide.

In the peroxide assay, excesses of all of the reactants participating in the procedure are introduced into a reaction zone, e.g., a test tube,



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along with the sample containing an unknown amount of the compound to be assayed. The peroxide produced is brought into contact with a known amount of the formula (1) compound. The peroxide, an oxidizing agent, breaks the carbon-boron bond of the ChromoBora or FluoroBora, releasing $Rp_1 - OH$, which is then contacted with a substance having a greater affinity for $Rp_1 - OH$ than for the formula (1) compound. The fluorescence or color intensity of the substance is then measured as a measure of the unknown amount of the compound being assayed.

The selective affinity substance can be a water-immiscible organic solvent which is capable of extracting $Rp_1 - OH$ away from the formula (1) compound. The solvent forms a fluorescent organic layer separate from the aqueous layer; if desired the two layers can then be separated to facilitate quantification of the fluorescence of the organic layer.

One factor which influences the choice of formula (1) compound when a water-immiscible organic solvent is to be used is the tightness of the complex between the ReportaBora and the CarrierCeptor. Compounds in which the complex is very tight are most preferred because a looser complex may be broken between the boronate and the CarrierCeptor, thus allowing the colored or fluorescent starting material, along the $Rp_1 - OH$, to be partially extracted by the solvent. Furthermore, in the case of a looser complex, the tendency of the organic solvent to push the



- 25 -

equation (3) equilibrium toward dissociation of the complex by extraction of the ReportaBora may overshadow a comparatively smaller peroxide effect.

Table V shows the extent to which three FluoraBoras, complexed with six CarrierCeptors in aqueous solution, are extracted into ethyl acetate. Of the compounds of Table V, those made with comparatively tightly-complexing TAPSO are most preferred for the peroxide assay because, absent peroxide (i.e., in a blank reaction), a comparatively large proportion of the fluorescence remains in aqueous solution, unextracted by ethyl acetate. Compounds in which the complex is even tighter, so that the blank extraction value is still lower, would be even more desirable.

Another factor influencing the aforesaid choice, when the reporter group of the formula (1) compound is a fluorophore, is the manner in which the organic solvent being used affects the properties of the fluorophore. Some fluorophores are enhanced, i.e., their fluorescence is made more intense, in a given organic solvent, while others exhibit diminished fluorescence (quenching). Some fluorophores may also exhibit altered emission spectra in certain organic solvents. Although any fluorescent formula (1) compound can be used, provided its fluorophore is not totally quenched in the organic solvent, both enhancement and spectrum alteration desirably heighten detectability.

Table VI shows the relative fluorescence of three FluoraBoras combined with TAPSO, compared to the fluorescence of equal concentrations of



- 26 -

FluoraBora in the water-immiscible organic solvent ethyl acetate. Because FBPA and FBI are enhanced (two and seven-fold, respectively), formula (1) compounds made with them are suitable for use in the peroxide assay described above. FBIII, on the other hand, is quenched to a large extent in ethyl acetate, and formula (1) compounds made with it are therefore not desirable for use in the assay in which ethyl acetate is employed.

10 Instead of a water-immiscible organic solvent, the selective affinity substance in the peroxide assay can be a water-miscible protein which has a greater affinity for Rp_1 than for the corresponding formula (1) compound being used.

15 Preferred proteins are those, e.g., serum albumin, which posses hydrophobic pockets which can attract Rp_1 .

 When such a protein is used, it is necessary that the formula (1) compound be

20 fluorescent, and that the protein be capable of producing an observable effect, preferably enhancement or, less preferably, quenching, of fluorescence, of Rp_1 , there being no easily observed water/protein phase separation. Table VII

25 shows the relative enhancement or inhibition with serum albumin, of the three fluorophores of eighteen formula (1) compounds. Table VII shows that, in general, FBI and, to a lesser extent, FBPA, are enhanced in serum albumin, while FBIII

30 exhibits diminished fluorescence.

 The extent to which each of the three fluorophores of Table VII is enhanced or inhibited



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depends, to a significant extent, on the CarrierCeptor with which it is combined. Thus, enhancement, in serum albumin, of FBPA and FBI, and inhibition of FBIII, is much greater if the formula

5 (1) compound has been made with loosely complexing MOPSO than with tightly complexing TAPSO. This is because the loose Rp_1 -MOPSO complex favors an equilibrium in which more free Rp_1 is available to seek the hydrophobic pockets of serum albumin,

10 where enhancement or inhibition of fluorescence occurs. Before performing an assay, therefore, a blank should always be run in which the peroxide-generating reagents are not added, so that enhancement or inhibition in the absence and

15 presence of those reagents can be compared.

The other observable effect which a water-miscible protein such as serum albumin can have on a fluorophore is a shift in the peak emission spectrum; such a shift can, as in the case

20 of a water-immiscible organic solvent, aid detectability. FBPA, for example, normally emits at 460 nm, while its dissociated fluorophore, in the hydrophobic pockets of serum albumin, emits at 427 nm.

25 Glucose is an example of a compound which can be measured using the above-described method. A sample containing an unknown amount of glucose is placed in a test tube with a known amount of Compound 1 (Table III) and a known amount of serum

30 albumin in aqueous solution at pH between 8 and 9. An excess of glucose oxidase is then added, and the increase in fluorescence compared to that of a



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no-enzyme, no-substrate blank is measured as a measure of the amount of peroxide produced, which in turn is a measure of the amount of glucose in the sample.

5 Here, glucose oxidase catalyzes the oxidation of glucose to gluconic acid by oxygen, forming H_2O_2 . The H_2O_2 oxidizes the C-B bond of FBI in the formula (1) complex, forming dansylamido phenol, which enters the serum albumin
10 pockets, where it exhibits enhanced fluorescence.

 Using principles similar to those just described, certain formula (1) compounds can also be used to measure an unknown amount of a compound which participates in an enzymatic procedure in
15 which an enzyme acts on a substrate. The method employs fluorescent formula (1) compounds in which Rp_1 includes a group, positioned between the boron atom and the fluorophore, capable of acting as a substrate for the enzyme. A desired enzyme
20 substrate can be incorporated into a desired formula (1) compound using conventional techniques.

 According to the assay methods, the formula (1) compound being used is introduced into a reaction zone, along with excesses of all of the
25 other participating reactants except the compound to be measured, and a sample containing the compound to be measured. The enzymatic reaction releases modified fluorophore which, after completion of the reaction, is contacted with a
30 water-immiscible organic solvent or a water-miscible protein, as described above for the peroxide assay. The extent of fluorophore



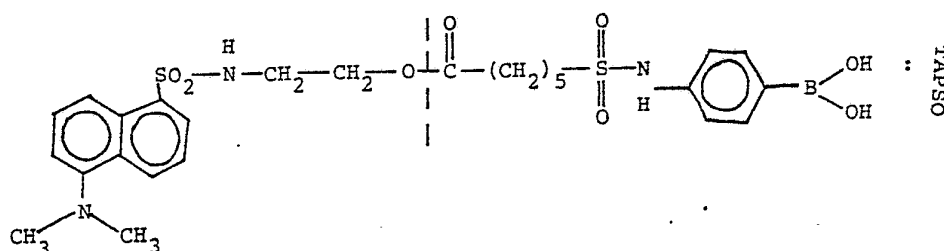
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extraction or fluorescence enhancement or inhibition is then measured versus a blank, as described above, as a measure of the unknown compound.

5 If a water-miscible protein is used, the rate of the enzymatic reaction can also be followed by measuring the rate at which fluorescence enhancement increases.

10 Esterases such as lipases are examples of compounds which can be assayed using this method. In a test tube, in aqueous solution, is placed a known amount of compound 6 (Table III), of the formula

15



A sample containing an unknown amount of lipase is then added. Lipase present in the sample breaks the ester bond (as indicated by the dotted line), releasing modified Rp_1 , containing the fluorescent dansyl group, which is then extracted or contacted with a known amount of serum albumin and fluorescence measured, as described above for the peroxide assay.

20

Antibody Assay

25 Another new assay employing formula (1) compounds is an antibody assay employing red blood

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cells. (Red Blood cells are commonly used in other antibody assays.) A formula (1) compound is used in which the reporter group of Rp_1 is a hapten capable of complexing with the antibody being
5 assayed. Red blood cells are contacted, in aqueous solution, with the formula (1) compound, which is in an equation (3)-type equilibrium with its starting materials. The red blood cell membrane selectively extracts hapten-containing ReportaBora,
10 thus becoming sensitized. The sensitized red blood cells are contacted with a sample containing an unknown amount of antibody, together with complement which is capable of participating in the complexation of the hapten with the antibody. The
15 antibody in the sample complexes with the hapten in the sensitized cell membrane, fixing complement and causing cell lysis and the release of hemoglobin from the cell. The amount of hemoglobin released is a measure of the amount of antibody in the
20 sample.

A modification of the above-described procedure can be performed using red blood cell ghosts or membranes rather than intact cells. According to this modification, the formula (1)
25 compound used contains two reporter groups; a hapten and a fluorophore, both of which become incorporated into the membranes of sensitized red cell ghosts by the BorAdept process. When the ghosts are contacted with antibody and complement,
30 lysis of the ghost stroma occurs, causing the release of soluble fragments containing fluorophore. If desired, the solution can contain



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a CarrierCeptor to facilitate solubilization of the fluorescent fragments. The fluorescence in solution is then measured as a measure of the antibody titer.

5 Solubilization of Therapeutic Agents

 The BorAdept system of the invention can greatly facilitate the administration of therapeutic agents such as drugs which have heretofor been difficult to administer because of
10 poor water solubility or difficult to test in cell culture systems. A desired therapeutic agent can be incorporated into either a formula (1) or (2) compound. In either case, by virtue of the equation (3) or (4) equilibrium, the agent is not
15 only solubilized, but is in a form which enables the agent to be presented to and taken up by cells to which it is desired to deliver the agent, by the same mechanism by which fluorophore reporter groups are taken up by cells to be stained.

20 Therapeutic formula (1) compounds are generally made in the same way fluorescent formula (1) compounds are made; i.e., the desired therapeutic agent or a suitable precursor or derivative thereof is first reacted with the
25 desired boronic acid to form a ReportaBora (in this case, a TheraBora), which is then reacted with the desired CarrierCeptor. The boronic acid is preferably chosen to include a group which renders the boronic acid reactive with the therapeutic
30 agent being used.

 Compound 4 of Table III is an example of a formula (1) compound containing a derivative of



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therapeutic agent, the water-insoluble anti-convulsant diphenyl hydantoin. Another example is compound 2 of Table III, containing a derivative of the water-soluble anti-epileptic drug carbamazepine.

5 This new compound is made according to the reaction scheme illustrated on the following page.

Therapeutic formula (2) compounds are generally made in the same way that fluorescent formula (2) compounds are made, i.e., the desired

10 therapeutic agent or a suitable precursor or derivative thereof is, if necessary, incorporated into a compound having a desired property to make Rp_2 , which is then reacted with a compound of the

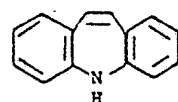
formula $\begin{array}{c} HX \\ HY \end{array} \rightarrow r$ to make the ReporterCeptor, which

15 is then reacted with the desired CarrierBora..

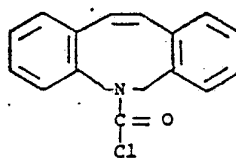
Compound 5 of Table IV is made according to the reaction scheme illustrated on the following pages:



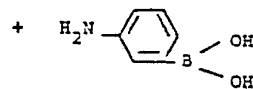
- 33 -

Iminostilbene
(commercially
available)

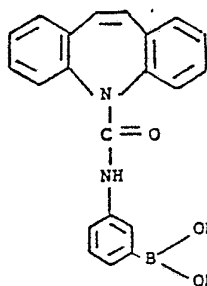
Phosgene



chlorocarboxyliminostilbene

m - aminophenyl
boronic acid

TAPSO



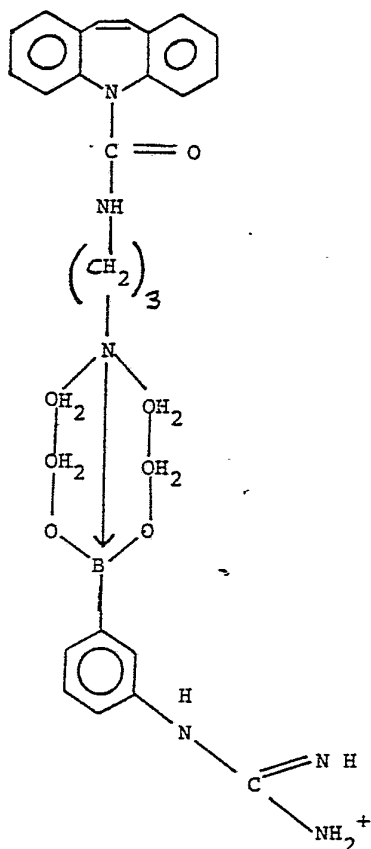
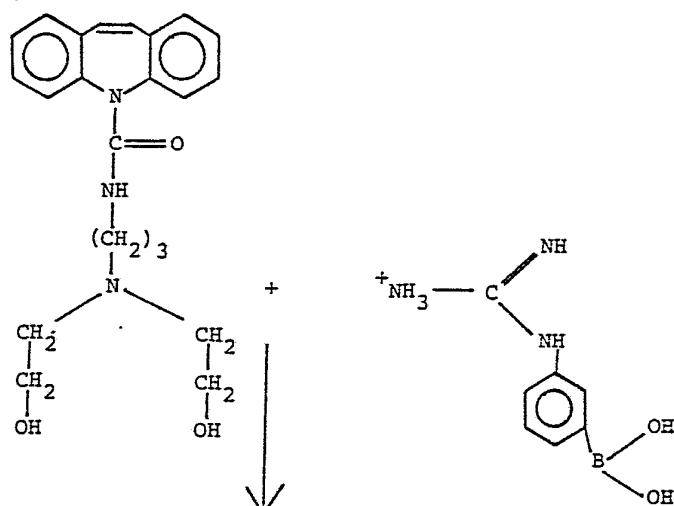
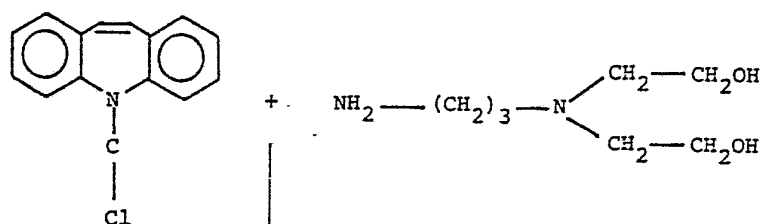
: TAPSO

carbamazepinophenyl boronic acid : TAPSO
(compound 2 of TABLE III)

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Synthesis of
Compound 5 of Table IV



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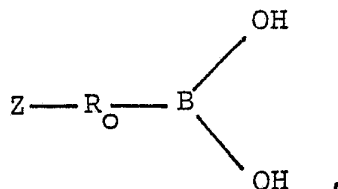
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ReportaBoras

Table I lists some preferred ReportaBoras of the invention. These new compounds, as is discussed above, are useful in making formula (1) BorAdept compounds. In addition, we have discovered that they have many applications by themselves, even when not complexed with a CarrierCeptor in a formula (1) compound. Many of these applications, e.g., the peroxide assay, are similar in principle to applications of formula (1) compounds.

The ReportaBoras of the invention are generally made by reacting a desired reporter group-containing compound (Rp_4) with a desired organic boronic acid capable of reacting with that Rp_4 .

Usually the organic boronic acid has the general formula,



where R_O is aryl, alkyl, heteroalkyl, heteroaryl, or aralkyl, and Z is a group capable of chemically combining with the Rp_4 being used. Preferably, R_O contains between 3 and about 25 carbon atoms. A preferred (because of ready availability) boronic acid is m-aminophenyl boronic acid, in which Z is NH and R_O is



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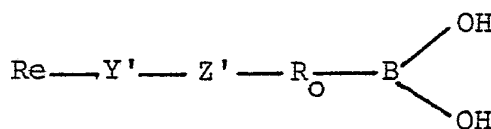
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Rp₄ usually has the formula



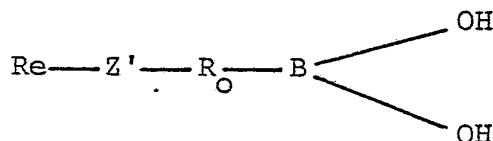
where Re is the reporter group and Y is a group capable of chemically combining with the Z portion of the boronic acid being used.

The ReportaBoras made by reacting the aforesaid boronic acids and Rp₄'s thus either have the formula



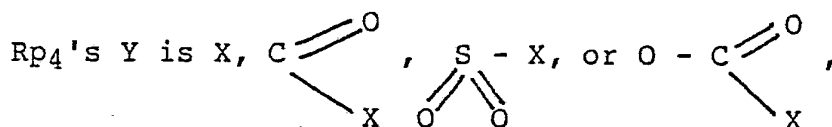
10

or



depending on whether Y is entirely lost in the course of the reaction.

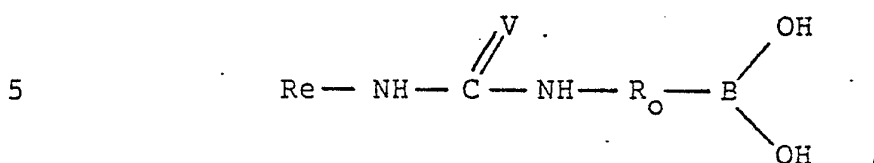
In some preferred boronic acids, Z is NH₂, OH, SH, or COOH, and in some preferred



X being any halogen. In ReportaBoras made from these starting materials, the carbon or sulfur atom of Y to which X was bonded bonds to Z, replacing an H. Z' is thus Z minus an H, and Y' is Y minus X. (there is no Y' when Y consists entirely of X).

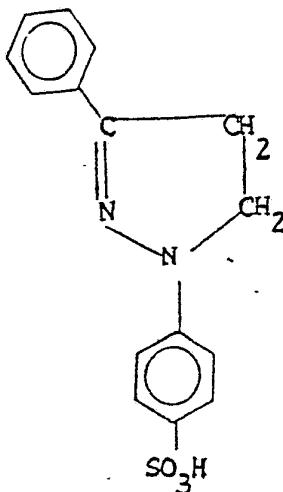
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The boronic acids wherein Z is NH_2 can also react with Rp_4 's in which Y is $\text{N}=\text{C}=\text{V}$, where V is O or S. The resulting ReportaBoras have the formula



Y' being $\text{NH}-\overset{\text{V}}{\underset{\text{||}}{\text{C}}}$ and Z' being NH.

One of the most preferred new ReportaBoras we have synthesized is referred to in Table I as "FBPA". This compound is made using a diaryl pyrazoline sulfonic acid, supplied to us as a gift by Ilford Limited, Basildon - Essex, England. Ilford's fluorescent compound has the formula



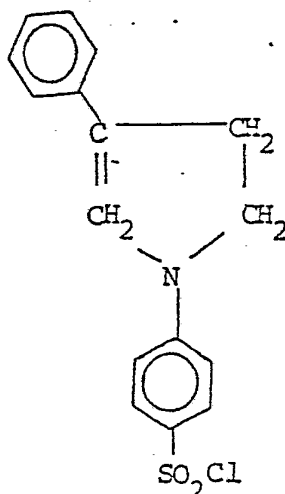
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The first step in our synthesis of FBPA is to make the sulfonyl chloride of Ilford's compound by reacting it with PCl_5 . The new sulfonyl chloride has the formula:

5



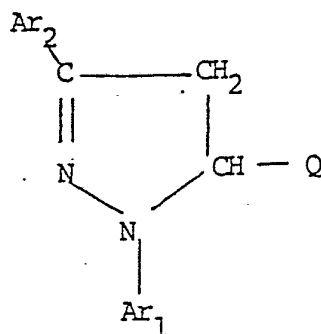
The new compound has a chemically stable crystalline structure with a melting point of 119°C . Besides being useful for making ReportaBoras, it is, also useful as a fluorescent reporter for labelling proteins such as antibodies and other compounds such as amino acids and polyamines. Because of its azure fluorescence, it can be used in conjunction with red and yellow fluorescent dyes in immunofluorescent labelling experiments.

Compound 21 is a diaryl sulfonyl chloride, and is one of a family of fluorescent compounds which we refer to as DARPSYL chlorides. These compounds are the sulfonyl chloride derivatives of compounds of the general formula



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where one of Ar_1 or Ar_2 is an aromatic group containing a sulfonyl chloride group and Q is hydrogen or an alkyl group. The compounds in which

5 Ar_1 contains the sulfonyl chloride group are the "A" compounds, and are derived from the DARPSYL compounds described in British Pat. No. 670,857. The sulfonyl chloride derivatives in which Ar_2 contains a sulfonyl chloride group are the "B"

10 compounds, and are also useful fluorescent stains.

The new A-DARPSYL chloride, (compound 21), as well as other DARPSYL-chlorides, can be combined with organic boronic acids to make useful FluoraBoras. To make FBPA (Table I), the A-DARPSYL

15 chloride described above is combined with m-aminophenyl boronic acid in DMF- H_2O . The mixture is then acidified, water is added causing FBPA to precipitate out and recrystallization is carried out, from cyclohexane.

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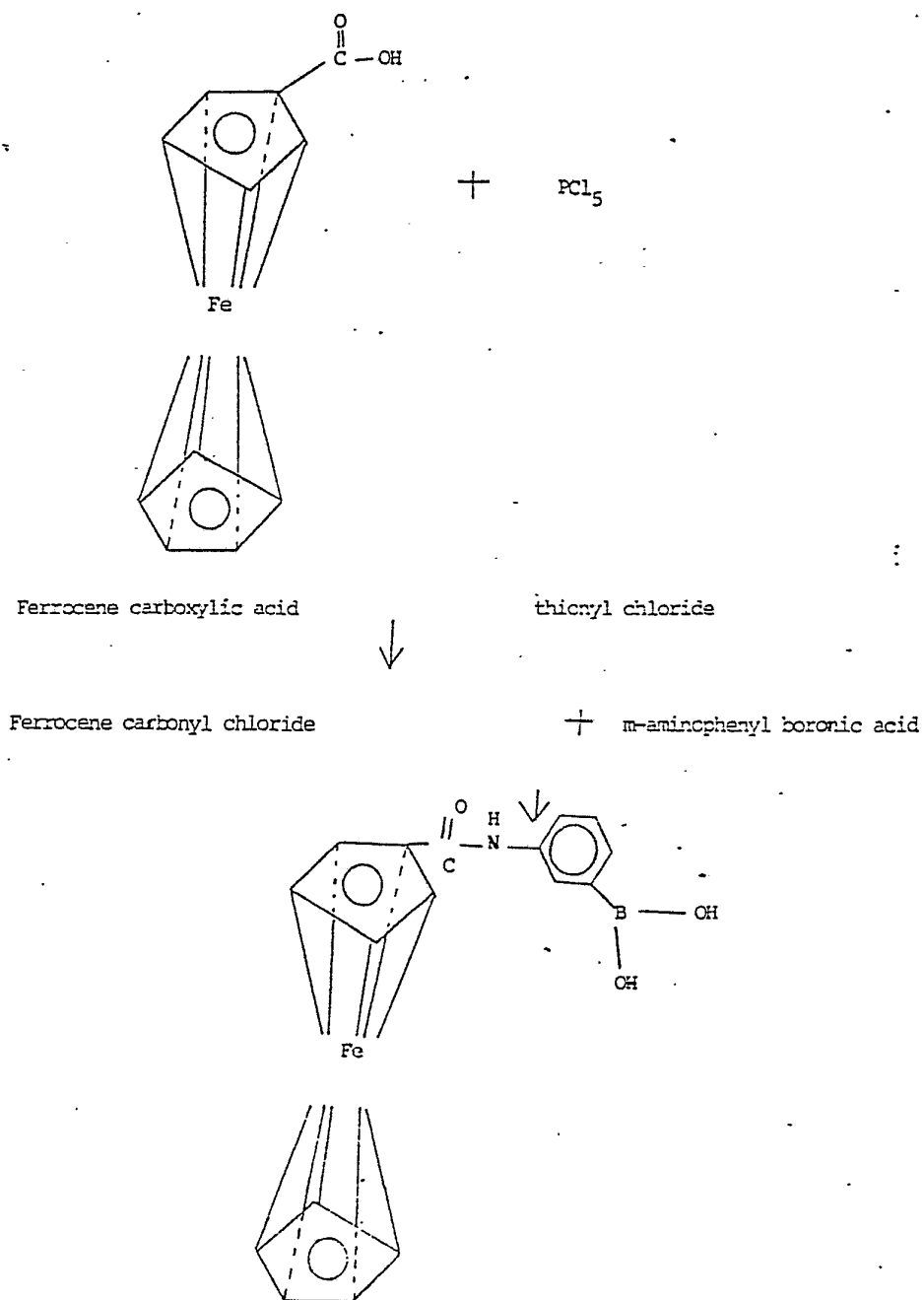
Another FluoraBora listed in Table I, FBI,
is made by adding an acetone solution of dansyl
chloride to an aqueous acetone solution of
m-aminophenyl boronic acid hemisulfate containing
5 2.5 equivalents of sodium bicarbonate, and then
acidifying the solution, adding more water causing
FBI to precipitate out and recrystallizing from
benzene.

FBII, another FluoraBora, is made by
10 adding solid fluorescein isothiocyanate to an
aqueous alkaline solution of m-aminophenyl boronic
acid.

Many of the ReportaBoras listed in Table I
are not fluorescent, but rather contain reporter
15 groups such as chromophores or derivatives of
therapeutic agents. The reporter group can also
be an antigen or an isotopically-labelled group, or
it can be an organometallic group, as in compounds
8 and 15 of Table III. Compound 12 of Table III
20 also contains an organometallic group, and in
addition contains the fluorescent dansyl reporter
group. Compound 8 is made according to the
reaction diagrammed below.



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In addition to the mercury and iron-containing complexes of the compounds of Table I, any desired organometallic complex can be used to make a ReporterBora, which in turn can be combined with a CarrierCeptor to make a BorAdept reagent. As is mentioned above, other useful organometallic complexes contain heavy, electron-opaque metals such as osmium, which are useful in staining for electron microscopy.

10 Cell Staining with ReportaBoras

Many of the ReportaBoras of Table I possess such poor water solubility that they cannot be presented to and taken up by living cells under non-toxic, physiological conditions. Such compounds, as has been discussed, are rendered useful for vital staining only when coupled to a CarrierCeptor.

Some ReportaBoras, e.g., FBI, however, are sufficiently water-soluble, and possess the necessary neutral or positive charge and are sufficiently hydrophobic, to be useful directly as vital stains. Such ReportaBoras are generally used for selective vital staining in the same manner as are BorAdept vital stains, described above.

ReportaBoras and ReporterCeptors can also be used to selectively stain fixed cells, using conventional techniques and solvents. As with vital staining, the selectivity provided by the boronic acid portion of the ReportaBoras can provide valuable diagnostic information.



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Certain non-toxic ReportaBoras are useful for vitally staining the surfaces of cells and extracellular areas, and are also useful for staining the eye surfaces of living animals to determine the location of surface irregularities such as scratches and lesions; the negatively charged ReportaBoras are particularly useful for the latter purpose. To stain eye surfaces, the desired ReportaBora, dissolved in a physiological buffer or CarrierCeptor as a formula (1) complex, is applied in any conventional manner, e.g., with an eyedropper.

Support-bound FluoraBoras

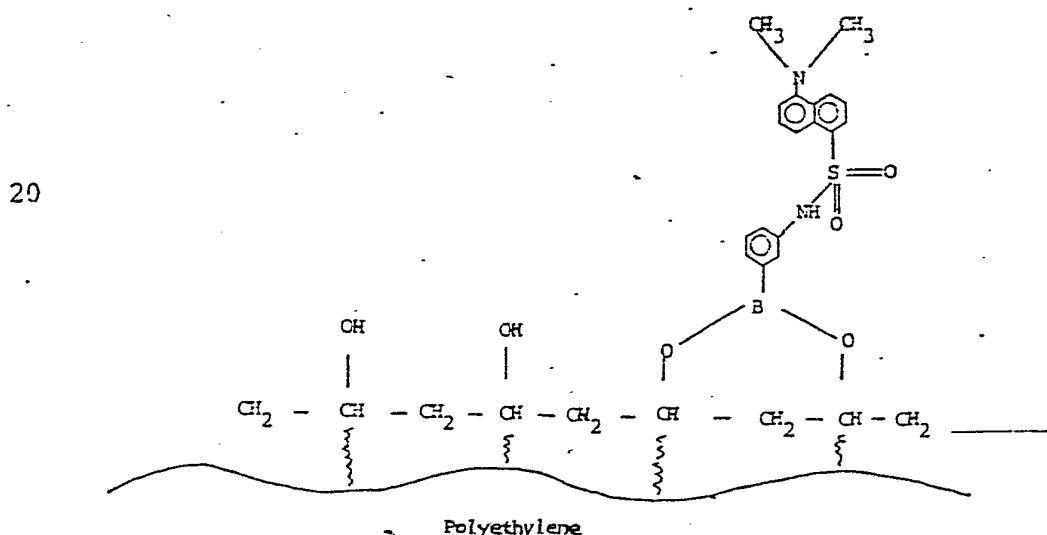
FluroaBoras can be bound to certain water-insoluble supports and used in peroxide and other assay methods. The supports which can be used include all organic, water-insoluble polymeric materials having hydroxyl groups which can complex with boronic acids. Examples of suitable supports are polymers containing polyglycerol methacrylate, and hydrophilic fibrous polyethylene containing attached polyvinyl alcohol; the latter support is available from Crown Zellerbach under the name "Fybrel". Support-bound FluoraBoras preferably are made by reacting a solubilized FluoraBora (in acetone, benzene, DMF, or the like) with the powdered form of an appropriate support material and then washing the powder with a solvent to remove excess FluoraBora. Alternatively, if the FluoraBora being used is water-insoluble, it is



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first complexed with a suitable CarrierCeptor to form a formula (1) BorAdept compound, as described above. If a CarrierCeptor is used, it and the support should be chosen so that the support tends to form stronger complexes with the boronate of the FluoraBora being used, at the pH under which the reaction is carried out, then does the CarrierCeptor at that pH. The equation (3) equilibrium then allows the support to act as a separatory funnel, complexing with dissociated FluoraBora. Usually a weakly complexing buffer such as MOPSO will be used in this situation.

FluoraBoras bonded to Fybrel are particularly preferred embodiments of this aspect of the invention. The structure of FBI so bound is shown below. It can be seen that the boron atom forms a stable 6-membered ring with three carbons and two oxygens of the polyvinyl alcohol.



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FBI bound to Fybrel is made as follows.
FBI is dissolved in acetone. Fybrel is ground to
coarse powder and shaken with the dissolved FBI for
5 minutes. After exhaustive washing with acetone,
5 support-bound FBI is stored in the dark at 5°C
until used, either dry or in an organic solvent.

Peroxide Assay

Flow Chart I (Fig. 1) diagrams a method
for assaying, in aqueous solution, a compound which
10 participates in a chemical procedure which results
in the production of peroxide. The basic principle
is the same as that of the BorAdept peroxide assay,
previously described. Excesses of all of the
reactants participating in the procedure are
15 introduced into a reaction zone, e.g., a test tube,
along with the sample containing an unknown amount
of the compound to be assayed. The peroxide
produced is brought into contact with a
support-bound FluoraBora, described above. The
20 peroxide, an oxidizing agent, breaks the
carbon-boron bond of the boronic acid, releasing
fluorescent compound into solution, where it can be
measured directly using, e.g., a fluorometer,
enhanced using a protein such as serum albumin, or
25 extracted using a water-immiscible organic solvent
such as ethyl acetate.

The rate of release of fluorescent
compound can be increased by maintaining the pH of
the reaction zone in the range of about 8 to 9.
30 The assay can be further modified by separating
fluorescent compound in solution from the insoluble
support following the oxidation of the
carbon-boron bonds by the peroxide. This



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separating can be carried out by filtering, by removing the insoluble support using centrifugation, or by extracting the dissolved fluorescent compound in an organic, water-immiscible solvent.

Glucose is an example of a compound which can be assayed using the above-described method. To a sample, in aqueous solution, containing an unknown amount of glucose are added aqueous buffer of pH 8 to 9, glucose oxidase, and an excess of FBI bound to Fybrel. After the release of fluorescent compound into solution has been completed, the Fybrel is filtered out and fluorophore is measured, using a fluorometer, as a measure of glucose in the sample.

Apparatus useful in the practice of the peroxide assay method is illustrated in Figure 2. The rim 6 of the plug 3 fits snugly but movably within outer tube 2. Plug 3, whose inner core contains filter 4, fits immovable in the end of inner tube 1.

The bottom of tube 2 contains an excess of a support-bound FluoraBora, described above. Into tube 1 are introduced, in aqueous solution, excesses of all of the reactants, except the compound to be assayed, necessary to produce peroxide in the procedure being measured. A sample containing an unknown amount of the compound to be assayed, e.g., glucose, is introduced into tube 1, which is then pulled up to allow the solution, including any peroxidase produced, to pass downward through filter 4 to contact the support-bound FluoraBora. After the release of fluorophore into



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solution has been completed, tube 1 is pushed down to allow the fluorophore in solution to pass upward through filter 4. The fluorescence of the solution, or of an extract of the solution, a
5 measure of the compound being assayed in the sample, is measured with a fluorometer.

Other Assays

The stable bond formed between FluoraBoras and supports containing hydroxyl groups and between
10 FluoraBoras and certain other compounds, e.g., mucopolysaccharides, provides the basis for an assay for compounds having an affinity for boronic acids. The desired FluoraBora is contacted with an unknown amount of a compound to be assayed having
15 an affinity for boronic acids. Another material having an affinity for boronic acids, e.g., one of the supports discussed above, is then added to the mixture. This second material binds with FluoraBora which did not complex with the compound
20 being assayed. The amount of FluoraBora bound to this second material is inversely proportional to the amount of unknown present.

Other Embodiments

Other embodiments are within the following
25 claims. For example, many more compounds than are listed in the Tables are within the scope of the invention. Also, the assays can be varied in many conventional ways without departing from the inventive concept. For example, FluoraBoras can be
30 used in standard displacement assays to measure compound having an affinity for boronic acids.



TABLE I: ReportaBoras

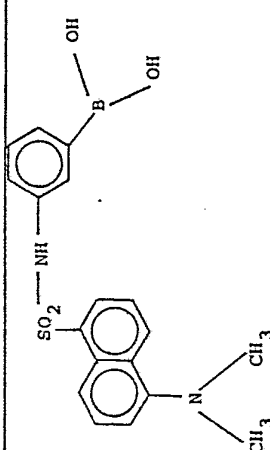
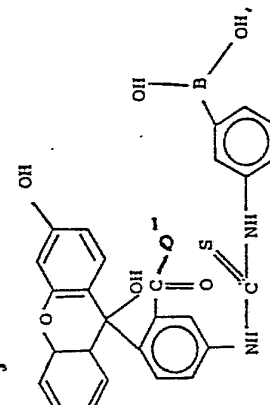
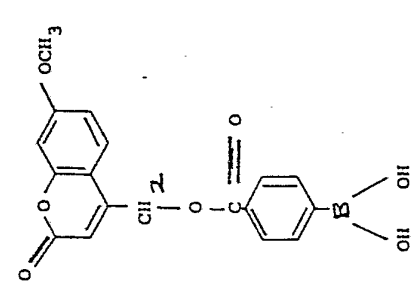
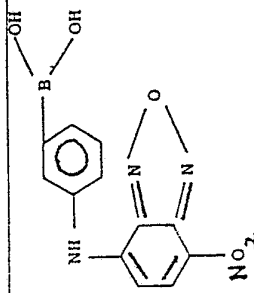
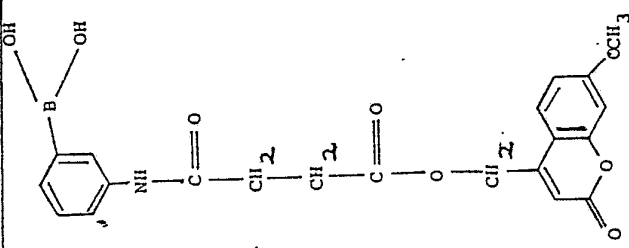
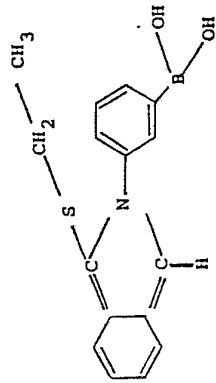
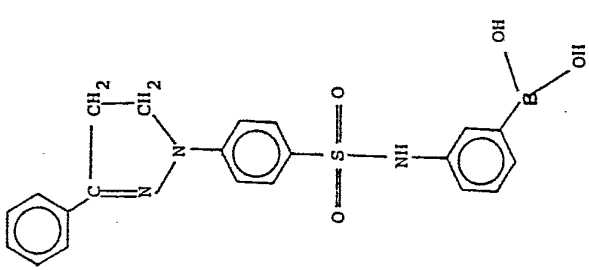
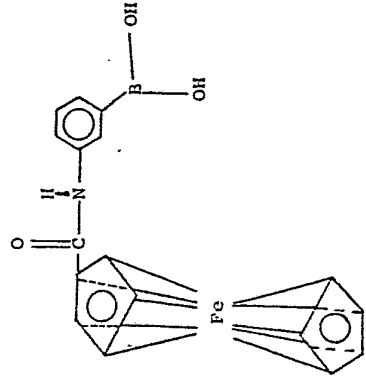
Structure	Trivial or Chemical Name	Utility of Reporter Group	Starting Materials
	FBI	fluorescent	Dansyl chloride, m-aminophenyl boronic acid
	FBI	fluorescent	Fluorescein isothiocyanate, m-aminophenyl boronic acid
	FBI	fluorescent	4-Bromomethyl-7-methoxycoumarin, 4-carboxyphenyl boronic acid

TABLE I: ReportaBoras

Structure	Trivial or Chemical Name	Utility of Reporter Group	Starting Materials
	FBIV	fluorescent	4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole, m-aminophenyl boronic acid
	FBV	fluorescent	4-Bromomethyl-7-methoxycoumarin, n-(succinamido)-3-aminophenyl boronic acid
	FBVI	fluorescent	O-phthalaldehyde, thiol, m-aminophenyl boronic acid

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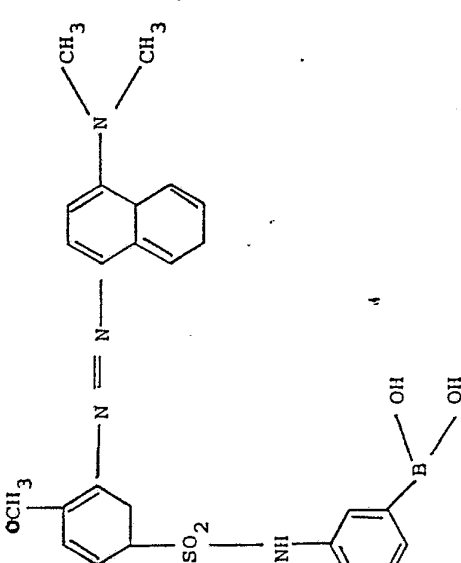
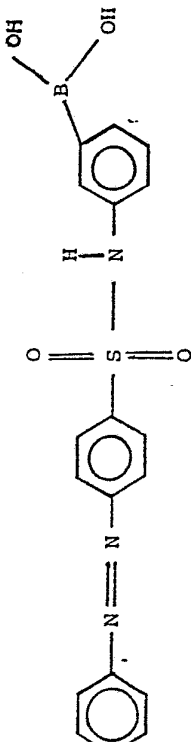
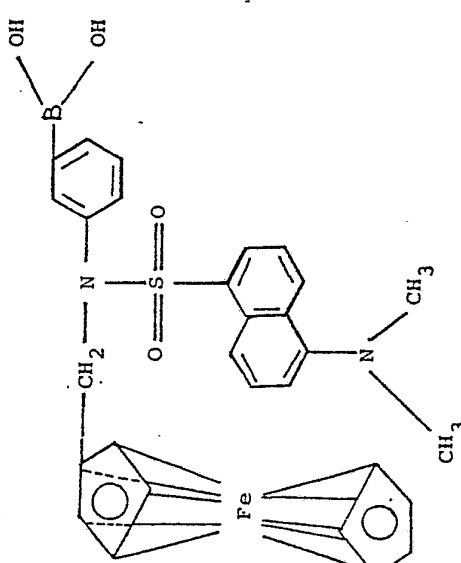
TABLE 1: ReportaBoras

Structure	Trivial or Chemical Name	Utility of Reporter Group	Starting Materials
	FPBA	fluorescent	A - DAPSYL chloride, m-aminophenyl boronic acid
	3-[Ferrocene carboxamido]phenyl boronic acid	electron-dense metal	Ferrocene carbonyl chloride, m-aminophenyl boronic acid

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TABLE I: ReportaBoras

Structure	Trivial or Chemical Name	Utility of Reporter Group	Starting Materials
 9	CBI	colored (brownish red),	Dimethylaminonaphthoazomethoxyphenyl-sulfonic acid, m-aminophenyl boronic acid
 10	CBIII	colored (reddish)	
 11	Metallo Fluorabora I (MFI)	electron-dense, fluorescent	Ferrocene carboxyaldehyde, m-aminophenyl boronic acid, dansyl chloride

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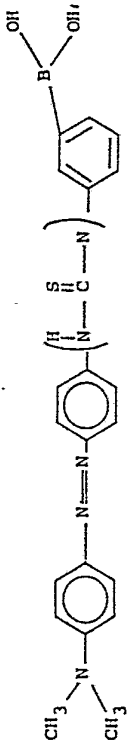
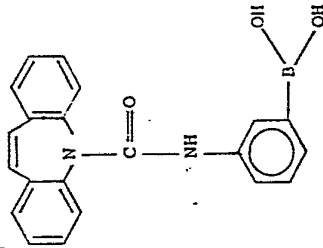
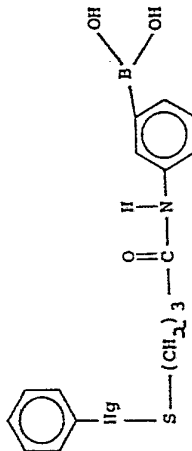
Structure	Trivial or Chemical Name	Utility or Reporter Group	Starting Materials
 12	CHII	colored (brown)	Dimethylaminophenylazo-phenylisothiocyanate, m-aminophenyl boronic acid
 13	carbamazepine phenylboronic acid	therapeutic	iminostilbene, phosphene, m-aminophenyl boronic acid
 14	MetalloBora I (MBI)	electron-dense metal	phenylmercurychloride, l-thiopropionic acid, m-amino phenyl boronic acid

Table II: CarrierCeptors

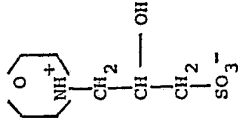
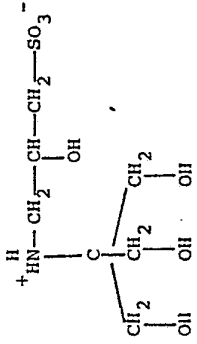
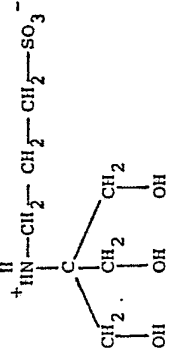
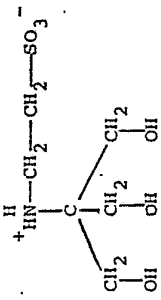
Structure	Trivial or chemical name	Relative strength of adduct with boronic acids	pKa
	MOPSO	Weak	6.75
	TAPSO	Strong	7.39
	TAPS	Fairly Strong	8.11
	TES	Fairly Strong	7.16

Table II: CarrierCeptors (cont.)

Structure	Trivial or chemical name	Relative strength of adduct with boronic acids	pKa
$ \begin{array}{c} \text{+H} \\ \\ \text{N}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{SO}_3^- \\ \quad \quad \quad \\ \text{CH}_2 \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{OH} \end{array} $	BES	moderate	6.88
$ \begin{array}{c} \text{+H} \\ \\ \text{N}-\text{CH}-\text{COO}^- \\ \quad \quad \quad \\ \text{CH}_2 \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{OH} \end{array} $	Bicine	moderate	8.04
$ \begin{array}{c} \text{+H} \\ \\ \text{N}-\text{CH}_2-\text{COO}^- \\ \quad \quad \quad \\ \text{CH}_2 \quad \text{CH}_2 \quad \text{CH}_2 \quad \text{OH} \end{array} $	Tricine	moderate	
$ \begin{array}{c} \text{H} \\ \\ \text{+H}-\text{N}-\text{C}-\text{COO}^- \\ \quad \\ \text{OH}-\text{CH}_2 \quad \text{H} \end{array} $	Serine	weak	

Table II: CarrierCeptors (cont.)

9	Structure	Trivial or chemical	Relative strength of adduct with boronic acids
	$\begin{array}{c} \text{H}^+ \\ \\ \text{HN} \\ \\ \text{CH}_3 - \text{CH} - \text{CH} - \text{COO}^- \\ \\ \text{OH} \end{array}$	Threonine	Weak
10	$\begin{array}{c} \text{NH}_3^+ - \text{C} - \text{NH} - (\text{CH}_2)_3 - \text{N} - \begin{array}{l} \text{CH}_2 - \text{CH}_2 - \text{OH} \\ \text{CH}_2 - \text{CH}_2 - \text{OH} \end{array} \\ \\ \text{Cl}^- \end{array}$		Moderate
11	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3 - \text{N}^+ - (\text{CH}_2)_3 - \text{N} - \begin{array}{l} \text{CH}_2 - \text{CH}_2 - \text{OH} \\ \text{CH}_2 - \text{CH}_2 - \text{OH} \end{array} \\ \\ \text{CH}_3 \end{array} \quad \text{I}^-$	trimethyl ammonium propyl diethanolamine	Moderate

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Table III: Formula (1) Compounds

Structure	Reporter Group or ReportaBora	Utility of Reporter Group	Trivial Name of CarrierCeptor
	chromophore	colored	TAPSO

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Table III: Formula (1). Compounds (cont.)

Structure	Reporter Group or Reporter	Utility of Reporter Group	Trivial Name of Carrier/Receptor
	4 diphenyl hydantoin derivative	therapeutic agent	TAPSO
	5 FBPA	fluorescent	TAPSO
	6 dansyl	fluorescent	TAPSO

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Table III: Formula (1) Compounds (cont.)

Structure	Reporter Group or Reportabore	Utility of Reporter Group	Trivial Name of Carrier
7 FBPA		fluorescent	quadinopropyl diethanolamine

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Table III: Formula (I) Compounds (cont.)

Structure	Reporter Group or Reportabara	Utility of Reporter Group	Trivial Name of CarrierCenter
	FBIII	fluorescent	MOPSO

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Table IV: Formula (2) Compounds

Structure	Name of Reporter Group	Utility of Reporter Group
	1 dansyl	fluorescent
	2 DARPSYL	fluorescent

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Structure	Name of Reporter Group	Utility of Reporter Group
	3	fluorescent
	4	electron-dense metal

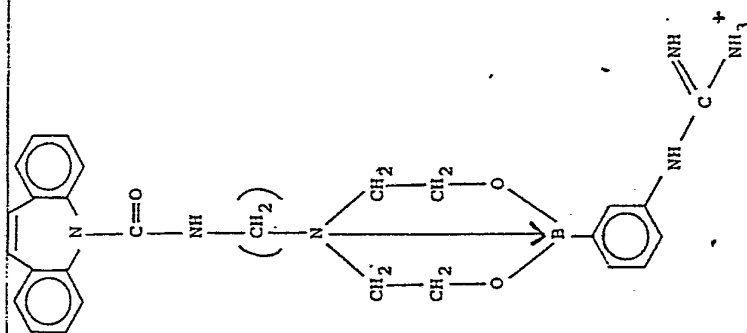
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therapeutic

5



* made from salicylidene, 2-Aminonaphthalene
(Frinton Laboratories); NaBH₄ reduction

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Table V: Extraction of Formula (1)
Compounds by Ethyl Acetate

		<u>Ethyl acetate: buffer partition</u>
5	FBPA complexed with	
	MOPSO	$\frac{87}{13}$
	BICINE	$\frac{93}{7}$
	BES	$\frac{72}{28}$
	TAPS	$\frac{90}{10}$
	TES	$\frac{92}{8}$
10	TAPSO	$\frac{67}{33}$
FBI complexed with	MOPSO	$\frac{92}{8}$
	BICINE	$\frac{71}{29}$
	BES	$\frac{81}{19}$
	TES	$\frac{93}{7}$
15	TAPS	$\frac{84}{16}$
	TAPSO	$\frac{28}{72}$
FBIII complexed with	MOPSO	$\frac{99}{1}$
	BICINE	$\frac{98}{2}$
	BES	$\frac{95}{5}$
	TES	$\frac{95}{5}$
20	TAPS	$\frac{99}{1}$
	TAPSO	$\frac{55}{45}$

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Table VI: Fluorescence of FluoraBoras
in TAPSO vs ethyl acetate

	<u>TAPSO pH 7.4</u>	<u>ETHYL ACETATE</u>
5 FBPA	1015 μ A/ μ mole (370/953)	2363 μ A/ μ mole (370/428)
FBI	8.8 μ A/ μ mole (340/560)	61.3 μ A/ μ mole (340/510)
FBIII	436 μ A/ μ mole (330/410)	Quenched

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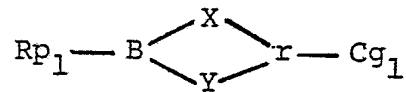
Table VII: Enhancement or inhibition
of formula (1) compounds in serum albumin

			<u>Fluorescence enhancement</u>
5	FBPA complexed with	MOPSO	2.8
		BICINE	2.4
		BES	1.9
		TAPS	1.7
		TES	1.5
10		TAPSO	1.6
15	FBI complexed with	MOPSO	6.8
		BICINE	5.6
		BES	5.0
		TES	3.8
		TAPS	3.2
		TAPSO	2.4
20	FBIII complexed with	MOPSO	0.33
		BICINE	0.39
		BES	0.40
		TES	0.63
		TAPS	0.68
		TAPSO	0.75

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Claims

1. A compound of the formula



wherein

5 Rp_1 is a group which includes a reporter group comprising a fluorophore, a chromophore, an organometallic group, a therapeutic agent, an antigen, or an isotopically-labelled group,

10 each of X and Y, independently, is N, O, or S,

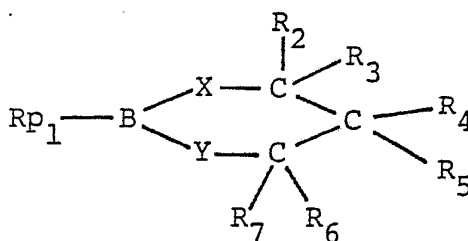
Cg_1 is a carrier group which includes a solubilizing group which is substantially ionized in water, and

15 r is a receptor group which, with X and Y, forms complexes with boronic acids.

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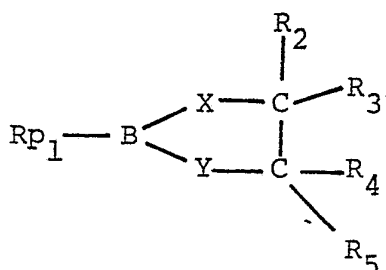


2. The compound of claim 1, of the formula



wherein each R₂ - R₇, independently, is hydrogen, aryl, alkyl, heteroalkyl, heteroaryl, aralkyl, or a solubilizing group which is substantially ionized in water, provided that the compound contains at least one solubilizing group.

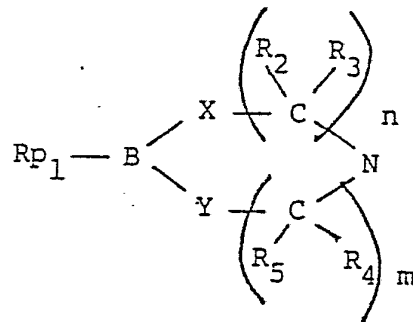
3. The compound of claim 1, of the formula



10 wherein each R₂ - R₅, independently, is hydrogen, aryl, alkyl, heteroalkyl, heteroaryl, aralkyl, or a solubilizing group which is substantially ionized in water, provided that the compound contains at least one solubilizing group.

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4. The compound of claim 1, of the formula



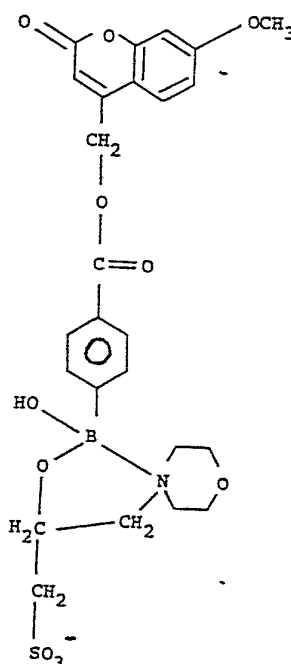
wherein

each $R_2 - R_5$, independently, is
 5 hydrogen, aryl, alkyl, heteroalkyl, heteroaryl,
 aralkyl, or a solubilizing group which is
 substantially ionized in water, provided that the
 compound contains at least one solubilizing group,
 and

10 each of n and m , independently, is 0, 2,
 or 3, provided that both cannot be 0, and provided
 that each $R_2 - R_5$ can be different in
 successive repeating units.

5. The compound of claim 2, of the formula

15

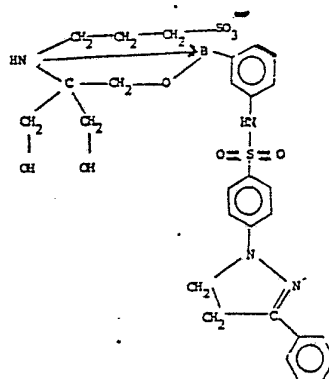


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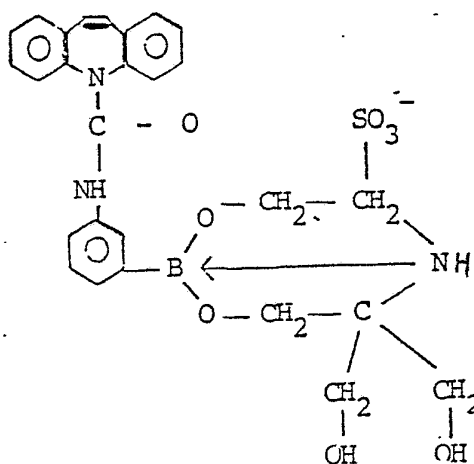


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6. The compound of claim 3, of the formula



7. The compound of claim 4, of the formula

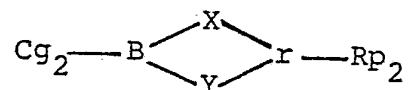


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8. A compound of the formula



wherein

5 Rp_2 is a group which includes a reporter group comprising a fluorophore, a chromophore, an organometallic group, a therapeutic agent, an antigen, or an isotopically-labelled group,

each of X and Y, independently, is N, O, or S,

10 Cg_2 is a carrier group which includes a solubilizing group which is substantially ionized in water, and

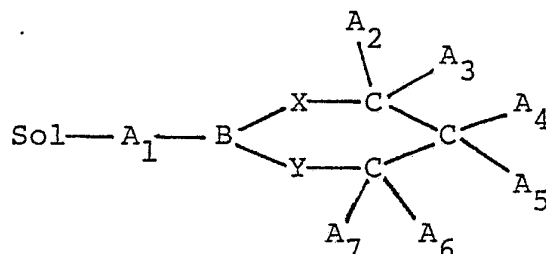
r is a receptor group which, with X and Y, forms complexes with boronic acids.

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9. The compound of claim 8, of the formula



wherein

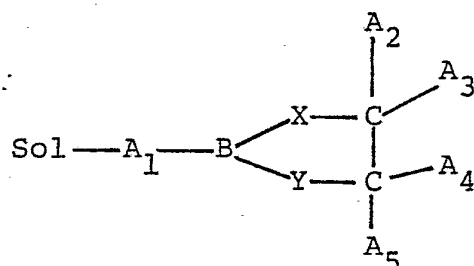
Sol is a solubilizing group which is
 5 substantially ionized in water,

A₁ is alkyl, aryl, heteroaryl,
 heteroalkyl, or aralkyl, and

each A₂ - A₇, independently, is
 hydrogen, aryl, alkyl, heteroalkyl, heteroaryl,
 10 aralkyl, or Rp₂, provided that the compound
 contains at least one Rp₂.

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10. The compound of claim 8, of the formula



wherein

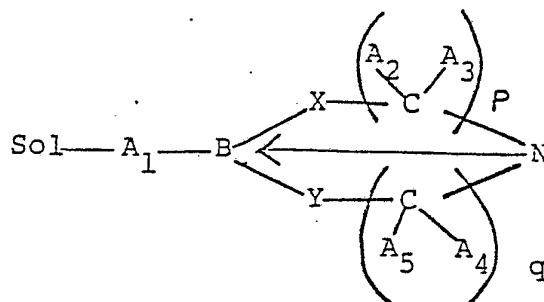
5 Sol is a solubilizing group which is substantially ionized in water,

A₁ is alkyl, aryl, heteroaryl, heteroalkyl, or aralkyl, and

10 each A₂ - A₅, independently, is hydrogen, aryl, alkyl, heteroalkyl, heteroaryl, aralkyl, or Rp₂, provided that the compound contains at least one Rp₂.

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11. The compound of claim 8, of the formula



wherein

5 Sol is a solubilizing group which is substantially ionized in water,

A₁ is alkyl, aryl, heteroaryl, heteroalkyl, or aralkyl,

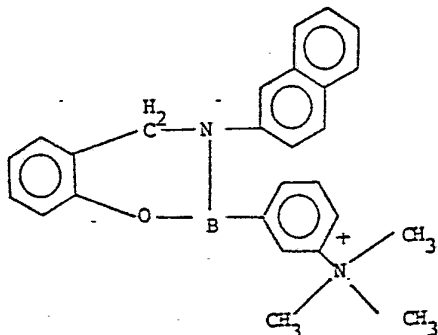
10 each A₂ - A₅, independently, is hydrogen, aryl, alkyl, heteroalkyl, heteroaryl, aralkyl, or Rp₂, provided that the compound contains at least one Rp₂, and

15 each of p and q, independently, is 0, 2, or 3, provided that both cannot be 0, and provided that each A₂ - A₅ can be different in successive repeating units.

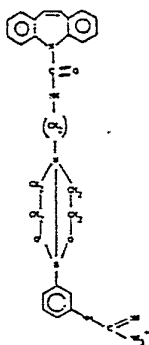


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12. The compound of claim 9, of the formula



13. The compound of claim 11, of the
5 formula



14. The compound of claim 1, said compound being chosen from the compounds listed in Table III herein.

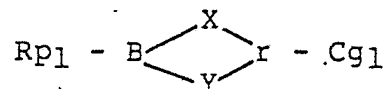
10 15. The compound of claim 1, said compound being the reaction product of a compound listed in Table I herein and a compound listed in Table II herein.



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16. The compound of claim 8, said compound being chosen from the compounds listed in Table IV herein.

17. A method of staining living cells
5 comprising contacting said cells with a compound of the formula



wherein

10 Rp_1 is a group which includes a reporter group comprising a fluorophore, a chromophore, an organometallic group, an antigen, or an isotopically-labelled group,

each of X and Y, independently, is N, O, or S,

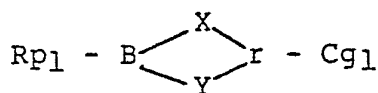
15 Cg_1 is a carrier group which includes a solubilizing group which is substantially ionized in water, and

r is a receptor group which, with X and Y, forms complexes with boronic acids.



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18. A method of staining a protein comprising contacting said protein with a compound of the formula



5 wherein

Rp_1 is a group which includes a reporter group comprising a fluorophore, a chromophore, an organometallic group, an antigen, or an isotopically-labelled group,

10 each of X and Y, independently, is N, O, or S,

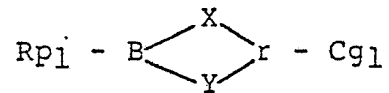
Cg_2 is a carrier group which includes a solubilizing group which is substantially ionized in water, and

15 r is a receptor group which, with X and Y, forms complexes with boronic acids.



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19. A method of staining a fabric or paper product comprising contacting said fabric or paper product with a compound of the formula



5 wherein

Rp_1 is a group which includes a reporter group comprising a fluorophore or a chromophore, each of X and Y, independently, is N, O, or S,

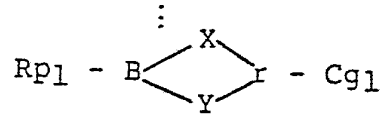
10 Cg_1 is a carrier group which includes a solubilizing group which is substantially ionized in water, and

r is a receptor group which, with X and Y, forms complexes with boronic acids.



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20. A method of brightening a fabric comprising contacting said fabric with a compound of the formula



5 wherein

Rp_1 is a group which includes a reporter group comprising a fluorophore,
each of X and Y, independently, is N, O,
or S,

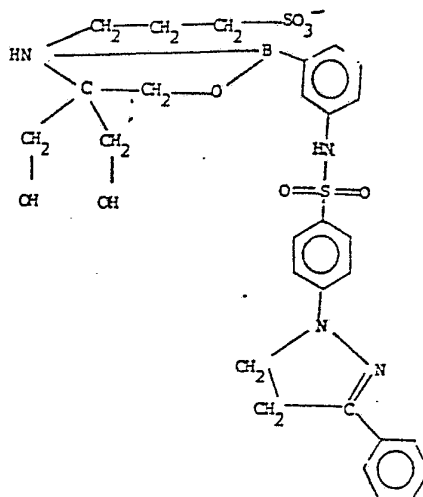
10 Cg_1 is a carrier group which includes a solubilizing group which is substantially ionized in water, and

r is a receptor group which, with X and Y, forms complexes with boronic acids.

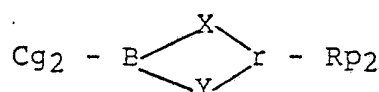


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21. The method of any one of claims 17-20 wherein said compound has the formula



22. A method of staining living cells.
5 comprising contacting said cells with a compound of the formula



wherein

10 Rp_2 is a group which includes a reporter group comprising a fluorophore, a chromophore, an organometallic group, an antigen, or an isotopically-labelled group,

each of X and Y, independently, is N, O, or S,

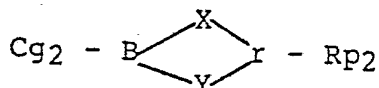
15 Cg_2 is a carrier group which includes a solubilizing group which is substantially ionized in water, and

r is a receptor group which, with X and Y, forms complexes with boronic acids.



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23. A method of staining a fabric or paper product comprising contacting said fabric or paper product with a compound of the formula



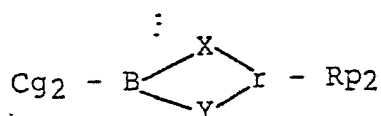
5 wherein

Rp_1 is a group which includes a reporter group comprising a fluorophore or a chromophore, each of X and Y, independently, is N, O, or S,

10 Cg_1 is a carrier group which includes a solubilizing group which is substantially ionized in water, and

r is a receptor group which, with X and Y, forms complexes with boronic acids.

15 24. A method of brightening a fabric comprising contacting said fabric with a compound of the formula



wherein

20 Rp_2 is a group which includes a reporter group comprising a fluorophore, each of X and Y, independently, is N, O, or S,

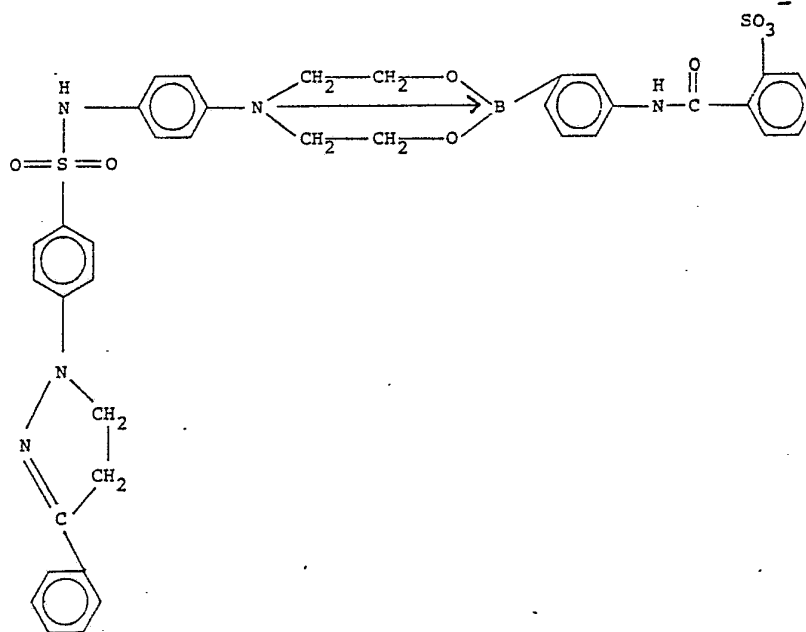
25 Cg_2 is a carrier group which includes a solubilizing group which is substantially ionized in water, and

r is a receptor group which, with X and Y, forms complexes with boronic acids.



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25. The method of any one of claims 22-24 wherein said compound has the formula



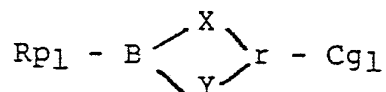
SUBSTITUTE SHEET



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26. A method of measuring, in aqueous solution, an unknown amount of a compound which participates in a chemical procedure which results in the production of peroxide comprising the steps of

5 providing a reaction zone,
 introducing into said reaction zone
 excesses of all reactants, except
 said compound to be measured, participating in said
 10 chemical procedure,
 a sample containing an unknown amount
 of said compound to be measured, and
 a compound of the formula



15 wherein

Rp_1 is a group which includes a reporter group comprising a fluorophore or a chromophore,

20 each of X and Y, independently, is N,
 O, or S,

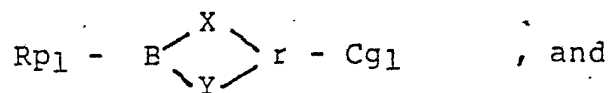
Cg_1 is a carrier group which includes a solubilizing group which is substantially ionized in water, and

25 r is a receptor group which, with X and Y, forms complexes with boronic acids,
 whereby said peroxide breaks the bond between Rp_1 and B, thereby releasing $\text{Rp}_1\text{-OH}$,



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contacting said released Rp_1 -OH with a substance having a greater affinity for Rp_1 -OH than for said compound of the formula



- 25 measuring the fluorescence or color intensity of said substance as a measure of the compound being measured.



- 81 -

27. The method of claim 26 wherein said substance is a water-immiscible organic solvent.

28. The method of claim 27 wherein said solvent is ethyl acetate.

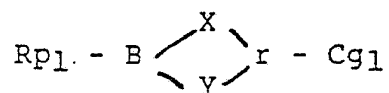
5 29. The method of claim 26 wherein said substance is a water-miscible protein.

30. The method of claim 29 wherein said protein is serum albumin.



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31. A method of measuring, in aqueous solution, an unknown amount of a compound which participates in an enzymatic procedure in which an enzyme acts on a substrate, said method comprising the steps of
- 5 providing a reaction zone,
introducing into said reaction zone
excesses of all reactants, except
said compound to be measured, participating in said
10 enzymatic procedure,
a sample containing an unknown amount
of said compound to be measured, and
a compound of the formula



15 wherein

Rp_1 is a group which includes a reporter group comprising a fluorophore or a chromophore and, positioned between the boron atom and said chromophore or fluorophore, said substrate,
20 each of X and Y, independently, is N, O, or S,

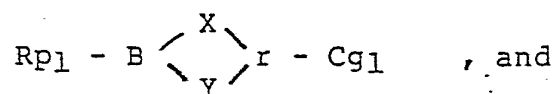
Cg_1 is a carrier group which includes a solubilizing group which is substantially ionized in water, and
25 r is a receptor group which, with X and Y, forms complexes with boronic acids,



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whereby said enzymatic procedure causes Rp_1 to break at said substrate, releasing fluorophore and chromophore,

30 contacting said fluorophore or chromophore with a substance having a greater affinity for the released fluorophore or chromophore than for said compound of the formula



35 measuring the fluorescence or color intensity of said substance as a measure of the compound being measured.



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32. The method of claim 31 wherein said substance is a water-immiscible organic solvent.

33. The method of claim 32 wherein said solvent is ethyl acetate.

5 34. The method of claim 31 wherein said substance is a water-miscible protein.

35. The method of claim 34 wherein said protein is serum albumin.



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36. A method of measuring an antibody in
 a sample comprising
 providing red blood cells,
 contacting said red blood cells, in
 5 aqueous solution, with a compound of the formula



wherein

Rp_1 is a group which includes a
 reporter group comprising a hapten capable of
 10 complexing with said antibody,
 each of X and Y, independently, is N,
 O, or S,

Cg_1 is a carrier group which
 includes a solubilizing group which is
 15 substantially ionized in water, and
 r is a receptor group which, with X
 and Y, forms complexes with boronic acids,
 whereby the membranes of said red
 blood cells extract hapten-containing boronic acid,
 20 thereby becoming sensitized,

contacting said sensitized red blood cells
 with said sample, together with complement capable
 of participating in the complexation of said hapten
 with said antibody,

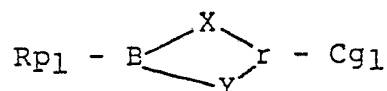
25 whereby said complexation occurs,
 causing the lysis of said red blood cells and the
 release of hemoglobin therefrom, and

measuring said released hemoglobin as a
 measure of said antibody in said sample.



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37. A method of measuring an antibody in
 a sample comprising
 providing red blood cells ghost,
 contacting said red blood cells ghost, in
 5 aqueous solution, with a compound of the formula



wherein

Rp_1 is a group which includes a
 reporter group comprising a hapten capable of
 10 complexing with said antibody, and a reporter group
 comprising a fluorophore,

each of X and Y, independently, is N,
 O, or S,

Cg_1 is a carrier group which
 15 includes a solubilizing group which is
 substantially ionizable in water, and

r is a receptor group which, with X
 and Y, forms complexes with boronic acids,

whereby the membranes of said red
 20 blood cell ghosts extract hapten and fluorophore-
 containing boronic acid, thereby becoming
 sensitized,

contacting said sensitized red blood cell
 ghosts with said sample, together with complement
 25 capable of participating in the complexation of
 said hapten with said antibody,

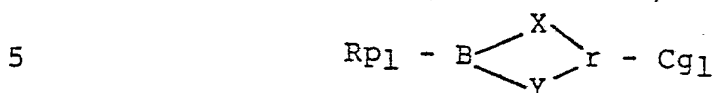
whereby said complexation occurs,
 causing the lysis of said red blood cell ghosts and
 the release of fluorophore therefrom, and

30 measuring released fluorescence as a
 measure of said antibody in said sample.



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38. A method of improving the water solubility of a therapeutic agent of poor water solubility comprising chemically incorporating said therapeutic agent into a compound of the formula



wherein

Rp_1 is a group which includes said therapeutic agent,

10 each of X and Y, independently, is N, O, or S,

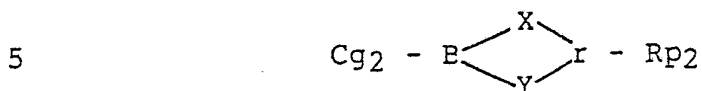
Cg_1 is a carrier group which includes a solubilizing group which is substantially ionizable in water, and

15 r is a receptor group which, with X and Y, forms complexes with boronic acids.



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39. A method of improving the water solubility of a therapeutic agent of poor water solubility comprising chemically incorporating said therapeutic agent into a compound of the formula



wherein

Rp₂ is a group which includes said therapeutic agent,

each of X and Y , independently, is $N, 0$,

10 or S,

Cg₂ is a carrier group which includes a solubilizing group which is substantially ionizable in water, and

15 r is a receptor group which, with X and Y,
forms complexes with boronic acids.

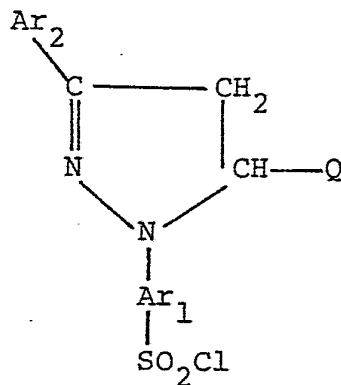
40. The method of claim 38 or claim 39 wherein said therapeutic agent is diphenyl hydantoin or a derivative thereof.

41. The method of claim 38 or 39 wherein
20 said therapeutic agent is carbamazepine or a
derivative thereof.



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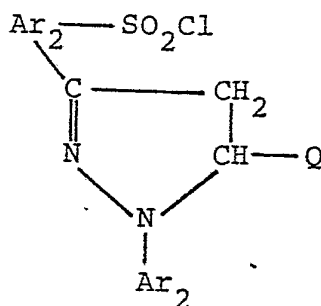
42. A compound of the formula



wherein

each of Ar₁, and Ar₂ is an aryl group,
 5 and Q is hydrogen or an alkyl group.

43. A compound of the formula



wherein,

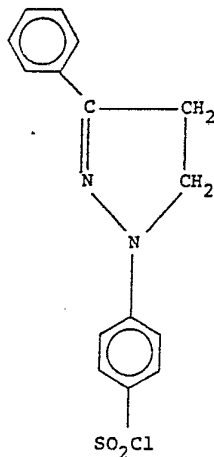
each of Ar₁, and Ar₂ is an aryl group,
 10 and Q is hydrogen or an alkyl group.

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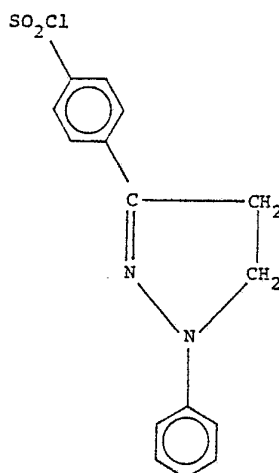


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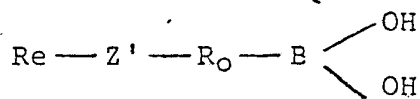
44. The compound of claim 42, of the formula



5 45. The compound of claim 43, of the formula



46. A compound of the formula



wherein

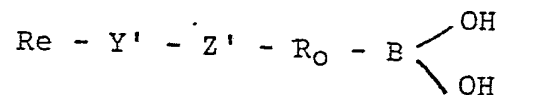
10 Re is a reporter group comprising a fluorophore, a chromophore, an organometallic group, a therapeutic agent, an antigen, or an isotopically-labelled group,

15 R₀ is aryl, alkyl, heteroalkyl, heteroaryl, or aralkyl, and

Z' is a group linking Re and R₀.

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47. The compound of claim 43, of the formula



wherein Y' is a group linking Re and Z'.

5 48. The compound of claim 46, said compound being chosen from the compounds listed in Table I herein.

49. The compound of claim 46 wherein Z' is NH, O, S, or COO.

10 50. The compound of claim 47 wherein

Y' is C = O, $\text{O}=\text{S}=\text{O}$, or COO.



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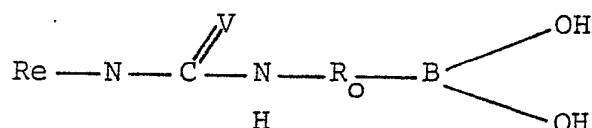
51. The compound of claim 47 wherein

Z' is NH, and

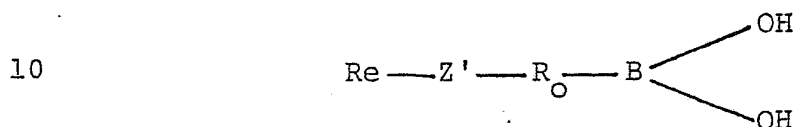
Y' is NH—C=V

V being O or S, whereby said compound

5 has the formula



52. A method of staining living cells comprising contacting said cells with a water-soluble, non-toxic compound of the formula



wherein

Re is a reporter group comprising a fluorophore, a chromophore, an organometallic group, an antigen, or an isotopically-labelled group,

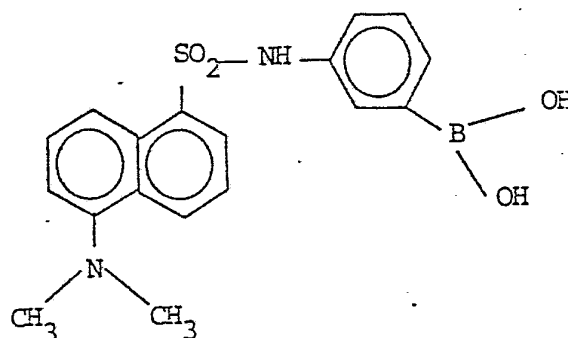
15

R_O is aryl, alkyl, heteroalkyl, heteroaryl, or aralkyl, and

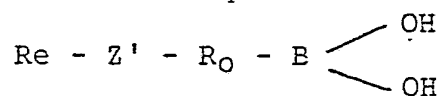
Z' is a group linking Re and R_O.

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53. The method of claim 52 wherein said compound has the formula



54. A method of staining the surfaces of
 5 eyes comprising contacting said eyes or living
 cells with a water-soluble, non-toxic compound of
 the formula



wherein

10 Re is a reporter group comprising a
 fluorophore or a chromophore,

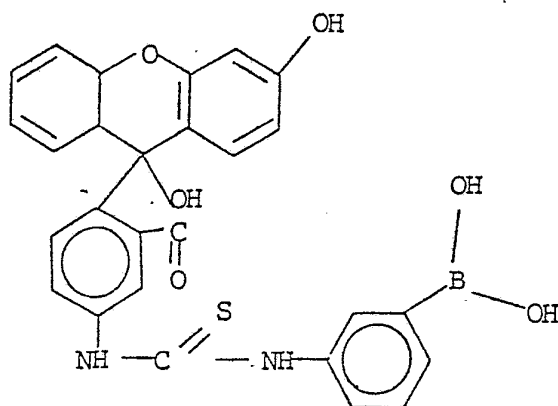
R₀ is aryl, alkyl, heteroalkyl,
 heteroaryl, or aralkyl, and

Z' is a group linking Re and R₀.



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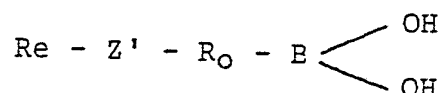
55. The method of claim 54 wherein said compound has the formula



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56. A method of measuring, in a sample, a compound having an affinity for boronic acids comprising

contacting said sample with a
5 compound of the formula



wherein

Re is a reporter group comprising a fluorophore, a chromophore, an organometallic
10 group, a therapeutic agent, an antigen, or an isotopically-labelled group,

R_O is aryl, alkyl, heteroalkyl, heteroaryl, or aralkyl, and

Z' is a group linking Re and R_O,
15 thereby to form a mixture,

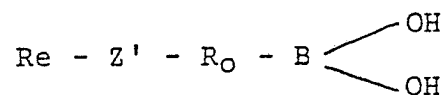
contacting said mixture with a material having an affinity for boronic acids, and

measuring the amount of compound of the aforesaid formula bound to said material, said
20 amount being inversely proportional to the amount of said compound having an affinity for boronic acids present in said sample.



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57. A composition comprising
 an organic, polymeric, water-insoluble
 support having hydroxyl groups capable of
 complexing with boronic acids, and
 5 bound to said support, a compound of the
 formula



wherein

- Re is a reporter group comprising a
 10 fluorophore, a chromophore, an antigen, or an
 isotopically-labelled group,

R_O is aryl, alkyl, heteroalkyl,
 heteroaryl, or aralkyl, and

Z' is a group linking Re and R_O.

- 15 58. The composition of claim 57 wherein
 said support is hydrophobic fibrous polyethylene
 containing attached polyvinyl alcohol.



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59. A method for assaying, in aqueous solution, a compound which participates in a chemical procedure which results in the production of peroxide comprising the steps of

5 providing a reaction zone,
introducing into said reaction zone
excesses of all reactants, except
said compound to be assayed,
a sample containing an unknown amount

10 of said compound to be assayed, and
the composition of claim 57, wherein
Re comprises a fluorophore,
whereby fluorophore is released into
solution, and

15 measuring released fluorescence as a
measure of said compound being assayed.

60. The method of claim 59, further comprising the step of separating said released fluorophore from said support.

20 61. The method of claim 60 wherein said separating is carried out by filtering.

62. The method of claim 60 wherein said separating is carried out by centrifugation.



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63. The method of claim 60 wherein said separating is carried out by dissolving said fluorophore compound in an organic, water-immiscible solvent.

5 64. The method of claim 59 wherein said compound to be assayed is glucose.

65. Apparatus for assaying, in aqueous solution, a compound which participates in a chemical procedure which results in the production
10 of peroxide comprising

the composition of claim 57, wherein Re comprises a fluorophore

means for providing a reaction zone for the production of said peroxide, and

15 means for contacting said peroxide with said composition of claim 57, thereby to release fluorophore into solution.

66. The apparatus of claim 65, further comprising all reactants, except said compound to
20 be assayed, which participate in said production of peroxide.



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67. The apparatus of claim 65, further comprising

means for maintaining the pH of said reaction zone in the range of about 8 to 9, and

5 means for separating said dissolved fluorophore from said insoluble support.

68. The apparatus of claim 65 wherein said compound to be assayed is glucose.

69. The apparatus of claim 67 wherein
10 said separating means comprises filtering means.

70. The apparatus of claim 67 wherein said separating means comprises an organic, water-immiscible solvent capable of dissolving said fluorophore.



1/1

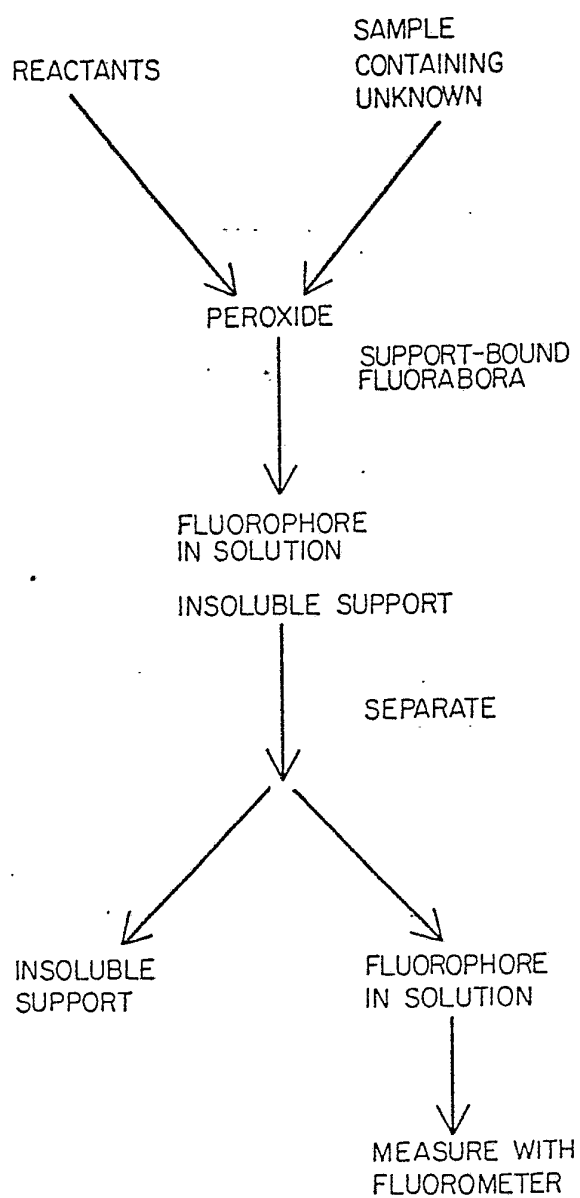


FIG 1

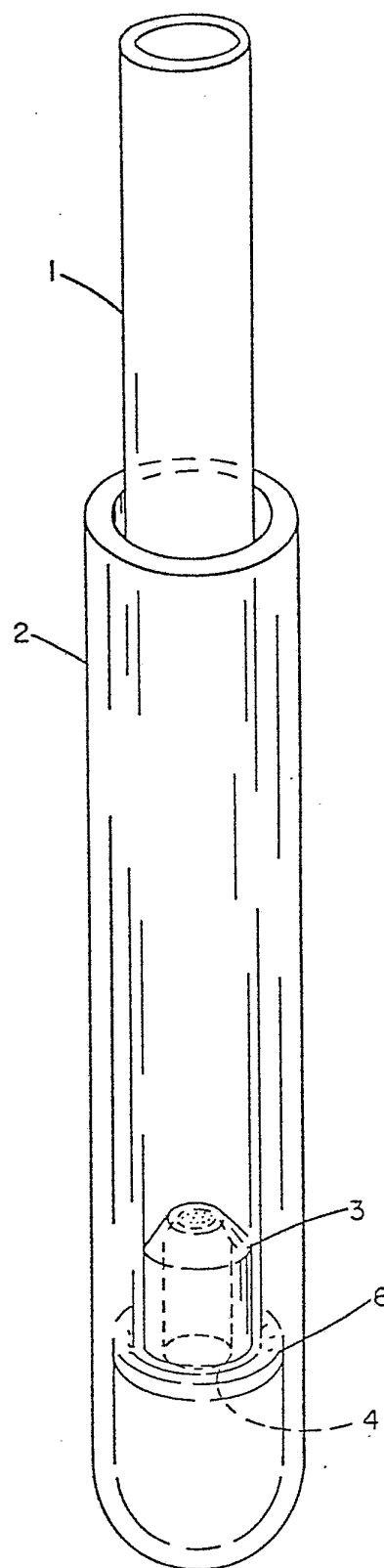


FIG 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 82/00725

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC		
INT. CL. ³ C07F 5/04		
US. CL. 260/462R; 260/462A; 260/462C; 8/517; 8/688; 8/690;*		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
US	260/462R, 462A, 462C	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵		
CHEMCON		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category [*]	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	US, A, 3,038,926, (Farthouat et al), 12 June 1962	8, 9
X	US, A, 3,131,208, (Woods) 28 April 1964	8, 9
X	GB, A 790,090, (Bataafsche Petroleum Maatschappij) 05 February 1958	8, 9
A	Chemical Abstracts Volume 88, no. 17 issued 24 April 1978, (Columbus, Ohio, U.S.A.) A.G. Smith et al, 'Gas Chromotography Che- mical Ionization Mass Spectrometry of Pro- staglandin Fa Cyclic Boronate Derivatives' see page 459, column 1, the abstract no. 120142m Biomed Spectom. 1977, 4(4) 258-64 (Eng). (section 22)	1, 2
[*] Special categories of cited documents: ¹⁵ "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ²	Date of Mailing of this International Search Report ²	
14 SEPTEMBER 1983	17 SEP 1982	
International Searching Authority ¹	Signature of Authorized Officer ²⁰	
ISA/US	Fred Edmundson FRED EDMUNDSON	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

23/230R; 23/230B; 23/230M; 260/190; 260/196;
260/205; 260/239D; 260/433; 260/439CY;
260/502.3; 422/56; 424/3; 424/7; 424/185;
428/288; 428/289; 435/7; 544/69; 544/107;
548/110; 548/379; 548/405; 549/213; 560/13

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

1, 2, 8 and 9

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☒ No protest accompanied the payment of additional search fees.