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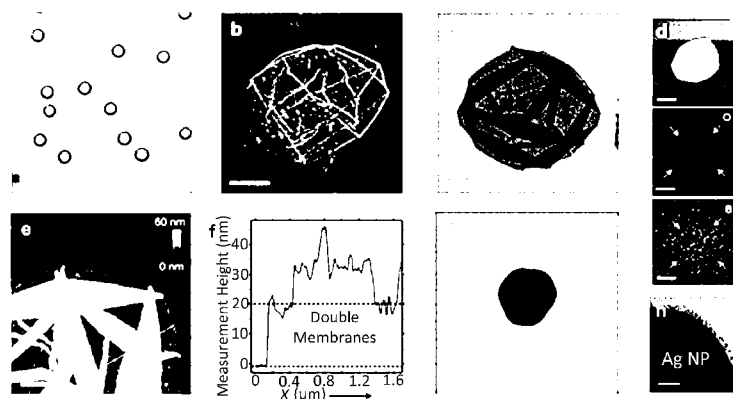


Figure 1

(S7) Abstract: The present invention relates to capsules, and the method for making such capsules, having a boronate linked organic network layer comprising (a) a plurality of boronic acid containing moieties and (b) a plurality of galloyl linking moieties, which capsules are responsive to externally applied stimuli, for applications including cell encapsulation, advanced drug delivery, biomedical diagnostics, micro-reactions, and the formation of biomimetic protocells.

Title of Invention

GLUCOSE SENSITIVE PHENYLBORATE ACID CAPSULES FOR INSULIN DELIVERY

Cross-Reference

[0001] This application claims priority from Australian Provisional Patent Application No. 2015902037 filed 2 June 2015, the contents of which should be understood to be incorporated into this specification by this reference.

Technical Field

[0002] Capsules and methods for making the same are of particular interest in the development of stimuli-responsive capsules for applications including cell encapsulation, advanced drug delivery, biomedical diagnostics, micro-reactions, and the formation of biomimetic protocells. "Smart" capsules have previously been prepared using layer-by-layer techniques involving multistep assembly. The present invention relates to capsules and the method for making capsules having a boronate linked organic network layer which are responsive to externally applied stimuli.

Background of Invention

[0003] Capsules and the methods for making the same have been described. Of particular interest are stimuli-responsive capsules ("Smart" capsules) which can selectively release an active agent for a variety of applications including drug delivery, therapeutic treatments and diagnostic imaging techniques. These smart capsules can be responsive to biological, chemical and other externally applied stimuli. Biological stimuli can include for example pH, redox reactions, and enzyme triggers. Chemical stimuli can include small molecules such as glucose or mannose. Smart capsules responsive to biological or chemical stimuli are desired due to functional similarity to dynamic biological systems found in nature including for example organelles, cells, and organs. In general, stimuli-responsive capsules are engineered for a single biological trigger, and therefore unable to respond to complex microenvironments as observed in nature. Accordingly, it is desirable to design dual- or multi-responsive capsules and to develop methods for making the same.

[0004] Metal-phenolic networks (MPNs) based on metal coordination between phenolic materials have been described for the production of thin films and preparing capsules which can be responsive. Natural phenolic building blocks are promising for biological applications, as many are generally recognized as safe (GRAS) by the U.S. Food and Drug Administration

(FDA). However, while the multivalent coordination property of MPNs allows capsules to disassemble under specific acidic conditions (e.g., < pH 4.0 for Fe^{III}-TA capsules), they are not responsive to multiple biological stimuli.

[0005] Some systems have been developed using multistep assembly of boronate-functionalized polymers with polyelectrolytes such as poly(sodium 4-styrene sulfonate) (PSS), mannan, poly(vinylalcohol) (PVA), and chitosan based on layer-by-layer (LbL) techniques. However, such boronate-functionalized capsules are only stable and responsive to vicinal diols (e.g., *cis*-diols) under alkaline pH conditions (e.g., pH 9–11), which limits their potential in biological applications.

[0006] In addition, crystalline porous boronate linked organic network systems for depositing onto a surface have been developed. These systems involve a saturated boronate ester linkage in a covalently bonded network formed between a polyfunctional boronic acid and a polyfunctional diol. However, these systems remain limited as they lack the demanded material functional properties used for potential applications in life science and biomedicine. Additionally, these systems are limited due to their time-consuming and complicated formation process as a result of the saturated crystalline structure and their lack of stimuli-responsive ability.

[0007] Accordingly, it is desirable to develop a method of production which could be used for the rapid synthesis of capsules and to produce capsules which can be stable, robust and have dual-response properties.

[0008] The discussion of documents, acts, materials, devices, articles and the like is included in this specification solely for the purpose of providing a context for the present invention. It is not suggested or represented that any or all of these matters formed part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

[0009] Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification (including the claims) they are to be interpreted as specifying the presence of the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components, or group thereof.

Summary of Invention

[0010] As a result of research into improving the efficiency of capsule forming techniques and to develop robust and stable capsules which can be responsive to multiple external

stimuli, the present applicants have identified i) a rapid assembly for the formation of a capsule and ii) stimuli-responsive capsules which are stable and robust under specific conditions utilising assembly of a boronate linked organic network layer in the presence of a template. This technique allows rapid assembly of capsules along with stimuli-responsiveness to external stimuli such as pH and *cis*-diols.

[0011] Accordingly, in one embodiment the present invention provides a method of forming a capsule comprising at least one boronate linked organic network layer the method comprising reacting a compound having a plurality of boronic acid moieties with a compound having a plurality of galloyl moieties in the presence of a template to form a capsule comprising at least one boronate linked organic network layer.

[0012] The method of the present invention is typically very rapid and therefore allows the rapid formation of a capsule in the presence of a template. As a result of this method, the present invention provides capsules which are stable and robust under specific conditions, for example, under alkaline conditions. Additionally, the boronate ester linkages contained in the boronate linked organic networks of the capsules (BPN capsules) are reversible under specific conditions and therefore can be used as dual-responsive capsules sensitive to pH and/or *cis*-diol external stimuli.

[0013] Advantageously, the use of a compound having a plurality of galloyl moieties in the method discussed above produces capsules which are more robust than capsules that are formed from polyfunctional diols.

[0014] Accordingly, in yet a further embodiment the present invention provides a capsule comprising at least one boronate linked organic network layer, wherein the boronate linked organic network layer comprises (a) boron containing moieties containing a plurality of boron atoms and (b) linking moieties containing a plurality of oxygen atoms, wherein the boron containing moieties are covalently bonded to the linking moieties via a boronate ester linkage wherein at least one of the oxygen atoms in the boronate ester linkage is located on a carbon atom adjacent to a carbon atom bearing a hydroxyl group.

[0015] In certain embodiments, the capsules provided by the present invention can be used in a number of applications, including use in the fabrication of sensors, responsive surfaces, self-healing materials and drug delivery systems. As stated previously, the capsules of the present invention can be responsive to external stimuli.

[0016] Accordingly, in some embodiments, the applicants have found that the capsules are stable in biological systems both *in vivo* and *in vitro* in certain circumstances and can

therefore be used in imaging and delivery of active agents loaded in the capsules such as insulin and therapeutic drugs when external stimuli is applied. In certain embodiments, the capsules are responsive to acidic pH and/or in the presence of external competing *cis*-diols at physiological pH.

Brief Description of Drawings

[0017] **Figure 1** shows structural characterization of BPN capsules. (a) DIC image of capsules after the removal of the templates. (b) SEM and (c) TEM images of air-dried capsules. (d) HAADF-STEM and EDX mapping images of capsules, oxygen (O, violet) and boron (B, white). (e) AFM image of capsules. (f) Height measurement in AFM image (e). (g) and (h) TEM images of silver nanoparticles coated with the BPN film. The scale bars are (a) 5.0 μm , (b)-(e) 500 nm, (g) 25 nm, and (h) 5 nm.

[0018] **Figure 2** shows the pH and *cis*-diol responsiveness of BPN capsules. (a) Disassembly profiles at pH 7.4 followed by decreasing the pH to 5.0, or after adding 100 mM mannitol. (b) Release profiles of DOX-loaded BPN capsules upon treatment with pH 5.0 solution and pH 7.4 solution. (c) Release profiles of DOX-loaded BPN and Fe^{III} -TA capsules after treatment with *cis*-diols at pH 7.4 and/or pH 5.0 after 17.5 h. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS, not significant (two-way ANOVA). Data are means $\pm$ SD, $n = 3$. (d) Intracellular disassembly of BPN capsules at different time points. Scale bars are 5.0 μm .

[0019] **Figure 3** shows a DIC image (a) and fluorescence microscopy image (b) of DOX-loaded BPN capsules in pH 7.4 PBS. Scale bars are 5.0 μm .

[0020] **Figure 4** shows deconvolution fluorescence microscopy images of DOX-loaded BPN capsules at 4 h incubation. The images are acquired with a standard FITC and tetramethylrhodamine isothiocyanate (TRITC) filter set. The blue nucleus stained with Hoechst 33342 was visualized with the DAPI filter. The arrows point to DOX-loaded BPN capsules. All the scale bars represent 10 μm .

[0021] **Figure 5** shows cytotoxicity of DOX-loaded capsules and free DOX as a function of DOX concentration evaluated by MTT assay. The cell viability of untreated cells was used as control and normalized as 100%. Data are means $\pm$ SD, $n = 12$.

[0022] **Figure 6** shows a MTT assay of BPN capsules using HeLa cells. Cells were incubated with different numbers of capsules for 48 h. The results are average values with standard deviations, means $\pm$ SD, $n = 12$.

[0023] **Figure 7** shows stability studies of the BPN capsules. (a) Capsules incubated in HBP with and without treatment with 100 mM mannitol: DIC images of capsules in HBP solution with or without 100 mM mannitol at different times. Scale bars are 2.0 μm . (b) The remaining capsule populations before and after 12 h incubation in HBP with and without 100 mM mannitol. $**P < 0.01$ (Student's t-test). (c) Tumor formation and injection of ^{64}Cu /BPN capsules into the tumor. (d) PET/CT image of mice 5 h and 12 h after capsules injection (maximum intensity projection). (e) Standard uptake values located at the tumor site at 5 h and 12 h post-injection. NS, not significant. Data are means $\pm$ SD, $n = 3$ in (b), and $n = 2$ in (e).

[0024] **Figure 8** shows the formation of BPN capsules with galloyl moieties (derived from tannic acid). The catechol moiety derived from HHTP cannot form capsules under similar conditions. Scale bars are 5.0 μm .

Detailed Description

[0025] As used herein the term network refers to an interconnected group of objects that form a structure significantly more extensive in size than the individual components from which it is made. In relation to the present invention a network refers to the structure formed between the compound having a plurality of boronic acid moieties with the compound having a plurality of galloyl moieties.

[0026] As discussed above, the present invention relates to a method of forming a capsule comprising at least one boronate linked organic network layer the method comprising reacting a compound having a plurality of boronic acid moieties with a compound having a plurality of galloyl moieties in the presence of a template to form a capsule comprising at least one boronate linked organic network layer.

[0027] The template may be of any suitable template material. In theory the template could be a solid, a liquid or a gas depending upon the exact nature of the template that is desired to be coated by the method of the invention. For example a liquid template (typically called an emulsion template) could be an oil particle (oil in water) or a silicon emulsion. In relation to gaseous templates a suitable example would be an air bubble immobilised in a permeable matrix. In certain embodiments the template is a solid template. In certain embodiments the template is a liquid template.

[0028] The method of the present invention is found to be applicable to templates made from a wide variety of materials. The nature of the template will depend on the identity of the template desired to be coated or whether there is an intention to remove or retain the

template (in the method of manufacturing a capsule from the method of the invention). In certain embodiments the template is selected from the group consisting of an organic particle, an inorganic particle, a biological particle and combinations thereof.

[0029] The identity of the template may also vary depending upon the intended end use of the capsule of the invention. For example if the intended end use of the capsule is to deliver an agent to a part of the body then in some embodiments of the invention it is desirable to incorporate the agent to be delivered on or into the template prior to the formation of the capsule. In these embodiments the template chosen is typically a porous template and the agent to be delivered is adsorbed/absorbed on or into the porous template. A skilled worker in the field would readily be able to determine a suitable porous template based on the desired agent to be incorporated in or onto the template.

[0030] Examples of suitable materials include inorganic oxides such as ceria, zirconia, titania and silica, metals such as gold, and organics such as certain polymeric materials like polystyrene, melamine formaldehyde or biological materials like alginate. Emulsion templates could also be used such as air bubbles, oil droplets or silicon based silane emulsions. Whilst the template may be made of any suitable material as discussed above it is commonly calcium carbonate based due to the relative ease of access of materials of this type and their relatively low cost.

[0031] In one embodiment the template is a solid template. The template may be a solid non porous template or a porous template. In one embodiment the template is a liquid template. In one embodiment the template is a porous template.

[0032] In certain embodiments the template is selected from the group consisting of polystyrene, glass, gold (Au), polydimethylsiloxane (PDMS), poly(lactic-co-glycolic acid) (PLGA), melamine-formaldehyde resin (MF), low-molecular-weight PDMS emulsion, silica (SiO_2), aminated SiO_2 , cetyltrimethylammonium bromide-capped Au nanoparticles (Au NPs) and calcium carbonate (CaCO_3). In one embodiment the template is a metallic particle. In one embodiment the template is a silica particle. In one embodiment the template is a calcium carbonate particle.

[0033] In one embodiment the template is made of a suitable material which allows for its subsequent removal during a template removal step. A skilled addressee in the art will readily understand the types of materials that can be used to form templates of this type depending upon the layers of material to be coated on the template. As will be readily appreciated if an optional template removal step is contemplated then the compound having a plurality of boronic acid moieties and the compound having a plurality of galloyl moieties used

to form the at least one boronate linked organic network layer that forms the capsule in the presence of the template must be compatible with (i.e. not degrade) under the conditions required for template removal.

[0034] The templates used may take any suitable shape and may be for example in the shape of, spheres, cubes, prisms, fibres, rods, tetrahedrons or irregular particles. Accordingly, the shape of each template is independently selected from the group consisting of a sphere, a cube, a prism, a fibre, a rod, a tetrahedron and an irregular shape. As will be appreciated by a skilled worker in the art the shape of the template will typically determine the shape of the capsule ultimately produced. It is typical, however, that the template is spherical or substantially spherical.

[0035] It will be convenient to describe the invention in terms of a spherical template, but it shall be kept in mind that any capsule produced by the method of the invention may be of any shape depending on the shape of the template. Thus in general the final shape of the capsules produced by the method of the invention will take the general shape of the template used in their production. Thus for example if the template is spherical then the final product will typically be spherical. If the template is a fibre then once again the final product will typically be a fibre. There may be some fluctuation in the size of the capsule compared to the size of the template, due to shrinkage and/or swelling of the capsule depending on the specific production conditions. A skilled worker in the field will typically be able to readily choose a suitable template shape for their desired end use application.

[0036] The template may be of any suitable size with the size being determined, in part by the desired size of the final capsule to be produced and by the availability of the desired template. In particular the methods of the present invention are found to be particularly applicable to templates of less than 1 cm. In one embodiment the template has a particle size of less than 1 mm. In one embodiment the template has a particle size of less than 500 μm . In one embodiment the template has a particle size of less than 100 μm . In one embodiment the template has a particle size of less than 1000 nm. In one embodiment the template has a particle size of less than 500 nm. In one embodiment the template has a particle size of less than 200 nm. In one embodiment the template has a particle size of less than 100 nm. In one embodiment the template has a particle size of less than 10 nm. In one embodiment the template has a particle size of less than 1 nm. In one embodiment the template has a particle size from 1 nm to 1 cm. In one embodiment the template has a particle size from 1 nm to 1 cm. In one embodiment the template has a particle size from 1 nm to 5 mm. In one embodiment the template has a particle size of from 1 nm to 1 mm. In one embodiment the template has a particle size of from 1 nm to 500 μm . In one

embodiment the template has a particle size of from 1 nm to 400 μm . In one embodiment the template has a particle size of from 1 nm to 300 μm . In one embodiment the template has a particle size of from 1 nm to 200 μm . In one embodiment the template has a particle size of from 1 nm to 100 μm . In one embodiment the template has a particle size of from 1 nm to 50 μm . In one embodiment the template has a particle size of from 1 nm to 1000 nm. In one embodiment the template has a particle size of from 1 nm to 500 nm. In one embodiment the template has a particle size of from 1 nm to 400 nm. In one embodiment the template has a particle size of from 1 nm to 300 nm. In one embodiment the template has a particle size of from 1 nm to 200 nm. In one embodiment the template has a particle size of from 1 nm to 100 nm. In one embodiment the template has a particle size of from 1 nm to 50 nm. In one embodiment the template has a particle size of from 20 nm to 500 nm. In one embodiment the template has a particle size of from 20 nm to 400 nm. In one embodiment the template has a particle size of from 20 nm to 300 nm. In one embodiment the template has a particle size of from 20 nm to 200 nm. In one embodiment the template has a particle size of from 20 nm to 100 nm. In one embodiment the template has a particle size of from 50 nm to 400 nm. In one embodiment the template has a particle size of from 50 nm to 300 nm. In one embodiment the template has a particle size of from 50 nm to 200 nm. In one embodiment the template has a particle size of from 50 nm to 100 nm. In one embodiment the template has a particle size of from 30 nm to 30 μm . In another embodiment the template has a particle size of from 200 nm to 6 μm . In another embodiment the template has a particle size of from 400 nm to 5 μm . In another embodiment the template has a particle size of from 500 nm to 4 μm . Once again a skilled worker will readily be able to choose a suitable sized template based on the desired size of the final particle.

[0037] The surface of the template may be modified by addition of additives. Any of a number of additives can be added to the template. In one embodiment, the additive can be poly(sodium styrene sulfonate) (PSS). Without wishing to be bound by any one theory, the addition of PSS stabilizes the templates to form monodisperse templates which have a high loading capacity for active agents.

[0038] In addition to the discussion of possible templates above it will be appreciated that in the method of making a capsule the template may be either a functional template or a sacrificial template. In one embodiment the template is a functional template. In another embodiment the template is a sacrificial template.

[0039] A functional template is a template that is intended to remain in the capsule after the capsule is produced to impart some functional property on the capsule. An example of such a functional template would be a metallic template that is used in imaging applications.

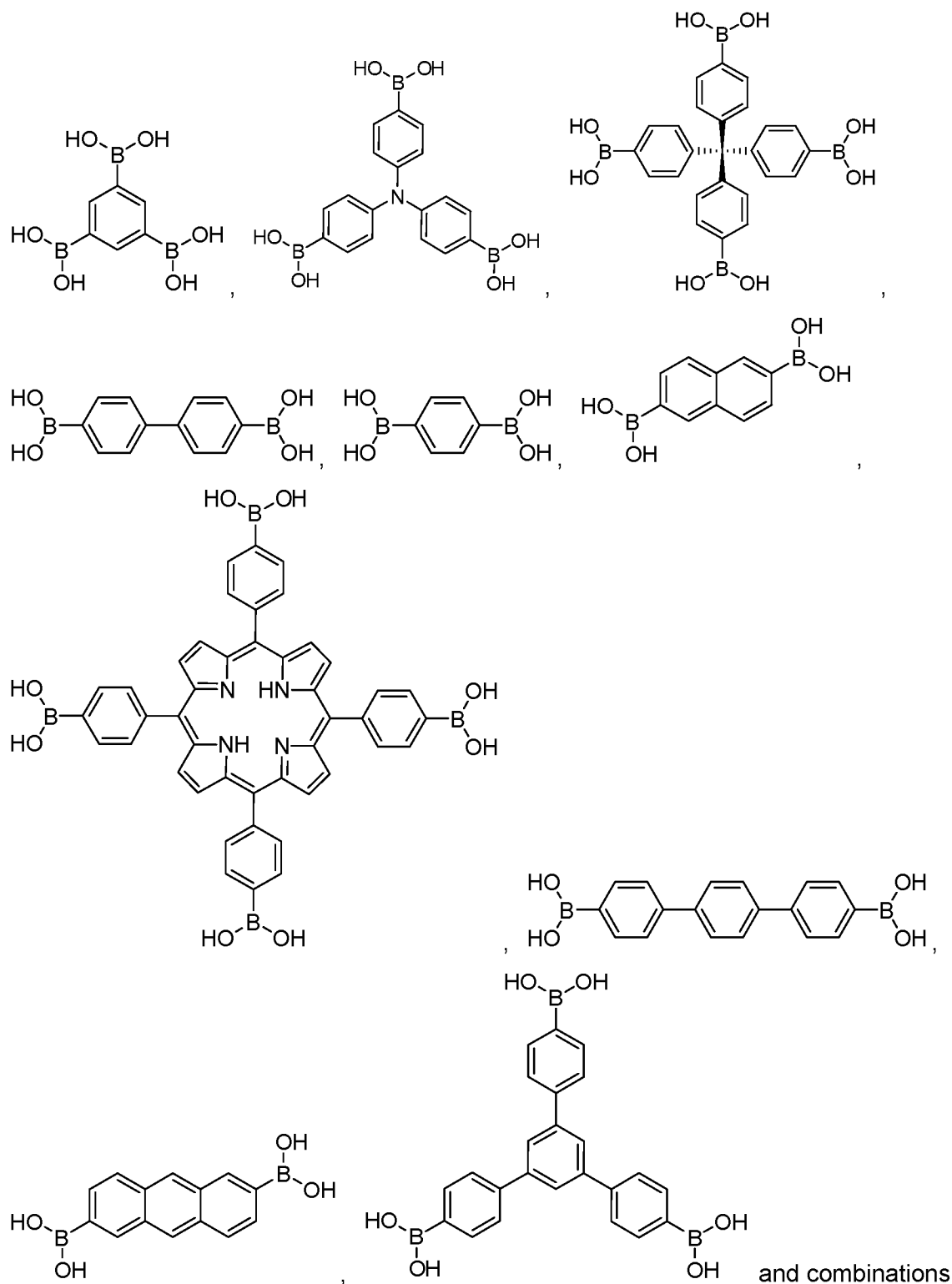
Another example of a functional template may be a radioactive metal template used in targeted chemotherapy applications. A skilled worker in the art can readily identify suitable materials for use as functional templates as the choice of functional template will typically be determined by the function required.

[0040] A sacrificial template is a template that is used during the production of the capsule but which is designed to be removed after capsule formation to form a hollow capsule. The choice of a sacrificial template is general relatively straightforward as the main consideration is the ability to remove the template without causing any damage to the at least one boronate linked organic network contained in a layer of the capsule.

[0041] It will be apparent to a skilled addressee that the compound having a plurality of boronic acid moieties must have at least two boronic acid moieties in order to form a network comprising at least one boronate linked organic network layer. It is to be understood that any compound having a plurality of boronic acid moieties may be used to form a capsule having at least one boronate linked organic network layer. In one embodiment, the compound having a plurality of boronic acid moieties is a phenylboronic acid moiety. In one embodiment, the compound having a plurality of boronic acid moieties is a diboronic acid, triboronic acid or a tetraboronic acid.

[0042] Without wishing to be bound by any one theory, in embodiments wherein the compound having a plurality of boronic acid moieties is a phenylboronic acid moiety, these compounds provide excellent building blocks for the formation of boronate ester linkages due to the favorable *syn-peri*-planar arrangement of the aromatic hydroxyl groups combined with their electron-donating properties.

[0043] In one embodiment, the compound having a plurality of boronic acid moieties is selected from the group consisting of:



thereof.

[0044] It will also be apparent to a skilled addressee that the compound having a plurality of galloyl moieties must have at least two galloyl moieties in order to form a network comprising at least one boronate linked organic network layer when it is reacted with the compound having a plurality of boronic acid moieties as discussed above. It is to be

understood that any compound having a plurality of galloyl moieties may be used to form a capsule having at least one boronate linked organic network layer.

[0045] In one embodiment, the compound having a plurality of galloyl moieties is selected from the group consisting of ellagitannins, tannic acid, 1,2,3,4,6-pentagalloyl glucose and combinations thereof. In one embodiment, the compound having a plurality of galloyl moieties is selected from the group consisting of tannic acid, tellimagrandin II, castalagin, castalin, casuarictin, grandinin, punicalagin, punicalin, roburin A, terflavin B, and combinations thereof.

[0046] In the method of forming a capsule of the present invention, the reaction between the compound having a plurality of boronic acid moieties and the compound having a plurality of galloyl moieties to form at least one boronate linked organic network layer can be performed by any technique known to a skilled worker in the art. In one embodiment, the method can be performed using a batch process or a continuous flow process. In one embodiment, the reacting step of the method of the invention comprises addition of a solution of a compound having a plurality of galloyl moieties to a solution of a template followed by addition of a solution of a compound having a plurality of boronic acid moieties. In an alternative embodiment, the reacting step of the method of the invention comprises addition of a solution of a compound having a plurality of boronic acid moieties to a solution of a template followed by addition of a solution of a compound having a plurality of galloyl moieties.

[0047] In the methods of the present invention, any suitable solvent or combinations of solvents may be used that are capable of facilitating reaction of a compound having a plurality of boronic acid moieties with a compound having a plurality of galloyl moieties in the presence of a template to form a capsule comprising at least one boronate linked organic network layer. It would be apparent to a skilled addressee to choose a solvent or combination of solvents capable of dissolving a compound having a plurality of boronic acid moieties and a compound having a plurality of galloyl moieties which is non-interfering to facilitate the reaction to form at least one boronate linked organic network layer. In one embodiment, the solution is an aqueous or organic solvent. In some embodiments, the solution is water or a buffered aqueous solution.

[0048] The solution of the compound having a plurality of boronic acid moieties may be at any suitable concentration. Indeed the concentration of the solution of the compound having a plurality of boronic acid moieties will be determined based on the concentration of the compound having a plurality of galloyl moieties to be used. It is to be understood that the

concentration should be sufficient to form a capsule having at least one boronate linked organic network layer. Typically however, the solution of the compound having a plurality of boronic acid moieties is at a concentration of less than 2 mM. In certain embodiments the concentration of the solution of the compound having a plurality of boronic acid moieties is from 0.1 to 1.5 mM. In certain embodiments the concentration of the solution of the compound having a plurality of boronic acid moieties is from 0.5 to 1.4 mM. In certain embodiments the concentration of the solution of the compound having a plurality of boronic acid moieties is from 1.1 to 1.3 mM.

[0049] The solution of the compound having a plurality of galloyl moieties may be at any suitable concentration. The concentration of the solution of the compound having a plurality of galloyl moieties will be determined based on the concentration of the compound having a plurality of boronic acid moieties to be used as discussed above. It is to be understood that the concentration should be sufficient to form a capsule having at least one boronate linked organic network layer. Typically however, the solution of the compound having a plurality of galloyl moieties is at a concentration of less than 2 mM. In certain embodiments the concentration of the solution of the compound having a plurality of galloyl moieties is from 0.1 to 1.5 mM. In certain embodiments the concentration of the solution of the compound having a plurality of galloyl moieties is from 0.1 to 1 mM. In certain embodiments the concentration of the solution of the compound having a plurality of galloyl moieties is from 0.1 to 0.3 mM.

[0050] Indeed while the discussion above has focussed on the individual concentrations of the compound having a plurality of boronic acid moieties and the compound having a plurality of galloyl moieties a skilled addressee will readily appreciate that the important variable is the molar ratio of the galloyl moiety to the boronic acid moiety. The ideal molar ratio will be determined by the individual compound having a plurality of boronic acid moieties and the compound having a plurality of galloyl moieties used in each instance as this will affect the reaction stoichiometry for formation of the capsule comprising at least one boronate linked organic network layer. Whilst the reaction will proceed under a wide variety of conditions there is no doubt that for any given combination of compound having a plurality of boronic acid moieties and compound having a plurality of galloyl moieties there is an optimal ratio that can be determined by a skilled worker in the art.

[0051] Nevertheless the present applicants have found that a suitable molar ratio of galloyl moiety to boronic acid moiety is in the ratio of 10:1 to 1:10. In some embodiments the molar ratio of galloyl moiety to boronic acid moiety is 8:1 to 1:8. In some embodiments the molar ratio of galloyl moiety to boronic acid moiety is 5:1 to 1:5. In some embodiments the

molar ratio of galloyl moiety to boronic acid moiety is 3:1 to 1:3. In some embodiments the molar ratio of galloyl moiety to boronic acid moiety is 1:1.

[0052] The reacting step may occur at any suitable temperature. In certain embodiments the reacting may occur at a temperature in the range of 10°C to 40°C. In certain embodiments the reacting may occur at a temperature in the range of 10°C to 30°C. In certain embodiments the reacting may occur at a temperature in the range of 15°C to 25°C. In certain embodiments the reacting may occur at a temperature in the range of 20°C to 25°C.

[0053] As discussed above, the method of the present invention allows rapid assembly of capsules comprising at least one boronate linked organic network layer. As such in some embodiments the reacting step occurs for less than 30 minutes. In some embodiments the reacting step occurs for less than 25 minutes. In some embodiments the reacting step occurs for less than 20 minutes. In some embodiments the reacting step occurs for less than 15 minutes. In some embodiments the reacting step occurs for less than 10 minutes. In some embodiments the reacting step occurs for less than 5 minutes. In some embodiments the reacting step occurs for less than 2 minutes. In some embodiments the reacting step occurs for less than 1 minute. In some embodiments the reacting step occurs for less than 50 seconds. In some embodiments the reacting step occurs for less than 40 seconds. In some embodiments the reacting step occurs for less than 30 seconds. In some embodiments the reacting step occurs for less than 20 seconds. In some embodiments the reacting step occurs for less than 10 seconds. In some embodiments the reacting step occurs for less than 5 seconds. In some embodiments the reacting step occurs for about 2 seconds.

[0054] In addition, in certain embodiments an alkaline pH assists the rapid assembly of capsules comprising at least one boronate linked organic network layer. In some embodiments, the reacting step occurs at an alkaline pH. In some embodiments, the reacting step occurs at a pH of from 7 to 14. In some embodiments, the reacting step occurs at a pH of from 7.5 to 13. In some embodiments, the reacting step occurs at a pH of from 7.5 to 12. In some embodiments, the reacting step occurs at a pH of from 7.5 to 11. In some embodiments, the reacting step occurs at a pH of from 7.5 to 10. In some embodiments, the reacting step occurs at a pH of from 8 to 10. In some embodiments, the reacting step occurs at a pH of from 8 to 9. In some embodiments, the reacting step occurs at a pH of 8.5.

[0055] In the methods of the present invention, an alkaline solution may be added to increase the pH of the solution during the reacting step. It would be apparent to a skilled person that any suitable alkaline solution may be used to form a capsule comprising at least one boronate linked organic network layer according to the methods of the present invention.

In some embodiments, the alkaline solution is selected from the group consisting of Tris-HCl buffer, TAPS buffer, Bicine buffer, Tricine buffer, TAPSO buffer, HEPES buffer, TES buffer, MOPS buffer and combinations thereof.

[0056] In certain embodiments the method of the invention for making capsules may involve the step of removal of the template to produce a capsule having a hollow core. If desired the template may be removed by exposure to a suitable agent that is capable of degrading the template. In general the agent will be chosen such that it is able to degrade the template but such that it will not damage the at least one boronate linked organic network layer of the capsule. An example of a suitable agent is ethylenediaminetetraacetic acid (EDTA). It has been found that the calcium carbonate template is readily degraded in EDTA.

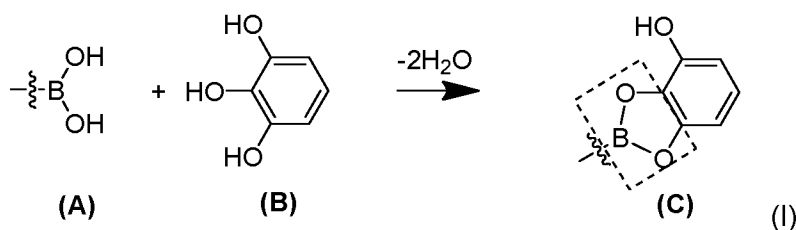
[0057] Typically EDTA may be of any concentration although it is convenient to use an EDTA concentration of from 0.05 to 10 M, more preferably about 200 mM. In some embodiments, the EDTA solution is applied as a buffered solution with MOPS buffer.

[0058] In one embodiment removal of the template comprises dissolving the template. The template may be dissolved in a number of ways and the exact method chosen will depend on the nature of the template to be dissolved. In one embodiment the template is dissolved by contacting the template with a solution of EDTA in MOPS buffer.

[0059] In principle any substance that can dissolve or degrade the template may be used in the step of removing the template and a skilled addressee would readily understand the required agent based on the template used at first instance.

[0060] Following optional removal of the template the resultant capsule is then typically washed to remove any excess template. The capsules may then be isolated by any technique known to the skilled address including filtration and/or centrifugation.

[0061] While the term "boronate" and "boronate ester" linkage used in the context of the present invention would be clear and understood to a skilled addressee, nevertheless for the purpose of clarity, the term "boronate" and "boronate ester" linkage is defined as follows in scheme (I):



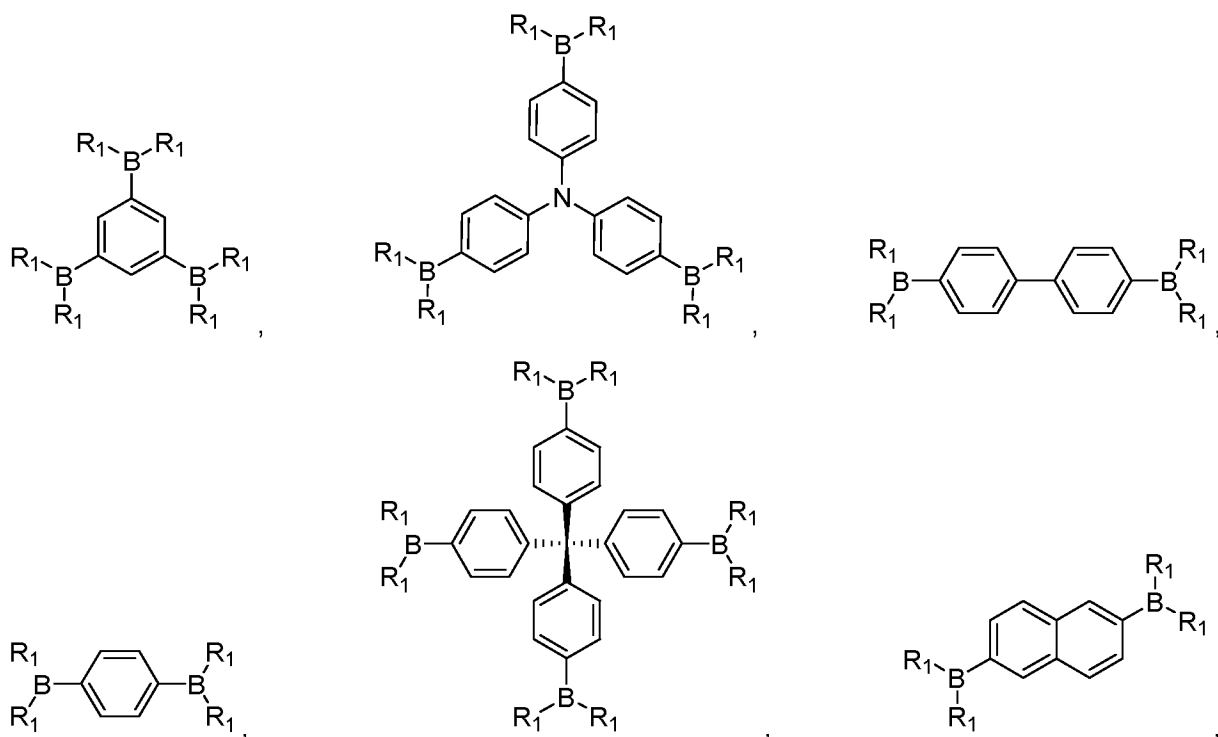
[0062]

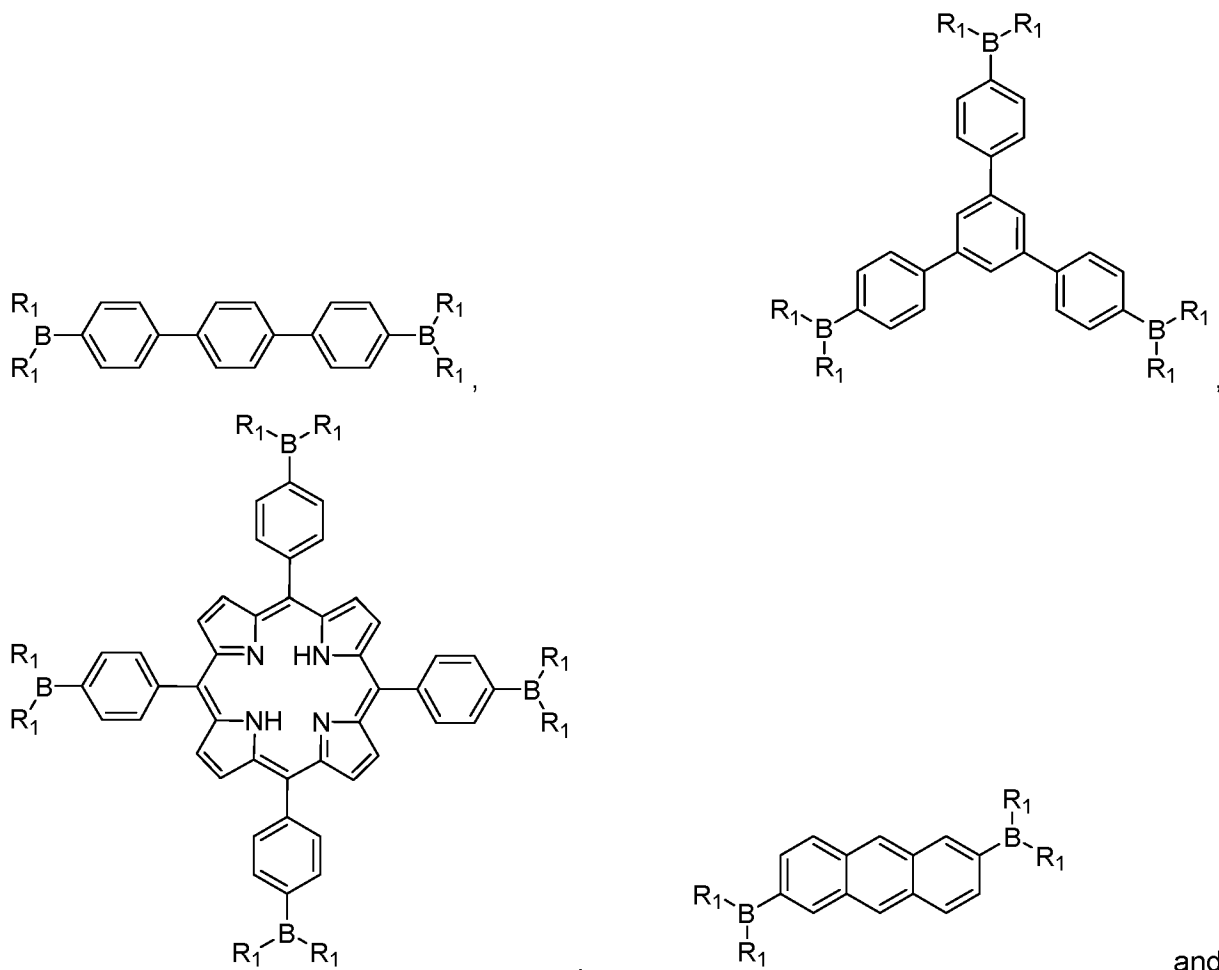
[0063] As shown in the scheme (I), a representative boronic acid (A) is reacted with a representative galloyl moiety (B) to form a representative boronate ester (highlighted) with loss of two water molecules.

[0064] The method of the present invention produces a capsule comprising at least one boronate linked organic network layer wherein the boronate linked organic network layer is the reaction product of a compound having a plurality of boronic acid moieties with a compound having a plurality of galloyl moieties as discussed above.

[0065] As discussed above, the present invention also relates to a capsule comprising at least one boronate linked organic network layer, wherein the boronate linked organic network layer comprises (a) boron containing moieties containing a plurality of boron atoms and (b) linking moieties containing a plurality of oxygen atoms, wherein the boron containing moieties are covalently bonded to the linking moieties via a boronate ester linkage wherein at least one of the oxygen atoms in the boronate ester linkage is located on a carbon atom adjacent to a carbon atom bearing a hydroxyl group.

[0066] It will be apparent to a skilled addressee that any boron containing moiety having a plurality of boron atoms must have at least two boron atoms in order for the final capsule to comprise a network comprising at least one boronate linked organic network layer. It is to be understood that any boron containing moiety containing a plurality of boron atoms may be used in the capsule having at least one boronate linked organic network layer. In one embodiment, the boron containing moieties are selected from the group consisting of:

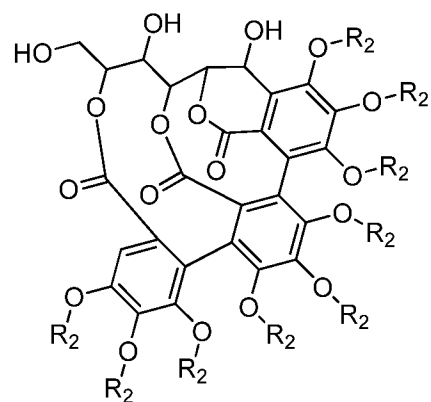
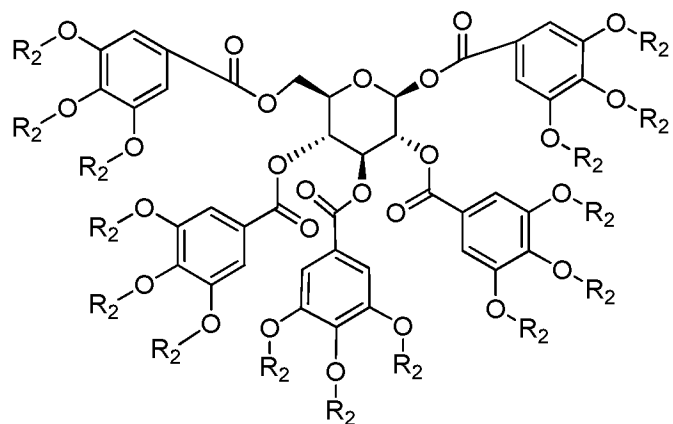
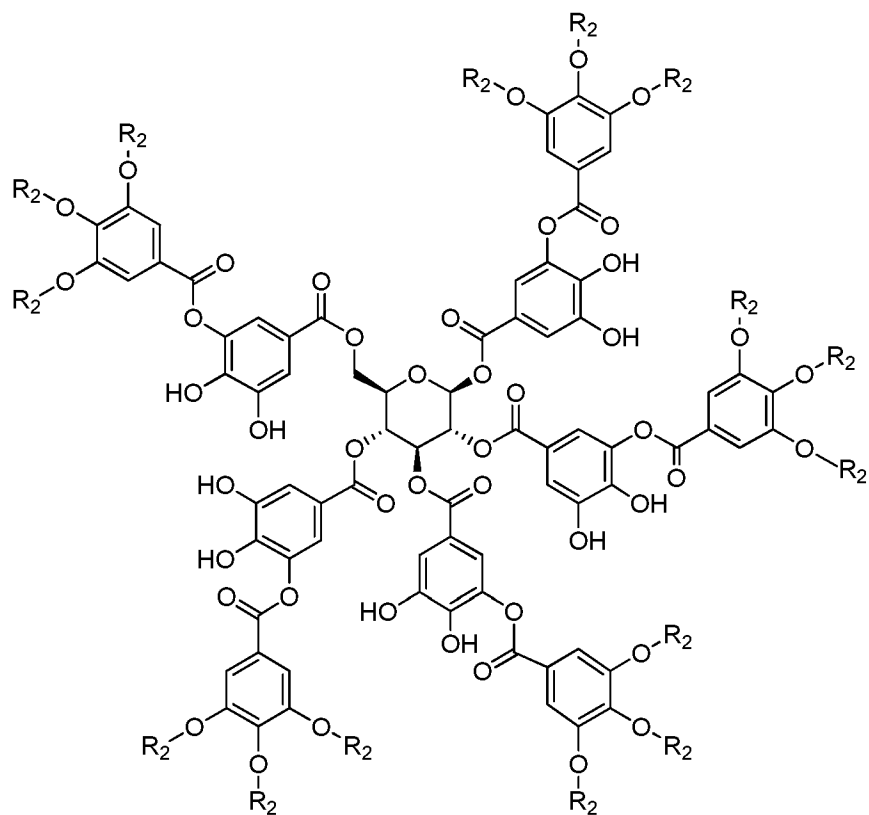


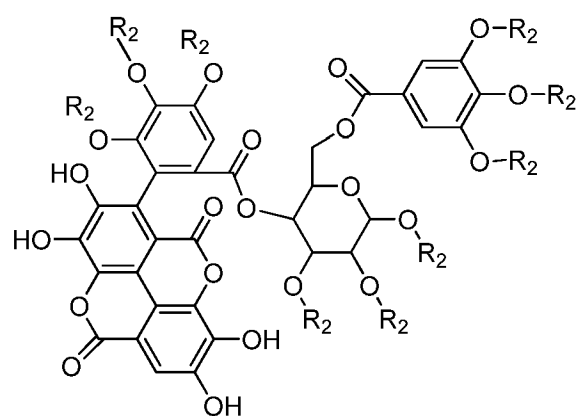
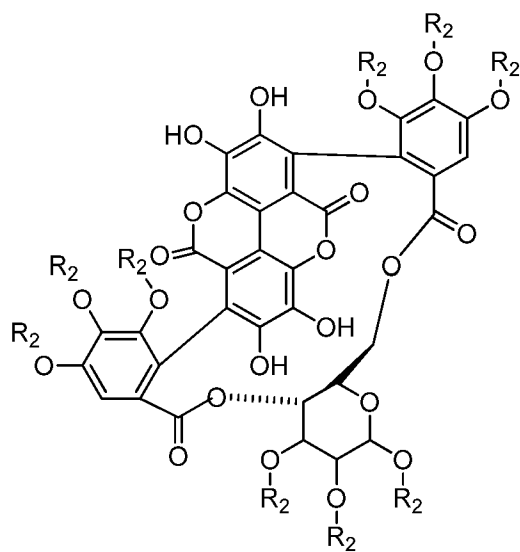
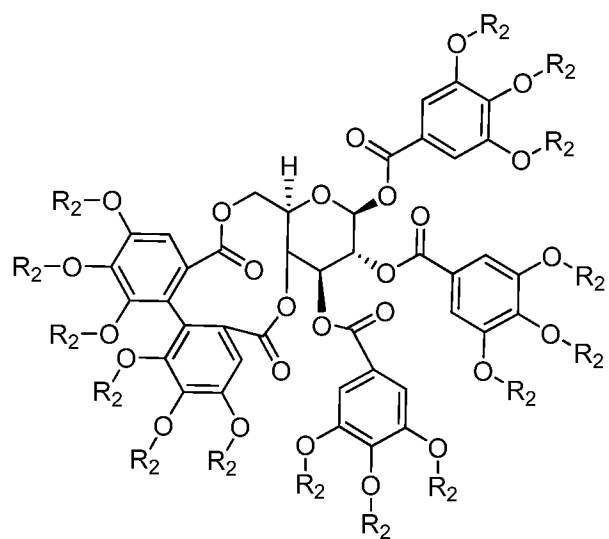
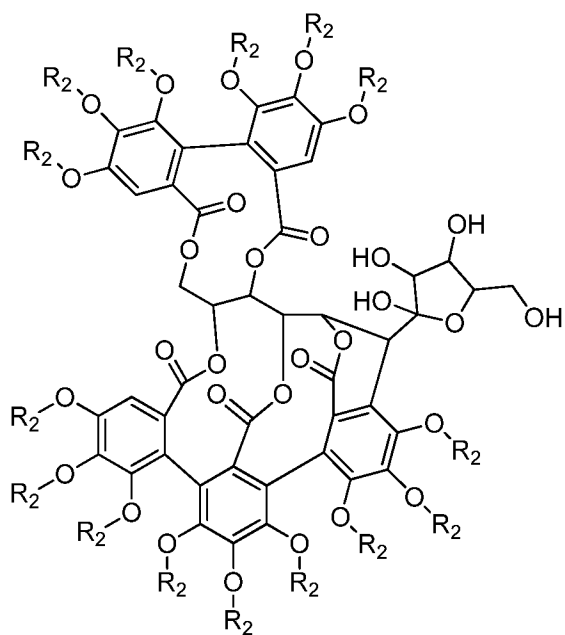
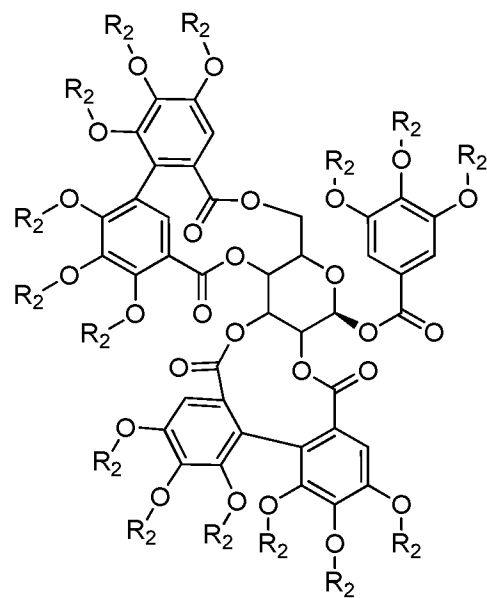
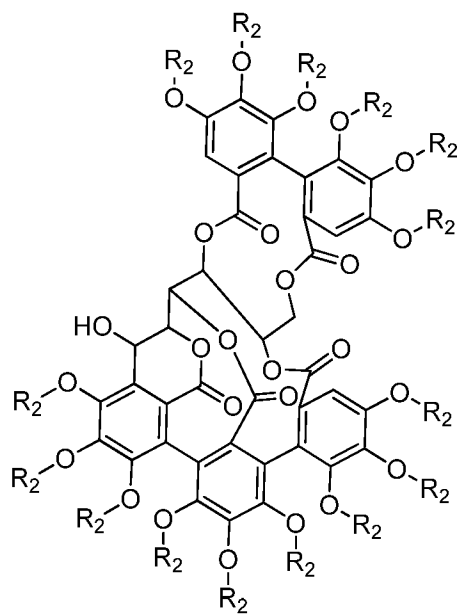


combinations thereof, wherein each R₁ is OH or when taken together with the R₁ on the same boron atom represents the point of attachment of the boron atom to the linking moiety.

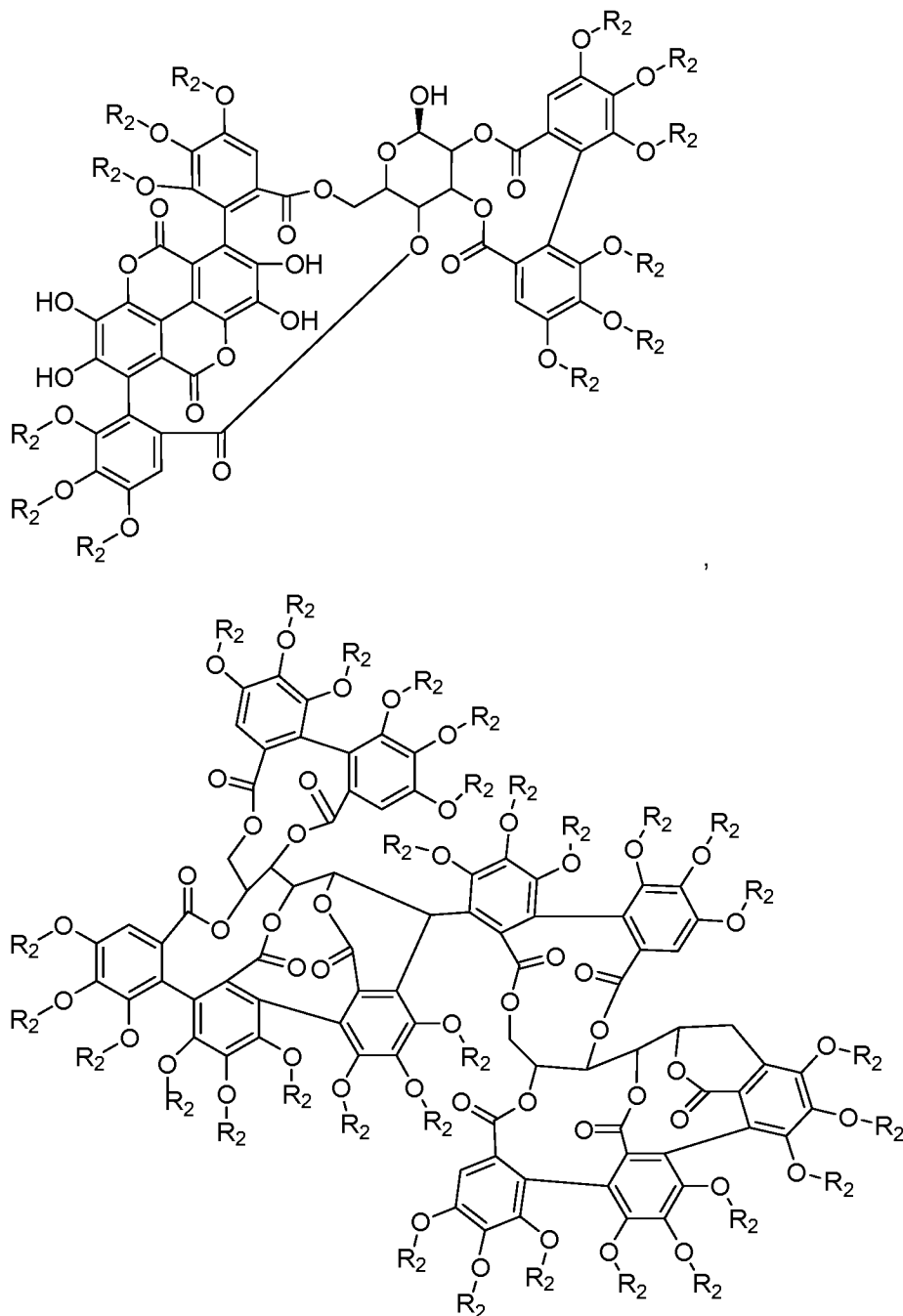
[0067] Similarly, it will be apparent to a skilled addressee that any linking moiety containing a plurality of galloyl groups must have at least six oxygen atoms in order for a capsule to comprise a network comprising at least one boronate linked organic network layer. It is to be understood that any linking moiety containing a plurality of galloyl groups may be used in the capsule having at least one boronate linked organic network layer. In one embodiment, the linking moieties are selected from the group consisting of:

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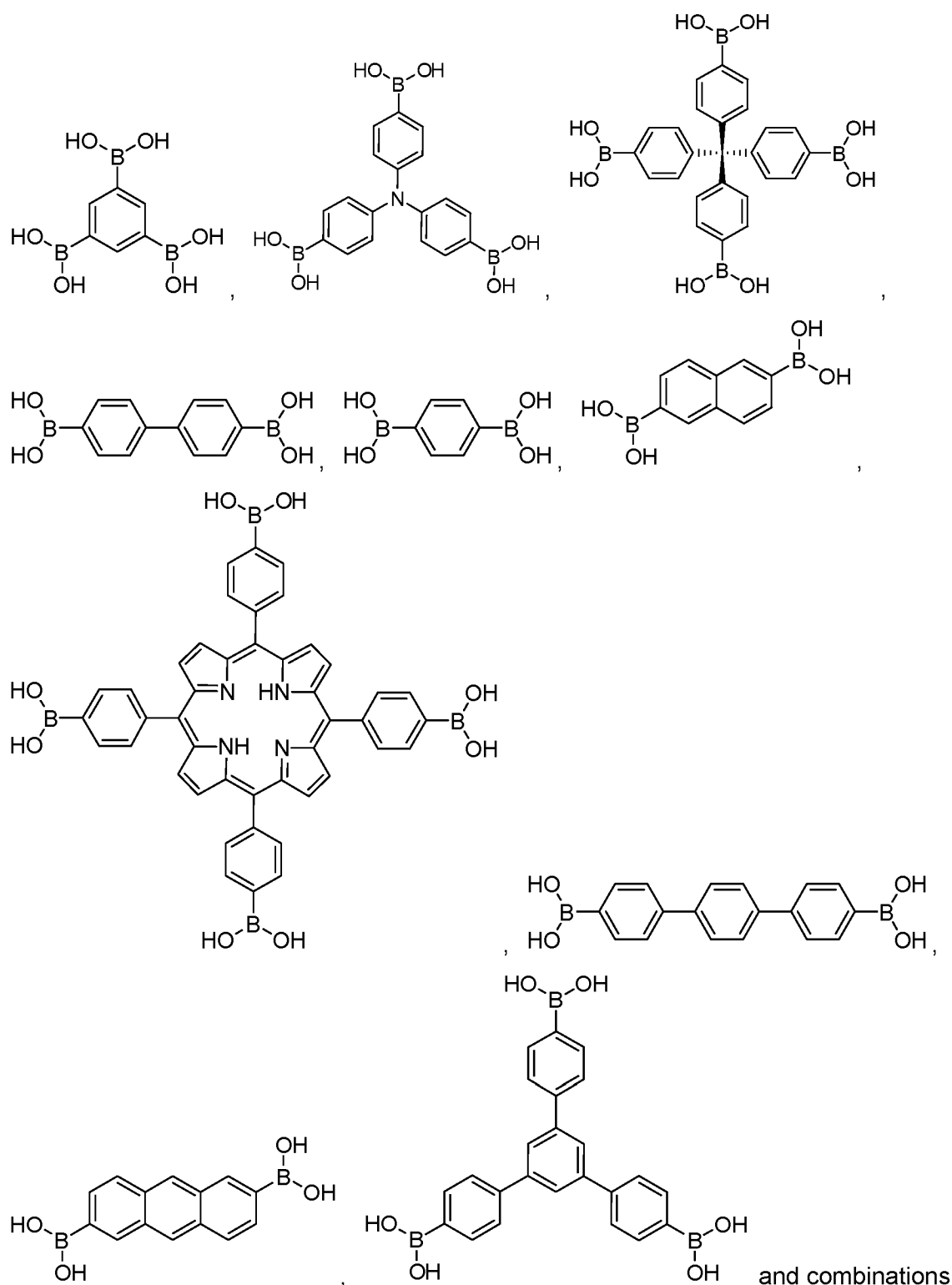
and combinations

thereof, wherein each R₂ is H or two R₂ on adjacent carbon atoms form a boronate linkage to a boron atom of the boron containing moiety.

[0068] The capsule comprising at least one boronate linked organic network layer of the present invention can be formed from the reaction product of a compound having a plurality of boronic acid moieties with a compound having a plurality of galloyl moieties. As previously discussed, any compound having a plurality of boronic acid moieties may be used to form the capsule having at least one boronate linked organic network layer. In one embodiment, the compound having a plurality of boronic acid moieties is a phenylboronic acid moiety. In one

embodiment, the compound having a plurality of boronic acid moieties is a diboronic acid, triboronic acid or a tetraboronic acid.

[0069] In one embodiment, the compound having a plurality of boronic acid moieties is selected from the group consisting of:



thereof.

[0070] In addition to the above, any compound having a plurality of galloyl moieties may be used to form a capsule having at least one boronate linked organic network layer.

[0071] In one embodiment, the compound having a plurality of galloyl moieties is selected from the group consisting of ellagitannins, tannic acid, 1,2,3,4,6-pentagalloyl glucose and combinations thereof. In one embodiment, the compound having a plurality of galloyl moieties is selected from the group consisting of tannic acid, tellimagrandin II, castalagin, castalin, casuarictin, grandinin, punicalagin, punicalin, roburin A, terflavin B, and combinations thereof.

[0072] In some embodiments, the capsule of the present invention is responsive to externally applied stimuli including pH and/or *cis*-diols. In some embodiments, the capsule of the present invention is multi-responsive. In some embodiments, the capsule of the present invention is dual responsive to both pH and *cis*-diol triggers.

[0073] Without being bound by any one theory, it is believed that the responsive properties of the capsules of the present invention are a result of the reversible nature of the boronate ester formed by the reaction of a compound having a plurality of boronic acid moieties and a compound having a plurality of galloyl moieties.

[0074] Accordingly, in some embodiments the capsule is stable under alkaline conditions. In some embodiments the capsule is stable in a pH range of from 7 to 14. In some embodiments the capsule is stable in a pH range of from 7.5 to 13. In some embodiments the capsule is stable in a pH range of from 7.5 to 12. In some embodiments the capsule is stable in a pH range of from 7.5 to 11. In some embodiments the capsule is stable in a pH range of from 7.5 to 10. In some embodiments the capsule is stable in a pH range of from 7.5 to 9.5. In some embodiments the capsule is stable in a pH range of from 8 to 10. In some embodiments the capsule is stable in a pH range of from 8 to 9.

[0075] As discussed above, the boronate ester linkages of the capsules comprising at least one boronate linked organic network layer can be responsive to pH and/or external vicinal diols (e.g. *cis*-diols). In some embodiments the capsule disassembles under acidic conditions. In some embodiments the capsule disassembles in a pH range of from 0 to 7. In some embodiments the capsule disassembles in a pH range of from 1 to 7. In some embodiments the capsule disassembles in a pH range of from 2 to 7. In some embodiments the capsule disassembles in a pH range of from 2 to 6.5. In some embodiments the capsule disassembles in a pH range of from 2 to 6.0. In some embodiments the capsule disassembles in a pH range of from 3 to 6. In some embodiments the capsule disassembles

in a pH range of from 4 to 6. In some embodiments the capsule disassembles in a pH range of from 4.5 to 5.5. In some embodiments the capsule disassembles in a pH of 5.0.

[0076] In addition, in some embodiments the capsule disassembles in the presence of any suitable compound comprising a vicinal diol as would be known to a person skilled in the art. In preferred embodiments, the capsule disassembles in the presence of mannitol, glucose or combination thereof.

[0077] The concentration of the compound comprising a vicinal diol may be at any suitable concentration to disassemble the capsules and will be determined based on the concentration and amount of the capsules used as well as the compound used. Typically however, the concentration of the compound comprising a vicinal diol is less than 500 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is less than 400 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is less than 300 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is less than 200 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is less than 100 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 1 to 100 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 10 to 100 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 20 to 30 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 20 to 40 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 20 to 50 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 20 to 60 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 50 to 100 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 60 to 100 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 70 to 100 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 80 to 100 mM. In certain embodiments the concentration of the compound comprising a vicinal diol is in the range of from 90 to 110 mM.

[0078] The capsules of the present invention can be used for a number of applications as discussed above. In particular, the applications include drug delivery, therapeutic treatments and diagnostic imaging techniques. In some embodiments, the capsules of the present invention are stable in the presence of carbohydrates in biological environments, which allows the capsules to be used in biological applications. For example, the capsules can be used in

intracellular drug delivery systems, extracellular remote-controlled drug delivery systems, closed-loop insulin delivery systems, biological targeting systems, and biomimetic protocells.

[0079] Accordingly, the capsules can be loaded with any suitable active agent known to a skilled person which will be determined based on the desired use of the capsule. As stated above in one embodiment the present invention therefore provides a capsule capable of delivering an active agent to a part of the body of a mammal, the method comprising encapsulating the active agent in a capsule comprising at least one boronate linked organic network layer formed from reacting a compound having a plurality of boronic acid moieties with a compound having a plurality of galloyl moieties in the presence of a template, and administering the capsule containing the active agent to the mammal.

[0080] The active agent may be any active agent that has a desired biological activity. The active agent may be a pharmaceutically active agent or a veterinary active agent. In principle any active agent that can be used can be delivered by the capsules of the present invention. Potential active agents may include proteins or protein crystals, peptides, DNA, polymer-drug conjugates, hydrophobic drugs, nanoparticles e.g. magnetite, and quantum dots. In some embodiments, the active agent is insulin, doxorubicin or combination thereof.

[0081] The first step in the method involves encapsulation of the active agent in the capsule produced by the method of the present invention. Whilst this may be achieved in a number of ways it is typical that the encapsulation method takes advantage of adsorption of the active agent through incubation. Once encapsulated in this way the active agent is then ready to be delivered.

[0082] The administration of the capsule containing the active agent to the mammal may be carried out in any way known in the art. In some embodiments, the route of administration of the capsule includes enteral, topical and parenteral administration. In some embodiments, the route of administration of the capsule include oral, topical, transmucosal, inhalation and injection administration. Indeed given that the materials are generally quite small in size a suitable means of administration is by oral administration or subcutaneous injection.

[0083] Pharmaceutical compositions containing an active agent encapsulated as discussed above for parenteral injection typically comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the

maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0084] These compositions may also contain adjuvants such as preservative, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of micro-organisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminium monostearate and gelatin.

[0085] Once administered to the body it is desirable that the active agent be able to be controllably released at the site of interest. One advantage of the capsules of the present invention is that they allow for release in this way as the boronate ester between a compound having a plurality of boronic acid moieties and a compound having a plurality of galloyl moieties is pH and/or vicinal diol responsive. As such when the capsule disassembles it can release the active agent at the site of interest.

[0086] The capsules of the present invention may also be used in diagnostic imaging as discussed above. Accordingly in some embodiments the present invention also provides a method of diagnostic imaging of a part of the body of a mammal, the method comprising administering a capsule to the mammal, the capsule comprising at least one boronate linked organic network layer formed from reacting a compound having a plurality of boronic acid moieties with a compound having a plurality of galloyl moieties in the presence of a template and containing a detectable moiety, and detecting the presence of the detectable moiety in the mammal. The capsules of the present invention can be used in a number of diagnostic imaging techniques including Positron emission topography (PET), magnetic resonance imaging (MRI) and fluorescence imaging. In one embodiment the imaging technique is PET imaging. In one embodiment the imaging technique is MRI imaging. In one embodiment the imaging technique is fluorescence imaging.

[0087] As stated above in order to be used for diagnostic imaging it is necessary that the capsule contains a detectable moiety and/or is loaded with a detectable moiety.

[0088] In certain embodiments a template may be used (and retained) such that the encapsulated template acts as the detectable moiety for the purposes of diagnostic imaging. Alternatively, a skilled addressee would readily be able to identify other suitable templates or active agents which may be loaded in the capsule that have this type of activity. For example, a template containing any radioisotope of a metal that may be imaged may be

incorporated into the capsules of the present invention for the purposes of radio-imaging. Examples of metals that may be incorporated into the capsules of the invention for use in radioimaging, for example, include Bismuth 213, Cobalt 57, Cobalt 60, Holmium 166, Lutetium 177, Rhenium 186, Technetium 99, Copper 64, Gallium 67, indium 111, and Thallium 201 is a detectable moiety for PET Imaging. In addition certain metals such as Fe^{III}, Mn^{II} and Gd^{III} can be used as MRI contrast agents merely by way of example.

[0089] Utilising the capsules of the invention in imaging typically relies on the presence of the template used in forming the capsule or a capsule loaded with an active agent being able to be detected by an imaging technique such as fluorescence or radioimaging or by incorporation of a detectable moiety that is fluorescent.

[0090] For example when the desired method of imaging is fluorescent imaging this is typically carried out by loading an active agent having a detectable moiety or a template having a detectable moiety retained within the capsule in order for the capsules to be imaged. Alternatively the fluorescence of the capsules may be inherent.

[0091] As would be appreciated by a skilled addressee any form of imaging is typically only useful if the imaging provides information to the person carrying out the analysis. As such in order to obtain the maximum amount of information using the capsules of the present invention it is preferred that they are attached to a biological entity prior to use. As will be appreciated in these cases diagnostic imaging will rely on the binding to the biological entity being involved in facilitating the localisation of the capsule in the desired tissues or organs of the subject being treated/imaged.

[0092] Thus for example in relation to the use of the capsules of the invention in imaging it is anticipated that these may be used by first binding them to a biological entity of interest followed by administration of an effective amount of the capsule to a subject followed by monitoring of the subject after a suitable time period to determine if the capsule has localised at a particular location in the body, whether the capsule has disassembled or whether the capsule is broadly speaking evenly distributed through the body. As a general rule where the capsule is localised in tissue or an organ of the body this is indicative of the presence in that tissue or organ of something that is recognised by the particular molecular recognition moiety used.

[0093] Accordingly judicious selection of a biological entity to connect the capsule is important in determining the efficacy of any of the capsules of the invention in diagnostic imaging applications. In this regard a wide range of biological entities that can act as molecular recognition moieties are known in the art which are well characterised and which

are known to selectively target certain receptors in the body. In particular a number of biological entities that can act as molecular recognition moieties or molecular recognition portions are known that target tissue or organs when the patient is suffering from certain medical conditions. Examples of biological entities that can act as molecular recognition moieties or molecular recognition portions that are known and may be used in this invention include Octreotate, octreotide, [Tyr³]-octreotate, [Tyr¹]-octreotate, bombesin, bombesin(7-14), gastrin releasing peptide, single amino acids, penetratin, annexin V, TAT, cyclic RGD, glucose, glucosamine (and extended carbohydrates), folic acid, neurotensin, neuropeptide Y, cholecystokinin (CCK) analogues, vasoactive intestinal peptide (VIP), substance P, alpha-melanocyte-stimulating hormone (MSH). For example, certain cancers are known to over express somatostatin receptors and so the molecular recognition moiety may be one which targets these receptors. An example of a molecular recognition moiety or molecular recognition portion of this type is [Tyr³]-octreotate. Another example of a molecular recognition moiety or molecular recognition portion is cyclic RGD which is an integrin targeting cyclic peptide. In other examples a suitable molecular recognition moiety or molecular recognition portion is bombesin which is known to target breast and pancreatic cancers.

[0094] The monitoring of the subject for the location of the capsule will typically provide the analyst with information regarding the location of the detectable moiety and hence the location of any material that is targeted by the molecular recognition moiety (such as cancerous tissue). An effective amount of the capsule of the invention will depend upon a number of factors and will of necessity involve a balance between the amount of detectable moiety required to achieve the desired imaging effect and the general interest in not exposing the subject (or their tissues or organs) to any unnecessary levels of radiation which may be harmful.

[0095] Accordingly, the present applicants have identified i) a rapid assembly for the formation of a capsule and ii) stimuli-responsive capsules which are stable and robust under specific conditions utilising assembly of a boronate linked organic network layer in the presence of a template. These capsules have numerous applications as discussed above and include a range of biological applications, such as closed-loop insulin delivery systems by glucose-activation, anticancer drug delivery by acidic pH-trigger, biological targeting by selective interaction with furanoside carbohydrates, or biomimic modeling as microreactors responsive to multiple environmental changes.

EXAMPLES

[0096] The present invention will now be described with reference to the following examples.

Materials

[0097] Tannic acid (TA), benzene-1,4-diboronic acid (BDDBA), gallic acid (GA), tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 3-(*N*-morpholino)propanesulfonic acid (MOPS), doxorubicin hydrochloride (DOX), D-glucose, D-mannitol, ethylenediaminetetraacetic acid (EDTA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), phosphate buffered saline (PBS), deuterium oxide (D₂O), deuterium chloride (DCI), sodium deuteroxide (NaOD), dimethyl sulfoxide-*d*₆ (DMSO-*d*₆), ammonium hydroxide (NH₄OH) solution and poly(sodium 4-styrene sulfonate) (PSS, *M_w* ~70 kDa), were purchased from Sigma-Aldrich (Australia). Wheat germ agglutinin Alexa Fluor® 488 conjugate and Hoechst 33342 were purchased from Invitrogen (Life Technologies, USA). All these materials were used as received. Calcium carbonate (CaCO₃) template particles and silver nanoparticles (Ag NPs) were synthesized according to literature methods. High-purity Milli-Q (MQ) water with a resistivity of 18.2 MΩ.cm was obtained from an inline Millipore RiOs/Origin water purification system.

Generation of ⁶⁴Cu for PET Imaging

[0098] No-carrier-added ⁶⁴Cu was produced with the IBA Nirta target by the ⁶⁴Ni(p,n)⁶⁴Cu reaction. The target was produced by direct electroplating of highly enriched ⁶⁴Ni (> 99%, Isoflex USA) onto an Ag disk (24 mm diameter and 1.0 mm thick disk). The plating cell was filled with a ⁶⁴Ni solution and NH₄OH (total 55 mL) and electroplating was carried out at 5.0 mA using a chopped saw tooth current for ~10 h to give an average 20 nm ⁶⁴Ni thickness. Targets were irradiated using an IBA 18/9 cyclotron with an incident beam of 14.9 MeV (18 MeV degraded by 0.5 mm aluminium foil). The irradiated disk was then loaded into an IBA Pinctada module and the ⁶⁴Ni plating was dissolved in recirculating 3 mL 12 M HCl at 70 °C using a peristaltic pump. Once dissolved, the solution was loaded onto an AG 1-X8 anion exchange cartridge for purification and the cartridge was washed with 12 M HCl and ethanol to elute impurities such as ⁶¹Co. ⁶⁴Cu was recovered with ~2 mL of water. Typical production yields average 30 mCi for 4 h irradiation at 35 μA.

Cell Culture

[0099] HeLa cell line was purchased from ATCC (Rockville, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO₂ and subcultured prior to confluence using trypsin.

Characterization

[0100] Differential interference contrast (DIC) images were obtained using an inverted Olympus IX71 microscope. Atomic force microscopy (AFM) experiments were carried out with a JPK NanoWizard II BioAFM. Typical scans were performed in intermittent contact mode with Mikromasch silicon cantilevers (NSC/CSC). The film thickness of the capsules was analyzed by using JPK SPM image processing software (version V.3.3.32). Transmission electron microscopy (TEM) images and corresponding energy dispersive X-ray spectroscopy (EDX) profiles were obtained by using a FEI Tecnai TF20 instrument with an operation voltage of 200 kV. The acquisition time for elements mapping is more than 50 min to ensure a sufficient and accurate signal is obtained from the samples. Scanning electron microscopy (SEM) images were acquired on a FEI Quanta 200 field emission scanning electron microscope operated at an accelerating voltage of 10 kV. In AFM, TEM and SEM characterizations, aqueous suspensions of capsules (1.0 µL) were allowed to air-dry on glass slides, formvar-carbon coated gold grids, and Piranha cleaned silicon wafers, respectively [(98% H₂SO₄:30% H₂O₂ (7:3)) – *Caution! Piranha solution is highly oxidizing and corrosive! Extreme care should be taken during preparation and use.*]. UV-Vis absorption measurements were conducted using an Infinite M200 microplate reader (Tecan, Switzerland). The zeta-potentials were measured using a Zetasizer Nano-ZS (Malvern Instruments, UK) with a 633 nm He-Ne ion laser. The capsules were suspended in 10 mM phosphate buffer (pH 7.4). The results are expressed as the mean and standard deviation obtained from three measurements. Flow cytometry assays were conducted on a Cyflow Space (Partec GmbH) flow cytometer. Cell imaging was performed using a DeltaVision deconvolution fluorescence microscope (Applied Precision) with a 60× 1.42 NA oil objective with a standard FITC/TRITC/DAPI filter set. Images were processed with Imaris (Bitplane) software using the maximum intensity projection. Deconvolution images were taken on a series of z-sections within the top and bottom of the cells. Each slice of the images (i.e., in the same z-plane) was analyzed to verify if the capsules were inside or outside of the cells.

Example 1. ^1H NMR Study of pH-Dependent Reversible Complexation and *cis*-Diol Competition

[0101] All ^1H NMR spectra were recorded on a Varian unity 400 spectrometer in D_2O solvent using residual solvent peak as a standard. The spectra were acquired over 64 acquisitions. Unless stated otherwise the spectra were taken 15 min after preparation of the sample. The desired final concentrations of GA (100 mg/mL, D_2O in stock solution) and BDBA (100 mg/mL, $\text{DMSO-}d_6$ in stock solution) were either 2.0 mg/mL or 10.0 mg/mL. As for *cis*-diol responsiveness, the desired final concentrations of GA (20.0 mg/mL, D_2O in stock solution) and BDBA (20.0 mg/mL, $\text{DMSO-}d_6$ in stock solution) were 2.0 mg/mL, and that of mannitol (100 mg/mL, D_2O in stock solution) was 10 mg/mL. The percentage of BDBA-GA complexes was determined by the integration of GA areas. The residual $\text{DMSO-}d_6$ solvent peak was used as reference for the integration. The pH of the solutions was adjusted by adding NaOD or DCl. Due to the changes of solubility of GA and BDBA with pH, the sum of integration of areas could be changed (< 10.8%).

[0102] We first characterized the pH-dependent reversible complexation of BDBA and gallic acid (GA) using ^1H NMR spectroscopy. GA is commonly used as a model compound for the study of polyphenols because it is highly soluble in water and bears the fundamental galloyl moiety. At pH 5.0, the mixture of GA and BDBA results in a two distinct peaks (7.80 and 7.05 ppm), corresponding to the individual compounds, which indicates a general lack of complexation between BDBA and GA. There were also three small peaks at 7.70, 7.62 and 6.99 ppm, which can be ascribed to 33% BDBA-GA complexes. At pH 8.5, significant changes are observed for the chemical shifts and peak splitting. The four BDBA protons (7.60–7.25 ppm) give rise to a complex splitting pattern, with the single peak at 7.48 ppm ascribed to binding between BDBA and GA in the molar ratio of 1 to 2. Additionally, the protons of complexed GA give rise to two individual signals at 6.84 and 6.73 ppm. To further study the pH-dependent reversibility, the solution was acidified from pH 8.5 to 5.0 and the peaks assigned to the BDBA-GA complex dramatically decreased, while the peaks attributed to the free individual BDBA and GA reappeared.

[0103] The competitive interaction between *cis*-diols and the BPN complex was also studied by using ^1H NMR spectroscopy. The BDBA peak split into three peaks (doublets at 7.68 and 7.61 ppm, and a singlet at 7.43 ppm), and two peaks appeared close to the free mannitol (4.31 ppm and 4.08 ppm). The peaks at 7.68, 7.61, 4.31, and 4.08 ppm resulted from complexation between mannitol and BDBA, and the single peak at 7.43 ppm corresponds to complexation in the molar ratio of 1:2. A pH 7.4 solution of BDBA-GA was mixed with mannitol, resulting in 76% uncomplexed GA, while prior to mixing, the BDBA-GA solution contained 28%

uncomplexed GA. The BDBA peaks clearly demonstrated complexation with both GA and mannitol. Moreover, the peaks corresponding to mannitol were similar to the peaks observed in a mixture of solely BDBA and mannitol. These results confirmed that mannitol could effectively compete with the capsules of the present invention (BPN capsules) (BDBA-GA) to form BDBA-mannitol complexes, thereby highlighting the fundamental mechanism of *cis*-diol responsiveness relevant for BPN capsules.

Example 2. Preparation of Boronate-Phenolic Network (BPN) Capsules

[0104] *Preparation of CaCO₃ templates:* In brief, 2.4 mL of sodium carbonate (1 M) and 200 mL of PSS (1 mg/mL) were mixed in a 200 mL beaker, after which 4.8 mL of CaCl₂ solution (1 M) was rapidly added under vigorous stirring. After stirring for 30 s, the CaCO₃ particles were observed under DIC microscopy. Afterwards, the resulting CaCO₃ particles with desired particle diameter (0.8 – 3.5 μ m) were washed three times with MQ water to remove unreacted substance and resuspended in MQ water. PSS was used for the template synthesis because it leads to the formation of monodisperse CaCO₃ particles that have a high loading capacity for active agents, and can release active agents *in vitro* based on the capping material used.

[0105] *Capsule formation:* 50 – 100 μ L of CaCO₃ template particles (10 – 45 mg/mL) were diluted to 470 μ L MQ water, and then 5 μ L of TA solution (24 mM, MQ water in stock solution) and 25 μ L of BDBA solution (24 mM, DMSO in stock solution) were added to the particle suspension to yield the following final concentrations: TA 0.24 mM and BDBA 1.2 mM in a total volume of 500 μ L. The at least one boronate linked organic network layer can be formed by adding 500 μ L of Tris-HCl buffer (100 mM, pH 8.5) to raise the pH of the suspension. The suspension was vigorously mixed by vortexing for 20 s immediately after the individual additions. The boronate coated particles were washed three times with MOPS buffer (100 mM, pH 8.0) to remove excess BPN complexes. In the washing step, the particles were spun down by centrifugation (2000 g, 60 s) and the supernatant was removed. Sonication was applied after each washing step to avoid the aggregation of particles. To obtain hollow capsules, the CaCO₃ templates were removed by adding 500 μ L of EDTA (200 mM, pH 8.0) into 500 μ L of particle suspension in MOPS buffer (100 mM, pH 8.0). Extra washing steps with EDTA (200 mM, pH 8.0) were performed to remove the template completely when the particle concentration was high. Finally, the hollow capsules were spun down by centrifugation (3000 g, 60–90 s), and the remaining pellet was washed and redispersed in desired buffer solutions. The formation of at least one boronate linked organic network layer on Ag NP ($D \approx 34.2 \pm 4.5$ nm) templates was carried out following the protocol

described for the CaCO_3 templates, except that in the washing step the BPN-Ag NPs were spun down at 8000 g for 5–10 min (depending on the nanoparticle concentration).

[0106] As discussed above, capsules of the present invention (BPN capsules) were fabricated by mixing BDBA and TA solutions in the presence of poly(sodium styrene sulfonate) (PSS)-stabilized calcium carbonate (CaCO_3) particulate templates at pH 8.5. PSS was used for template synthesis to form monodisperse CaCO_3 particles with a high loading capacity for active agents. The covalent binding of BDBA and TA in the capsules was assessed using Fourier transform infrared (FTIR) spectrometry. Compared with the spectrum of TA, a new absorption peak appeared at 1370 cm^{-1} in the spectrum of the capsules due to the B–O stretching vibration. The peak at 1625 cm^{-1} , corresponding to the O–H bending vibration of hydroxyl groups in TA, decreased. The formation of BPN capsules on CaCO_3 particles shifted the particle zeta potential from -5 ± 5 to -40 ± 2 mV, which is more than a two-fold shift compared to previously reported Fe^{III} -TA coating (-18 ± 4 mV). Without being bound by any one theory, the negative zeta potential was likely due to the phenolic building blocks and charged BDBA, which should be able to further bind with competing *cis*-diols. Finally, the featureless X-ray diffraction (XRD) data indicated that the films were amorphous, similar to metal-phenolic networks (MPNs), but different to the crystalline structure of covalent organic frameworks (COFs).

[0107] Monodisperse, spherical capsules were readily observed under differential interference contrast (DIC) microscopy as shown in Fig. 1a. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) showed that dried capsules of the present invention had features similar to polymeric capsules, such as folds and creases, due to collapse during the air drying process as shown in Fig. 1b,c. High-angle annular dark field (HAADF) imaging and atomic force microscopy (AFM) imaging showed the smooth surface of the capsules, suggesting that no excess complexation was occurring (Fig. 1d,e). Energy-dispersive X-ray spectroscopy (EDX) mapping revealed the elemental compositions of BPN capsules. The boronate linked organic network layer thickness (10.5 ± 1.2 nm) is similar to that reported for MPN capsules (Fig. 1f). As shown in Fig. 1g,h, the formation of BPN films can also be performed on silver nanoparticles, implying an important extension of this technique towards forming capsules in the presence of nanoparticles, where the template can either be retained as a functional template, or removed to form nanoscale hollow capsules.

Example 3. Disassembly Experiments in Response to pH and *cis*-Diols

[0108] Capsule disassembly experiments were carried out in physiological pH 7.4 PBS buffer solution in the initial 4 h. After 4 h, the capsules were washed and dispersed into pH

5.0 sodium acetate buffer solutions (50 mM), or pH 7.4 PBS solutions with 100 mM mannitol, followed by incubation up to 11 h. The suspensions of capsules were constantly incubated on a thermo-shaker (Eppendorf Thermomixer Comfort) at 37 °C and 600 rpm. At different time points, 5 µL of capsule suspension was collected and diluted with 995 µL of PBS buffer solution for flow cytometric analysis. Samples were measured on a Partec CyFlow Space (Partec GmbH, Germany) flow cytometer to count the number of capsules. The disassembly experiments were performed with three different batches of the CaCO₃ templates, and the results were shown as the mean ± SD from three independent experiments. The data points at 10 h contain error bars.

[0109] To demonstrate the dual-responsiveness of the BPN capsules in biological conditions, the capsules were first incubated under physiological pH 7.4 for 4 h and then triggered with acidic pH 5.0 or through the addition of 100 mM mannitol. After 6 h at pH 5.0 the number of remaining capsules dropped below 10%, suggesting that the boronate linked organic network layer rapidly dissociated when a critical percentage of boronate ester bonds was hydrolyzed. This is in contrast with Fe^{III}-TA capsules, which take approximately 10 days at pH 4.0 to reach 10%. Mannitol could also cleave the crosslinks of the BPN, as evidenced by the decrease in the capsule population. These results correspond well with the NMR studies of Example 1 competitive interactions. Previously reported boronate-functionalized capsules obtained through LbL assembly only degraded under alkaline pH conditions (e.g., pH 9–11), because only the tetravalent charged borate moiety, which exists at high pH, could bind with *cis*-diols to form a stable complex. However, phenolic building blocks of the present invention can form a stable boronate linked organic network layer with BDBA and dissociate in the presence of competing excess *cis*-diols at physiological pH. Based on the zeta-potential and NMR studies, this could be because the complexation of BDBA and TA induces the formation of thermodynamically stable, charged BDBA in the capsules, which is favorable for binding with excess *cis*-diols.

Example 4. Release Experiments in Response to pH and *cis*-Diols

[0110] DOX was adsorbed into PSS-stabilized CaCO₃ particles through incubation for 5 h, followed by washing with MQ water 3 – 5 times to remove excess and loosely adsorbed DOX. The preparation of DOX-loaded capsules is the same as the protocol described in Example 2.

[0111] *pH-Triggered release*: DOX-loaded BPN capsules were incubated in PBS (pH 7.4) or sodium acetate buffer (pH 5.0, 50 mM) in a total volume of 150 µL at 37 °C under soft, constant shaking at 600 rpm. At determined time intervals, the capsule suspensions were

centrifuged at 3000 *g* for 30 s, and 100 μL of the supernatant was carefully collected for DOX quantification by absorbance measurement. Afterwards, the capsule suspensions were topped up to 150 μL by using fresh PBS (pH 7.4) or sodium acetate buffer (pH 5.0, 50 mM) for the subsequent release studies.

[0112] *cis-Diols-triggered release*: To examine the *cis*-diols responsiveness of BPN capsules, DOX-loaded BPN capsules and Fe^{III} -TA capsules were first incubated in PBS (pH 7.4) for the initial 17.5 h to reach an equilibrium state. The Fe^{III} -TA capsules were used as a representative of metal-phenolic network (MPN) capsules. During the period from 17.5 h to 47.5 h, the medium of DOX-loaded BPN capsule suspensions was washed and changed to PBS (pH 7.4) with 27.5 mM glucose, or PBS (pH 7.4) with 100 mM mannitol. The medium of loaded Fe^{III} -TA capsules was also washed and changed to PBS (pH 7.4) with 100 mM mannitol. In the final stage from 47.5 h to 56 h, the media of DOX-loaded BPN capsule suspensions were washed and changed to sodium acetate buffer (pH 5.0, 50 mM) with 27.5 mM glucose or sodium acetate buffer (pH 5.0, 50 mM) with 100 mM mannitol to further study the effect of combination of acidic pH and *cis*-diols. The capsule suspensions were topped up to 150 μL by fresh buffer solutions for the subsequent release studies. At determined time intervals, the capsule suspensions were centrifuged at 3000 *g* for 30 s.

[0113] The DOX concentrations in the supernatant were examined based on the absorbance at a wavelength of 490 nm by using an Infinite M200 microplate reader (Tecan, Switzerland). DOX release percentage $r(i)$ at each time point was calculated as:

$$r(1)(\%) = \frac{(\Delta A(1) - A_b)}{A_t} \times 100\% \quad (i = 1)$$

$$r(i)(\%) = \frac{\left((\Delta A(i) - A_b) + \frac{100}{150} \times \sum_{i=1}^{i-1} \Delta A(i) \right)}{A_t} \times 100\% \quad (i \geq 2)$$

where $\Delta A(i)$ is the absorbance of collected supernatant at each time point, A_b is the background absorbance of buffer solutions, A_t is the initial absorbance of the DOX-loaded capsule suspension which represents the total loading amount of DOX in the capsules (it was very close to the absorbance of totally released DOX after disrupting the capsules by acidification and sonication, data not shown). The values of A_t used in the study were in the range from 0.3 to 0.6. The absorbance of DOX (0.04 to 0.6) has linear correlation with DOX concentration (data not shown). It is noted that in Fig. 2, the disassembly does not fully correlate with DOX release. Without being bound by any one theory, it is believed that this is

because encapsulation of DOX could stabilize the capsules and the disassembly kinetics of the capsules may change. The release experiments were performed with three different batches of CaCO_3 templates, and the data were analyzed to give the means $\pm$ SD from three independent experiments.

[0114] To demonstrate encapsulation and release from the BPN capsules, doxorubicin hydrochloride (DOX) was chosen as an exemplary active agent and loaded into CaCO_3 particles. As shown in Fig. 2b and Fig. 3, the release of DOX was negligible at pH 7.4, however when the pH was decreased to 5.0 the DOX release was dramatically accelerated. This demonstrates the potential of these capsules for achieving intracellular endocytic pH-triggered active agent release. To verify the *cis*-diol responsiveness, the DOX-loaded BPN capsules were triggered with glucose or mannitol. Fe^{III} -TA capsules were chosen as representative control MPN capsules without *cis*-diols responsiveness. As shown in Fig. 2c, DOX release from the BPN capsules and Fe^{III} -TA capsules was slow at the initial 17.5 h. At physiological pH with the presence of 27.5 mM glucose (containing one *cis*-diol), there was a minimal change in the release kinetics of the BPN capsules, while 100 mM mannitol led to a moderate increase in the release kinetics. The release from the Fe^{III} -TA capsules was not sensitive to 100 mM mannitol even after 35 h. The DOX release kinetics from the BPN capsules could be further accelerated by the combination of *cis*-diols with acidic pH. This three-stage release experiment demonstrated that the BPN capsules exhibited stimuli-response to acidic pH and *cis*-diols, making it possible to tune the release kinetics of active agents to suit biological variations, such as in acidic endocytic compartments and external systemic administration of mannitol.

Example 5. Intracellular Disassembly and Active Agent Release

[0115] HeLa cells were seeded in an 8-well Lab-Tek I chambered coverglass slides (Thermo Fisher Scientific, Rochester) at a density of 3×10^4 cells per well and allowed to adhere for 12 h. Afterwards, cells were incubated with DOX-loaded BPN capsules (at a capsule-to-cell ratio of 100:1) for 4 h, 8 h and 24 h followed by washing three times with DPBS. Cells were fixed with 3% paraformaldehyde for 15 min at room temperature. Afterwards, cell membranes were stained with wheat germ agglutinin Alexa Fluor® 488 (0.01 mg/mL) at room temperature for 20 min, and cell nuclei were stained using Hoechst 33342 (2.5 $\mu\text{g/mL}$) at room temperature for 15 min.

[0116] The cellular interactions between the BPN capsules and HeLa cells were studied to investigate the intracellular disassembly of the capsules (Fig. 2d and Fig. 4).

Example 6. Capsule Cytotoxicity Assay

[0117] HeLa cells were used to evaluate the cytotoxicity of blank and DOX-loaded BPN capsules by MTT assays. Cells were seeded into 96-well plates (Costar 3596, Corning, USA) at a density of 5×10^3 cells/well in 100 μ L of DMEM supplemented with 10% FBS. After incubation overnight, culture media were replaced with 100 μ L of fresh media containing a different number of blank BPN capsules (capsule-to-cell ratio: 1:1, 25:1, 50:1, 75:1, and 100:1), or containing DOX-loaded BPN capsules or free DOX with various DOX concentrations of 0.05, 0.1, 0.5, 1.0 and 5.0 μ g/mL. After 48 h of incubation, 10 μ L of MTT solution in DPBS (5 mg/mL) was added to each well, and further incubated for 4 h. Then the medium was aspirated, the MTT-formazan generated by live cells was dissolved in 100 μ L of DMSO and measured on an Infinite M200 microplate reader (Tecan, Switzerland) at a wavelength of 570 nm. Cell viability was expressed as a relative percentage of untreated cells. The results are average values with standard deviations, means $\pm$ SD, n = 12.

[0118] The cytotoxic effect of the DOX-loaded capsules approached that of free DOX on HeLa cells (Fig. 5). BPN capsules showed negligible influence on the viability of HeLa cells even at high capsule dosages (Fig. 6).

Example 7. Stability Experiments in the Presence of Human Blood Plasma

[0119] BPN capsules were incubated in normal human blood plasma (50%) with or without 100 mM mannitol in a total volume of 20 μ L at 37 $^{\circ}$ C under soft, constant shaking to simulate the blood conditions *in vivo*. For the quantification of capsule number, 0.5 μ L of capsule suspension was taken from the stock suspension, and the representative DIC microscopy images were acquired at 0, 5, and 12 h. The number of capsules was counted in each image manually under a 60 $\times$ object lens. Data were analyzed to give the means $\pm$ SD from three independent experiments. It is noted that the DIC images are only used to show the representative conditions of the capsules, but cannot be used to show the complete condition of capsules at different time points.

[0120] It has been disclosed that carbohydrates are actively involved in a wide range of biological processes, such as intercellular recognition. These carbohydrates might be an unexpected trigger for BPN capsule disassembly *in vivo*, which would potentially limit their biological applications. Therefore, the stability of BPN capsules were studied in the presence of competing carbohydrates to simulate conditions expected *in vivo*. As shown in Fig. 7a,b, the capsules remained spherical and intact in human blood plasma (HBP) even for 12 h. In contrast, when the HBP was doped with 100 mM mannitol, the number of capsules decreased. This indicated that the BPN capsules are stable, even in the presence of the competing

carbohydrates present in blood, and are still capable of disassembling in the presence of excess mannitol.

Example 8. *In Vivo* Stability Experiments by PET/CT Imaging

[0121] 2×10^6 human prostatic adenocarcinoma cells (PC3) were implanted subcutaneously in immunosuppressed mice, and the tumor was allowed to grow for 10 days. After tumor formation, ^{64}Cu /BPN capsules were injected locally and the capsule stability was evaluated by PET/computed tomography (CT) imaging 5 h and 12 h post-injection. PET scans were acquired by using a NanoPET/CT In Vivo Preclinical Imager (Mediso, Budapest, Hungary) with a PET acquisition time of 10 min and coincidence relation, 1-3 was performed. PET scan image reconstruction was performed with the following parameters: OSEM with SSRB 2D LOR, energy window, 400 – 600 keV; filter Ram-Lak cut off 1, number of iteration/subsets, 8/6. During the imaging, the animals were placed in a Minerve imaging chamber and anesthetized with a mixture of 2.5% isoflurane in oxygen (1 L min^{-1}). Anaesthesia was monitored by measuring respiratory frequency and body temperature was kept at 37°C with a heating pad underneath the animal to prevent hypothermia. In order to add anatomical information to the molecular PET, computed tomography (CT) scans were acquired directly after the PET scan. For the scans, an X-ray voltage of 45 kVp, an exposure time of 900 ms and a pitch of 0.5 were used. A total projection of 240 projects over 360° of rotation was acquired. Projection data were rebinned by 1:4 and reconstructed using a Ram-Lak filter into a matrix having an isotropic voxel size of $96 \mu\text{m}$. Image files of PET and CT scan were fused and analyzed using the analysis software InVivoScope version 2.00. Without being bound by any one theory, this experiment was based on the hypothesis that if the capsules were disassembled over time by unexpected triggers *in vivo*, the PET signal of free ^{64}Cu or capsule fragments would have moved from the tumor to the liver because of the clearance effects of the reticuloendothelial system. The color scale for all PET image data shows radiotracer uptake with white corresponding to the highest activity and blue to the lowest activity. It is noted that it is not exactly known what local environment the capsules were exposed to at the tumor site, and future investigations will be necessary to fully understand the *in vivo* performance of the BPN capsules for theranostic applications.

[0122] As discussed above, positron emission tomography (PET) was used as a preliminary means to evaluate the *in vivo* stability of these capsules in a tumor mice model to determine if the carbohydrate metabolism of tumors would make the capsules unstable *in vivo* (Fig. 7c). ^{64}Cu was incorporated into the BPN capsules at pH 8.0 through free hydroxyl groups, resulting in PET-active ^{64}Cu /BPN capsules. After tumor formation, ^{64}Cu /BPN capsules were injected locally and the stability was evaluated by PET/computed tomography (CT) imaging.

Fig. 7d,e showed that even after 12 h the PET signal from the capsules was still mainly located at the tumor site. This suggested that these capsules exhibit good stability *in vivo*, which will be useful for exploring future biological applications.

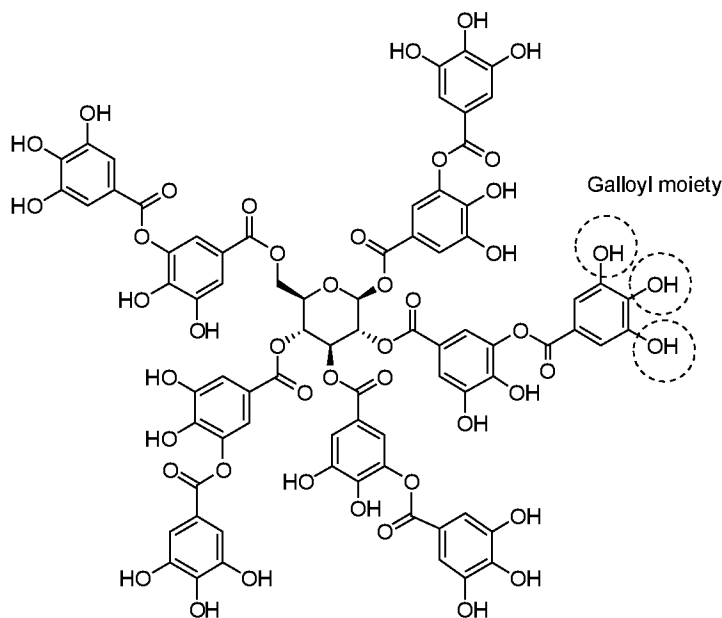
Example 9. Statistical Analysis

[0123] All results are expressed as the mean $\pm$ standard deviation. In Fig. 2a, the statistical significance in the comparison of before and after the triggers was estimated by two-way analysis of variance (ANOVA), where $^{**}P < 0.01$, and $^{***}P < 0.001$. In Fig. 2b,c, the statistical significance in the comparison among the groups at different stages was estimated by two-way analysis of variance (ANOVA), where $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. In Fig. 7b, the statistical significance in the comparison before and after the triggers was estimated by student's t-test, where $^{**}P < 0.01$, and *NS* denotes not significant. In Fig. 7e, the statistical significance in the comparison in different time was estimated by student's t-test, where *NS* denotes not significant.

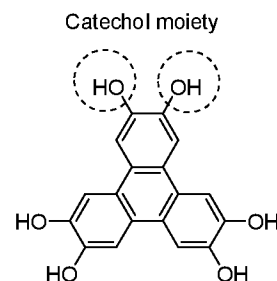
Comparative Example 1. Capsule Stability using Galloyl Moieties versus Catechol Moieties

[0124] In this comparative example, the higher stability of galloyl-based boronate networks relative to catechol-based boronate networks was demonstrated. In addition, it is shown that the higher stability of capsules using compounds having a plurality of galloyl moieties produced more stable and robust capsules comprising at least one boronate linked organic network layer than capsules using compounds having a plurality of catechol moieties. The capsules of the present invention (BPN capsules) can exhibit dual-responsive properties to acidic pH and *cis*-diol stimuli, as discussed above.

[0125] In this comparative example, the compound having a plurality of galloyl moieties is tannic acid (TA), and the comparative compound having a plurality of catechol moieties is 2,3,6,7,10,11-Hexahydroxytriphenylene Hydrate (HHTP). For ease of comparison, both TA and HHTP were chosen as they have a similar dendronized molecular structure, as shown in Scheme (II). The main difference between these two compounds is that TA contains a plurality of galloyl moieties whereas HHTP contains a plurality of catechol moieties.



(D)



(E)

(II)

[0126] Molecular structure of tannic acid (TA) (D) and 2,3,6,7,10,11-Hexahydroxytriphenylene Hydrate (HHTP) (E) showing the galloyl moiety and catechol moiety, respectively.

[0127] Both of TA and HHTP were used to study formation of capsules comprising at least one boronate linked organic network layer. CaCO_3 templates were suspended in 470 μL MQ water, and then 5 μL of TA (24 mM, MQ water in stock solution) or HHTP solution (24 mM, ethanol in stock solution) and 25 μL of BDBA solution (24 mM, DMSO in stock solution) were added to the particle suspension to yield the following final concentrations: TA and HHTP 0.24 mM and BDBA 1.2 mM in a total volume of 500 μL . The capsules can be formed by adding 500 μL of MOPS buffer (100 mM, pH 8.0) to raise the pH of the suspension. The suspension was vigorously mixed by vortexing for 20 s immediately after the individual additions. The BPN capsules were washed three times with MOPS buffer (100 mM, pH 8.0) to remove excess BPN complexes. In the washing step, the particles were spun down by centrifugation (2000 g , 60 s) and the supernatant was removed. Sonication was applied after each washing step to avoid the aggregation of particles. To obtain hollow capsules, the CaCO_3 templates were removed by adding 500 μL of EDTA (200 mM, pH 8.0) into 500 μL of particle suspension in MOPS buffer (100 mM, pH 8.0). Finally, the hollow capsules were spun down by centrifugation (3000 g , 60–90 s), and the remaining pellet was washed and redispersed in desired buffer solutions.

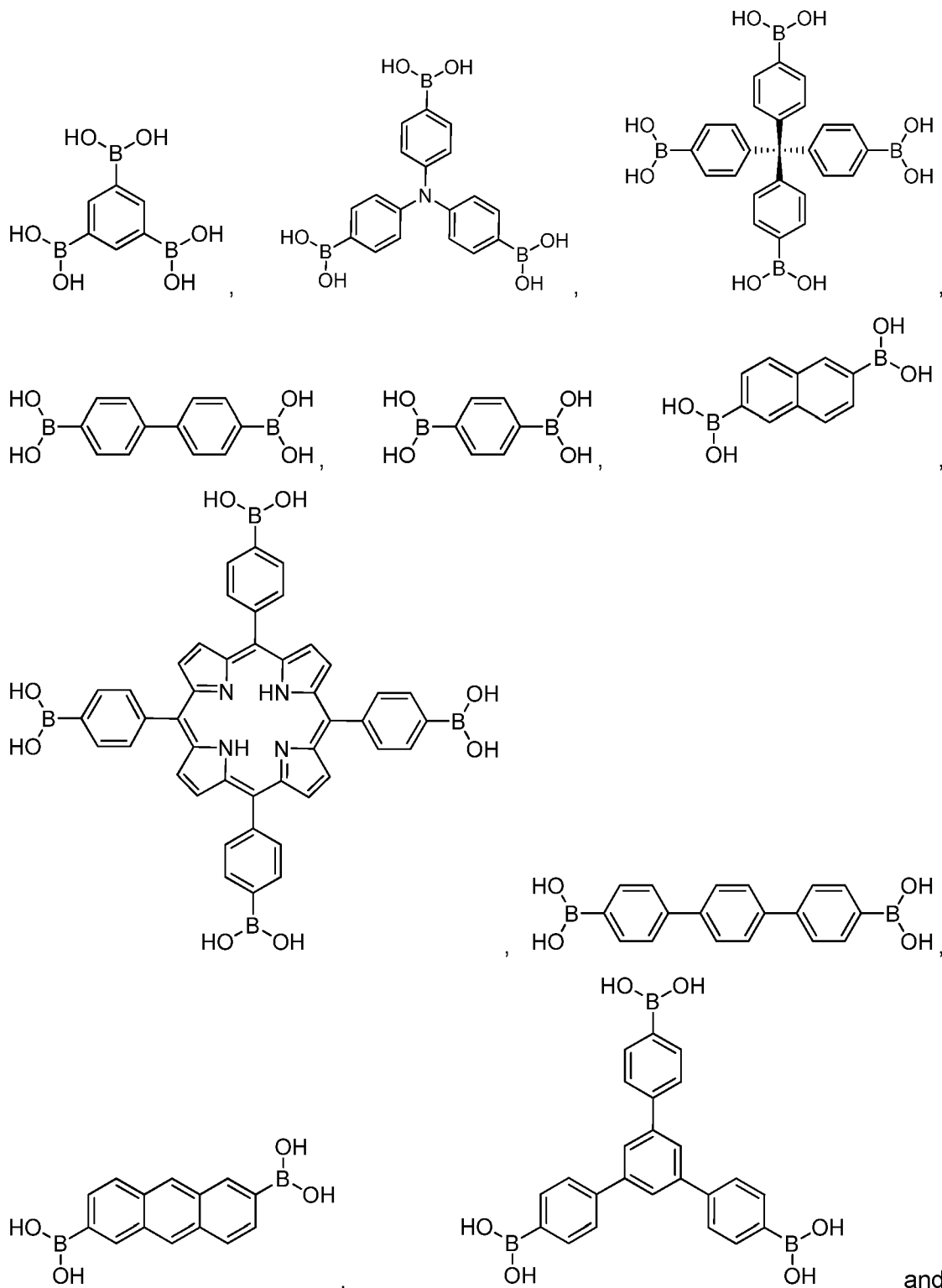
[0128] The formation of capsules is observed under the DIC microscopy. As shown in Fig. 8, the boronic acid moiety (derived from BDBA) and galloyl moiety (derived from TA) can form monodispersed capsules, which indicates that the capsules of the present invention are robust and stable.

[0129] However, when the comparative compound comprising a plurality of catechol moieties (derived from HHTP) were used, the HHTP-BDBA networks cannot form capsules after the removal of CaCO_3 templates, which means that the HHTP-BDBA networks cannot form robust and stable capsule under similar conditions. Without being bound by any one theory, the applicants note that HHTP can form capsules when coordinated with Fe^{3+} (data not shown) and it is believed that the reason that HHTP-BDBA networks cannot form capsules is because of the weak interaction of catechol moieties with boronic acid moieties and not because of the relatively smaller dendronized molecular structure to that of TA.

CLAIMS

1. A method of forming a capsule comprising at least one boronate linked organic network layer the method comprising reacting a compound having a plurality of boronic acid moieties with a compound having a plurality of galloyl moieties in the presence of a template to form a capsule comprising at least one boronate linked organic network layer.
2. A method according to claim 1 wherein the reacting step comprises addition of a solution of a compound having a plurality of galloyl moieties to a solution of a template followed by addition of a solution of a compound having a plurality of boronic acid moieties.
3. A method according to claim 1 wherein the reacting step comprises addition of a solution of a compound having a plurality of boronic acid moieties to a solution of a template followed by addition of a solution of a compound having a plurality of galloyl moieties.
4. A method according to any one of claims 1 to 3 further comprising addition of an alkaline solution.
5. A method according to claim 4 wherein the alkaline solution is selected from the group consisting of Tris-HCl buffer, TAPS buffer, Bicine buffer, Tricine buffer, TAPSO buffer, HEPES buffer, TES buffer, MOPS buffer and combinations thereof.
6. A method according to any one of claims 1 to 5 wherein the reacting step occurs at an alkaline pH.
7. A method according to claim 6 wherein the reacting step occurs at a pH of from 8 to 10.
8. A method according to claim 6 wherein the reacting step occurs at a pH of from 8 to 9.
9. A method according to claim 6 wherein the reacting step occurs at a pH of 8.5.
10. A method according to any one of claims 1 to 9 wherein the compound having a plurality of boronic acid moieties is a phenylboronic acid moiety.
11. A method according to any one of claims 1 to 10 wherein the compound having a plurality of boronic acid moieties is a diboronic acid, triboronic acid or a tetraboronic acid.

12. A method according to any one of claims 1 to 9 wherein the compound having a plurality of boronic acid moieties is selected from the group consisting of:

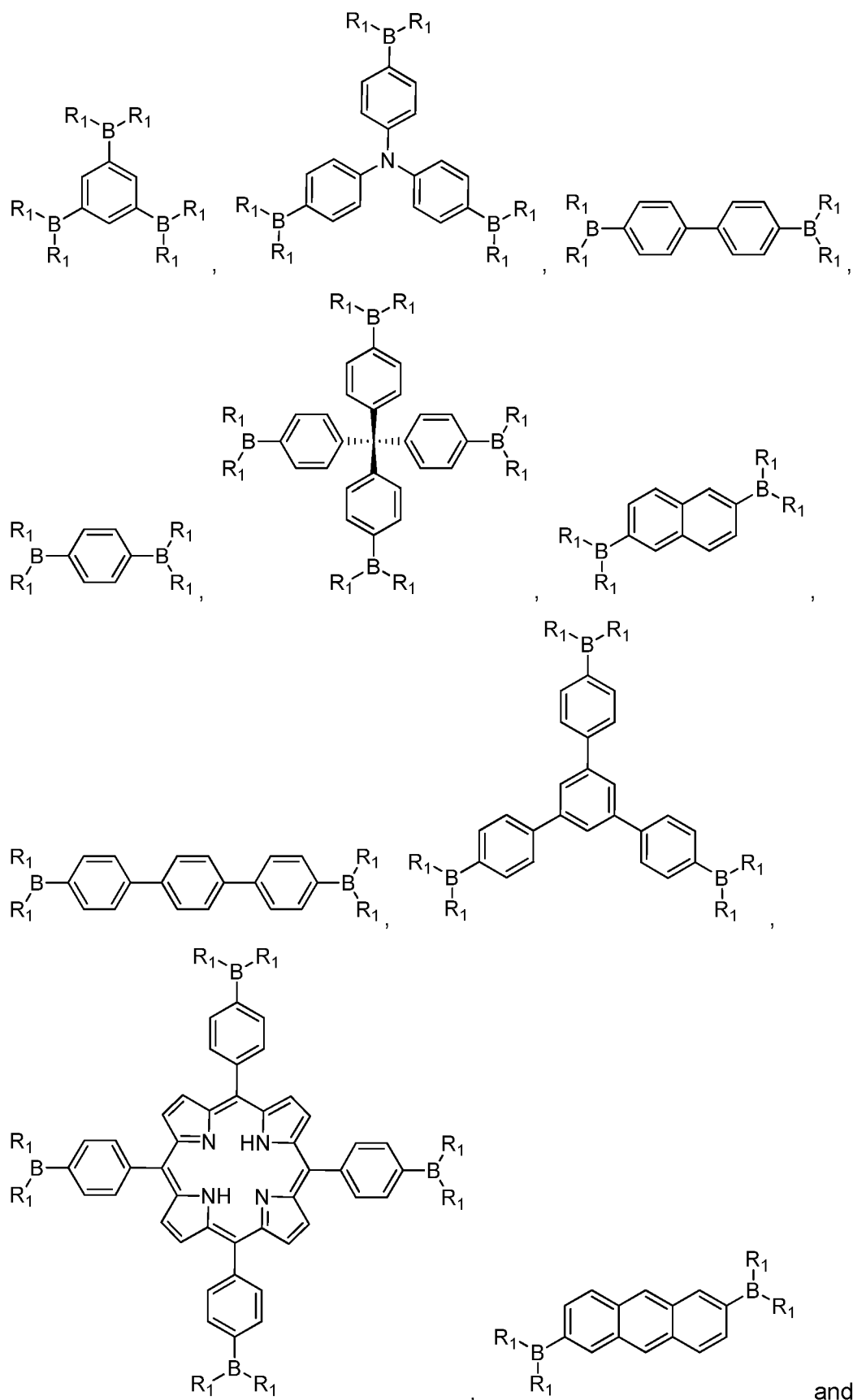


combinations thereof.

13. A method according to any one of claims 1 to 12 wherein the compound having a plurality of galloyl moieties is selected from the group consisting of ellagitannins, tannic acid, 1,2,3,4,6-pentagalloyl glucose and combinations thereof.

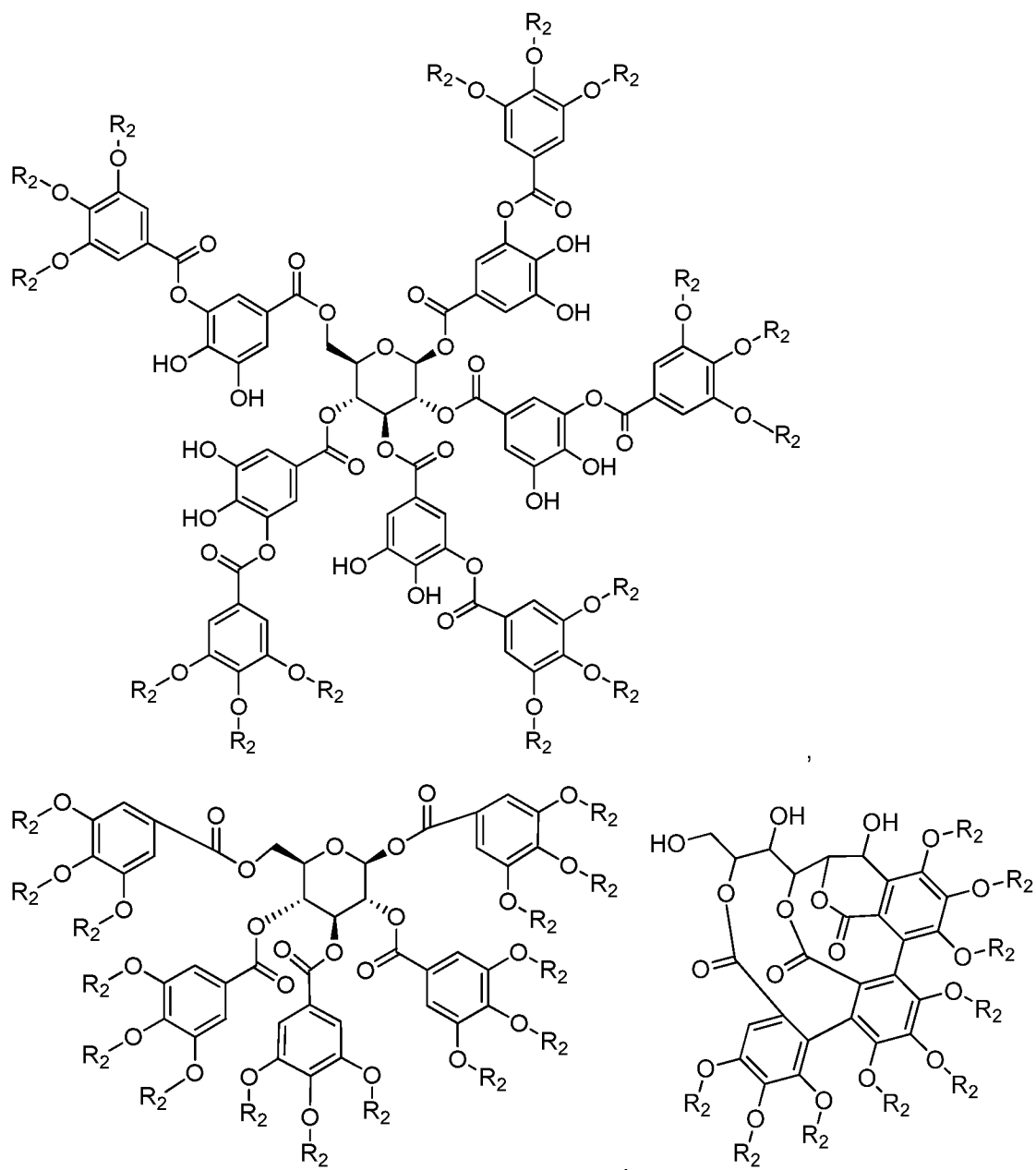
14. A method according to any one of claims 1 to 12 wherein the compound having a plurality of galloyl moieties is selected from the group consisting of tannic acid, tellimagrandin II, castalagin, castalin, casuarictin, grandinin, punicalagin, punicalin, roburin A, terflavin B, and combinations thereof.
15. A method according to any one of claims 1 to 14 wherein the reacting step occurs at a temperature of 10 to 40 °C.
16. A method according to any one of claims 1 to 15 wherein the molar ratio of the galloyl moiety to the boronic acid moiety is in the ratio of 10:1 to 1:10.
17. A method according to any one of claims 1 to 15 wherein the molar ratio of the galloyl moiety to the boronic acid moiety is in the ratio of 5:1 to 1:5.
18. A method according to any one of claims 1 to 15 wherein the molar ratio of the galloyl moiety to the boronic acid moiety is in the ratio of 1:1.
19. A method according to any one of claims 1 to 18 further comprising the step of removing the template.
20. A capsule comprising at least one boronate linked organic network layer, wherein the boronate linked organic network layer comprises (a) boron containing moieties containing a plurality of boron atoms and (b) linking moieties containing a plurality of oxygen atoms, wherein the boron containing moieties are covalently bonded to the linking moieties via a boronate ester linkage wherein at least one of the oxygen atoms in the boronate ester linkage is located on a carbon atom adjacent to a carbon atom bearing a hydroxyl group.
21. A capsule according to claim 20 wherein the boronate linked organic network layer is the reaction product of a compound having a plurality of boronic acid moieties with a compound having a plurality of galloyl moieties.
22. A capsule according to claim 20 wherein the boron containing moieties are selected from the group consisting of:

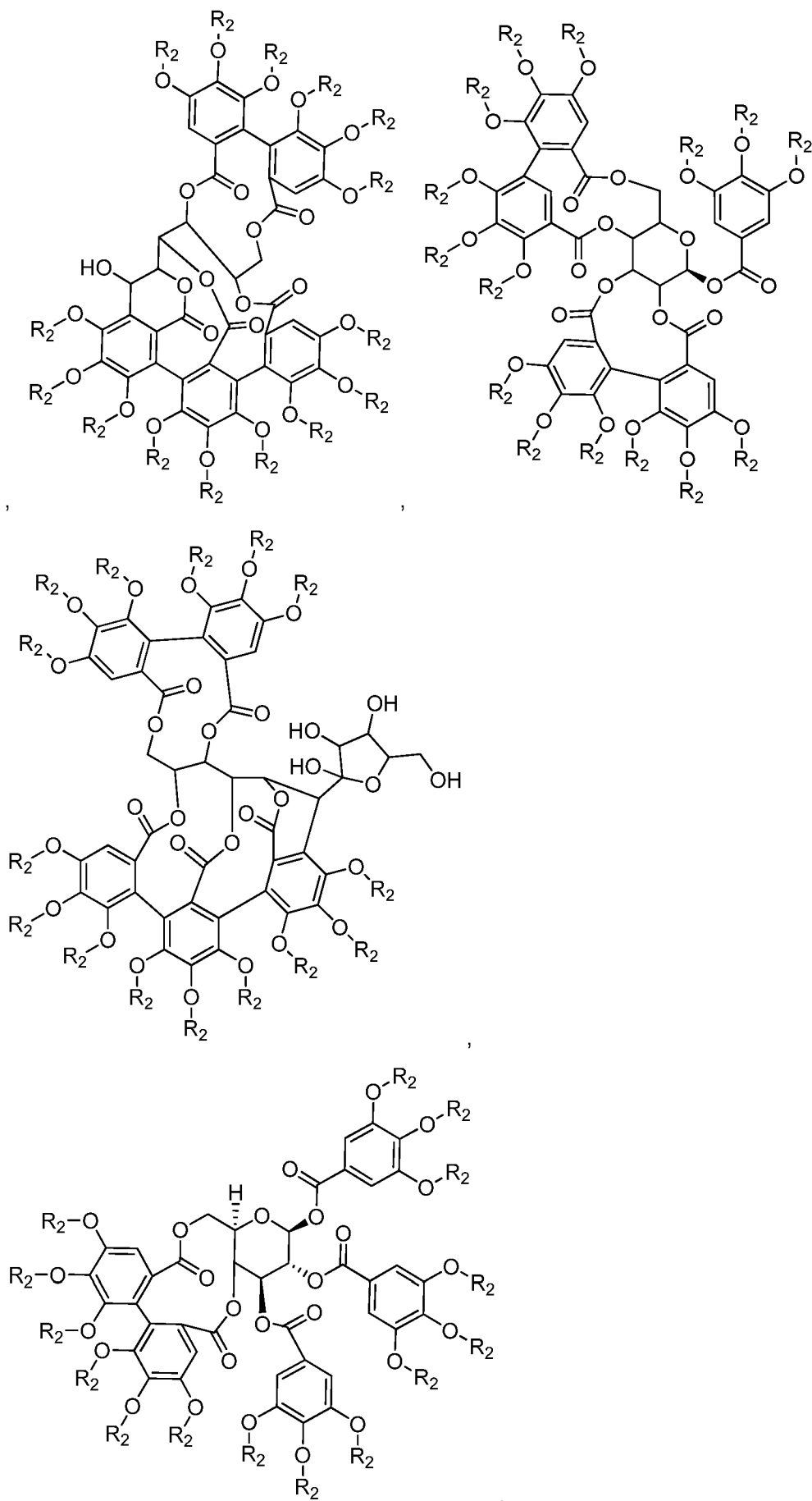
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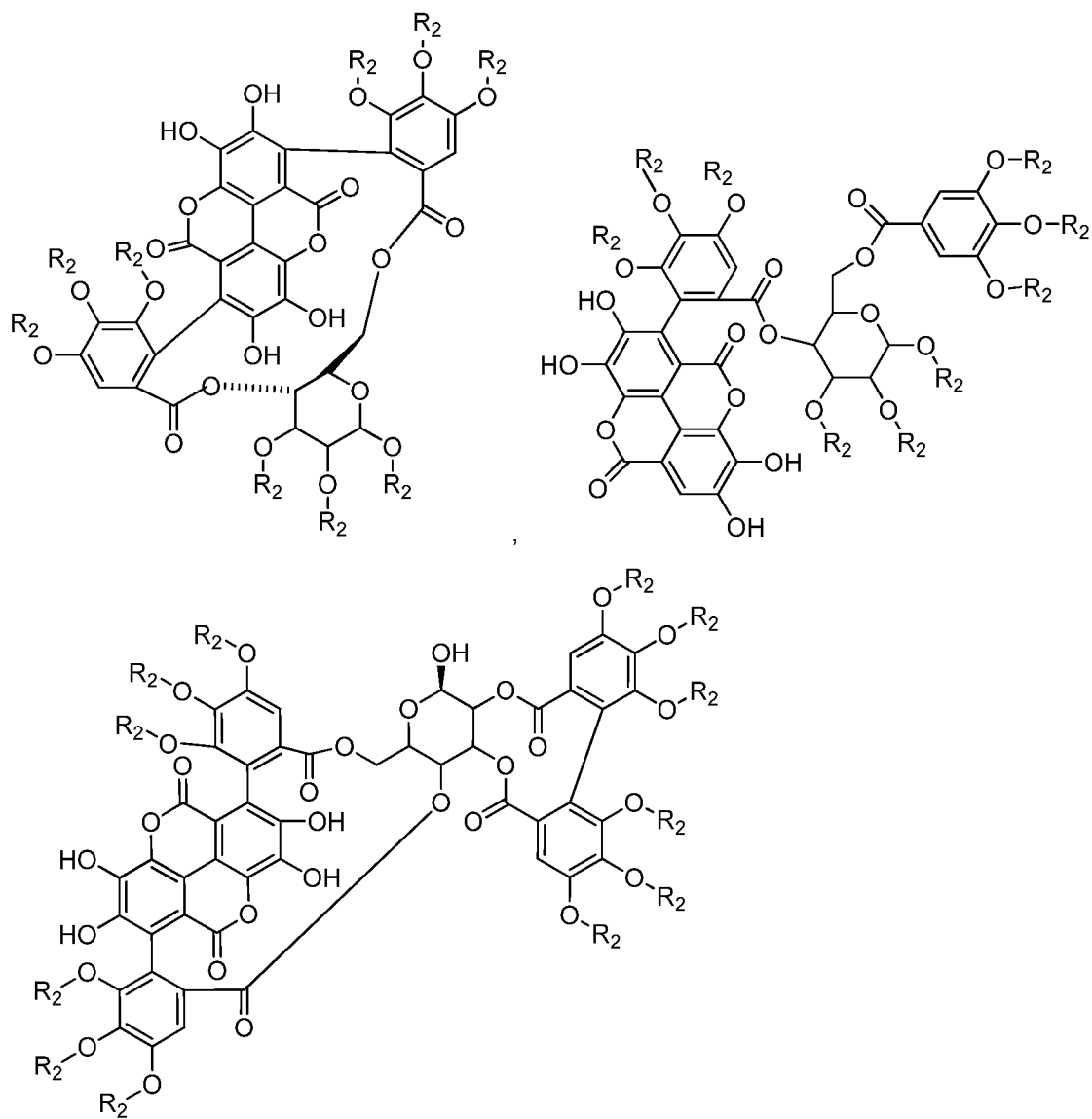


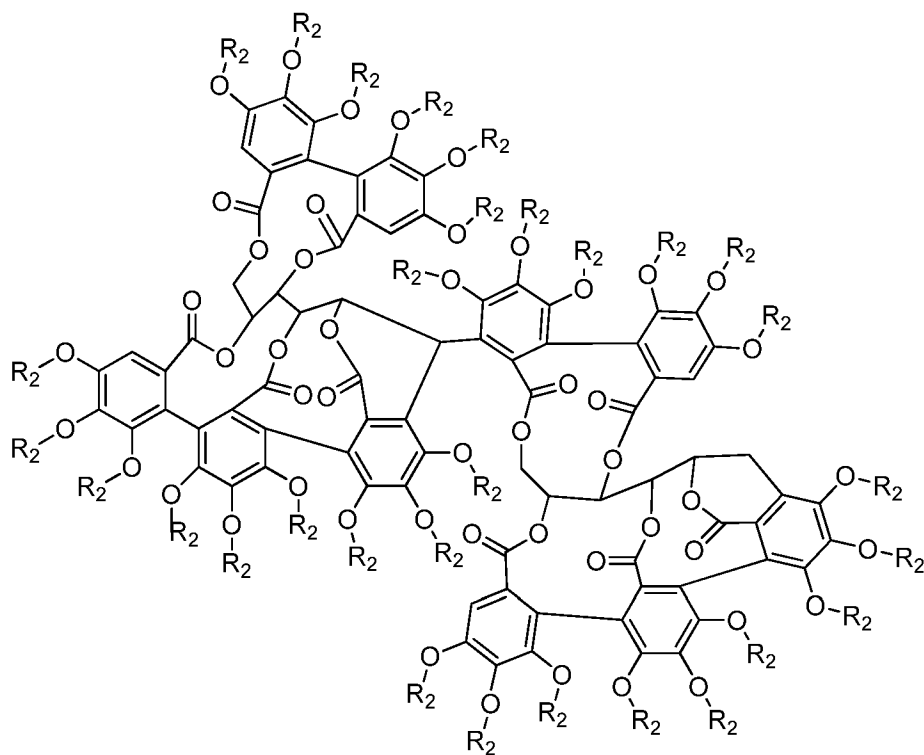
combinations thereof, wherein each R_1 is OH or when taken together with the R_1 on the same boron atom represents the point of attachment of the boron atom to the linking moiety.

23. A capsule according to claim 20 or 22 wherein the linking moieties are selected from the group consisting of:





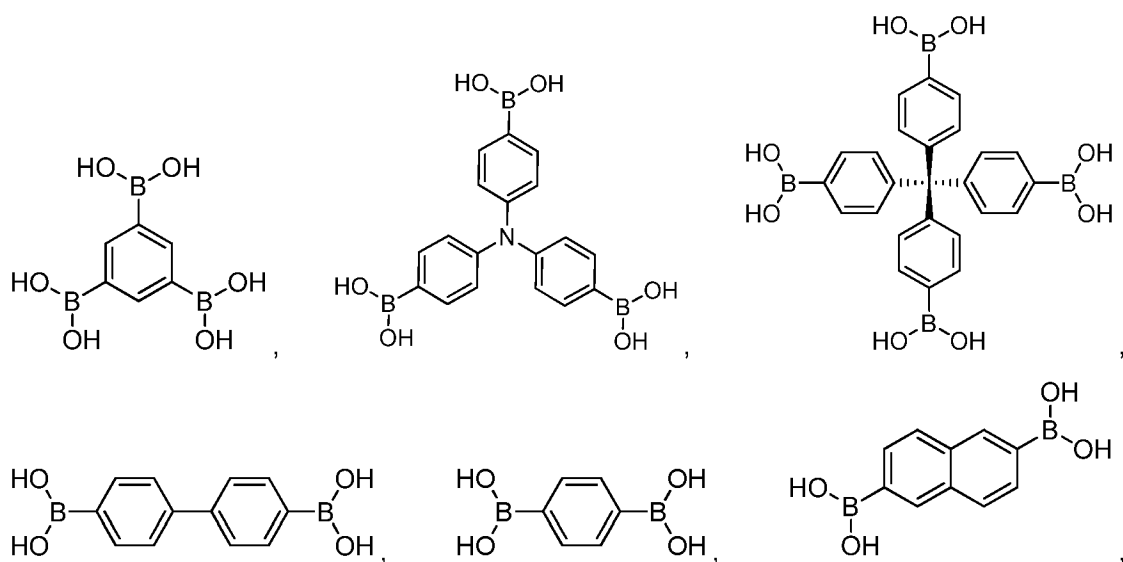


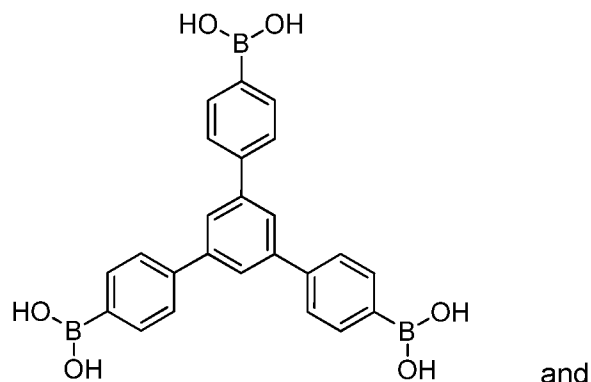
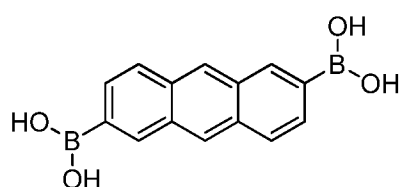
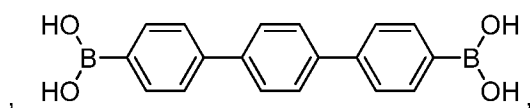
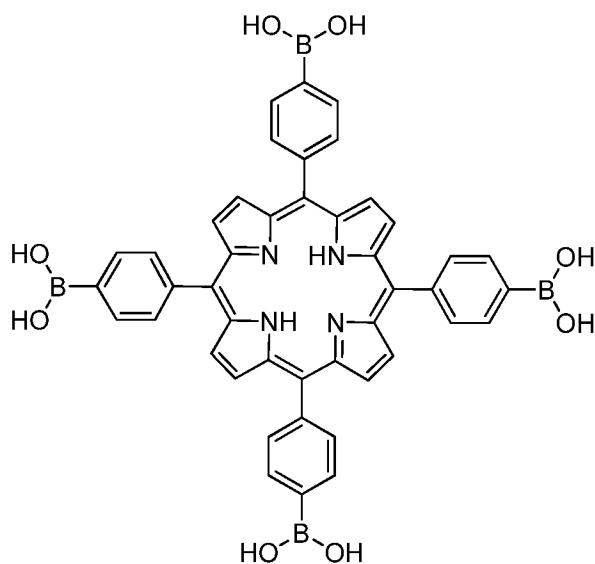


and

combinations thereof, wherein each R_2 is H or two R_2 on adjacent carbon atoms form a boronate linkage to a boron atom of the boron containing moiety.

24. A capsule according to claim 21 wherein the compound having a plurality of boronic acid moieties is selected from the group consisting of:





combinations thereof.

25. A capsule according to claim 21 or 24 wherein the compound having a plurality of galloyl moieties is selected from the group consisting of ellagitannins, tannic acid, 1,2,3,4,6-pentagalloyl glucose and combinations thereof.
26. A capsule according to any one of claims 21, 24 and 25 wherein the compound having a plurality of galloyl moieties is selected from the group consisting of tannic acid, tellimagrandin II, castalagin, castalin, casuarictin, grandinin, punicalagin, punicalin, roburin A, terflavin B, and combinations thereof.
27. A capsule according to any one of claims 20 to 26 wherein the capsule is stable under alkaline conditions.
28. A capsule according to claim 27 wherein the capsule is stable in a pH range of from 7.5 to 9.5.
29. A capsule according to any one of claims 20 to 28 wherein the capsule disassembles under acidic conditions.

30. A capsule according to claim 29 wherein the capsule disassembles in a pH range of from 2 to 6.5.
31. A capsule according to any one of claims 20 to 30 wherein the capsule disassembles in the presence of a compound having a vicinal diol moiety.
32. A capsule according to claim 31 wherein the compound is selected from the group consisting of glucose, mannitol or combination thereof.
33. A capsule according to any one of claims 20 to 32 wherein the capsule is loaded with an active agent.

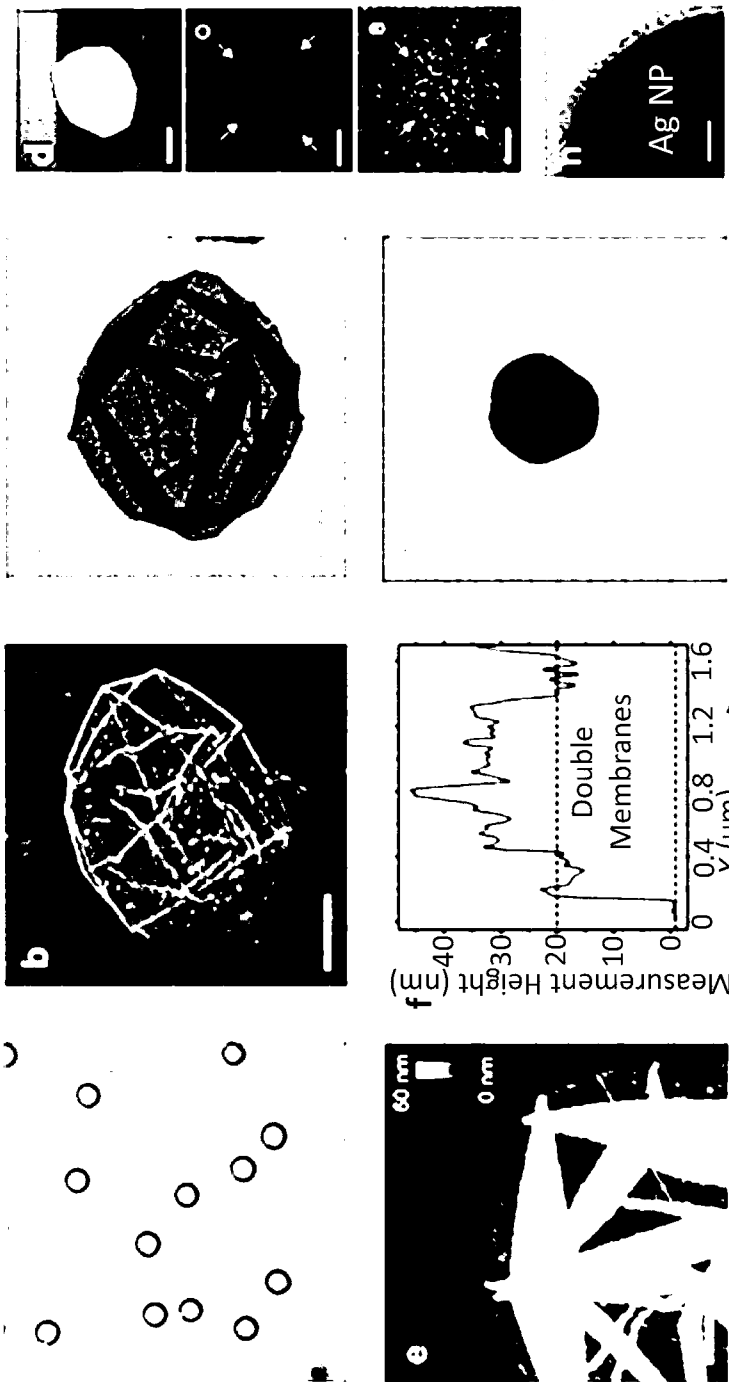
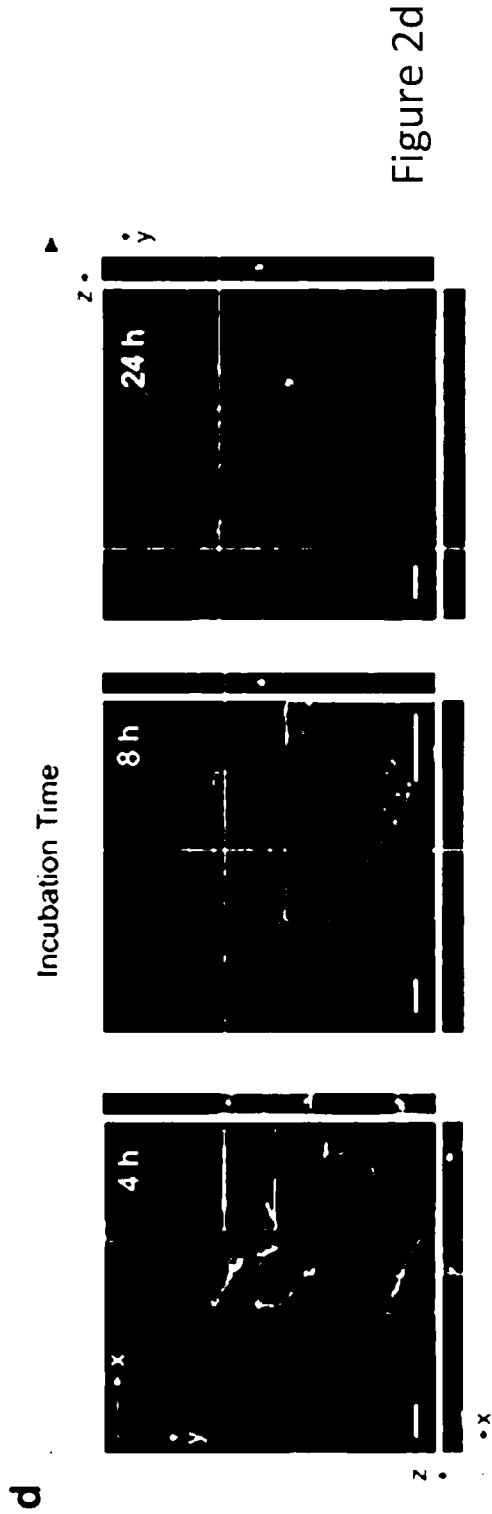
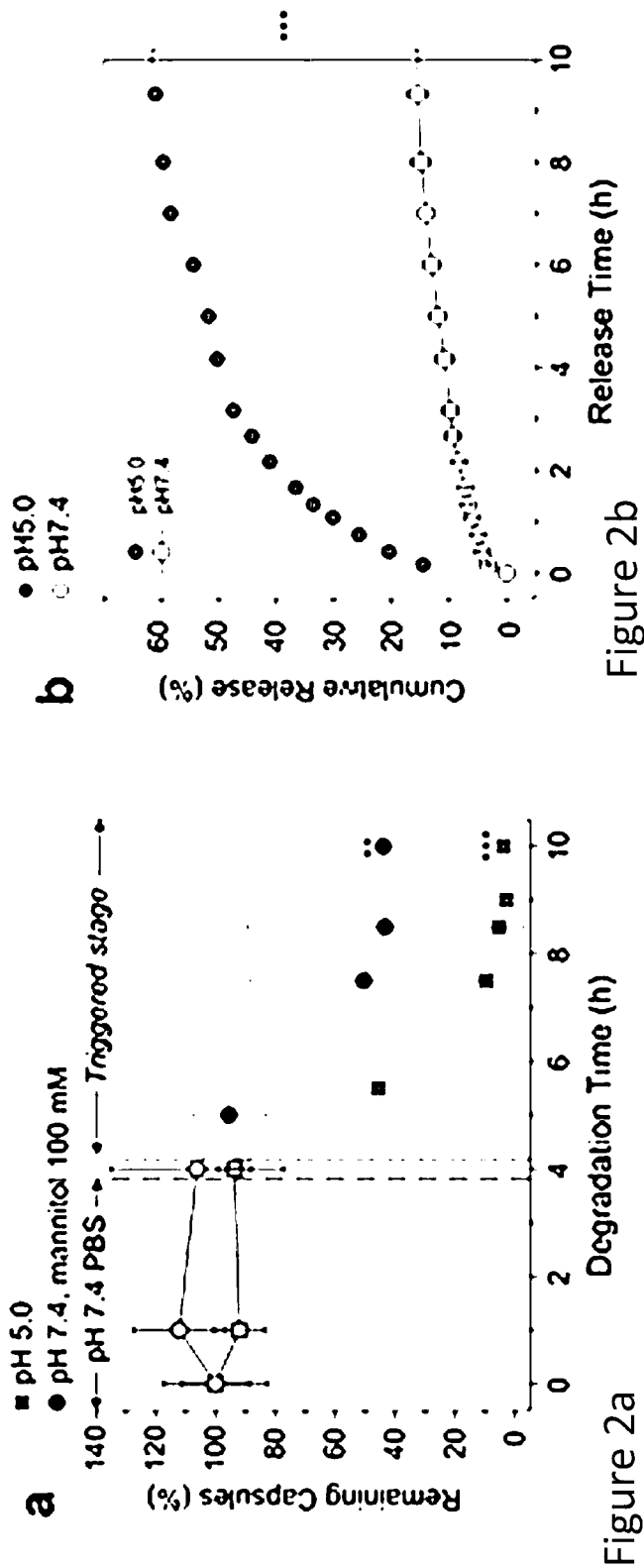


Figure 1



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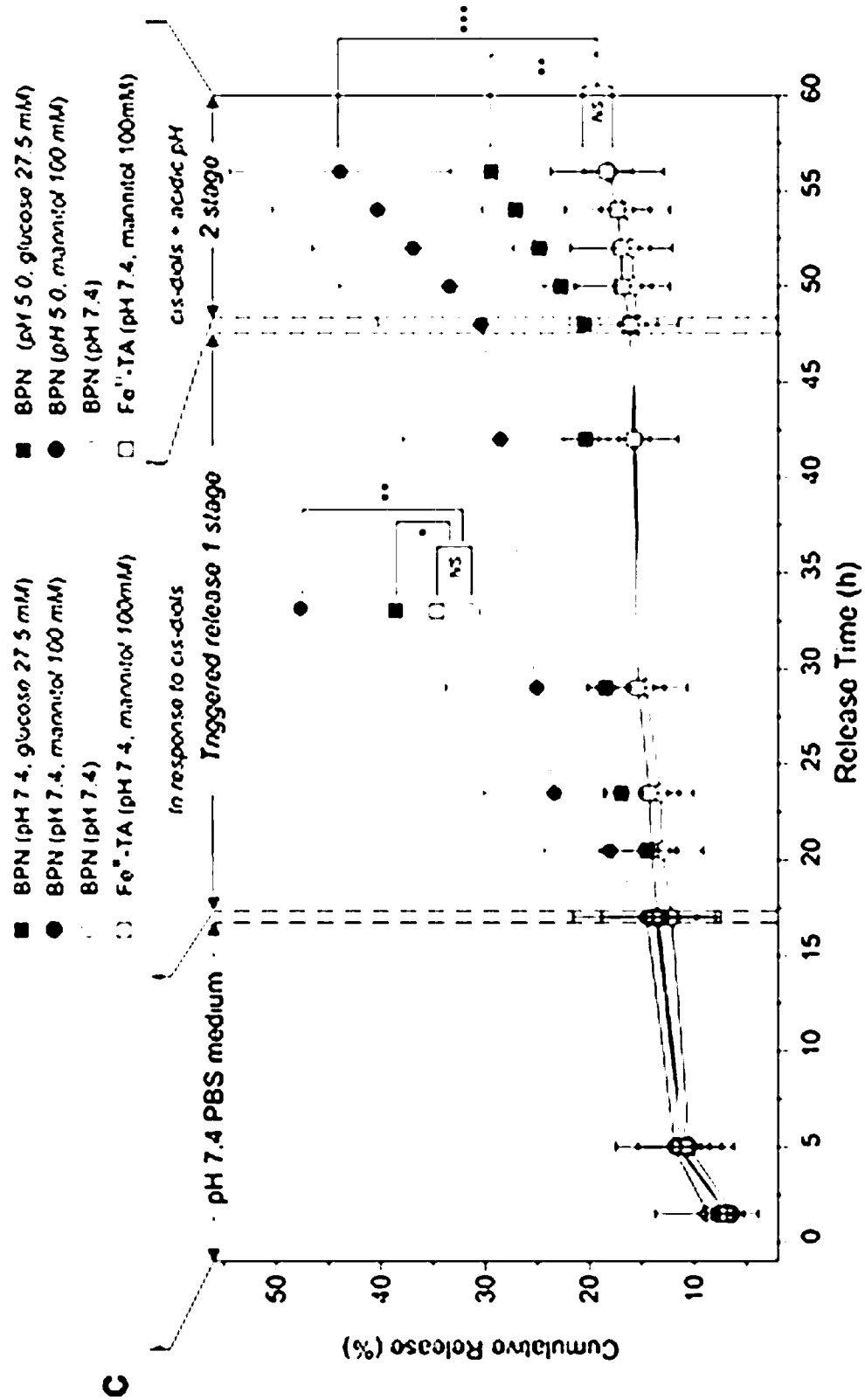


Figure 2c

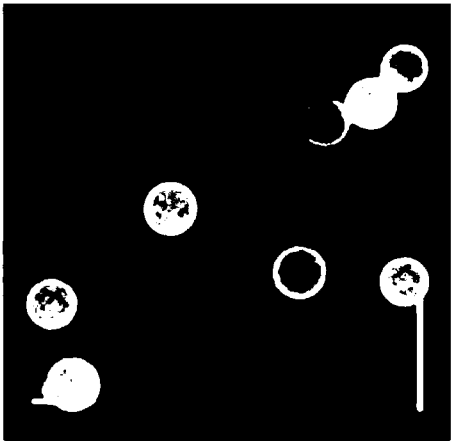


Figure 3

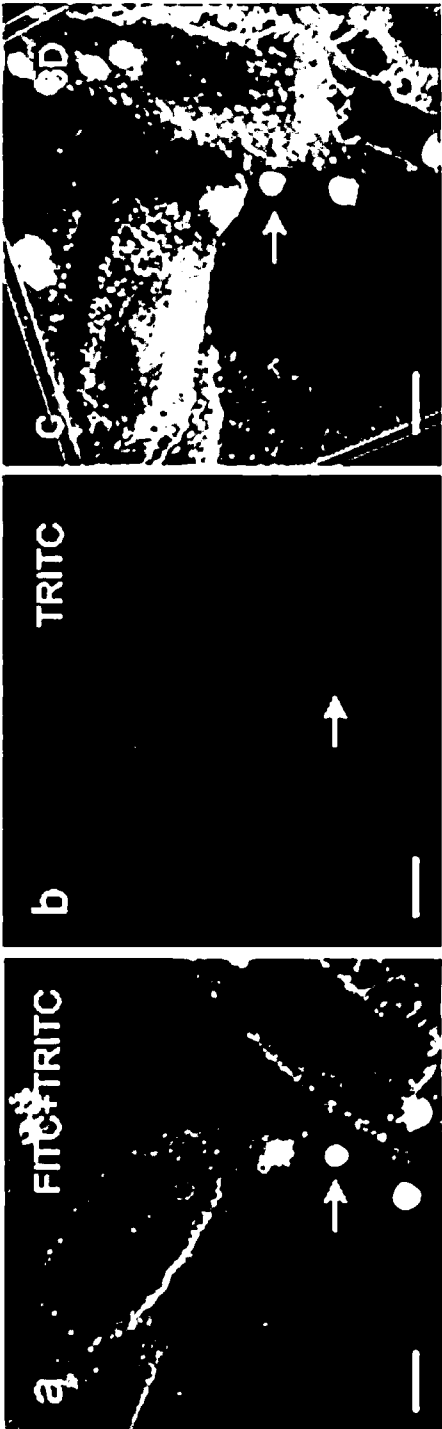


Figure 4

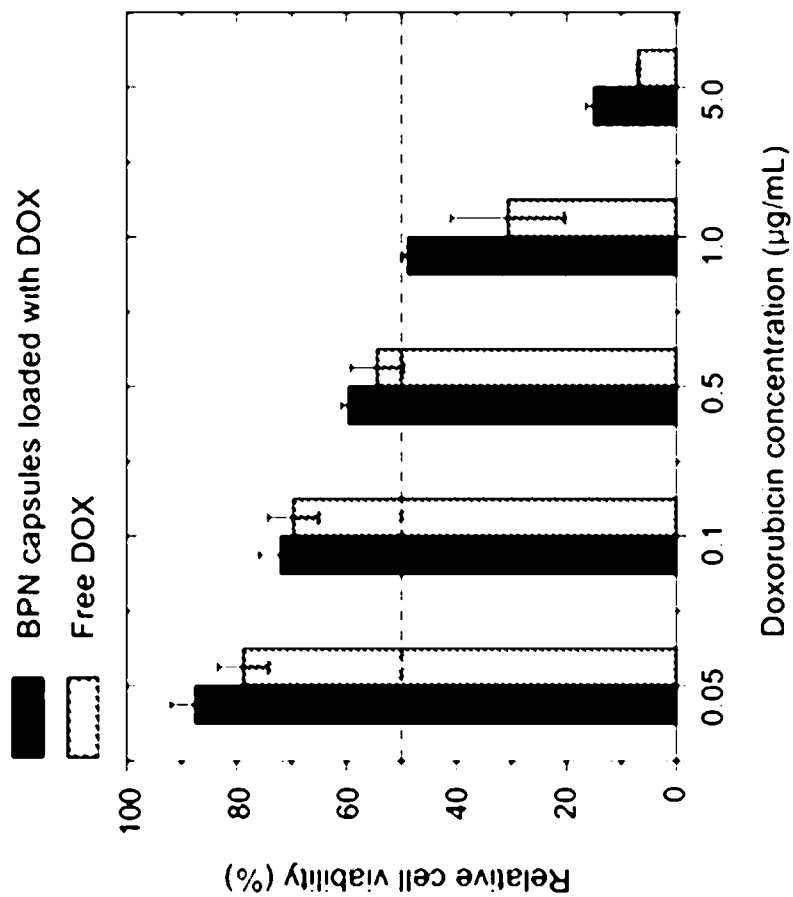


Figure 5

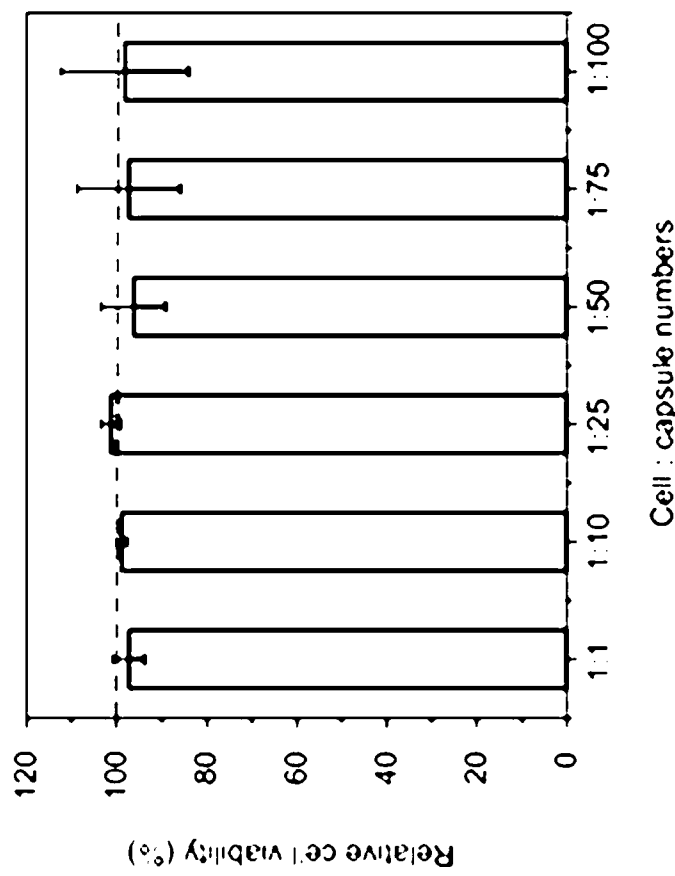


Figure 6

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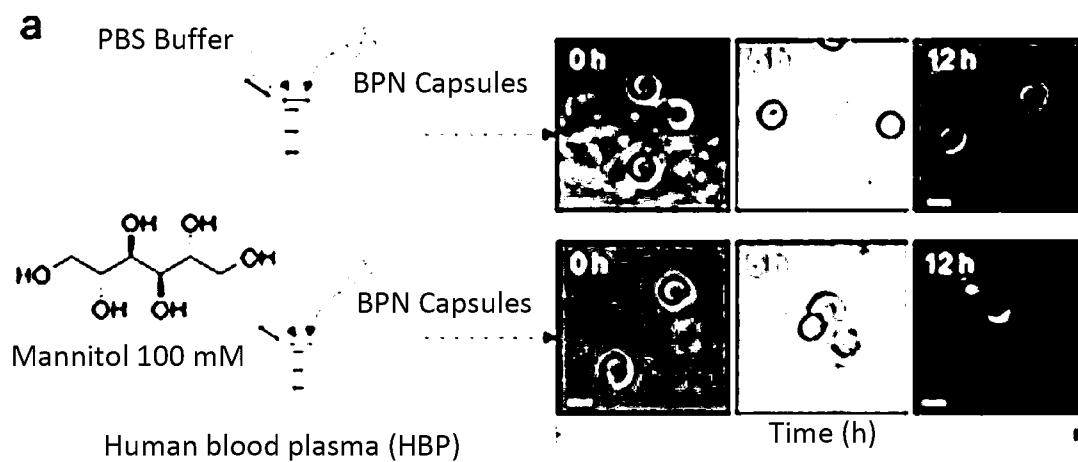


Figure 7a

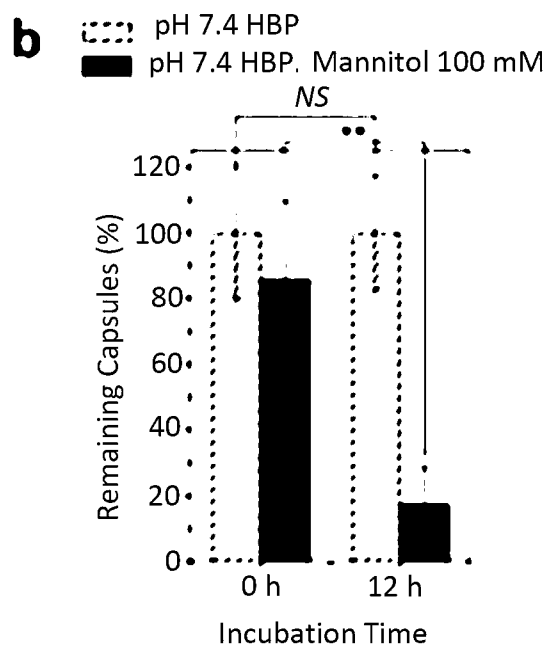


Figure 7b

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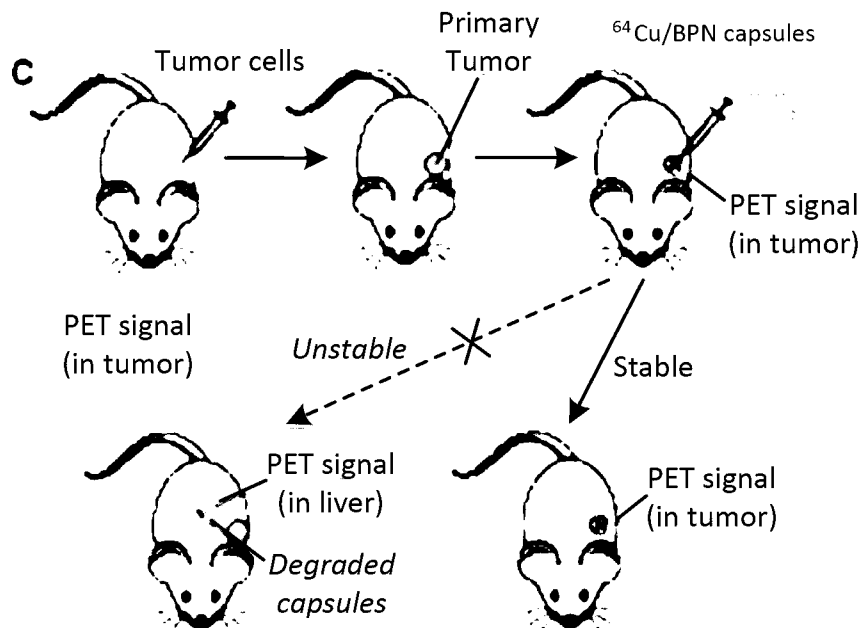


Figure 7c

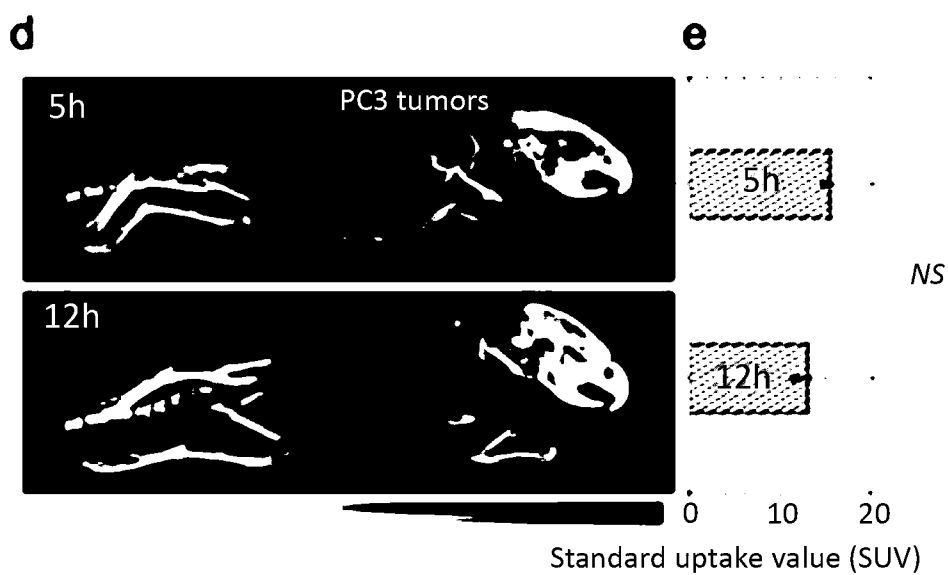


Figure 7d/7e

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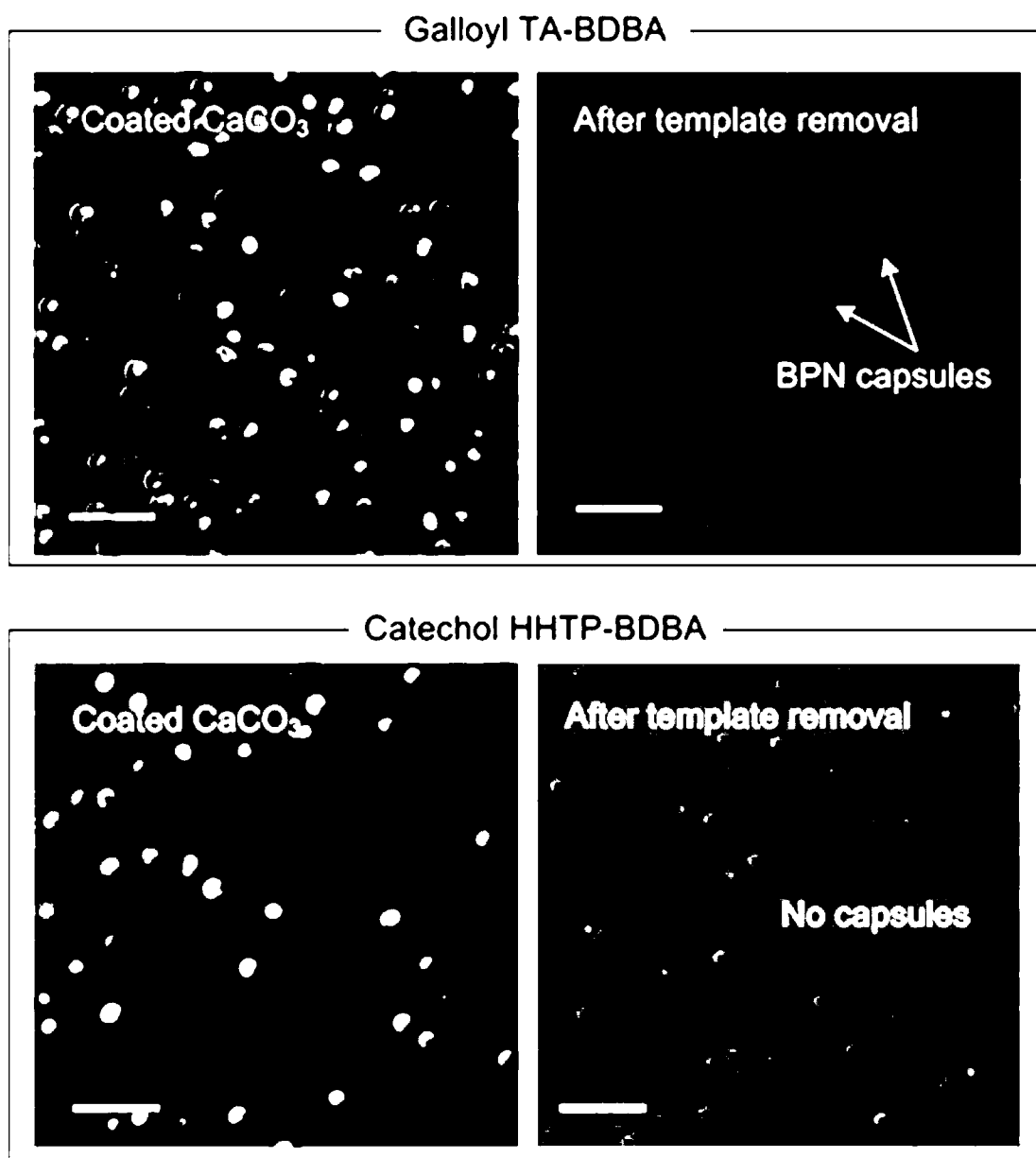


Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2016/050441

A. CLASSIFICATION OF SUBJECT MATTER

A61K 9/52 (2006.01) A61K 9/51 (2006.01) A61K 31/69 (2006.01) A61K 47/12 (2006.01) A61P 3/10 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PATENTSCOPE, ESPACE, AUSPAT: University of Melbourne, Caruso, Guo or Ejima

MEDLINE, EPODOC and WPIAP (Epoque) and Medline, CA Plus and Embase (STN): boronate, boronic, galloyl, ellagitannins, tannic acid, tellimagrandin, castalagin, castalin, casuarictin, grandinin, punicalagin, punicalin, roburin, terflavin, capsule and similar terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"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)	"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
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 27 June 2016		Date of mailing of the international search report 27 June 2016	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au		Authorised officer Grant McNeice AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262832617	

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2016/050441
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHANG, J. et al., "A colorimetric method for alpha-glucosidase activity assay and its inhibitor screening based on aggregation of gold nanoparticles induced by specific recognition between phenylenediboronic acid and 4-aminophenyl-alpha-D-glucopyranoside" Nano Research, March 2015, vol. 8, issue 3, pages 920-930 Page 921, col. 1, last para.; page 922; page 923, col. 1, para. 1 and Scheme 1; and page 925	20, 22 and 27-33
X	WO 2009/029317 A2 (GEORGIA STATE UNIVERSITY RESEARCH FOUNDATION, INC.) 05 March 2009 Page 13, lines 8-16; Page 18, lines 27-31; page 19, lines 17-18; page 25, Examples 3 and 5; page 26, lines 1-5; Figure 1; and claims 1, 2 and 4	20, 22 and 27-33

Form PCT/ISA/210 (fifth sheet) (July 2009)

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/AU2016/050441	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2009/029317 A2	05 March 2009	WO 2009029317 A2	05 Mar 2009
		EP 2155705 A2	24 Feb 2010
		EP 2529793 A2	05 Dec 2012
		US 2010137249 A1	03 Jun 2010
		US 8653258 B2	18 Feb 2014
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			