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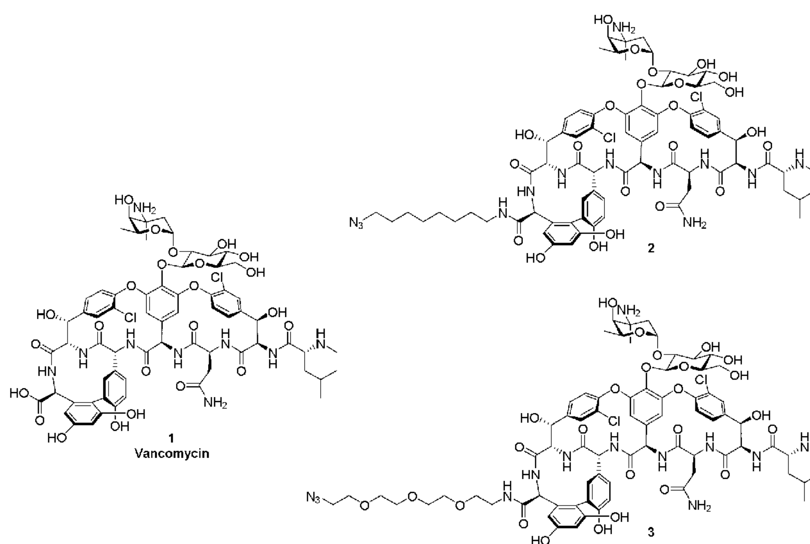

FIG. 1

(57) Abstract: A visualization construct comprising: (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii) is provided. The visualization component may be a fluorescent component, a quantum dot, an MRI visualization component, a PET or SPECT visualization component, an MPI visualization component, or a radiographic visualization component. The glycopeptide antibiotic may be vancomycin, teicoplanin, oritavancin; telavancin, chloroeremomycin, or balhimycin. The first linker may comprises a polyethylene glycol (PEG) moiety, or a linear carbon chain of greater than four carbons. Also provided are associated methods of producing and using related compounds, adducts, and constructs, such as methods of visualizing microorganisms.

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TITLE

VISUALIZATION CONSTRUCTS

TECHNICAL FIELD

THE present invention relates to constructs comprising a glycopeptide antibiotic and a visualization component. In some forms, the invention relates to visualizing of microorganisms or components thereof bound to such constructs. The invention also relates to use of such constructs for the diagnosis or monitoring of diseases related to bacterial infections.

BACKGROUND

Microorganisms such as bacteria cause a vast range of diseases or conditions of living organisms, including plants and animals such as humans. Since the middle of the last century, antibiotics have been effectively used for control of bacterial diseases. However, bacteria have substantial capacity to develop resistance to antibiotics, and disease caused by antibiotic resistant bacteria is currently considered a major global crisis.

Bacteraemia and sepsis (or septicaemia) are related conditions in which microorganisms (typically bacteria) are present in blood. In bacteraemia, a microorganism enters the blood, e.g. through a wound, infection, or surgical procedure. In sepsis, a microorganism enters and replicates in the blood. Sepsis is typically considered a very serious condition, and is frequently life-threatening. Sepsis is commonly caused by the Gram positive cocci bacteria *Staphylococcus* species, particularly *S. aureus*. However, other Gram positive bacteria including *Streptococcus*, and *Enterococcus* species can cause sepsis, as can certain Gram negative bacteria (e.g. *Escherichia*, *Pseudomonas*, and *Klebsiella* species) and fungi, e.g. *Candida* species. For treatment of sepsis, diagnosis of the site of infection leading to the sepsis can be important.

Vancomycin, a glycopeptide antibiotic, is commonly used for treatment of infections by multidrug-resistant Gram-positive bacteria. Vancomycin is generally considered as the first-line treatment for serious MRSA infections. However, resistance to glycopeptide antibiotics is being observed, for example in Enterococci species. Reduced susceptibility of MRSA to glycopeptide antibiotics, known as glycopeptide-intermediate *S. aureus* (GISA) has also been observed and cases of vancomycin-resistant *S. aureus* (VRSA) have begun to be reported.

Technologies used to visualize cellular structure and dynamics in living cells enable scientists to understand interaction and function of biomolecules within the complex system. In particular, understanding the cellular complexity of bacteria is important in developing new strategies to combat antibiotic-resistant bacteria. Additionally, such technologies may assist in identifying the site of infection in certain conditions such as sepsis.

In consideration of the above, new approaches for studying bacteria to understand their cellular complexity and interaction with antibiotics, which may assist in the development of new antibiotics, are highly desirable. Furthermore, new approaches for visualising the presence and location of an infection, which may help in determining treatment options, would be of great benefit.

SUMMARY

In one broad form, the present invention is directed to constructs comprising an optionally derivatized glycopeptide antibiotic connected to a visualization component.

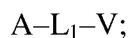
In a first aspect of this first broad form, there is provided a construct comprising:

(i) an optionally derivatized glycopeptide antibiotic;

(ii) a visualization component; and

(iii) a first linker connecting (i) and (ii).

Constructs of this aspect may have the following general structure:



$$A–L_1–V;$$

wherein:

A is the optionally derivatized glycopeptide antibiotic;

L_1 is the first linker; and

V is the visualization component.

In some preferred embodiments of the first aspect, the construct comprises:

(i) an optionally derivatized glycopeptide antibiotic;

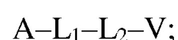
(ii) a visualization component;

(iii) a first linker connected to (i); and

(iv) a second linker connected to (ii),

wherein (iii) is connected to (iv) and (i) is connected to (ii).

Constructs of this embodiment may have the following general structure:



$$A–L_1–L_2–V;$$

wherein:

A is the optionally derivatized glycopeptide antibiotic;

L_1 is the first linker;

L_2 is the second linker; and

5 V is the visualization component.

Suitably, the construct of this aspect is for binding to a microorganism, wherein the optionally derivatized glycopeptide antibiotic binds to the microorganism.

Preferably, the microorganism is a Gram positive bacteria.

Suitably, the visualization component facilitates visualization of a 10 microorganism bound to the construct.

In a preferred embodiment, the visualization component is a light-emitting component.

In some embodiments, the light emitting component emits light in the visual spectrum, i.e. in a wavelength range of about 400 nm to about 700 nm. In some 15 embodiments, the light emitting component emits light in the infrared spectrum, i.e. in a wavelength range of about 700 nm to about 1mm.

Preferably, the light emitting component is a luminescent component. In some preferred embodiments, the light emitting component comprises a fluorescent compound. In preferred embodiments, the fluorescent compound is an organic or 20 carbon-containing compound. In certain particularly preferred embodiments, the organic fluorescent compound comprises one or more heterocyclic groups. In some embodiments, the heterocyclic groups are N or O-containing heterocyclic groups.

In some embodiments, the fluorescent component is or comprises a quantum dot semiconductor particle.

25 In some preferred embodiments, the fluorescent compound has a neutral charge.

In some preferred embodiments the fluorescent compound has a molecular weight between about 100 and about 500 Da. Preferably, the fluorescent compound has a molecular weight between about 150 and about 250 Da.

30 In one particularly preferred embodiment, the fluorescent compound is optionally derivatized 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD). In one particularly preferred embodiment, the fluorescent compound is optionally derivatized 7-(dimethylamino)-coumarin-4-acetic acid (DMACA). In one particularly preferred embodiment, the fluorescent compound is optionally derivatized 5-(dimethylamino)-1

naphthalene-1-sulfonyl (dansyl). In one particularly preferred embodiment, the fluorescent compound is optionally derivatized 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY). In one particularly preferred embodiment, the fluorescent compound is optionally derivatized (*E*)-2-(2-(6-hydroxy-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-3,3-dimethyl-1-propyl-3*H*-indol-1-ium iodide (HXPI). In one particularly preferred embodiment, the fluorescent compound is optionally derivatized (*E*)-3,3-dimethyl-2-(2-(6-(prop-2-yn-1-yloxy)-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-1-propyl-3*H*-indol-1-ium iodide (PXPI).

In an embodiment, the visualization component is a Magnetic Resonance Imaging (MRI) visualization component. In some embodiments, the MRI component comprises ^{19}F (e.g. as part of an organic compound, although without limitation thereto). In some embodiments, the MRI component comprises gadolinium, preferably Gd(III). Preferably the Gd(III) is of a macrocycle complex.

In an embodiment, the visualization component is a Positron Emission Tomography (PET) visualization component. In some embodiments, the PET visualization component comprises ^{64}Cu. In some embodiments, the PET visualization component comprises ^{68}Ga. Preferably, in embodiments comprising ^{64}Cu or ^{68}Ga, the ^{64}Cu or ^{68}Ga is contained within a macrocycle complex. In some embodiments, the PET visualization component contains ^{18}F. In some embodiments, the PET visualization component contains ^{11}C. In some embodiments, the PET visualization component contains ^{124}I. In some embodiment, the PET visualization component contains ^{99m}Tc. The ^{18}F; ^{11}C; ^{124}I; or ^{99m}Tc as per these embodiments may be present as part of an organic compound, although without limitation thereto.

Suitably, the PET visualization component may comprise a chelating compound. In one preferred embodiment, the chelating compound is optionally derivatized 1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA), such as 2,2',2''-(10-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA-alkyne). In one preferred embodiment, the chelating compound is optionally derivatized 2-[4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl]acetic acid (NOTA), such as 2-[4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl]acetic acid propargylamide (NOTA-alkyne) or optionally derivatized NOTA analogs such as 2-[4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl]butanoic acid or 2-[4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl](4-carboxy-4-butanoic acid), and their corresponding propargylamide derivatives. In one preferred embodiment, the

chelating compound is optionally derivatized 2,2′-(7-(4-(2-((2-aminoethyl)amino)-2-oxoethyl)benzyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (NODA) such as 2,2'-(7-(4-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)benzyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (NODA-alkyne) and related analogs such as 2,2'-(7-(4-(2-oxo-2-(prop-2-yn-1-
5 ylamino)ethyl)benzyl)-1,4,7-triazonane-1,4-diyl)diacetic acid.

In an embodiment, the visualization component is a radiographic visualization component. In some embodiments, the radiographic visualization component contains ^{123}I. In some embodiments, the radiographic visualization component contains ^{99m}Tc.

In an embodiment, the visualization component is a Magnetic Particle Imaging
10 (MPI) visualization component. In some embodiments, the MPI component comprises a magnetic nanoparticle. In a preferred embodiment the magnetic nanoparticle has been optimised for MPI.

In certain preferred embodiments, the glycopeptide antibiotic of the construct is selected from the group consisting: of vancomycin; teicoplanin; oritavancin;
15 telavancin; chloroeremomycin; and balhimycin. In one particularly preferred embodiment, the glycopeptide antibiotic is vancomycin.

In some embodiments, the first linker of the construct is at least partially hydrophilic. In preferred such embodiments, the first linker comprises a polyethylene glycol (PEG) moiety. Preferably, the PEG moiety is at least PEG3. In some
20 particularly preferred embodiments, the PEG moiety is PEG3 or PEG4.

In some embodiments, the first linker of the construct is hydrophobic.

In some embodiments wherein the first linker is hydrophobic, the first linker comprises a linear carbon chain of greater than four carbons. Preferably the linear carbon chain is C4-C12. In one particularly preferred embodiment, the linear carbon
25 chain is C8. In another particularly preferred embodiment, the linear carbon chain is C11.

In preferred embodiments, the first linker comprises one or more nitrogen-containing moieties. Preferably, the one or more nitrogen-containing moieties include an amine-derived moiety and/or an azide-derived moiety.

30 Preferably, the first linker comprises a nitrogen-containing moiety at a first end of the linker. Preferably, the moiety is an amine-derived moiety. Preferably the moiety connects the linker to the glycopeptide antibiotic. The nitrogen-containing moiety may be an amide bond formed between an amine group from a first end of a

precursor to the first linker and the C-terminal carboxy moiety from the glycopeptide antibiotic.

Preferably, the first linker comprises a nitrogen-containing moiety at a second end of the linker. Preferably, the first linker is connected to the visualization
5 component via the nitrogen-containing moiety. In preferred embodiments, this moiety is an azide-derived moiety. Preferably, this moiety is a triazole moiety.

In some embodiments, as hereinabove described, the first linker may be connected to the visualization component via a second linker. In preferred such embodiments, the second linker comprises a linear carbon chain. Preferably, the linear
10 carbon chain is a C1 to C4 linear carbon chain. Preferably, the second linker is connected to the first linker via an azide-derived moiety at the second end of the first linker. Preferably, the azide-derived moiety is a triazole. Suitably, the triazole moiety may be formed between an azide group from a second end of a precursor to the first linker and an alkyne group of a precursor to the second linker.

15 The visualization component is preferably connected to the first and/or second linker via a moiety selected from the group consisting of an amide; amine; sulphide; urethane; urea; sulphonamide; ether; or thioester moiety. Preferably, the moiety is of the second linker. In one particularly preferred embodiment, the moiety is an amide moiety. In another particularly preferred embodiment, the moiety is an amine moiety.

20 In a second aspect there is provided a method of producing a construct, the method including the steps of obtaining (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker, and connecting (i) and (ii) using (iii).

In a preferred embodiment of the second aspect, the method includes the step
25 of:

(a) obtaining (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; (iii) a first linker; and (iv) a second linker;

(b) connecting (i) to (iii);

(c) connecting (ii) to (iv); and

30 (d) connecting (iii) to (iv).

Preferably the construct produced according to the method of this aspect is the construct of the first aspect.

In a third aspect there is provided a method of binding a construct to a microorganism or component thereof, the construct comprising (i) an optionally

derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii), the method including the steps of:

(a) combining the construct and a microorganism or component thereof; and

(b) selectively binding the glycopeptide antibiotic of the construct with the 5 microorganism or component thereof,

to thereby bind the construct to the microorganism or component thereof.

Preferably, the construct is the construct of the first or second aspect.

In some embodiments the microorganism of step (a) is a Gram positive bacteria. In some preferred embodiments the microorganism is a pathogenic 10 microorganism.

In a fourth aspect there is provided a method of visualizing a microorganism or component thereof, the method including the steps of:

(a) combining a construct with the microorganism or component thereof, the construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a 15 visualization component; and (iii) a first linker connecting (i) and (ii);

(b) selectively binding the microorganism or component thereof to the glycopeptide antibiotic of the construct; and

(c) visualizing the construct bound to the microorganism or component thereof using the visualization component,

20 to thereby visualize the microorganism or component thereof using the construct.

Preferably, the construct of step (a) is the construct of the first or second aspects. Preferably the microorganism of step (a) is as described for the third aspect.

Visualization according to the method of the fourth aspect may be by any 25 suitable approach. In embodiments wherein the visualization component of the construct is a fluorescent probe, preferably the visualization is by fluorescence detection. In embodiments wherein the visualization component of the construct is an MRI visualization component, preferably the visualization is by MRI. In embodiments wherein the visualization component of the construct is a PET 30 visualization component, preferably the visualization is by PET. In embodiments wherein the visualization component is an MPI visualization component, preferably visualization is by MPI. In embodiments, wherein the visualization component is a radiographic visualization component, preferably the visualization is by a radiological approach.

Visualization according to the method of the fourth aspect may be *in vitro* visualization, or *in vivo* visualization.

In embodiments wherein the visualization is *in vitro* visualization, suitably, step (a) will include adding the construct to a sample containing the microorganism. In these embodiments, the sample of step (a) may be a sample obtained from a biological subject. Preferably, the subject is a human or an animal. The sample may be urine, sputum, blood or a blood product including platelets, plasma, and/or serum.

In preferred embodiments the sample of step (a) is a blood sample. Preferably, the blood sample is human blood. In some embodiments, the blood sample may comprise aggregated red and/or white blood cells.

In embodiments wherein the visualization is *in vivo* visualization, suitably, step (a) will include administering the construct to a biological subject. Preferably, the subject is a human or an animal.

In a fifth aspect, there is provided a method of analysing a microorganism or component thereof, the method including the steps of:

(a) combining a construct with a microorganism or component thereof, the construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii);

(b) selectively binding the microorganism or component thereof to the glycopeptide antibiotic of the construct;

(c) visualizing the construct bound to the microorganism or component thereof using the visualization component; and

(d) analysing the microorganism or component thereof based on the visualization of step (c).

In preferred embodiments, steps (a)-(c) are as set forth for the fourth aspect.

Analysis of step (d) may be *in vitro* or *in vivo* analysis.

In embodiments wherein the analysis is *in vitro* analysis, the visualization of step (c) is will be *in vitro* visualization.

In embodiments wherein the analysis is *in vivo* analysis, the visualization of step (c) will be *in vivo* visualization. The *in vivo* analysis will be performed in a subject to which the construct has been administered for the visualization of step (c).

In a sixth aspect there is provided a method of diagnosing and/or monitoring a disease, disorder or condition, the method including the steps of analysing a microorganism or component thereof according to the method of the fifth aspect, and

diagnosing a disease, disorder or condition based on the analysis of the microorganism.

Preferably the disease, disorder or condition is caused by a Gram positive bacteria. In one preferred embodiment the disease, disorder or condition is a Gram positive bacterial infection. In one particularly preferred embodiment the disease, disorder or condition is sepsis. In another particularly preferred embodiment the disease, disorder or condition is a urinary tract infection.

In a seventh aspect there is provided a method of treating a disease, disorder or condition, the method including the steps of diagnosing a disease, disorder or condition according to the method of the sixth aspect, and treating the disease or condition based on the diagnosis.

In an eighth aspect there is provided a method of inhibiting, controlling, or killing a microorganism, the method including the step of contacting a construct of the first aspect with a microorganism, to thereby inhibit, control, or kill the microorganism.

Preferably, the step of contacting the construct with the microorganism includes the step of selectively binding the glycopeptide antibiotic of the construct to the microorganism.

Preferably, the microorganism is a Gram positive bacteria.

In certain embodiments the Gram positive bacteria is an antibiotic resistant bacteria. In certain embodiments, the microorganism shows at least partial resistance to the glycopeptide antibiotic of the construct when present as a free antibiotic.

In a ninth aspect there is provided a composition for diagnosing, treating or preventing a disease, disorder, or condition in a subject, the composition comprising a construct of the first aspect, or a construct produced according to the method of the second aspect.

In a tenth aspect there is provided a method of diagnosing, treating or preventing a disease, disorder, or condition in a subject, the method including the step of administering to a subject an effective amount of the construct of the first aspect, a construct produced according to the method of the second aspect, or a composition of the ninth aspect, to thereby treat or prevent the disease, disorder, or condition in the subject.

In an eleventh aspect the invention provides for use of a construct of the first aspect, or a construct produced according to the method of the second aspect, in the

manufacture of a composition for the diagnosis, treatment or prevention of a disease, disorder, or condition in a subject.

Preferably, the disease, disorder, or condition according to the ninth to eleventh aspects is a disease caused by a Gram positive bacteria. In some particularly
5 preferred embodiments, the disease is bacterial sepsis. In other preferred embodiments the disease is a urinary tract infection.

In a twelfth aspect, the invention is directed to a method of increasing the activity or efficacy of a glycopeptide antibiotic, the method including the step of connecting a glycopeptide antibiotic to a visualization component by a first and/or
10 second linker. In preferred embodiments, the glycopeptide antibiotic, the visualization component, and the first and/or second linker are as set forth for the first aspect.

In a thirteenth aspect, there is provided a method of assessing a compound for activity in disrupting an outer cell membrane of a Gram negative microorganism, the method including the steps of:

15 (a) combining a microorganism with the compound to be assessed;

(b) combining a construct with the microorganism, the construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii); and

(c) determining if the construct is bound to the microorganism by visualization
20 of the construct, wherein

the degree of binding of the construct to the microorganism is related to the degree of activity of the compound in disrupting the cell membrane of the microorganism.

In a second broad form, the present invention relates to glycopeptide antibiotic
25 adducts.

A first aspect of the second broad form provides a glycopeptide antibiotic adduct comprising an optionally derivatized glycopeptide antibiotic bound to a first linker. Compounds of this broad form will be suitable for connection to a visualization component, to form constructs of the first aspect of the first broad form.

30 Preferably, the glycopeptide antibiotic of the second broad form is selected from the group consisting of vancomycin; teicoplanin; oritavancin; telavancin; chloroeremomycin; and balhimycin. Preferably the glycopeptide antibiotic is vancomycin.

Preferably, the first linker of the second broad form comprises a PEG group, preferably at least PEG3.

In some embodiments the first linker comprises a linear carbon chain of greater than four carbons. Preferably the linear carbon chain is C4-C12. In one
5 particularly preferred embodiment, the linear carbon chain is C8. In another particularly preferred embodiment, the linear carbon chain is C11.

In some embodiments, the first linker comprises one or more nitrogen-containing compounds. Preferably, the first linker comprises an amine-derived moiety. Preferably, the amine-derived moiety is at a first end of the first linker.
10 Preferably, the amine-derived moiety is an amide bond connecting the linker to the glycopeptide antibiotic.

In one preferred embodiment, the first linker comprises an azide moiety. Preferably, the azide moiety is at a second end of the first linker opposite the glycopeptide antibiotic.

15 In a second aspect of the second broad form there is provided a method of inhibiting, controlling, or killing a microorganism, the method including the step of contacting an adduct of the first aspect of the second broad form with a microorganism, to thereby inhibit, control, or kill the microorganism.

In a third aspect of the second broad form there is provided a composition for
20 treating or preventing a disease, disorder, or condition in a subject, the composition comprising a glycopeptide antibiotic adduct of the first aspect of the second broad form.

In a fourth aspect of the second broad form, there is provided a method of treating or preventing a disease, disorder, or condition in a subject, the method
25 including the step of administering to a subject an effective amount of the adduct of the first aspect of the second broad form, or a composition of the third aspect of the second broad form, to thereby treat or prevent the disease, disorder, or condition in the subject.

In a fifth aspect of the second broad form, the invention provides for use of an
30 adduct of the first aspect of the second broad form in the manufacture of a composition for the treatment or prevention of a disease, disorder, or condition in a subject.

In a sixth aspect of the second broad form, the invention is directed to a method of increasing the activity or efficacy of a glycopeptide antibiotic, the method

including the step of connecting a glycopeptide antibiotic to a first linker. Preferably, the first linker comprises a linear carbon chain. In one particularly preferred embodiment, the linear carbon chain is C8.

It will be appreciated that the indefinite articles “a” and “an” are not to be read
5 herein as singular indefinite articles or as otherwise excluding more than one or more than a single subject to which the indefinite article refers. For example, “an” antibiotic includes one antibiotic, one or more antibiotics, or a plurality of antibiotics.

As used herein, unless the context requires otherwise, the words “comprise”, “comprises” and “comprising” will be understood to mean the inclusion of a stated
10 integer or group of integers but not the exclusion of any other integer or group of integers.

BRIEF DESCRIPTION OF THE FIGURES

In order that the invention may be readily understood and put into practical effect, preferred embodiments will now be described by way of example with
15 reference to the accompanying figures.

Figure 1 sets forth the structure of vancomycin (1) and the glycopeptide antibiotic adducts N_3-C8-Van (2) and N_3-PEG3-Van (3).

Figure 2 sets forth (A) the synthesis of NBD-alkyne (10) and DMACA-alkyne (11); and (B) the structure of constructs NBD-Tz-C8-Van (12), DMACA-Tz-C8-Van
20 (13), and NBD-Tz-PEG3-Van (14), and a schematic representation of the synthesis of these constructs from N_3-C8-Van (2) or N_3-PEG3-Van (3), using NBD-alkyne (10) or DMACA-alkyne (11), respectively. For (A), reagents and conditions are (i) propargylamine, HATU, DIPEA, DMF, rt; and (ii) propargylamine, Cs2CO3, THF.

Figure 3 sets forth vancomycin labelled with keys for NMR elucidation, with
25 reference to the data provided in FIGS. 4 and 7.

Figure 4 sets forth NMR data of glycopeptide antibiotic adducts N_3-C8-Van (2) and N_3-PEG3-Van (3). Multiplicity abbreviations: br = broad; s = singlet; d = doublet; t = triplet; q = quartet; quin = quintet; m = multiplet; v br = very broad; o = obscured. Note; HMBC did not show correlation to all carbonyl carbon.

30 Figure 5 sets forth MS/MS data for N_3-C8-Van (2).

Figure 6 sets forth MS/MS data for N_3-PEG3-Van (3).

Figure 7 sets forth NMR data of constructs NBD-Tz-C8-Van (12), DMACA-Tz-C8-Van (13), and NBD-Tz-PEG3-Van (14). Multiplicity abbreviations: br = broad; s = singlet; d = doublet; t = triplet; q = quartet; quin = quintet; m = multiplet; v

br = very broad; o = obscured. Note; HMBC did not show correlation to all carbonyl carbon.

Figure 8 sets forth MS/MS data for NBD-Tz-C8-Van (12).

Figure 9 sets forth MS/MS data for DMACA-Tz-C8-Van (13).

5 Figure 10 sets forth MS/MS data for NBD-Tz-PEG3-Van (14).

Figure 11 sets forth antimicrobial activity (measured in MIC) of glycopeptide antibiotic adducts N_3-C8-Van, N_3-C3-Van, and N_3-PEG3-Van, and constructs NBD-Tz-C8-Van, DMACA-Tz-C8-Van, and NBD-Tz-PEG3-Van against Gram-positive bacteria. Note that there is some overlap in the data presented in the respective charts.

10 Figure 12 sets forth SR-SIM fluorescence imaging of *S. aureus* (ATCC 25923) stained with (A) Green; NBD-Tz-C8-Van (12), Red; FM4-64FX (bacterial membrane), Blue; Hoechst 33342 (nucleic acid) (B) Blue; DMACA-Tz-C8-Van (13), Red; FM4-64FX (bacterial membrane), Green; SYTO 21 (nucleic acid), (C) Green; NBD-Tz-PEG3-Van (14), Red; FM4-64FX (bacterial membrane), Blue; Hoechst 15 33342 (nucleic acid).

Figure 13 sets forth SR-SIM fluorescence imaging of *S. aureus* cell division (A) Green; NBD-Tz-C8-Van (12), Red; FM4-64FX (bacterial membrane), Blue; Hoechst 33342 (nucleic acid) (B) Blue; DMACA-Tz-C8-Van (13), Red; FM4-64FX (bacterial membrane), Green; SYTO 21 (nucleic acid) (C) Green; NBD-Tz-PEG3-20 Van (14), Red; FM4-64FX (bacterial membrane), Blue; Hoechst 33342 (nucleic acid).

Figure 14 sets forth a cross section of fluorescent imaging of *S. aureus* (ATCC 25923) (A) Blue; DMACA-Tz-C8-Van (13), Red; FM4-64FX (bacterial membrane), Green; SYTOX green (nucleic acid); (B) Combination of DMACA-Tz-C8-Van (13) (Blue) with TMP-NBD probe (Green) (C) Combination of DMACA-Tz-C8-Van (13) 25 with linezolid-NBD probe (Green). Co-stained with Red; FM4-64FX (bacterial membrane).

Figure 15 sets forth structures of HXPI (A), PXPI (B), and DOTA-alkyne (C).

Figure 16 sets forth structures of PXPI-Tz-C8-Van (A), PXPI-Tz-PEG3-Van (B).

30 Figure 17 sets forth structures of DOTA-Tz-C8-Van (A) and DOTA-Tz-PEG3-Van.

Figure 18 sets forth the degree of construct binding to *E. coli* exposed to various compounds.

Figure 19 sets forth structures of bisamineoxime, tetraamine, NODA, and NOTA derivatives.

DETAILED DESCRIPTION

This invention relates to the design and production of constructs comprising a glycopeptide antibiotic and a visualization component. Such constructs include constructs for microorganism visualization, and constructs with antimicrobial activity. The invention also relates to adducts comprising an optionally derivatized glycopeptide antibiotic bound to a linker, suitable for connection to a visualization component to form such constructs.

The invention is at least partly predicated on the recognition that such constructs may offer important advantages for visualization of microorganisms or components thereof in biological samples. Furthermore, the invention is at least partly predicated on the surprising discovery that certain constructs comprising a glycopeptide antibiotic and a visualization component, and/or certain glycopeptide antibiotic adducts, may have substantially increased activity or efficacy as compared to the corresponding free antibiotic itself.

The invention is also at least partly predicated on the discovery of design parameters with surprising benefits for binding of Gram positive bacteria using constructs as herein described. In particular, it has been surprisingly found that the use of certain linkers to connect glycopeptide antibiotics to visualization components has particular advantages for binding efficiency of such constructs against Gram positive bacteria. Such advantages for binding efficiency may lead to advantages for visualization of Gram positive bacteria using constructs of the invention, and/or for antimicrobial activity of constructs of the invention against Gram positive bacteria. Preferred constructs may have particular advantages with regards to selectivity for binding to bacteria in preference to mammalian cells.

Constructs

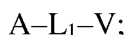
Constructs of the invention will comprise an optionally derivatized glycopeptide antibiotic connected to a visualization component. As used herein, unless the context requires otherwise, the terms "*connect*", "*connection*", "*connected*" etc., will be understood to encompass direct connection (e.g. direct binding or direct linkage), or indirect connection (e.g. connection via one or more other molecules).

Suitably, the construct of this aspect is for binding to a microorganism or component thereof, wherein the optionally derivatized glycopeptide antibiotic binds to the microorganism or component thereof.

One aspect of the invention relates to a construct comprising: (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii).

As used herein, a "*derivatized*" glycopeptide antibiotic broadly encompasses glycopeptide antibiotics comprising one or more modifications or alterations such as transformations of existing functional groups and introduction of temporary protecting groups and the like. The term is also considered to include all salt forms. Preferably, the derivatized glycopeptide antibiotic will be a biologically active derivative, which retains at least a part of one or more biological activities of a corresponding non-derivatized or unmodified glycopeptide antibiotic. Biological activities of a glycopeptide antibiotic may include Gram positive bacteria and/or antimicrobial activity; and/or peptidoglycan and/or Lipid II binding, although without limitation thereto. In some preferred embodiments the derivatized glycopeptide antibiotic retains at least: 10%; 20%; 30%; 40%; 50%; 60; 70%; 80%; or 90% of one or more biological activities. For an example of modifications of glycopeptide antibiotics that may result in biologically active derivatives, the skilled person is directed to Malabarba *et al.* (1997) Medicinal Research Reviews, 17(1) 69–137, incorporated herein by reference.

Constructs of this aspect may have the following general structure:



$$A–L_1–V;$$

wherein:

A is the optionally derivatized glycopeptide antibiotic;

L_1 is the first linker; and

V is the visualization component.

It will be appreciated that the respective connections between A and L_1; and L_1 and V, may be direct or indirect connections. In a preferred embodiment, the connection between A and L_1 is a direct connection. In a preferred embodiment, the connection between L_1 and V is an indirect connection.

In some preferred embodiments of the first aspect, the construct comprises:

(i) an optionally derivatized glycopeptide antibiotic;

(ii) a visualization component;

(iii) a first linker connected to (i); and

(iv) a second linker connected to (ii),

wherein (iii) is connected to (iv) and (i) is connected to (ii).

Constructs of these embodiments may have the following general structure:

5 $$A–L_1–L_2–V$$

wherein:

A is the optionally derivatized glycopeptide antibiotic;

L_1 is the first linker;

L_2 is the second linker; and

10 V is the visualization component.

It will be appreciated that the respective connections between A and L_1; L_1 and L_2; and L_2 and V may be direct or indirect connections. In preferred embodiments, the connection between A and L_1 is a direct connection. In preferred embodiments, the connection between and L_1 and L_2 is a direct connection. In preferred embodiments,

15 the connection between L_2 and V is a direct connection.

In certain preferred embodiments, the construct of this aspect may be depicted as follows:

$$A\text{-}W\text{-}L_1\text{-}X\text{-}L_2\text{-}Y\text{-}V$$

wherein:

20 A is the optionally derivatized glycopeptide antibiotic

W is a functionality of the first linker (L_1) linking L_1 to A;

X is a functionality of L_1 linking L_1 to the second linker (L_2);

Y is a functionality of L_2 linking L_2 to V; and

V is the visualization component.

25 Preferably:

W comprises an amide bond;

X comprises a triazole; and

Y comprises an amide, amine, sulphide, or thioester, urethane, urea, sulphonamide, or ether moiety

30 Alternatively, in certain embodiments, constructs of the invention may be depicted as follows:

$$A\text{-}W\text{-}R_1\text{-}X\text{-}R_2\text{-}Y\text{-}V$$

wherein:

A is the optionally derivatized glycopeptide antibiotic;

W-R_1-X is the first linker comprising functional groups W and X on first and second ends, respectively, and an internal moiety R_1;

R_2-Y is the second linker comprising moiety R_2 and functional group Y; and

V is the visualization component.

5 Preferably:

W is an amide bond connecting the first linker to A;

R_1 comprises a PEGN moiety (as hereinbelow described) and/or a linear carbon chain;

X is a triazole connecting the first linker and R_2 of the second linker;

10 R_2 comprises a linear carbon chain of at least C1; and

Y is an amine, amide, or sulphonamide bond connecting the second linker to V.

The structure of particularly preferred constructs of the invention may be depicted as follows:

15 A-W-PEG3-X-C1-Y-V

wherein:

A, W, X, and Y are as described for the directly preceding embodiment;

W-PEG3-X is the first linker comprising W (amide bond) and X (triazole) on first and second ends respectively, and an internal PEG3 moiety.

20 C1-Y is the second linker comprising a C1 chain and Y (amine or amide bond); and

V is a fluorescent visualization component selected from the group consisting of optionally derivatized: NBD; DMACA; dansyl and BODIPY.

Exemplary embodiments of constructs in the form A-W-PEG3-X-C1-Y-V are
25 set forth in FIG. 2.

It will be appreciated that functional groups (e.g. W, X, and Y set forth above) joining individual components (e.g. L_1; L_2; A; and V set forth above) of constructs of this aspect may be described herein as functional groups of or belonging to a particular component of the construct. It will be understood that this designation is not
30 limiting with respect to precursor components that may be used to form the construct, such as in preferred embodiments as herein described. That is, description of a functional group as belonging to a given component does not imply any specific relationship of the functional group with a precursor of that component.

Visualization

Certain preferred embodiments of constructs of the invention are designed for binding to a microorganism or a component thereof to facilitate visualization or imaging of the microorganism or component thereof. As used herein, in the context of constructs of the invention, the terms "*visualization*", "*visualize*", and "*visualized*" etc., and "*imaging*", "*image*", and "*imaged*" etc., refer to the making of a visual representation. Broadly, binding of preferred constructs to a microorganism or component thereof will provide some suitable signal or tag for visual representation. The visualization may be direct, i.e. facilitated by an emission of light in the visual spectrum from the construct. The visualization may also be indirect, i.e. facilitated by emission of a signal from the construct that can be converted into a visual representation by suitable processing.

Preferred constructs of the invention are particularly suited to visualization of Gram positive bacteria. However, it will be appreciated that visualization of other microorganisms which bind to glycopeptide antibiotics is also within the scope of the invention. By way of non-limiting example, it will be appreciated that certain glycopeptide antibiotics have been shown to inhibit fungal growth, although the mechanism of inhibition (and in particular, whether this involves binding to fungal components) is presently unclear in at least many instances.

As hereinbelow described, preferred constructs of this aspect bind Lipid II and/or peptidoglycan, wherein the optionally derivatized glycopeptide antibiotic binds to the Lipid II and/or peptidoglycan. Although such constructs of this aspect that bind Lipid II and/or peptidoglycan will typically be suitable for visualization of Gram positive microorganisms as set forth above, it will be readily appreciated that such constructs will also typically be suited to visualization of Lipid II and/or peptidoglycan itself, regardless of origin, provided that the optionally derivatized glycopeptide antibiotic of the construct can access and bind the Lipid II and/or peptidoglycan. It will be appreciated that the Lipid II and/or peptidoglycan may be contained within any suitable microorganism. It will be further appreciated that the Lipid II and/or peptidoglycan may be visualized after being obtained or produced by any suitable means, such as extraction from a microorganism, or by synthetic production.

It will be further appreciated that construct of this aspect may have application in visualization of Gram negative bacteria in certain circumstances. In particular, it has been determined for this invention that visualization constructs of this aspect can

be desirable for the assessment of outer membrane damage to Gram negative bacteria. As will be understood by the skilled person, cell walls of Gram negative bacteria also comprise peptidoglycan, however this is located underneath an outer membrane, and typically not accessible to binding by antibiotics while the outer membrane is intact.

5 As set forth in further detail below, and with reference to Example 3, constructs of the invention can be applied to the assessment of membrane damage in Gram negative bacteria, wherein binding of the construct the Gram negative bacteria indicates that the Gram negative bacteria has incurred membrane damage, and peptidoglycan in the cell wall has been exposed.

10 In some embodiments, visualization using constructs of the invention is *in vitro* visualization. As used herein, "*in vitro*" visualization will be understood to refer to any visualization that occurs outside of a biological subject. *In vitro* visualization may be performed using any suitable sample containing a suitable microorganism or component thereof, and optionally other sample components. Such samples may 15 include laboratory samples, such as artificial cultures; and environmental samples such as soil and water samples. Such samples may also include samples obtained or removed from a biological subject, including samples from plants and animals.

In some preferred embodiments related to *in vitro* visualization the sample is an animal sample. The sample may be from a human; a primate (e.g. apes and 20 monkeys); a canine; a feline; an ungulate (e.g. equine, bovine, and swine); or an avian, although without limitation thereto. The animal may be livestock (e.g. horses, cattle and sheep), a companion animals (e.g. dogs and cats), a laboratory animals (e.g. mice, rats and guinea pigs) or a performance animal (e.g. racehorses, greyhounds and camels), although without limitation thereto. The animal sample may be of a 25 mammalian or non-mammalian species. Preferably, the animal sample is a human sample.

In some embodiments, visualization using constructs of the invention is *in vivo* visualization. As used herein, "*in vivo*" visualization will be understood to refer to any visualization that occurs within a biological subject. For the purposes of this 30 specification, *in vivo* visualization will exclude analysis performed within a sample obtained or removed from a biological subject, which will be considered to be *in vitro* visualization, as hereinabove described.

Preferably, *in vivo* visualization is performed within an animal. The animal may be a human; a primate (e.g. apes and monkeys); a canine; a feline; an ungulate

(e.g. equine, bovine, and swine); or an avian, although without limitation thereto. The animal may be livestock (e.g. horses, cattle and sheep), a companion animals (e.g. dogs and cats), a laboratory animals (e.g. mice, rats and guinea pigs) or a performance animal (e.g. racehorses, greyhounds and camels), although without limitation thereto.
5 The animal sample may be of a mammalian or non-mammalian species.

Preferably, *in vivo* visualization is performed within a human.

Glycopeptide antibiotics

As used herein a "*glycopeptide antibiotic*" will be understood to be a glycosylated peptide with at least some capacity to (i) bind and/or (ii) inhibit growth,
10 proliferation or viability of a microorganism. As used herein, the ability to inhibit growth, proliferation and/or viability of a microorganism may be referred to generally as "*antimicrobial*" activity.

Typically, glycopeptide antibiotics comprise cyclic or polycyclic peptides. Glycopeptide antibiotics typically have properties consistent with microbial,
15 nonribosomal origin. However, it will be appreciated that all suitable compounds regardless of origin, e.g. both isolated natural compounds and synthetically produced or recombinant compounds, are encompassed by the term glycopeptide antibiotic as used herein. For the purposes of this invention, by "*isolated*" is meant material that has been removed from its natural state or otherwise been subjected to human
20 manipulation. Isolated material may be substantially or essentially free from components that normally accompany it in its natural state, or may be manipulated so as to be in an artificial state together with components that normally accompany it in its natural state.

Glycopeptide antibiotics of constructs of the invention preferably have
25 capacity to bind Gram positive bacteria. It will be appreciated by the skilled person that glycopeptide antibiotics typically bind to peptidoglycan in the bacterial cell wall and to the Lipid II precursor of peptidoglycan. Typically, glycopeptide antibiotics bind selectively to Gram positive bacteria, with limited or absent binding activity towards Gram negative bacteria.

30 The glycopeptide antibiotic of the construct may be any suitable glycopeptide antibiotic. In preferred embodiments, the glycopeptide antibiotic is selected from the group consisting: of vancomycin; teicoplanin; oritavancin; telavancin; dalbavancin; chloroeremomycin; and balhimycin. In one particularly preferred embodiment, the glycopeptide antibiotic is vancomycin.

It will be appreciated that, typically, binding of peptidoglycan of the cell wall of Gram positive bacteria by glycopeptide antibiotics occurs in a substantially non-species or strain -specific manner. That is, glycopeptide antibiotics will generally have broad binding activity for Gram positive bacteria as a group. It will be further understood that glycopeptide antibiotics will generally bind to Gram positive bacteria regardless of whether the bacteria is sensitive or resistant to the antibiotic.

It will be appreciated that binding of the glycopeptide antibiotic may or may not inhibit growth, proliferation, or viability of the bound microorganism. It will be appreciated that, in some embodiments, the viability of a microorganism bound using the construct may be of limited or no significance for the desired visualization application. For other applications, viable or non-viable microorganisms may be preferred. Accordingly, for these embodiments, a suitable antibiotic that is optimized to either maintain or disrupt viability of a microorganism of interest may be selected.

It will be further appreciated that constructs according to certain embodiments of the invention are designed to inhibit or kill microorganisms to which the glycopeptide antibiotic of the construct binds. Accordingly, for these embodiments, a suitable antibiotic that is optimized to have at least partial antimicrobial activity against a microorganism of interest may be selected.

Visualization components

As used herein, a "*visualization component*" broadly includes any entity with the capacity to be connected to a glycopeptide antibiotic to form a construct of the invention, and to facilitate visualization or imaging using the construct. In particular, visualization components of constructs of the invention will be designed to facilitate imaging of microorganisms or components thereof bound to the constructs. Visualization components of the construct of the invention may take a range of suitable forms.

In some preferred embodiments, the visualization component is a light-emitting component. Light-emitting components broadly include luminescent and incandescent sources of light. Preferably, the light emitting component is a luminescent component. In some preferred embodiments, the light emitting component comprises a fluorescent compound. In some preferred embodiments, the light emitting component comprises a quantum dot. For a review of the application of quantum dots to fluorescent detection of biological materials, the skilled person is

directed to Medintz *et al* (2005) Nature Materials, 4 435 – 446, which is incorporated herein by reference.

In some embodiments, the light emitting component emits light in the visual spectrum, i.e. in a wavelength range of about 400 nm to about 700 nm. Visualization components comprising fluorescent compounds emitting light in the visual spectrum may be referred to alternatively herein as "*fluorescent probes*".

In some embodiments, the light emitting component emits light in the infrared spectrum, i.e. in a wavelength range of about 700 nm to about 1mm.

In some embodiments, the infrared spectrum is a near infrared spectrum. As used herein, the "*near infrared*" spectrum will be understood to include the wavelength range of about 700 nm to about 1500 nm. In some embodiments, the infrared spectrum is a mid infrared spectrum. As used herein, the "*mid infrared*" spectrum will be understood to include the wavelength range of about 1500 nm to about 5 μm.

In some embodiment, the infrared spectrum is a far infrared spectrum. As used herein, the "*far infrared*" spectrum will be understood to include the wavelength range of about 5 μm to about 1 mm.

Visualization components comprising fluorescent compounds emitting light in the infrared spectrum may be referred to alternatively herein as "*infrared probes*".

In some preferred embodiments, the fluorescent compound has a neutral charge.

In some preferred embodiments the fluorescent compound has a molecular weight between about 100 and about 500 Da. Preferably the molecular weight is between about 160 and about 250 Da.

In preferred embodiments, the fluorescent compound is an organic or carbon-containing compound. In certain particularly preferred embodiments, the organic fluorescent compound comprises one or more heterocyclic groups. In some embodiments, the heterocyclic groups are N or O –containing heterocyclic groups.

In one preferred embodiment, the heterocyclic group is or comprises a benzofurazan group. In one preferred embodiment, the heterocyclic group is or comprises a coumarin group. In one preferred embodiment, the heterocyclic group is or comprises a naphthalene group. In one preferred embodiment, the heterocyclic group is or comprises an isochromene group.

In one particularly preferred embodiment, the fluorescent compound is optionally derivatized 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD). In one particularly preferred embodiment, the fluorescent compound is optionally derivatized 7-(dimethylamino)-coumarin-4-acetic acid (DMACA). In one particularly preferred
5 embodiment, the fluorescent compound is optionally derivatized 5-(dimethylamino)-1 naphthalene-1-sulfonyl (dansyl). In one particularly preferred embodiment, the fluorescent compound is optionally derivatized 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY).

In an embodiment, the visualization component is a Magnetic Resonance
10 Imaging (MRI) visualization component. Suitable components to facilitate visualization by MRI have been described in the art. The skilled person is directed to Pierre *et al* (2014) Journal of Biological and Inorganic Chemistry, 19 127-131 (incorporated herein by reference) in this regard.

In some embodiments, the MRI visualization component comprises ^{19}F. In
15 some embodiments, the MRI visualization component comprises a gadolinium complex. Preferably, the gadolinium is Gd(III). In some embodiments, the MRI visualization component comprises a Europium complex. Preferably, the Europium is Eu(II). In some embodiments, the MRI visualization component comprises diethylenetriamine penta-acetic acid (DTPA), such as Gd-DTPA or Eu-DTPA. In
20 some embodiments the MRI visualization component comprises a chemical exchange transfer (CEST) agent. In some embodiments the MRI visualization component comprises a paramagnetic chemical exchange saturation transfer (PARACEST) agent. Visualization components that are MRI visualization components may be referred to alternatively herein as "*MRI probes*".

25 In an embodiment, the visualization component is a Positron Emission Tomography (PET) visualization component. Suitable components to facilitate visualization by PET have been described in the art. The skilled person is directed to Serdons *et al* (2009) Methods, 48(2) 104-111 (incorporated herein by reference) in this regard.

30 In some embodiments, the PET visualization component comprises a ^{64}Cu complex. In some embodiment, the PET visualization component comprises a ^{68}Ga complex. In some embodiments, the PET visualization component comprises ^{11}C. In some embodiments, the PET visualization component comprises ^{18}F. In some

embodiments, the PET visualization component comprises ^{124}I. In some embodiment, the PET visualization component contains ^{99m}Tc.

Suitably, the PET visualization component may comprise a chelating compound, such as part of a metal complex. Structures of some suitable chelating
5 compounds for PET applications are set forth in FIG. 19.

In one preferred embodiment, the chelating compound is optionally derivatized 1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA), such as 2,2',2''-(10-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA-alkyne).

10 In one preferred embodiment, the chelating compound is optionally derivatized 2-[4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl]acetic acid (NOTA), such as 2-[4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl]acetic acid propargylamide (NOTA-alkyne) or optionally derivatized NOTA analogs such as 2-[4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl]butanoic acid or 2-[4,7-bis(carboxymethyl)-
15 1,4,7-triazonan-1-yl](4-carboxy-4-butanoic acid), and their corresponding propargylamide derivatives.

In one preferred embodiment, the chelating compound is optionally derivatized 2,2′-(7-(4-(2-((2-aminoethyl)amino)-2-oxoethyl)benzyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (NODA), such as 2,2'-(7-(4-(2-oxo-2-(prop-2-yn-1-
20 ylamino)ethyl)benzyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (NODA-alkyne) and related analogs such as 2,2'-(7-(4-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)benzyl)-1,4,7-triazonane-1,4-diyl)diacetic acid.

The skilled person will appreciate that there is some overlap in nomenclature of certain chelating compounds such as some of the compounds set out above. For
25 example, both 2-[4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl](4-carboxy-4-butanoic acid) and 2,2′-(7-(4-(2-((2-aminoethyl)amino)-2-oxoethyl)benzyl)-1,4,7-triazonane-1,4-diyl)diacetic acid are sometime referred to as NODA. As used herein, unless the context requires otherwise, NODA will refer to the later compound.

In an embodiment, the visualization component is a Single photon emission
30 computed tomography (SPECT) visualization component. Suitable components to facilitate visualization by SPECT have been described in the art. The skilled person is directed to Mariani *et al* (2010) European Journal of Nuclear Medicine and Molecular Imaging, 10(37) 1959-1985 (incorporated herein by reference) in this regard.

Suitably, the SPECT visualization component may comprise a chelating compound, such as part of a metal complex. In one preferred embodiment, the chelating compound is optionally derivatized bisamineoxime (FIG. 19). In one preferred embodiment, the chelating compound is optionally derivatized tetraamine 5 (FIG. 19).

Visualization components that are PET or SPECT visualization components may be referred to alternatively herein as "*PET probes*" or "*SPECT probes*", respectively.

In some embodiments, the visualization component is a radiographic 10 visualization component. In some embodiments, the radiographic visualization component contains ^{123}I. In some embodiments, the radiographic visualization component contains ^{99m}Tc. Visualization components that are radiographic visualization components may be referred to alternatively herein as "*radiographic probes*".

15 In an embodiment, the visualization component is a Magnetic Particle Imaging (MPI) visualization component. Suitable components to facilitate visualization by MPI have been described in the art. The skilled person is directed to Panagiotopoulos *et al* (2015) International Journal of Nanomedicine, 10 3097–3114 (incorporated herein by reference) in this regard.

20 The MPI visualization component is preferably a magnetic nanoparticle. Preferably, the magnetic nanoparticle is a superparamagnetic nanoparticle. In particularly preferred embodiments, the MPI visualization component is superparamagnetic iron oxide nanoparticle (SPION). Visualization components that are MPI visualization components may be referred to alternatively herein as "*MPI* 25 *probes*".

Linkers

Linkers of constructs of the invention facilitate connection between the glycopeptide antibiotic and the visualization component.

It has been surprisingly found that constructs comprising certain linkers are 30 advantageous for binding to microorganisms such as Gram positive bacteria, to facilitate visualization. Certain linkers which are at least partially hydrophilic may be particularly effective for binding and visualization using constructs of the invention.

Without wishing to be bound by theory, based on experimental observations it is considered that molecules which are at least partially hydrophilic may be

particularly effective for binding to microorganisms to facilitate visualization using constructs of the invention. In particular, partially hydrophilic or hydrophilic linkers e.g. those comprising PEG as herein described, may provide an extended tether for the glycopeptide antibiotic, with limited or absent coiling occurring in an aqueous
5 environment.

In particularly preferred embodiments wherein the first linker is at least partially hydrophilic, the linker comprises a polyethylene glycol (PEG) group. As will be readily understood by the skilled person, PEG molecules may be expressed, when located internally within a larger molecule as in constructs of the invention, in the
10 form R_a–(O-CH_2-CH_2)n–, where 'n' is the number of PEG monomers and R_a is a carbon chain connecting the PEG. As used herein, PEG molecules may be expressed in the form 'PEGN', wherein 'N' is the number of PEG monomers.

It is particularly preferred that R_a comprises a C2-C4, or more preferably C2 linear carbon chain. Such PEG-containing moieties may take the form $–(CH_2)_m(O$-
15 CH_2-$CH_2)_n$–, where 'm' is 2 to 4 carbons e.g. –CH_2-CH_2-(O-CH_2-$CH_2)_3$–. It will be appreciated that a determination of the particular side or end of the PEG moiety to which a linear carbon chain, such as a C2 chain, is adjacent, may be dependent upon the end of the combined PEG-linear carbon chain molecule that is considered to be the 'start', or 'first end' of the PEG-linear carbon chain molecule.

20 Preferably, the PEG group is at least PEG3. Preferably the PEG group is in the range PEG3 to PEG10, including PEG4; PEG5; PEG6; PEG7; PEG8; and PEG9. In some particularly preferred embodiments, the PEG group is PEG3 or PEG4.

It will be appreciated that partially hydrophilic first linkers may comprise other components. In some embodiments, the partially hydrophilic first linker may
25 comprise a hydrophobic component. In some embodiments, the hydrophobic component may be a linear carbon chain. The linear carbon chain may be a C2-C15 chain, including C3; C4; C5; C6; C7; C8; C9; C10; C11; C12; C13; and C14, although without limitation thereto.

Certain hydrophobic linkers may also be suitable according to constructs of
30 the invention. It has been recognised that, where hydrophobic linkers are used, molecules comprising at least a particular linear chain length may be more effective for capture using constructs of the invention.

In certain embodiments, the linker is a hydrophobic molecule comprising a linear carbon chain of at least four carbons. Preferably the linear carbon chain is C4-

C20, including C5; C6; C7; C8; C9; C10; C11; C12; C13; C14; C15; C16; C17; C18; and C19, or preferably C6-C10.

In some embodiments, the linear carbon chain may be C6-C16; C6-C14; C8-C14; C8-12; C8-10; or C10-12. In one particularly preferred embodiment, the linear carbon chain is C8. In another particularly preferred embodiment the linear carbon chain is C11. It will be appreciated that the linear carbon chain may be an alkane, an alkene, or an alkyne, although without limitation thereto.

It is preferred according to the invention that the first linker is directly connected or directly bound to the glycopeptide antibiotic (see, e.g. FIGS. 1-2).

In some embodiments of the construct of the invention, the first linker may be connected to the glycopeptide antibiotic via reaction with a glycopeptide moiety selected from the group consisting of: a C-terminal carboxy moiety; a primary or secondary N-terminal moiety; a hydroxyl moiety; a phenolic moiety; and an amine moiety. Preferably, the first linker is connected to the glycopeptide antibiotic via an amide group formed from the C-terminal carboxy moiety.

In preferred embodiments, the first linker comprises one or more nitrogen-containing moieties. The nitrogen-containing moieties may be any suitable moieties. The nitrogen-containing moieties may contain one or a plurality of nitrogens. In some preferred embodiments, the nitrogen-containing moieties contain between 1 and 6 nitrogens, or preferably between 1 and 3 nitrogens. The nitrogen-containing moieties may be linear or cyclic moieties, including heterocyclic moieties.

In particularly preferred embodiments, the one or more nitrogen-containing moieties include an amine-derived moiety and/or an azide-derived moiety. By amine-derived moiety and azide-derived moiety it is intended that, whatever the actual functionality of the relevant connecting bond or moiety in the construct, it was at least in part derived by the coming together of an amine or an azide with a complimentary reactive functional group. For example, an amide bond in the construct could be termed an amine-derived moiety as it may have been formed from reaction of an amine with a carboxy group. The terms cover situations where the amine or other relevant group was present on precursors to the linker, glycopeptide antibiotic or visualization component prior to reaction to form the final construct.

Preferably, the first linker comprises a nitrogen-containing moiety at a first end of the linker. Preferably, the moiety is an amine-derived moiety. Preferably the

moiety connects the linker to the glycopeptide antibiotic. Preferably the connection is a direct connection.

It will be understood that the amine-derived moiety will be derived from an amine upon binding to another component forming part of the construct. It will be further understood that the particular identity of the amine-derived moiety will typically depend upon the component of the construct to which the amine-derived moiety is bound.

In preferred embodiments wherein the amine-derived moiety is bound to the glycopeptide antibiotic by the C-terminal carboxy moiety of the glycopeptide antibiotic, the amine-derived moiety will be an amide (see, e.g. FIG. 2).

In some embodiments wherein the amine-derived moiety is bound to the glycopeptide antibiotic by a primary or secondary amine group the amine-derived moiety will be a urea.

In some embodiments wherein the amine-derived moiety is bound to the glycopeptide antibiotic by a hydroxyl or phenolic group, the amine-derived moiety will be a urethane.

In some embodiments, as hereinabove described, the first linker may be connected to the visualization component via a second linker. In preferred such embodiments, the second linker comprises a linear carbon chain. Preferably, the linear carbon chain is C1-C10, including C2; C3; C4; C5; C6; C7; C8; and C9. It will be understood that, as used in this context, a single internal carbon group will be considered a ‘linear C1 carbon chain’.

Preferably the linear carbon chain is C1 to C4. In one particularly preferred embodiment, the linear carbon chain is C1 (see, e.g., FIG. 2).

Preferably, the second linker is connected to the first linker via an azide-derived moiety at the second end of the first linker. Preferably, the azide-derived moiety is a triazole. Suitably, the triazole moiety may be formed between an azide group from the second end of a precursor to the first linker and an alkyne group of a precursor to the second linker (see, e.g. FIG. 2). However, it will be readily understood that the triazole moiety may be alternatively formed, e.g. between an alkyne group from the second end of the first linker and an azide group of a precursor to the second linker. Preferably, the formation of the triazole from the precursors of the first and second linkers is achievable by rapid or ‘click’ reaction chemistry (see, e.g., the Examples).

For a summary of click chemistry, including in the context of triazole formation, the skilled person is directed to Kolb *et al* (2003) Drug Discovery Today, 8(24) 1128–1137. Although it will be appreciated that the formation of the triazole from the precursors of the first and second linkers as set forth in the Examples
5 involves the use of copper (Cu) catalysis, it will be appreciated that in some embodiments formation of the triazole may not require Cu catalysis. As will be understood by the skilled person, the use of an alkyne group within certain moieties, e.g. a strained cyclooctyne, can be particularly favourable for formation of a triazole even in the absence of Cu catalysis, such as under biological conditions.

10 The visualization component is preferably connected to the first and/or second linker via a moiety selected from the group consisting of an amide, amine, sulphide, urethane, urea, ether, or thioester moiety. It will be understood that such moieties may be formed by reaction of an amine, hydroxyl or thiol group that was present on the second end of a precursor to the first or second linker, with a group (e.g. a leaving
15 group or carboxyl group) present on a precursor to the visualisation component as present in the construct.

Preferably, the visualization component is connected to a moiety of the second linker. In one particularly preferred embodiment, the moiety is an amide moiety (see, e.g., FIG. 2). In another particularly preferred embodiment, the moiety is an amine
20 moiety (see, e.g. FIG. 2).

A related aspect of the invention is directed to a method of producing a construct, the method including the steps of obtaining (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker, and connecting (i) and (ii) using (iii).

25 In a preferred embodiment of the second aspect, the method includes the step of:

(a) obtaining (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; (iii) a first linker; and (iv) a second linker;

(b) connecting (i) to (iii);

30 (c) connecting (ii) to (iv); and

(d) connecting (iii) to (iv).

Preferably the construct produced according to the method of this aspect is the construct of the first aspect.

It will be appreciated that, the method of this aspect may be or involve *in vitro* synthesis, such as described in the Examples.

Additionally, *in vivo* production of the construct from component parts is within the scope of this aspect of the invention. By way of non-limiting example, a component of the construct comprising the optionally derivatized glycopeptide antibiotic connected to a precursor to the first linker; and a component of the construct comprising the visualization component connected to a precursor to the second linker may be administered separately *in vivo*, wherein these respective components connect to form the final construct *in vivo*. Suitably, in such embodiments, the structure of the respective ends of the first and second linkers will be such that connection (e.g. by way of formation of a triazole group as herein described) occurs by click or rapid chemistry, under biological conditions. By way of non-limiting example, use of an alkyne group of a strained cyclooctyne for the formation of a triazole connecting the first and second linker may be particular desirable for such embodiments.

It will be readily appreciated that the following methods encompass embodiments wherein *in vivo* production of the construct is performed. In these embodiments, the microorganisms may be bound to the glycopeptide antibiotic before or after connection of components of the construct to form the final construct *in vivo*.

Method of Binding a Microorganism to a Construct

The invention further provides a method of binding a construct to a microorganism or component thereof, the construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii), the method including the steps of:

(a) combining the construct and a microorganism or component thereof; and

(b) selectively binding the glycopeptide antibiotic of the construct with the microorganism or component thereof,

to thereby bind the construct to the microorganism or component thereof.

Preferably, the construct is a construct as hereinabove described.

Preferably, in embodiments of this aspect directed to binding of a microorganism, the microorganism is a Gram positive microorganism. Preferably, in embodiments of this aspect directed to binding of a microorganism component, the microorganism component is peptidoglycan and/or Lipid II. It will be appreciated that, in this context, any peptidoglycan and/or Lipid II will be considered a 'microorganism component'. That is, although peptidoglycan and/or Lipid II will

typically be derived from a suitable microorganism, peptidoglycan and/or Lipid II obtained by any other suitable means (e.g. synthetically) also falls within the scope of this aspect.

It will be appreciated that, in the context of step (b) of the method of this 5 aspect, by "*selectively binding*" or "*selectively bound*" etc., is meant that the microorganism or component thereof is bound with at least partial specificity as compared to other sample components and/or microorganisms. It will be further appreciated that, as hereinabove described, glycopeptide antibiotics typically bind to Gram positive bacteria as a group selectively (as compared, for example, to Gram 10 negative bacteria, or non-Gram staining bacteria). As such, unless the context requires otherwise, in the context of binding to Gram positive bacteria "*selectively binding*" etc. will refer to binding to Gram positive bacteria as a group with at least partial specificity (rather than binding to a particular Gram positive bacterial species or strain).

15 According to step (a) of this aspect, the construct and the microorganism may be combined *in vitro* or *in vivo*, as hereinabove described.

In particularly preferred embodiments the microorganism bound according to step (b) of this aspect is a pathogenic Gram positive bacteria. Preferably, the pathogenic Gram positive pathogenic bacteria is selected from the group consisting 20 of: *Bacillus*; *Clostridium*; *Corynebacterium*; *Enterococcus*; *Listeria*; *Staphylococcus*; and *Streptococcus*. Preferably, the Gram positive bacteria is a cocci e.g. *Staphylococcus*; or *Streptococcus*.

In some preferred embodiments, the *Staphylococcus* is *S. aureus*. The *S. aureus* may be, without limitation thereto, a glycopeptide-sensitive strain (such as 25 ATCC 25923); an MRSA stain (such as ATCC 43300); a glycopeptide-intermediate (GISA) strain (such as NRS 17); or a vancomycin-resistant (VRSA) strain (such as NARSA VRS4). The *S. aureus* may also be an NARSA VRS 10 strain; a NARSA VRS 3b strain; or an NRS 1 strain.

In some embodiments, the *Staphylococcus* is *S. epidermis*. The *S. epidermis* 30 may be, without limitation thereto, a glycopeptide-sensitive strain (such as ATCC 12228); or glycopeptide-intermediate (GISE) strain (such as NARSA NRS60).

In some preferred embodiments wherein the *Streptococcus* is *S. pneumoniae*. The *S. pneumoniae* may be, without limitation thereto, a glycopeptide sensitive strain (such as ATCC 33400); or a glycopeptide resistant strain (such as ATCC 700677).

In some embodiments, the *Enterococcus* is *E. faecium*. The *E. faecium* may be, without limitation thereto, a vancomycin A (Van A) resistant strain (such as ATCC 51559) or a vancomycin B (Van B) resistant strain (such as ATCC 51299).

In some embodiments, the *Enterococcus* is *E. faecalis*. The *E. faecalis* may be, 5 without limitation, a vancomycin sensitive strain (such as ATCC29212) or a vancomycin resistant strain.

Method of Visualizing a Microorganism

Another aspect of the invention relates to a method of visualizing a microorganism or component thereof. The method will include the steps of:

10 (a) combining a construct with a microorganism or component thereof, the construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii);

(b) selectively binding the microorganism or component thereof to the glycopeptide antibiotic of the construct; and

15 (c) visualizing the construct bound to the microorganism or component thereof using the visualization component,

to thereby visualize the microorganism or component thereof using the construct.

Preferably, steps (a) and (b) are as described above for methods of binding a 20 microorganism.

Visualization according to step (c) of the method of this aspect may be by any suitable approach. In embodiments wherein the visualization component of the construct is a fluorescent probe or infrared probe, preferably the visualization is by fluorescence detection.

25 A range of techniques for fluorescence detection have been described in the art. Such techniques include microscopy techniques and spectroscopy techniques, although without limitation thereto. In this regard, the skilled person is directed to '*Fluorescence Detection Techniques*' in Introduction to Fluorescence Sensing (Springer, 2009, Alexander P. Demchenko Ed.), and '*Fluorescence Microscopy for* 30 *Biological Imaging*' in Physics and Biology From Molecules to Life (World Scientific, 2014, Jean François Allemand and Pierre Desbiolles Eds.), which are incorporated herein by reference.

In a preferred embodiment wherein the visualization component is a fluorescent probe, the visualization is by Super-Resolution Structured Illumination

Microscopy (SR-SIM) or another high resolution fluorescent microscopy technique. The skilled person is directed to Huang *et al* (2009) Annual Reviews in Biochemistry, 78 993-1016; and Boutros *et al* (2015) Cell, 163(6) 1314–1325 (incorporated herein by reference). In another embodiment wherein the visualization component is a
5 fluorescent probe, the visualization is by Fluorescence Activated Cell Sorter (FACS) technique. The skilled person is directed to Herzenberg *et al* (2002) Clinical Chemistry 48(10) 1819-1827, incorporated herein by reference.

In embodiments wherein the visualization component of the construct is an MRI component or MRI probe, preferably the visualization is by MRI. Techniques for
10 MRI are known in the art. In particular regard to MRI approaches using probes to visualize biological targets (such as microorganisms) the skilled person is directed to Pierre *et al*, *supra*, and Sosnovik *et al* (2007) Current Opinion in Biotechnology, 18(1) 4-10 (incorporated herein by reference).

In embodiments wherein the visualization component of the construct is PET
15 component, preferably the visualization is by PET. Techniques for PET are known in the art; the skilled person is directed to Positron Emission Tomography: Basic Sciences (Springer-Verlag, 2009, Bailey, Townsend, Valk, and Maisey Eds.), incorporated herein by reference. In particular regard to PET approaches using probes to visualize biological targets (such as microorganisms) the skilled person is directed,
20 by way of example, to Gowrishankar *et al* (2014) PLOS ONE, 9(9) e107951 (incorporated herein by reference).

In embodiments wherein the visualization component is an MPI component, preferably visualization is by MPI. Techniques for MPI are known in the art. The skilled person is directed Panagiotopoulos *et al*, *supra*, and Magnetic Particle Imaging
25 (Elsevier, 2010, Kevin R. Minard), incorporated herein by reference, in this regard.

In embodiments wherein the visualization component is a radiographic visualization component, preferably the visualization is radiological visualization. Techniques for radiological visualization are known in the art. In particular regard to approaches using radiographic probes for visualization of biological components, the
30 skilled person is directed to Signore *et al* (2014) Current Pharmaceutical Design, 20(14) 2338-2345 (incorporated herein by reference). The visualization using a radiographic visualization component may be by conventional nuclear medicine planar imaging or SPECT (Single-photon emission computed tomography), although without limitation thereto.

As hereinabove described, visualization according to the method of this aspect may be *in vitro* visualization, or *in vivo* visualization.

In embodiments wherein the visualization is *in vitro* visualization, suitably, step (a) will include adding the construct to a sample containing the microorganism or 5 component thereof. In these embodiments, preferably the sample of step (a) is a sample obtained from a biological subject. Preferably the subject is an animal, as hereinabove described. Preferably the animal is a human.

The biological sample may take any suitable form. For example, the animal or human sample may be a tissue sample including a muscle; epithelial; connective; or 10 nervous tissue sample. The sample may further be a waste product such as urine, or an excretion such as sputum. In some preferred embodiments, the sample is a blood sample. Preferably, the blood sample is human blood.

In embodiments wherein the visualization is *in vivo* visualization, suitably, step (a) will include administering the construct to a biological subject. Preferably the 15 subject is a human or an animal, as hereinabove described.

The construct may be administered to the biological subject in the form of a pharmaceutically acceptable composition as hereinbelow described.

Any safe route of administration may be employed for providing a patient with constructs or compositions of the invention for *in vivo* visualization. For example, 20 enteral, oral, rectal, parenteral, sublingual, buccal, intravenous, intra-articular, intra-muscular, intra-dermal, subcutaneous, inhalational, intraocular, intraperitoneal, intracerebroventricular, transdermal and the like may be employed.

Preferably, the administration occurs directly to a site within the body wherein the *in vivo* visualization is to occur. Additionally or alternatively, the administration 25 may occur to a site within the body outside of where the *in vivo* visualization is to occur. In these embodiments, the construct will be transported to the site wherein the *in vivo* visualization is to occur, typically by bodily processes (e.g. blood flow; lymph flow; digestive movement etc.).

In one preferred embodiment, the construct is administered to the blood.

30 In embodiments wherein the subject is a human or an animal, the visualization may occur in any part of the body of the human or animal. By way of non-limiting example, the *in vivo* visualization may occur within the respiratory system (e.g. the lungs); the digestive system (e.g. the stomach or intestines); the nervous system (e.g.

the spine or brain); or the endocrine system (e.g. the pancreas). Preferably, the *in vivo* visualization occurs in the circulatory system.

It will be appreciated that the *in vivo* visualization may occur within one or more tissue types of the biological subject. For *in vivo* visualization in human and 5 animals, the visualization may occur in muscle; epithelial; connective; or nervous tissue, although without limitation thereto. In some preferred embodiments, *in vivo* visualization occurs in blood.

Method of Analysing a Microorganism

The invention further provides a method of analysing a microorganism or 10 component thereof. The method will include the steps of:

(a) combining a construct with a microorganism or component thereof, the construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii);

(b) selectively binding the microorganism or component thereof to the 15 glycopeptide antibiotic of the construct; and

(c) visualizing the construct bound to the microorganism or component thereof using the visualization component; and

(d) analysing the microorganism or component thereof based on the visualization of step (c).

20 In preferred embodiments, steps (a)-(c) are as set forth for the directly preceding aspect directed to methods of visualizing a microorganism or component thereof.

Analysis according to step (d) may be any suitable analysis. It will be appreciated that the analysis as per step (d) may be an *in vitro* analysis or an *in vivo* 25 analysis.

The skilled person will readily appreciate that visualization using constructs of the invention may facilitate a range of suitable analyses. Non-limiting examples of such analyses include the following.

(i) identification of microorganisms

30 (ii) analysis of cellular structures microorganisms

(iii) analysis of microorganism cellular dynamics (e.g. bacterial cell and/or cell wall division)

(iv) analysis of the interaction of glycopeptide antibiotics of the construct with microorganisms;

(v) assessment of the mode of action of the glycopeptide antibiotic of the construct on microorganisms

(vi) identification of resistance mechanisms of microorganisms towards the glycopeptide antibiotic of the construct;

5 (vii) assessment of the interaction of the glycopeptide antibiotic of the construct in combination with other antibiotics or antibiotic probes in the context of (ii)-(vi) above.

In regard to (ii) and (iii), the skilled person is directed to Wheeler *et al* (2011) Molecular Microbiology 82 1096-1109; Turner *et al* (2013) Nature Communications 10 4 1496; Tiyanont *et al* (2006) Proceedings of the National Academy of Sciences 103, 11033-11038; Daniel *et al* (2003) Cell 113, 767-776; and Turner *et al* (2010) Nature Communications 1 26, which are incorporated herein by reference. In regard to (iv)-(vi), the skilled person is further directed to Pereira *et al* (2007) Antimicrobial Agents and Chemotherapy 51 3627-3633, incorporated herein by reference.

15 It will be appreciated that analysis of step (d) may be *in vitro* or *in vivo* analysis. In embodiments wherein the analysis is *in vitro* analysis, the visualization of step (c) is will be *in vitro* visualization. Preferably, the *in vitro* analysis is of a sample obtained from a biological subject. Preferably the sample from the biological subject is a human or animal subject as described for the directly preceding aspect.

20 In embodiments wherein the analysis is *in vivo* analysis, the visualization of step (c) will be *in vivo* visualization. The *in vivo* analysis will be performed in a subject to which the construct has been administered for the visualization of step (c). Preferably, administration for the visualization of step (c) is as described for the directly preceding aspect. Preferably, the visualization will occur within the body of a 25 human or animal subject as described for the directly preceding aspect.

Methods of Assessing Activity of a Compound for Disrupting Cell Membranes

In another aspect, the invention provides for a method of assessing a compound for activity in disrupting a cell membrane of a microorganism.

The method will include the steps of:

30 (a) combining a microorganism with the compound to be assessed;

(b) combining a construct with the microorganism, the construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii); and

(c) determining if the construct is bound to the microorganism by visualization of the construct, wherein

the degree of binding of the construct to the microorganism is related to the activity of the construct in disrupting the cell membrane of the microorganism.

5 Suitably, said degree of binding is positively related to said activity of the compound.

The method of this aspect is particularly adapted for screening of compounds for activity in disrupting outer cell membranes of Gram negative microorganisms. As will be appreciated by the skilled person, Gram negative microorganisms have a cell
10 wall within which peptidoglycan is covered by an outer cell membrane.

Typically, peptidoglycan-binding antibiotics such as those of constructs described herein do not substantially bind to intact Gram negative microorganisms with intact out cell membranes. However, disruption of the outer cell membrane, such as by exposure of the microorganism to a compound with relevant activity, can result
15 in peptidoglycan of the Gram negative cell wall becoming accessible for binding. Accordingly, it will be readily understood by the skilled person that visualization of a Gram negative microorganism exposed to a compound according to the method of this aspect is indicative of disruption of the outer cell membrane by activity of the compound.

20

In a preferred embodiment, the microorganism according to the method of this aspect is *E. coli*.

An embodiment of the method of this aspect is set out in Example 3.

Methods of Diagnosing and/or Monitoring a Disease or Condition

25 Another aspect of the invention is directed to a method of diagnosing and/or monitoring a disease or condition. The method will include the steps of analysing a microorganism or component thereof in a sample of a subject, as described for the directly preceding aspect, and diagnosing and/or monitoring a disease or condition based on the analysis of the microorganism or component thereof. The analysis may
30 include identification of the microorganism. The analysis may be an *in vitro* or *in vivo* analysis, as hereinabove described.

Preferably the disease or condition is an infection with a Gram positive bacteria. Preferably, the pathogenic Gram positive pathogenic bacteria is selected from the group consisting of: *Bacillus*; *Clostridium*; *Corynebacterium*; *Enterococcus*;

Listeria; *Staphylococcus*; and *Streptococcus*. Preferably, the Gram positive bacteria is a cocci e.g. *Staphylococcus*; or *Streptococcus*.

In some preferred embodiments, the *Staphylococcus* is *S. aureus*. The *S. aureus* may be, without limitation thereto, a glycopeptide-sensitive strain (such as ATCC 25923); an MRSA stain (such as ATCC 43300); a glycopeptide-intermediate (GISA) strain (such as NRS 17); or a vancomycin-resistant (VRSA) strain (such as NARSA VRS4).

In some embodiments, the *Staphylococcus* is *S. epidermis*. The *S. epidermis* may be, without limitation thereto, a glycopeptide-sensitive strain (such as ATCC 12228); or glycopeptide-intermediate (GISE) strain (such as NARSA NRS60).

In some preferred embodiments wherein the *Streptococcus* is *S. pneumoniae*. The *S. pneumoniae* may be, without limitation thereto, a glycopeptide sensitive strain (such as ATCC 33400); or a glycopeptide resistant strain (such as ATCC 700677).

In some embodiments, the *Enterococcus* is *E. faecium*. The *E. faecium* may be, without limitation thereto, a vancomycin A (Van A) resistant strain (such as ATCC 51559) or a vancomycin B (Van B) resistant strain (such as ATCC 51299).

In some embodiments, the *Enterococcus* is *E. faecalis*. The *E. faecalis* may be, without limitation, a vancomycin sensitive strain (such as ATCC29212) or a vancomycin resistant strain.

In certain embodiments, the disease or condition may be selected from the group consisting of a bacterial infection of: the respiratory system (e.g. pneumonia); the digestive tract (e.g. gastroenteritis); the sinus (e.g. sinusitis); the ears (e.g. otitis media); the nervous system (e.g. meningitis); the skin (e.g. cellulitis); or the endocrine system (e.g. bacterial pancreatitis).

In one particularly preferred embodiment the disease or condition is bacteraemia. In another particularly preferred embodiment the disease or condition is bacterial sepsis. In one preferred embodiment, the disease or condition is bacterial sepsis caused by *S. aureus*.

Preferably the subject is a human or an animal subject as hereinabove described. In particularly preferred embodiments the subject is a human.

It will be readily appreciated that diagnosing and/or monitoring a condition according to this aspect may involve visualizing infections *in vivo* within any suitable location within the animal or human body. It will be appreciated that a disease or

condition may be monitored by assessing replication or reproduction of a microorganism within the body.

It will be further appreciated that diagnosing and/or monitoring a condition according to this aspect may involve identifying the location or source of an infection
5 within the human or animal body by visualizing a microorganism according to the method of this aspect.

Also within the scope of this aspect is monitoring for potential infection during or after a surgical procedure. By way of non-limiting example, *in vivo* visualization may be used to identify a microorganism to identify contamination
10 occurring during a surgical procedure. By way of another non-limiting example, *in vivo* visualization may be used to identify a potentially contaminated surgical implant that has been placed within the body.

It will be understood that current methods for diagnosis of bacterial infections, such as bacteraemia and/or bacterial sepsis, and also many other bacterial diseases,
15 typically rely on bacterial culture. Methods involving bacterial culture can have significant disadvantages, including substantial periods of time for culture and diagnosis, for example 10-24 hours, and relatively high rates of false positives.

Embodiments of the invention may have particular advantages with respect to time to analyse a microorganism or component thereof in a sample and/or to diagnosis
20 a disease or condition based on this identification.

In some preferred embodiments, analysis of a microorganism or component thereof as per the directly preceding aspect of the invention can be performed in less than 10 hours; less than 9 hours; less than 8 hours; less than 7 hours; less than 6 hours; less than 5 hours; less than 4 hours; less than 3 hours; less than 2 hours; or less than 1
25 hours.

In some particularly preferred embodiments, diagnosis of a disease or condition according to the method of this aspect can be performed in in less than 10 hours; less than 9 hours; less than 8 hours; less than 7 hours; less than 6 hours; less than 5 hours; less than 4 hours; less than 3 hours; less than 2 hours; or less than 1
30 hours.

A related aspect of the invention provides a method of treating a disease or condition, the method including the steps of diagnosing a disease or condition as hereinabove described, and treating the disease or condition based on the diagnosis.

It will be appreciated that the particular form of treatment that will be suitable according to the method of this aspect will be related to the particular disease that is diagnosed. Often, in preferred embodiments wherein the disease is a Gram positive bacterial infection, the treatment will comprise administration of an antibiotic. In some preferred embodiments, the antibiotic is selected from the group consisting of cloxacillin; dicloxacillin; methlocillin; nafcillin; oxacillin; cefazolin; cefoxitin; cefuroxime; cefepime; cefoperazone; cefotaxime; ceftazidime; ceftizoxime; ceftriaxone; trimethoprim; sulfamethoxazole; amoxicillin; clavulanate; penicillin; penicillin G; streptomycin; amoxicillin; clindamycin; doxycycline; etronidazole; rifampin; and vancomycin, or combinations thereof. In some particularly preferred embodiments the antibiotic is vancomycin.

In certain preferred embodiments wherein the disease or condition is a *S. aureus* infection, the antibiotic is selected from the group consisting of trimethoprim; sulfamethoxazole; clindamycin; vancomycin; doxycycline; minocycline; linezolid; and rifampin, or combinations thereof.

In some embodiments of this aspect, the treatment may additionally or alternatively involve surgical intervention.

The method of this aspect may have particular advantages with respect to the time taken for diagnosis and initial treatment, e.g. initial administration of an antibiotic. In some preferred embodiments, diagnosis and initial treatment according to the method of this aspect can be performed in less than 10 hours; less than 9 hours; less than 8 hours; less than 7 hours; less than 6 hours; less than 5 hours; less than 4 hours; less than 3 hours; less than 2 hours; or less than 1 hour.

Glycopeptide Antibiotic Adducts

The invention also provides compounds comprising an optionally derivatized glycopeptide antibiotic bound to a first linker. Such compounds may be referred to herein as 'glycopeptide antibiotic adducts'. Such compounds of the invention will be suitable for connection to a visualization component, to form constructs as hereinabove described.

Preferably, the glycopeptide antibiotic of such is selected from the group consisting of vancomycin; teicoplanin; oritavancin; telavancin; chloroeremomycin; and balhimycin. Preferably the glycopeptide antibiotic is vancomycin.

Preferably, the first linker of the second broad form comprises a PEG group, preferably at least PEG3.

In some embodiments the first linker comprises a linear carbon chain greater than four carbons, preferably wherein the linear carbon chain C4-C12. In one preferred embodiment the linear carbon chain is C8. In another preferred embodiment the linear carbon chain is C11.

5 In preferred embodiments, the first linker comprises an amine-derived moiety. Preferably the amine-derived group is at an end of the first linker. Preferably the amine-derived moiety is an amide bond connecting the first linker to the glycopeptide antibiotic.

In one preferred embodiment, the first linker comprises an azide moiety. 10 Preferably, the azide moiety is at an end of the linker opposite the glycopeptide antibiotic.

Methods of Increasing the Activity of a Glycopeptide antibiotic

In one aspect, the invention provides a method of increasing or enhancing the activity or efficacy of a glycopeptide antibiotic, the method including the step of 15 connecting a first linker to the glycopeptide antibiotic.

In a related aspect, the invention provides a method of increasing or enhancing the activity or efficacy of a glycopeptide antibiotic, the method including the step of connecting the glycopeptide antibiotic to a visualization component using a first linker.

20 In preferred embodiments of the above aspect, the glycopeptide antibiotic is hereinabove described in relation to constructs of the invention.

As set forth in the Examples, it has been surprisingly determined that glycopeptide antibiotics connected to particular linkers demonstrate increased activity towards at least certain microorganisms, as compared to the corresponding 25 'unconnected' or 'free' glycopeptide antibiotic. Furthermore, it has been surprisingly determined that constructs of the invention comprising particular linkers and a visualization component demonstrate increased activity towards at least certain microorganisms, as compared to the corresponding unconnected or free glycopeptide antibiotic of the construct.

30 Preferably, the first linker comprises a linear carbon chain of at least four carbons. In a particularly preferred embodiment, the first linker comprises C8.

Preferably the activity or efficacy of the glycopeptide antibiotic is towards a Gram positive bacteria. Preferably the bacteria is a pathogenic Gram positive bacteria. Particular preferred Gram positive bacteria according to this aspect are as described

above in relation to methods of diagnosing a disease or condition according to the invention.

In certain embodiments the Gram positive bacteria is a vancomycin resistant bacteria. The vancomycin resistant bacteria may be a Van A or Van B resistant
5 bacteria, although without limitation thereto. Preferably the vancomycin resistant bacteria is selected from the group consisting of *S. aureus*; *E. faecium*; and *E. faecalis*. In certain embodiments, the microorganism shows at least partial resistance to the free or unconnected glycopeptide antibiotic.

In some preferred aspects, the increase or enhancement of the activity or
10 efficacy or the antibiotic is a decrease in the Minimum Inhibitory Concentration (MIC) of the antibiotic for a particular microorganism, such as a Gram positive microorganism as described above.

In some preferred embodiments, the decrease in MIC is a fold decrease of between about 1.5 and about 5 to the free or unconnected antibiotic, including a fold
15 decrease of at least: 2; 2.5; 3; 3.5; 4; or 4.5.

Methods of Inhibiting a Microorganism

Another aspect of the invention provides a method of inhibiting, controlling, or killing a microorganism, the method including the steps of contacting a microorganism with (i) a construct of the invention; or (ii) a glycopeptide antibiotic
20 adduct of the invention, to thereby inhibit, control, or kill the microorganism.

Preferably the microorganism is a Gram positive bacteria. Preferably, the Gram positive bacteria is a pathogenic Gram positive bacteria. Particularly preferred Gram positive bacteria according to this aspect are as described above in relation to methods of diagnosing a disease or condition according to the invention.

25 Compositions and Methods for Diagnosis, Treatment or Prevention of Disease

In a further aspect, the invention provides a composition for diagnosing, treating or preventing a disease, disorder, or condition in a subject, the composition comprising (i) a construct of the invention; and/or (ii) a glycopeptide antibiotic adduct of the invention, as hereinabove described.

30 The composition may suitably contain one or more pharmaceutically-acceptable carriers, diluents or excipients. By "*pharmaceutically-acceptable carrier, diluent or excipient*" is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in systemic administration.

Depending upon the particular route of administration, a variety of carriers, well known in the art, may be used. These carriers may be selected from a group including sugars, starches, cellulose and its derivatives, malt, gelatine, talc, calcium sulphate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline and salts such as mineral acid salts including hydrochlorides, bromides and sulphates, organic acids such as acetates, propionates and malonates and pyrogen-free water. A useful reference describing pharmaceutically acceptable carriers, diluents and excipients is Remington's Pharmaceutical Sciences (Mack Publishing Co. N.J. USA, 1991) which is incorporated herein by reference.

Dosage forms of compositions according to this aspect include tablets, dispersions, suspensions, injections, solutions, oils, syrups, troches, capsules, suppositories, aerosols, transdermal patches and the like. These dosage forms may also include injecting or implanting controlled releasing devices designed specifically for this purpose or other forms of implants modified to act additionally in this fashion. Controlled release of the therapeutic agent may be effected by coating the same, for example, with hydrophobic polymers including acrylic resins, waxes, higher aliphatic alcohols, polylactic and polyglycolic acids and certain cellulose derivatives such as hydroxypropylmethyl cellulose. In addition, the controlled release may be effected by using other polymer matrices, liposomes and/or microspheres.

Compositions of the present invention suitable for enteral, oral or parenteral administration may be presented as discrete units such as capsules, sachets or tablets each containing a pre-determined amount of one or more therapeutic agents of the invention, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association one or more agents as described above with the carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the agents of the invention with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

Also provided is a method of treating or preventing a disease, disorder, or condition in a subject in need thereof, the method including the step of administering to the subject an effective amount of (i) a construct of the invention; (ii) a compound

of the invention comprising a glycopeptide antibiotic and a first linker; or (iii) a composition of the invention, as hereinabove described.

Any safe route of administration may be employed for providing a patient with constructs, adducts, or compositions of the invention. For example, enteral, oral, rectal, parenteral, sublingual, buccal, intravenous, intra-articular, intra-muscular, intra-dermal, subcutaneous, inhalational, intraocular, intraperitoneal, intracerebroventricular, transdermal and the like may be employed.

The construct, adduct or composition may be administered in a manner compatible with the dosage formulation, and in such amount as is pharmaceutically-effective. The dose administered to a patient, in the context of the present invention, should be sufficient to effect a beneficial response in a patient over an appropriate period of time. The quantity of agent(s) to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof, factors that will depend on the judgement of the practitioner.

It will also be appreciated that treatment methods and pharmaceutical compositions may be applicable to prophylactic or therapeutic treatment animals, inclusive of humans and non-human mammals such as livestock (*e.g.* horses, cattle and sheep), companion animals (*e.g.* dogs and cats), laboratory animals (*e.g.* mice, rats and guinea pigs) and performance animals (*e.g.* racehorses, greyhounds and camels), although without limitation thereto.

Preferably the disease or condition according to these embodiments is caused by a Gram positive bacteria. Particular preferred Gram positive bacteria according to this aspect are as described above in relation to methods of diagnosing a disease or condition according to the invention.

In certain embodiments, the disease or condition may be selected from the group consisting of a bacterial infection of: the respiratory system (e.g. pneumonia); the digestive tract (e.g. gastroenteritis); the urinary tract (e.g. a urinary tract infection); the sinus (e.g. sinusitis); the ears (e.g. otitis media); the nervous system (e.g. meningitis); the skin (e.g. cellulitis); or the endocrine system (e.g. bacterial pancreatitis).

In one particularly preferred embodiment the disease or condition is bacteraemia. In another particularly preferred embodiment the disease or condition is bacterial sepsis. In one preferred embodiment, the disease or condition is bacterial sepsis caused by a *S. aureus*.

EXAMPLES

Example 1. Production of Constructs and Glycopeptide Antibiotic Adducts

This Example describes particular preferred constructs of the invention, comprising a glycopeptide antibiotic in the form of vancomycin; a first linker; a second linker; and a fluorophore. The Example also describes particular preferred glycopeptide antibiotic adducts, comprising vancomycin and a first linker. As described, the glycopeptide antibiotic adducts were used for production of the constructs.

All materials, unless otherwise noted, were obtained from commercial suppliers and used without further purification. Non-aqueous reactions were conducted under an inert atmosphere of nitrogen. Analytical LCMS was performed on a Shimadzu LCMS 2020 using 0.05% formic acid in water as solvent A and 0.05% formic acid in acetonitrile as solvent B. LCMS conditions (solvent A = H_2O + 0.05% formic acid, solvent B = acetonitrile + 0.05% formic acid): Column Zorbax Eclipse XDB-Phenyl, 3.0 × 100 mm, 3.5 µ: Flow: 1 mL/min: Gradient timetable: 0.5 min, 5% B; 8.5 min, 100% B; 2.0 min, 100% B; 0.2 min, 5% B. Biotage Initiator microwave was used for Cu-catalyzed azide-alkyne cycloaddition. Column chromatography was performed using silica gel 60 (0.063–0.200 mm), 70–230 mesh ASTM. Agilent 1260 Infinity Preparative HPLC with a G1365D multiple wavelength detector set at λ= 210 nm and Grace Reveleris X2 chromatography systems were used for compound purification. Commercially available cartridges were used for MPLC chromatography (Reveleris C18 Reversed-Phase 12 g cartridge and 40 g cartridge), while HPLC purifications used an Agilent Eclipse XDB-Phenyl column 30 x 100 mm, 5 µm particle size. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra were obtained using a Bruker Avance-600 spectrometer equipped with a TXI cryoprobe. Chemical shifts are reported relative to the residual solvent signals in parts per million (δ) ($CDCl_3$: ^{1}H: δ 7.27, ^{13}C: δ 77.2; DMSO-d_6: ^{1}H: δ 2.50, ^{13}C: δ 39.5). High resolution mass spectrometry (HRMS) was performed on a Bruker Micro TOF mass spectrometer (Ultimate 3000) using (+)-ESI calibrated to HCOONa. The MS/MS was performed on SCIEX X500R QTOF.

Vancomycin Adducts

The glycopeptide antibiotic vancomycin can be modified at a number of regions that do not substantially interfere with binding of vancomycin. These sites

have accessible functional groups including the C-terminal carboxy group, primary and secondary amine groups, and hydroxyl and phenolic groups, all of which have been used to generate vancomycin derivatives. Binding of vancomycin depends mainly on the heptapeptide backbone. For this example, vancomycin was modified at
5 the C-terminal carboxy group, as modification of this site should not interfere with binding, nor with vancomycin dimerization, an additional component of vancomycin's mode of action.

A Cu-catalyzed azide-alkyne cycloaddition (CuAAC) reaction strategy was used production for production of glycopeptide adducts. The CuAAC reaction is
10 compatible with the multiple unprotected functional groups presented in antibiotics, especially the amine, hydroxyl, and amide groups on vancomycin. Glycopeptide adducts containing vancomycin and different linkers were synthesized.

As set forth in FIG. 1, two vancomycin adducts were produced, labelled '2' and '3'. FIG. 2 illustrates the synthesis of the vancomycin adducts. Vancomycin
15 adducts (2 and 3, respectively) were synthesized via amide coupling between an azido-alkyl-amine (N_3-C8-NH_2) or azido-PEG3-amine (N_3-PEG3-NH_2) and the carboxy group of vancomycin. Vancomycin (1) was reacted with azide-linkers in the presence of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and N,N-diisopropylethylamine (DIPEA) in DMF, providing azide-
20 derivatised vancomycin adducts.

Specifically, vancomycin hydrochloride (2 g, 1.35 mmol), PyBOP (770 mg, 1.48 mmol) in DMF (100 mL) was stirred until fully dissolved. DIPEA (1.88 mL, 10.08 mmol) was added to the reaction mixture. After 30 sec, linkers (2.03 mmol) in DMF (4 mL) was immediately added to the reaction mixture. The reaction was stirred
25 at room temperature for 16 h, and then concentrated under reduced pressure. The crude compounds were pre-purified by MPLC over C18 silica gel (Grace Reveleris, A: H_2O (0.1% TFA), B: ACN (0.1% TFA), 0 $\rightarrow$ 100% B over 8 min) to give white solid 2-3. The compound 2-3 were repurified by Prep HPLC (flow 20 mL/min, mobile phases A = 0.1% TFA in water and B = 0.1% TFA in acetonitrile, gradient 5$\rightarrow$100%
30 B over 20 min) and then lyophilized to give white powder.

Vancomycin adduct 2 is hereinafter referred to as N_3-C8-Van, or alternatively Van-8C-N_3. Vancomycin adduct 3 is hereinafter referred to as N_3-PEG3-Van, or alternatively Van-PEG3-N_3.

- Characteristics of N_3-C8-Van (2) obtained were as follows

Yield: 865 mg, 45%

LCMS: R_t = 3.61 min, @ 200 nm, $[M+2H]^{2+}$ = 801.6, Purity by UV @ 200 nm > 95% .

5 (+)-ESI-HRMS calc for $C_{74}H_{93}Cl_2N_{13}O_{23}$ $[M+2H]^{2+}$: 800.7942, found 800.7907.

^{1}H NMR and JMOD NMR (150 MHz, DMSO-d_6) FIGS. 3-4.

(+)-ESI-TOF-MS/MS: FIG. 5.

- Characteristics of N_3-PEG3-Van (3) obtained were as follows

10 Yield: 760 mg, 34%

LCMS: R_t = 2.9 min, @ 200 nm, $[M+3H]^{3+}$ = 550.8, Purity by UV @ 200 nm > 95%

(+)-ESI-HRMS calc for $C_{74}H_{93}Cl_2N_{13}O_{26}$ $[M+2H]^{2+}$: 824.7866, found 824.7861.

15 ^{1}H NMR and JMOD NMR (150 MHz, DMSO-d_6) FIGS. 3-4.

(+)-ESI-TOF-MS/MS: FIG. 6.

Synthesis and Characterization of Constructs

Fluorescent vancomycin probes were prepared by a single step synthesis using vancomycin N_3-C8-Van and N_3-PEG3-Van, set forth in FIG. 2. To avoid the 20 steric or electronic interaction of components with charged cell walls, fluorophores 7-nitrobenzofurazan (NBD) and 7-(dimethylamino)-coumarin-4-acetic acid (DMACA) were utilised as they have low molecular weight and/or no electronic charges, and it was considered likely that these would cause minimal disturbance to antimicrobial activity. These fluorophores have previously been successfully used to prepare other 25 fluorescent antibiotics [1]-[2]. Fluorophores NBD and DMACA were selected to prepare two colours (green and blue) of fluorescent vancomycin probes.

As set forth in FIG. 2, NBD-alkyne (labelled '10') and DMACA-alkyne (labelled '11') precursors were produced. A schematic of the production of 10 and 11 is provided in FIG. 2A. Details of the synthesis are as follows.

30 To produce NBD-alkyne (10), to a solution of 4-chloro-7-nitrobenzo[*c*][1,2,5]oxadiazole (300 mg, 1.5 mmol) in THF (10 mL) was added a solution of propargyl amine (110 μL, 1.65 mmol), Cs_2CO_3 (480 mg, 1.5 mmol). The reaction mixture was stirred at 50 °C for 4 h. After completion of the reaction, the reaction mixture was diluted with EtOAc (50 mL), washed with H_2O (30 mL), brine

(30 mL). The organic phase was separated, dried ($MgSO_4$), and evaporated to give the residue. The residue was purified by Si column chromatography (petroleum ether/EtOAc, 7:3) to give 10 (240 mg, 75%).

To produce DMACA-alkyne (11), a solution of the 2-(7-(dimethylamino)-2-oxo-2H-chromen-4-yl)acetic acid (0.3 g, 1.21 mmol) in DMF (5 mL) was added HATU in DMF (5 mL) followed by DIPEA (386 μL), and propargylamine (71 μL, 1.1 mmol). The solution was stirred at RT overnight. The reaction was evaporated under reduced pressure to remove DMF. The residue was diluted with water and extracted with ethyl acetate, dried over $MgSO_4$, and concentrated under reduced pressure. The crude compound was recrystallized in CH_2Cl_2. The solid was filtrated and washed with CH_2Cl_2 to give DMACA-alkyne (11) (0.149 g, 48%) as a green solid.

As set forth in FIG. 2, constructs (labelled '12', '13'. and '14') were synthesised then using NBD-alkyne (10) and DMACA-alkyne (11), via CuAAC reactions. $CuSO_4 \cdot 5H_2O$ and sodium ascorbate at 40 °C in DMF/ H2O (1:9) for 20 hours was used for conjugation [3]. It has been reported that acetic acid as a proton source accelerated the conversion of C-Cu bond-containing intermediate (Cu (I) acetylide and 5-cuprated 1,2,3-triazole) in CuAAC reaction, leading to reduction of byproduct since byproduct is increased by prolonging the reaction time. In addition, combination of $CuSO_4$/ NaASb is normally used in aqueous *t*-BuOH. Therefore, CuAAC reaction of vancomycin probes was optimized by using the solvent mixture of DMF/*t*-BuOH/H_2O and adding acetic acid to accelerate the reaction. It is known that the electronic effect of the alkyne influences the formation of Cu (I) acetylide. We also found that adding excess amount of catalysts did not improve the outcome of the reaction but increased unidentified by-product. Thus, quantities of $CuSO_4$ and NaASb were evaluated for each fluorophore.

Specifically, a mixture of azido-vancomycin (1 eq.) and alkyne-fluorophore (1.5 eq.) were dissolved in DMF (5 mL), followed by adding *t*-BuOH (5 mL) and H_2O (5 mL). Aqueous $CuSO_4$ (0.2 eq. for NBD, 0.5 eq. for DMACA, dissolved in 500 μL of water), aqueous sodium ascorbate (0.4 eq. for NBD, 1 eq. for DMACA, dissolved in 500 μL of water), and acetic acid (10 eq. for NBD, 20 eq. for DMACA) were added to the reaction mixture. The reaction mixture was stirred in a microwave reactor at 100 °C for 15 min. The reaction mixture was concentrated under reduced pressure to

yield the crude product. The crude compounds were pre-purified by MPLC over C18 silica gel (Grace Reveleris, A: H_2O (0.1% TFA), B: ACN (0.1% TFA), 0 $\rightarrow$ 100% B over 8 min). The constructs 12-14 were repurified by Prep HPLC (flow 20 mL/min, mobile phases A = 0.1% TFA in water and B = 0.1% TFA in acetonitrile, gradient 5 5$\rightarrow$100% B over 20 min) and then lyophilized to yield final constructs 12-14.

Quantity of $CuSO_4$/NaASb for NBD-alkyne (10) conjugation with the linker was less than for DMACA-alkyne (11), indicating that 10 is more reactive than 11 in this context. Under microwave conditions, CuAAC reactions of azido-vancomycin and fluorophores dissolved in DMF/*t*-BuOH/H_2O with $CuSO_4$, NaASb, HOAc as 10 catalyst were completed within 15 min at 100 °C.

N_3-C8-Van (150 mg, 9.37 x 10^{-5} mol) was reacted with NBD-alkyne (10) to give visualization construct 12 in the form of an orange powder (34 mg, 20%). Construct 12 is herein referred to as Vanco-8C-Tz-NBD, or alternatively NBD-Tz-C8-Van.

15 N_3-C8-Van (113 mg, 7.03 x 10^{-5} mol) was reacted with DMACA-alkyne (11) to give visualization construct 13 in the form of a green powder (22 mg, 16%). Construct 13 is herein referred to as Vanco-8c-Tz-DMACA, or alternatively DMACA-Tz-C8-Van.

N_3-PEG3-Van (150 mg, 9.09 x 10^{-5} mol) was reacted with NBD-alkyne (10) to 20 give visualization construct 14 in the form of an orange powder (34 mg, 18%). Visualization construct 14 is herein referred to as Vanco-PEG3-Tz-NBD, or alternatively NBD-Tz-PEG3-Van.

- Characteristics of NBD-Tz-C8-Van (12) obtained were as follows

LCMS: R_t = 3.74 min, @ 200 nm, $[M+2H]^{2+}$ = 607.5, Purity by UV @ 25 200 nm > 95%

(+)-ESI-HRMS calc for $C_{83}H_{100}Cl_2N_{17}O_{26}$ $[M+3H]^{3+}$: 606.8801, found 606.8801.

^{1}H NMR and JMOD NMR (150 MHz, DMSO-d_6) FIGS. 3 and 7.

(+)-ESI-TOF-MS/MS: FIG. 8.

30 - Characteristics of DMACA-Tz-C8-Van (13) obtained were as follows

LCMS: R_t = 3.71 min, @ 200 nm, $[M+2H]^{2+}$ = 629.6, Purity by UV @ 200 nm > 95%

(+)-ESI-HRMS calc for $C_{90}H_{110}Cl_2N_{15}O_{26}$ $[M+3H]^{3+}$: 628.9041, found 628.9051.

^1H NMR and JMOD NMR (150 MHz, DMSO-d_6) FIGS. 3 and 7.

(+)-ESI-TOF-MS/MS: FIG. 9.

5 - Characteristics of NBD-Tz-PEG3-Van (14) obtained were as follows

LCMS: R_t = 3.24 min, @ 200 nm, $[M+2H]^{2+}$ = 623.5, Purity by UV @ 200 nm > 95%

(+)-ESI-HRMS calc for $C_{83}H_{100}Cl_2N_{17}O_{29}$ $[M+3H]^{3+}$: 622.8750, found 622.8773.

10 ^1H NMR and JMOD NMR (150 MHz, DMSO-d_6) FIGS. 3 and 7.

(+)-ESI-TOF-MS/MS: FIG. 10.

Example 2. Assessment of Constructs and Adducts

Biological Activity

The synthesized constructs and glycopeptide adducts were tested for 15 antimicrobial activity against a panel of Gram-positive bacteria including ATCC reference strains and clinical isolates of *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Enterococcus faecalis* (*E. faecalis*), and *Enterococcus faecium* (*E. faecium*) (FIG. 11). Vancomycin and a vancomycin adduct comprising a first linker containing a 3 carbon linear chain (Van-3C-N_3) were 20 included as controlled.

Specifically, bacteria were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA), Merck Sharp & Dohme (Kenilworth, NJ), the Coli Genetic Stock Center (CGSC, Yale University), and independent academic clinical isolate collections. Bacteria were cultured in Muller Hinton broth (MHB) 25 (Bacto Laboratories, Cat. no. 211443) at 37 °C overnight. A sample of each culture was then diluted 50-fold in MHB and incubated at 37 °C for 1.5-3 h. The constructs and compounds were serially diluted two-fold across the wells, with concentrations ranging from 0.06 μg/mL to 128 μg/mL, plated in duplicate. The resultant mid-log phase culture was diluted to $1x10^6$ CFU/mL, then 50 μL was added to each well of the 30 compound-containing 96-well plates (Corning; Cat. No 3641, NBS plates), giving a cell density of 5×10^5 CFU/mL, and a final compound concentration range of 0.03 μg/mL to 64 μg/mL. All the plates were covered and incubated at 37 °C for 18 h with

the Minimum Inhibitory Concentration (MIC) defined as the lowest compound concentration at which no bacterial growth was visible (n = 4).

Surprisingly, NBD-Tz-C8-Van (12) and DMACA-Tz-C8-Van (13) showed generally increased activity against this Gram-positive panel compared to 5 vancomycin, while NBD-Tz-PEG3-Van maintained antimicrobial potency (FIG. 11). Among these results, it was surprisingly observed that NBD-Tz-C8-Van (12) and DMACA-Tz-C8-Van (13) exhibited increased activity, as compared to vancomycin, against VRSA (vancomycin; MIC = 64 μg/mL, 12; MIC = 16 μg/mL, 13; MIC = 32 μg/mL) and VRE (vancomycin; MIC = >64 μg/mL, 12; MIC = 32 μg/mL, 17; MIC = 10 32 μg/mL).

It was also surprisingly observed that the glycopeptide adduct N_3-C8-Van exhibited generally increased activity against the Gram positive panel (FIG. 11), including increased activity against VRSA and VRE.

These results demonstrate that combining vancomycin with particular linkers 15 (e.g. linkers comprising C8) may result in glycopeptide adducts with increased activity towards Gram positive bacteria, as compared to the free glycopeptide antibiotic.

Additionally, these results demonstrate that constructs of the invention comprising fluorescent probes with small molecular size and/or neutral charge 20 coupled to a glycopeptide antibiotic (vancomycin) may be particularly suitable for binding to Gram positive bacteria. Furthermore, these results demonstrate that constructs of the invention comprising such fluorescent probes linked to a glycopeptide antibiotic (vancomycin) by particular linkers (e.g. linkers comprising C8) may result in constructs with increased activity towards Gram positive bacteria, 25 as compared to the corresponding free glycopeptide antibiotic.

Microorganism visualization

Super-resolution structured illumination microscopy (SR-SIM) has been used to successfully show bacterial cell structures. Thus, an assessment of fluorescent imaging of *S. aureus* labeled with constructs 12-14 using SR-SIM was conducted 30 (FIG. 12).

The constructs showed strong fluorescence at the dividing septum compared to the lateral wall, confirming binding of the construct via the glycopeptide antibiotic to the nascent peptidoglycan Lipid II. Constructs comprising linkers comprising PEG3 (i.e. NBD-Tz-PEG3-Van; 14) and C8 (i.e. NBD-Tz-C8-Van; 12) did not show

substantial differences in selective binding. Notably however, construct 12 was approximately 4-fold more active than construct 14 against the Gram-positive panel.

Vancomycin probes have been used to study mechanism of bacterial cell division such as co-labelling with PG synthesis proteins [5], fluorescence microscopy of vancomycin-labelled bacteria showed the dynamics of peptidoglycan assembly in ovococci [6] and the PG biosynthesis in *B. subtilis* [10]. Most bacterial cells grow and divide by building a new cell wall disc called the septum, which is the structure that forms in the middle of the mother cell by invagination of the cell membrane and ingrowth of the cell wall, which splits the mother cell into two identical daughter cells [8] Thus, during cell division, ingrowth of the new PG, invagination of the cell membrane, and chromosome segregation were shown by labelling with vancomycin probes, membrane-labeling probe, nucleic acid-labelling probe, respectively (FIG. 13). Visualization of *S. aureus* cell by 3D-SIM clearly showed the dividing septum ring.

The vancomycin probes are able to spatially discriminate the peptidoglycan layer from the bacterial cell membrane as shown by cross-section measurements in FIG. 14A, which clearly show the peak of probe intensity outside of the peak intensity of a membrane-selective probe. Thus, vancomycin probes are potentially useful for assessing the target sites of other antibiotic probes such as TMP and linezolid probes. Co-staining of DMACA-Tz-C8-Van (13) (blue) with TMP and linezolid NBD probes (green) showed that the linezolid probe and TMP probe penetrated within the bacterial cell membrane into the cytosol. Both TMP and linezolid generally showed co-localisation with FM4-64FX (membrane dye) indicating that the membrane dye was quickly endocytosed in *S. aureus* (FIG. 14B-C).

Overall, the results of this example indicate that constructs as described herein as suitable for specific fluorescent labelling of Gram-positive bacteria.

Example 3. Production and Assessment of Further Compounds, Constructs and Glycopeptide Antibiotic Adducts

HXPI

To produce fluorescent compound (*E*)-2-(2-(6-hydroxy-2,3-dihydro-1*H*-xanthen-4-yl)vinyl)-3,3-dimethyl-1-propyl-3*H*-indol-1-ium iodide (HXPI), potassium carbonate (134 mg, 0.99 mmol) was suspended in 5 mL ACN and put under nitrogen. Resorcinol (104.6 mg, 0.95 mmol) was added and the white suspension stirred at room temperature for 15 mins. IR-780 (239.4 mg, 0.36 mmol) was suspended in 5

mL ACN, then added to the resorcinol suspension, and the dye flask rinsed with an additional 5 mL ACN. The combined green suspension was heated to 50°C for 1.5 h, then cooled and dry loaded onto silica. Flash chromatography on silica gel was carried out from 0 - 100% MeOH in DCM, then pure HXPI was concentrated under to
5 reduced pressure to form an iridescent blue solid (152.5 mg, 79%).

LCMS analysis was performed: R_t = 6.27 min, C4T11, $[M]^+$ = 412.2.

The structure of HXPI is given in FIG. 15(A).

PXPI

To produce fluorescent compound *(E)*-3,3-dimethyl-2-(2-(6-(prop-2-yn-1-
10 yloxy)-2,3-dihydro-1H-xanthen-4-yl)vinyl)-1-propyl-3H-indol-1-ium iodide (PXPI), HXPI (180 mg, 0.333 mmol) was dissolved in 10 mL dry DMF and put under an atmosphere of nitrogen. Sodium hydride (60% in mineral oil, 115 mg, 1.66 mmol) was added and the reaction stirred for 15 mins, during which time the colour changed from dark blue to pink. Propargyl bromide (0.1 mL, 0.9 mmol) was added and the
15 reaction heated to 60°C for 16 h. The resulting yellow/green mixture was cooled, quenched with a few drops of methanol, and concentrated under reduced pressure. Crude product was purified by normal phase silica chromatography (0 - 100% MeOH in DCM) to give PXPI as an iridescent blue solid (28.3 mg, 15%).

LCMS analysis was performed: R_t = 7.23 min, C4T11, $[M]^+$ = 450.2.

20 ^{1}H NMR ($CDCl_3$, 600 MHz) analysis was also performed: δ 8.64 (d, $^3J_{14,13}$ = 14.7 Hz, 1H, H-14), 7.50 (d, $^3J_{5,4}$ =7.4 Hz, 1H, H-5), 7.47 (d, $^3J_{4,5}$ =7.4 Hz, 1H, H-4), 7.42-7.39 (m, 2H, H-26 + H-27), 7.22 (s, 1H, H-21), 6.95 (m, 1H, H-24), 6.93 (m, 1H, H-2), 6.63 (d, $^3J_{13,14}$ = 14.4 Hz, 1H, H-13), 4.85 (s, 2H, H-28), 4.53 (m, 2H, H-10), 2.79 (m, 2H, H-16), 2.73 (m, 2H, H-18), 2.63 (s, 1H, H-30), 1.98 (m, 2H, H-11), 1.93
25 (m, 2H, H-17), 1.80 (s, 6H, H-9), 1.09 (t, $^3J_{12,11}$ = 6.9 Hz, 3H, H-12); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 177.82 (C, C-8), 161.30 (C, C-20), 154.28 (C, C-23), 145.98 (CH, C-14), 133.14 (CH, C-21), 129.37 (CH, C-3), 127.50 (CH, C-4), 122.63 (CH, C-5), 116.56 (C, C-22), 115.35 (C, C-15), 113.30 (CH, C-2), 113.12 (CH, C-26 +C-27), 105.10 (CH, C-13), 102.41 (CH, C-24), 77.43 (CH, C-30), 76.69 (C, C-29), 56.73
30 (CH_2, C-28), 50.80 (C, C-29), 47.66 (CH_2, C-10), 29.46 (CH_2, C-18), 28.47 (CH_3, C-9), 24.90 (CH_2, C-16), 21.61 (CH_2, C-11), 20.44 (CH_2, C-17), 11.79 (CH_3, C-12).

The structure of PXPI is given in FIG. 15(B).

DOTA-alkyne

To produce chelating compound 2,2',2''-(10-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA-alkyne), The NHS ester of DOTA (104.7 mg, 0.137 mmol) was dissolved in 3 mL DMF under argon, then propargylamine (17 μL, 0.27 mmol) was added. The yellow solution was agitated at room temperature overnight, then concentrated under reduced pressure to give a yellow oil. Crude product was purified by MPLC (0 - 100% ACN in water) then lyophilised to give 2,2',2''-(10-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid as an off white solid (89.4 mg, 97%).

LCMS analysis was performed: $R_t = 0.71$ min, C4T11, $[M+H]^+ = 442.2$.

The structure of DOTA-alkyne is given in FIG. 15(C).

N_3-C8-Van

An additional batch of N_3-C8-Van adduct was produced as follows. Vancomycin-HCl (511 mg, 0.344 mmol) was dissolved in 25 mL DMF, and PyBOP (199 mg, 0.383 mmol) was added, followed by DiPEA (0.44 mL, 2.53 mmol). 8-Azido-octylamine (142 mg, 0.686 mmol) was dissolved in 1 mL DMF, then added to the vancomycin solution. The reaction was stirred at room temperature for 16 h, then quenched with MeOH and concentrated to roughly half the original volume under reduced pressure. The concentrated reaction was injected directly onto a MPLC reverse phase column (0 - 100% ACN (0.1% TFA) in water (0.1% TFA)) to give vanco-C_8-N_3 as a white solid.

LCMS analysis was performed: $R_t = 3.57$ min, C4T09, $2[M+H]^+ = 801.5$.

^{1}H NMR (d6-DMSO, 600 MHz) was also performed: δ 9.31 (s, 1H, H-7e-OH), 8.98 (s, 1H, H-5d-OH), 8.97 (s, 1H, H-5d-OH), 8.67 (s, 1H, H-W5), 8.42 (s, 1H, H-W7), 7.86 (s, 1H, H-6b), 7.85 (s, 1H, H-NH-L1), 7.54 (d, J = 8.3 Hz, 1H, H-2f), 7.45 (s, 1H, H-2b), 7.44 (s, 1H, H-6f), 7.33 (d, J = 8.3 Hz, 1H, H-6e), 7.19 (s, 1H, H-5b), 7.18 (s, 1H, H-2e),7.05 (s, 2H, C3'-NH_2), 6.75 (d, J = 8.6 Hz, 1H, H-5f), 6.72 - 6.65 (m, 2H, H-5e + H-W6), 6.54 (d, J = 1.8 Hz, 1H, H-W3), 6.25 (d, J = 1.9 Hz, 1H, H-7f), 5.95 (s, 1H, H-Z2-OH), 5.86 (d, J = 6.3 Hz, 1H, H-Z6-OH), 5.75 (d, J = 8.1 Hz, 1H, H-X4), 5.60 (s, 1H, H-4b), 5.46 (d, J = 6.7 Hz, 1H, H-V4-OH), 5.36 (d, J = 5.9 Hz, 1H, H-G3-OH), 5.24 (m, 3H, H-Z6 + H-G1 + H-V1), 5.18 (s, 2H, H-Z2 + H-4f), 5.10 (d, J = 3.8 Hz, 1H, H-G4-OH), 4.92 (s, 1H, H-X2), 4.68 (d, J = 6.8 Hz, 2H, H-V5), 4.44 (s, 1H, H-X5), 4.38 (d, J = 5.8 Hz, 1H, H-X7), 4.20 (s, 1H, H-X3), 4.19

(s, 1H, H-X6), 4.03 (t, J = 5.5 Hz, 1H, H-G6-OH), 3.68 (d, J = 8.0 Hz, 2H, H-G6), 3.54 (s, 1H, H-G2) 3.53 (s, 1H, H-G6), 3.30 (complex m, H_2O + H-G3 + H-L8-N3), 3.27 (s, 2H, H-G4 + H-G5), 3.17 (d, J = 6.9 Hz, 1H, H-V4), 3.12 (q, J = 7.2 Hz, 1H, H-L1), 2.61 (s, 4H, H-1e + H-3a), 2.15 (d, J = 9.0 Hz, 1H, H-3a), 1.94 - 1.85 (m, 1H, H-V2), 1.80 - 1.70 (m, 2H, H-V2 + H-1a), 1.65 (s, 1H, H-1b), 1.52 (q, J = 7.3 Hz, H-L3 + H-1a), 1.46 (d, J = 6.8 Hz, 1H, H-L2), 1.29 (m, H-L4 + H-V7), 1.07 (d, J = 6.4 Hz, 3H, H-V6), 0.91 (s, 3H, H-1c), 0.91 (s, 1H, H-5d), 0.86 (s, 3H, H-1d), 0.85 (s, 1H, H-C3); ^{13}C NMR (d6-DMSO, 150 MHz) δ 170.5 (3xC), 169.9 (C), 169.2 (3xC), 167.9 (6xC), 157.1 (C, C-7e), 156.3 (C, C-7c), 155.1 (CH, C-5d), 152.6 (C, C-4c), 151.3 (C, C-4e), 150.0 (C, C-2d), 148.2 (C, C-6d), 142.6 (C, C-6a), 139.9 (C, C-2a), 137.8 (C, C-7a), 135.6 (CH, C-5b), 134.9 (C, C-4a), 131.9 (C, CH-4d), 128.6 (CH, C-2b), 127.5-127.2 (4xC, C-6b + C-6f + C-2f + C-2c), 126.3 (C, C-6c), 126.2 (C, C-5a), 125.3 (CH, C-5f), 124.4 (CH, C-2e), 123.4 (CH, C-6e), 121.9 (C, C-5c), 118.0 (C, C-7b), 116.4 (CH, C-5e), 107.4 (CH, C-4b), 106.5 (CH, C-7f), 104.7 (CH, C-4f), 106.5 (CH, C-7f), 104.7 (CH, C-4f), 102.1 (C, C-7d), 101.3 (CH, C-G1), 96.8 (CH, C-V1), 78.2 (CH, C-G2), 77.1 (CH, C-G3), 76.8 (CH, C-G5), 71.5 (CH, C-Z6), 71.3 (CH, C-Z2), 70.8 (CH, C-V4), 70.1 (CH, C-G4), 63.1 (CH, C-V5), 62.1 (CH, C-X6), 61.3 (CH_2, C-G6), 59.3 (CH, C-X1), 58.9 (CH, C-X2), 57.5 (CH, C-X7), 55.0 (CH, C-X4), 53.9 (C, C-V3), 53.7 (CH, C-X5), 51.0 (CH, C-X3), 50.6 (CH_2, C-L8), 39.5 (CD_3OD + C-1a), 38.9 (CH_2, C-L1), 33.2 (CH_2, C-V2), 30.7 (CH_3, C-1e), 29.2 (CH_2, C-L2), 28.7 (CH_2, C-L3), 28.5 (CH_2, C-L4), 28.2(CH_2, C-L5), 26.4 (CH_2, C-L6), 26.1 (CH_2, C-L7), 23.8 (CH, C-1b), 22.8 (CH_3, C-1d), 22.4 (CH_3, C-V7), 22.3 (CH_3, C-1c), 16.8 (CH_3, C-V6).

PXPI-Tz-C8-Van

Visualization construct PXPI-Tz-C8-Van was produced by dissolving N_3-C8-Van (17.5 mg, 0.0109 mmol) in 8 mL DMF, and PXPI alkyne (28.32 mg, 0.0491 mmol) was added and the blue solution stirred at 50°C for 1 hour. CuI (37.6 mg, 0.197 mml), then DiPEA (200 μL, 1.2 mmol), then AcOH (200 μL, 3.6 mmol) were added and the reaction stirred for 25 mins. The blue mixture was cooled and injected directly onto a MPLC reverse phase column (0 - 100% ACN (0.1% FA) in water (0.1% FA)) to give semi-pure vanco-C_8-PXPI as a blue solid. This was then further purified using HPLC (0 - 100% ACN (0.1% TFA) in water (0.1% TFA)) to give pure PXPI-Tz-C8-Van as a blue solid (9.6 mg, 41%).

The structure of this construct is given in FIG. 16(A).

PXPI-Tz-PEG3-Van

Visualization construct PXPI-Tz-PEG3-Van was produced similarly as described for PXPI-Tz-C8-Van, with the exception that N_3-PEG3-Van was used in
5 place of N_3-C8-Van.

The structure of this construct is given in FIG. 16(B).

DOTA-Tz-C8-Van

Visualization construct DOTA-C8-Van was produced by dissolving N_3-C8-Van (10.13 mg, 0.00632 mmol) in 6 mL DMF, and DOTA-alkyne (13.83 mg, 0.0314
10 mmol) was added and stirred at 50°C for 30 min. CuI (25.26 mg, 0.133 mml), then DiPEA (130 μL, 0.75 mmol), then AcOH (130 μL, 2.2 mmol) were added and the reaction stirred for 10 mins. The reaction was cooled and injected directly onto a MPLC reverse phase column (0 - 100% ACN (0.1% FA) in water (0.1% FA)) and all peaks after the initial 0% flush were combined and lyophilised. The resulting solid
15 was dissolved in 1:1 *v/v* ACN/H_2O, and Na_2S-$9H_2O$ was added. The suspension was vortexed and centrifuged to precipitate copper sulfide species. This process was repeated until no more precipitation was observed.

The structure of this construct is given in FIG. 17(A).

DOTA-Tz-PEG3-Van

20 Visualization construct DOTA-Tz-PEG3-Van was produced similarly as described for DOTA-Tz-C8-Van, with the exception that N_3-PEG3-Van was used in place of N_3-C8-Van.

The structure of this construct is given in FIG. 17(B).

Example 4. Assessment of Membrane Damage in Gram Negative Bacteria using
25 Visualization Constructs

An assessment of the use of visualization constructs to detect membrane damage in Gram negative bacteria was performed. More particularly, *Escherichia coli* (strain ATCC 25922) was exposed to various compounds (as set out in Table 1), and binding of visualization constructs to the bacteria was subsequently assessed. Where
30 binding was detected, this was indicative of activity of the compound to disrupt the Gram negative outer membrane, and allow the visualization construct access to peptidoglycan within the *E. coli* cell wall.

Minimum Inhibitory Concentration

To assess whether the compounds tested had inhibitory activity on *E. coli*, MIC analysis using a micro-broth dilution assay was performed. Vancomycin HCl and visualization construct NBD-Tz-PEG3-Van were included as controls.

- Compound preparation

5 TMP (trimethoprim) was in 20% DMSO/H2O; all other compounds were supplied 1.28 mg/mL in water. The maximum concentration used for screening was 64 μg/ml for erythromycin and citropin 1.1 (and the vancomycin HCl and NBD-Tz-PEG3-Van controls) and 32 μg/ml for all other antibiotics.

- Micro-broth Dilution Assay

10 Bacteria was cultured in Mueller Hinton broth (MHB) (Bacto laboratories, Cat. no. 211443) and Mueller Hinton Broth Cation adjusted (CaMHB) (Bacto laboratories, Cat. no. 212322) at 37 °C overnight. The culture was then diluted 50-fold in fresh CaMHB/MHB broth and incubated at 37 °C for 2-3 hrs.

The compound was serially diluted two-fold across the wells of 96-well micro-
15 titre plates (corning; Cat. No 3370, non-treated polystyrene plate) and NBS 96-well micro-titre plates (corning; Cat. No 3461, non-binding surface), with concentrations ranging from 0.06 μg/mL to 128 μg/mL, plated in duplicate. The resultant mid-log phase culture was diluted to 1×10^6 CFU/mL, then 50 μL was added to each well of the compound-containing 96-well plates giving a cell density of 5×10^5 CFU/mL, and
20 a final compound concentration range of 0.03 μg/mL to 64 μg/mL for citropin 1.1 and of 0.015 μg/mL to 32 μg/mL for other antibiotics.

The plates were covered and incubated at 37 °C for 22 h. MICs were determined visually, being defined as the lowest concentration showing no visible growth. MICs were determined visually at 22 h incubation and the MIC was defined
25 as the lowest concentration with which no growth was visible after incubation.

-Results

Results of the MIC assessment are set forth in Table 1.

Detection of Membrane Damage

- Compound preparation

30 All compounds were supplied 1.28 mg/mL in water, TMP and erythromycin were dissolved in 20% DMSO/H2O. Compounds were then diluted with water to get desired concentrations:

- NBD-Tz-PEG3-Van at 32 μg/mL in HBSS: 500 μL for one sample

2. - Each antibiotic compound at 0.125, 1.25, 6.25, 12.5, 125 μg/mL in HBSS: 1 mL for one sample.

- Permeability test in E. coli (ATCC 25922)

E. coli were cultured in LB at 37°C overnight. A sample of each culture was then diluted 50-fold in LB and incubated at 37°C for 1.5-2 h. The resultant mid log phase cultures were harvested at 4000 rpm for 15 min, washed once with HBSS (4000 rpm, for 15 min), and resuspended in HBSS to an OD600 of 1.

- Compound treatment

(1) A solution (1 mL) antibiotics at 0.125, 1.25, 6.25, 12.5, 125 μg/mL was added to an Eppendorf tube containing bacteria pellets, left for 1 hour at 37 °C with shaking, and then centrifuged (14,000 g, 2 min) and washed once with HBSS. 3 replicates for each concentration;

(2) A solution (500 μL) of NBD-Tz-PEG3-Van (32 μg/mL in HBSS) was added to an Eppendorf tube containing bacteria pellets, left for 30 min at 37 °C, and then centrifuged and washed once with HBSS.

(3) Pellets were suspended in 1 mL of HBSS for FACS

- Flow cytometry

For fluorescence intensity measurement using the flow cytometer (Gallios flow cytometer; Beckman Coulter), bacteria cell pellets were resuspended in 1 mL of PBS. A total of 40,000 events were collected, and the data were analyzed using Kaluza Analysis 1.5 software. Fluorescent intensity from FL1 (excitation, 488 nm; emission, 525/20 nm) was plotted against the number of events count on a histogram. Gates were created based on fluorescent intensity to discriminate between unstained (no permeabilisation), partially stained (partial permeabilisation) and completely stained (full permeabilisation) cells. The percent of these gated events were plotted against the concentration of antibiotics.

- Results

Results of the fluorescence intensity assessment using flow cytometry are set out in Figure 18. A summary of assay results for the eight antibiotic compounds PMXB (polymyxin B) sulphate, Colistin sulphate, OCT C4 TFA (octapeptin C4 trifluoroacetate salt), Tachyplesin-1, Arenicin-3, Gentamicin, TMP, Citropin 1.1, and Erythromycin is set forth in Table 2.

Notably, there is significantly greater fluorescence signal from compounds known to act by membrane disruption, such as polymyxin B, colistin, octapeptin,

tachyplesin, arenicin and citropin, compared to compounds known to kill bacteria by different mechanisms (gentamicin, erythromycin, trimethoprim). Also, compounds of a similar class (e.g. polymyxin B and colistin, or arenicin and tachyplesin) gave similar results, indicating that this method could be potentially applied to determining 5 the mechanism by which unknown compounds act.

Throughout the specification, the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Various changes and modifications may be made to 10 the embodiments described and illustrated without departing from the present invention.

The disclosure of each patent and scientific document, computer program and algorithm referred to in this specification is incorporated by reference in its entirety.

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TABLES

Table 1. Minimum Inhibitory Concentration (MIC) in µg/mL of antibiotics against *E. coli* strains in MHB or CaMHB using non-treated polystyrene plates or NBS plates.

Compound ID	Batch	Compound	non-treated polystyrene plates *E. coli* ATCC 25922		NBS plates
			MHB	CaMHB	MHB
MCC_000636	003	PMXB sulphate	0.125	0.125	0.03
MCC_000094	002	Colistin sulphate	0.25	0.25	0.06-0.25
MCC_000631	008	OCT C4 TFA	4	4	1
MCC_008385	003	Tachyplesin-1	2-4	2-8	0.125-0.25
NZ_17000		Arenicin-3	2	1-4	0.25-0.5
MCC_000437	003	Gentamicin sulfate	2	1	1
MCC_000191	002	TMP	1	1-2	1
	004	Citropin 1.1	32	64	32
MCC_008136	001	Erythromycin	64	64	nd
MCC_00095	010	Vancomycin HCl	>64	>64	>64
MCC_008777	001	Van-3PEG-Tz-NBD	>64	>64	>64

Table 2. Compounds tested for activity in disrupting Gram negative bacterial membranes using constructs of the invention, and assay results.

Compound	Mode of action of compound	Assay result
polymyxin B (PMXB) sulphate	Membrane disrupting peptide	Positive
Colistin sulphate	Membrane disrupting peptide	Positive
octapeptin C4 trifluoroacetate salt (OCT C4 TFA)	Membrane disrupting peptide	Positive
Tachyplesin-1	Membrane disrupting peptide	Positive
Arenicin-3	Membrane disrupting peptide	Positive
Gentamicin	Inhibit protein synthesis	Negative
TMP	Inhibit DHFR in the folic acid synthesis pathway	Negative
Citropin 1.1	Membrane disrupting peptide (more active against Gram-positive bacteria)	Positive (at high concentration)
Erythromycin	Inhibit protein synthesis	Negative

CLAIMS

1. A construct comprising:
   (i) an optionally derivatized glycopeptide antibiotic;
5 (ii) a visualization component; and
   (iii) a first linker connecting (i) and (ii).

2. The construct of claim 1, wherein the visualization component is a light-emitting component.

10

3. The construct of claim 2, wherein the light-emitting component emits light in the visual spectrum.

4. The construct of claim 3, wherein the light-emitting component emits light in
15 the infrared spectrum.

5. The construct of any one of claims 2-4, wherein the light emitting component is or comprises a fluorescent component.

20 6. The construct of claim 5, wherein the fluorescent component is an organic compound.

7. The construct of claim 6, wherein the fluorescent component has a neutral charge.

25

8. The construct of any one of claims 5-7, wherein the fluorescent component has a molecular weight of about less than 500 Da.

9. The construct of any one of claims 5-8, wherein the fluorescent component is
30 selected from the group consisting of NBD, DMACA, dansyl, BODIPY, HXPI, PXPI, or derivatives thereof.

10. The construct of any one of claims 1-9, wherein the light emitting component is or comprises a quantum dot.

11. The construct of claim 1, wherein the visualization component is an MRI visualization component.

5 12. The construct of claim 1, wherein the visualization component is a PET or SPECT visualization component.

13. The construct of claim 12, wherein the visualization component is selected from the group consisting of DOTA, NOTA, NODA-alkyne, bisamineoxime, and
10 tetraamine, or derivatives thereof.

14. The construct of claim 1, wherein the visualization component is an MPI visualization component.

15 15. The construct of claim 1, wherein the visualization component is a radiographic visualization component.

16. The construct of any preceding claim, wherein the glycopeptide antibiotic of the construct is selected from the group consisting: of vancomycin; teicoplanin;
20 oritavancin; telavancin; chloroeremomycin; and balhimycin.

17. The construct of claim 16, wherein the glycopeptide antibiotic is vancomycin.

18. The construct of any preceding claim, wherein the first linker comprises a
25 polyethylene glycol (PEG) moiety.

19. The construct of claim 18, wherein the PEG moiety is at least PEG3.

20. The construct of claim 19 wherein the PEG moiety is PEG3 or PEG4.

30

21. The construct of any one of claims 1-17, wherein the first linker comprises a linear carbon chain of greater than four carbons.

22. The construct of claim 21, wherein the linear carbon chain is C4-C15.

23. The construct of claim 22, wherein the linear carbon chain is C8 or C11.

24. The construct of any preceding claim, wherein the first linker comprises a
5 nitrogen-containing moiety at a first end of the linker.

25. The construct of claim 24, wherein the nitrogen-containing moiety is an amine-derived moiety.

10 26. The construct of claim 25, wherein the nitrogen-containing moiety is an amide bond connecting the first linker to the glycopeptide antibiotic.

27. The construct of any preceding claim, wherein the first linker comprises a nitrogen-containing moiety at a second end.

15

28. The construct of claim 27, wherein the nitrogen-containing moiety is an azide-derived moiety.

29. The construct of claim 28, wherein the nitrogen-containing moiety is a triazole
20 moiety.

30. The construct of any preceding claim, wherein the first linker is connected to the visualization component via a second linker.

25 31. The construct of claim 30, wherein the second linker comprises a linear carbon chain.

32. The construct of claim 31, wherein the linear carbon chain is C1-C4.

30 33. The construct of claim 31 or claim 32, wherein the second linker is connected to the first linker via a triazole moiety at the second end of the first linker.

34. The construct of any one of claims 30-33, wherein the visualization component is connected to the second linker via a moiety selected from the group

consisting of an amide, amine, sulphide, thioester, urethane, urea, sulphonamide, and ether.

35. The construct of claim 34, wherein the moiety is an amide moiety or an amine
5 moiety.

36. A method of binding a construct to a microorganism or a component thereof, the construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii), the method
10 including the steps of:

(a) combining the construct and a microorganism or component thereof; and

(b) selectively binding the glycopeptide antibiotic of the construct with the microorganism or component thereof,

to thereby bind the construct to the microorganism or component thereof.

15

37. A method of visualizing a microorganism or component thereof, the method including the steps of:

(a) combining a construct with the microorganism or component thereof, the construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a
20 visualization component; and (iii) a first linker connecting (i) and (ii);

(b) selectively binding the microorganism or component thereof to the glycopeptide antibiotic of the construct; and

(c) visualizing the construct bound to the microorganism or component thereof using the visualization component,

25 to thereby visualize the microorganism or component thereof.

38. A method of analysing a microorganism or component thereof, the method including the steps of:

(a) combining a construct with a microorganism or component thereof, the
30 construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii);

(b) selectively binding the microorganism or component thereof to the glycopeptide antibiotic of the construct; and

(c) visualizing the construct bound to the microorganism or component thereof using the visualization component; and

(d) analysing the microorganism or component thereof based on the visualization of step (c).

39. A method of diagnosing and/or monitoring a disease, disorder or condition, the method including the steps of analysing a microorganism or component thereof according to claim 38, and diagnosing a disease or condition based on the analysis of the microorganism or component thereof.

40. A method of treating a disease, disorder or condition, the method including the steps of diagnosing a disease, disorder or condition according to the method of claim 39, and treating the disease or condition based on the diagnosis.

41. A method of assessing a compound for activity in disrupting an outer cell membrane of a Gram negative microorganism, the method including the steps of:

(a) combining a microorganism with the compound to be assessed;

(b) combining a construct with the microorganism, the construct comprising (i) an optionally derivatized glycopeptide antibiotic; (ii) a visualization component; and (iii) a first linker connecting (i) and (ii); and

(c) determining the degree to which the construct is bound to the microorganism by visualization of the construct, wherein

the activity of the compound in disrupting the cell membrane of the microorganism is related to the degree to which the construct is bound to the microorganism.

42. The method of claim 41, wherein the Gram negative bacteria is *E. coli*.

43. A glycopeptide antibiotic adduct comprising an optionally derivatized glycopeptide antibiotic bound to a first linker.

44. The adduct of claim 43, wherein the glycopeptide antibiotic is selected from the group consisting of vancomycin; teicoplanin; oritavancin; telavancin; dalbavancin; chloroeremomycin; and balhimycin.

46. The adduct of claim 45, wherein the glycopeptide antibiotic is vancomycin.

47. The adduct of any one of claims 43-46, wherein the first linker comprises a
5 PEG group of at least PEG3.

48. The adduct of any one of claims 43-47, wherein the first linker comprises a linear carbon chain, wherein the linear carbon chain is C4-C15.

10 49. The adduct of any one of claims 43-48, wherein the first linker comprises an amine-derived moiety.

50. The adduct of any one of claims 43-49, wherein the first linker comprises an azide moiety.

15

51. A method of inhibiting, controlling, or killing a microorganism, the method including the step of contacting the construct of any one of claims 1-35 or the adduct of any one of claims 43-50 with a microorganism, to thereby inhibit, control, or kill the microorganism.

20

52. A composition for treating or preventing a disease, disorder, or condition in a subject, the composition comprising the construct of any one of claims 1-35, or the adduct of any one of claims 43-50.

25 53. A method of treating or preventing a disease, disorder, or condition in a subject, the method including the step of administering to a subject an effective amount of the construct of any one of claims 1-35, or the adduct of any one of claims 43-50, to thereby treat or prevent the disease, disorder, or condition in the subject.

30 54. Use of the construct of any one of claims 1-35, or the adduct of any one of claims 43-50, in the manufacture of a composition for the treatment or prevention of a disease, disorder, or condition in a subject.

55. A method of increasing the activity or efficacy of a glycopeptide antibiotic, the method including the step of connecting a glycopeptide antibiotic; a linker; and/or a visualization component to the glycopeptide antibiotic, to thereby increase the activity or efficacy of the glycopeptide antibiotic.

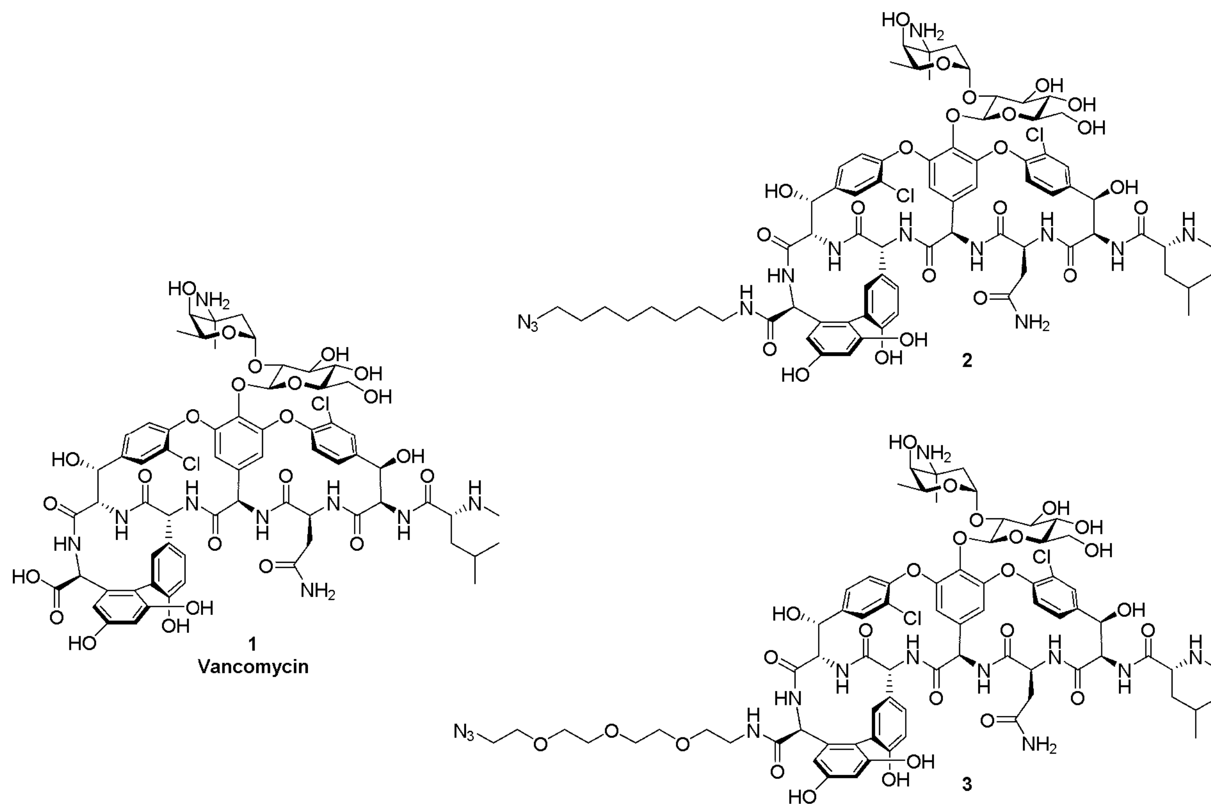
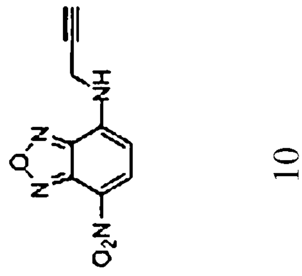
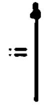
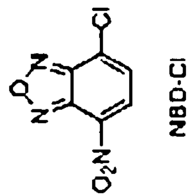
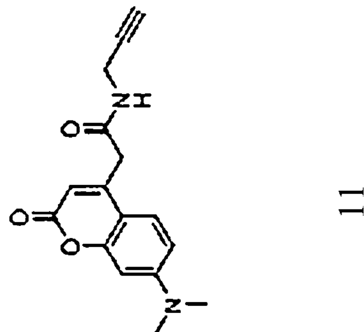


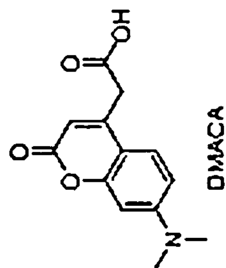
FIG. 1





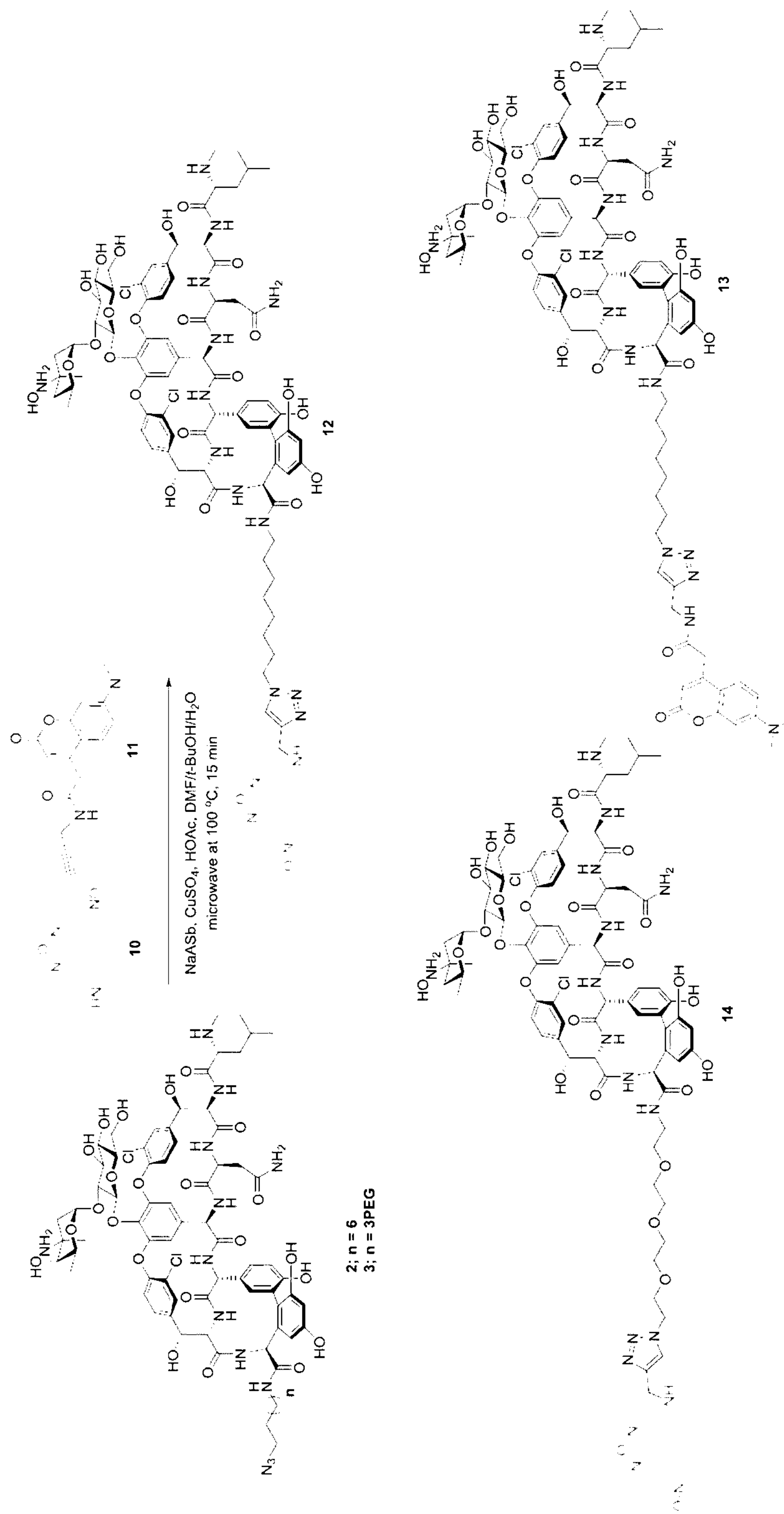






(A)

FIG. 2



(B)

FIG. 2 cont'd

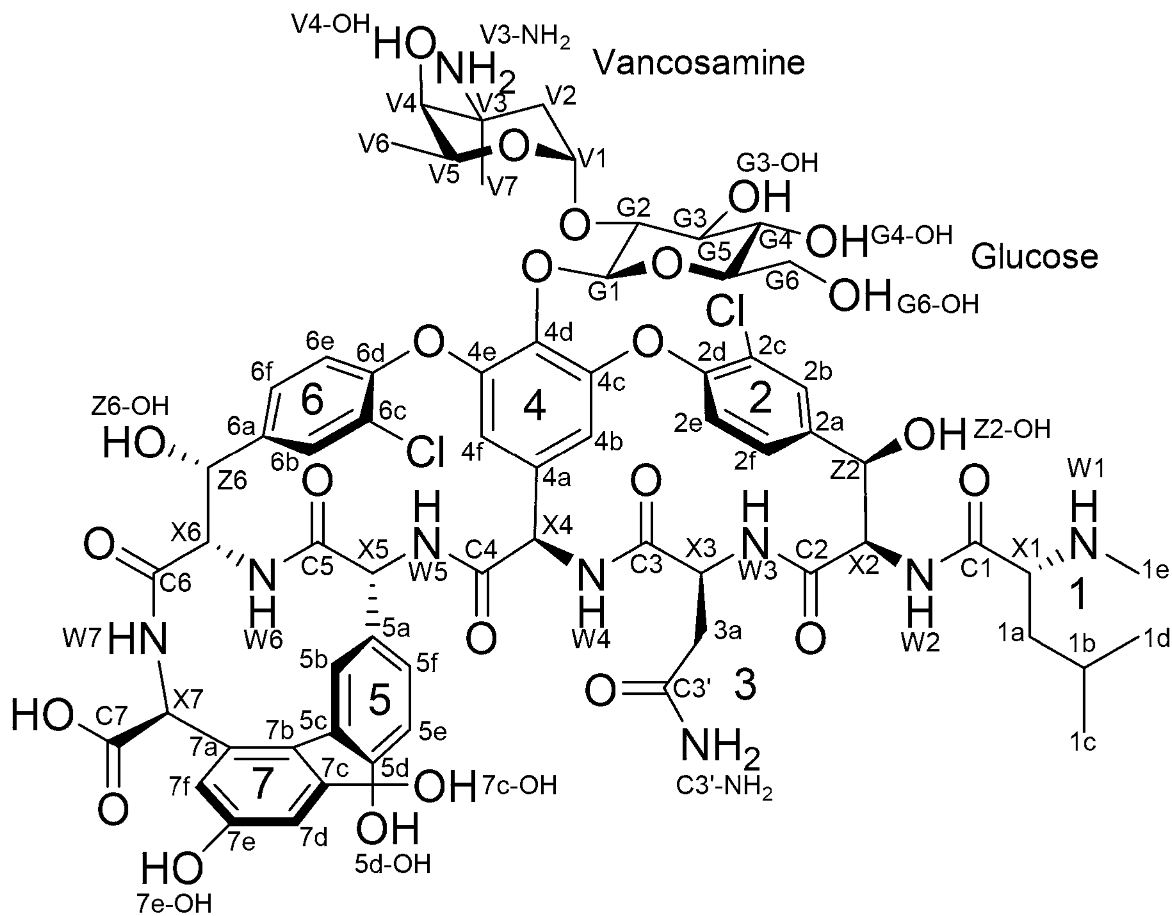


FIG. 3

Position		Vancomycin TFA		2		3
	JMOD	^{1}H	JMOD	^{1}H	JMOD	^{1}H
1						
C1			167.9			
X1	59.7	3.89 (br s)	59.6	3.95 (br s)	59.6	3.94 (s)
W1		n/o		n/o		n/o
1a	39.4	1.69-1.61 (m) 1.57-1.55 (m)	39.40	1.56-1.50 (m) 1.7-1.67 (m)	39.1	1.69-1.66 (m) 1.58-1.55 (m)
1b	23.7	1.69-1.61 (m)	23.70	1.65-1.61 (m)	23.7	1.64-1.61 (m)
1c	22.3	0.91 (d, 6.2)	22.30	0.91 (d, 6.2)	22.3	0.91 (d, 6.2)
1d	22.8	0.85 (d, 6.1)	22.8	0.85 (d, 6.1)	22.8	0.85 (d, 6.2)
1e	31.4	2.62 (s)	31.20	2.64 (s)	31.2	2.64 (s)
2						
C2						
X2	58.9	4.93 (br s)	58.90	4.93 (s)	58.9	4.94 (br s)
W2		n/o		n/o		n/o
Z2	71.3	5.19 (s)	71.30	5.19 (s)	71.3	5.19 (s)
Z2-OH		5.97 (s)		5.99 (s)		5.99 (d, 3.2)
2a	139.4		139.80		139.7	
2b	128.6	7.47 (br s)	128.60	7.46 (o)	128.6	7.46 (s)
2c	126.3		127.20		127.2	
2d	149.9		149.90		149.9	
2e	124.3	7.18 (d, 8.4)	124.30	7.18 (d, 8.7)	124.3	7.19 (d, 8.5)
2f	127.3	7.55 (d, 8.3)	127.30	7.55 (d,m 8.7)	127.3	7.55 (d, 8.3)
3						
C3	170.5		170.50			
X3	51.0	4.26 (v br s)	51.00	4.21 (v br s)	51	4.24 (br s)
W3		6.55 (v br s)		6.58 (br s)		6.55 (v br s)
3a	36.6	2.56 (o) 2.16 (br d, 14.7)	cannot see peak from HSQC	2.56 (o) 2.14 (br dd, 14.8, 6.0)	Cannot see from HSQC	2.57 (o) 2.16 (dd, 15.3, 9.4)
C3'	170.5		170.50			
C3'-NH2		7.04 (br s)		7.06 (v br s)		7.05 (s)
4						
C4	169.1		169.10		169.1	
X4	54.9	5.76 (d, 7.8)	54.90	5.76 (d, 7.8)	54.9	5.76 (d, 7.6)
W4		n/o		n/o		n/o
4a	134.8		134.80		134.8	
4b	107.3	5.58 (s)	107.30	5.59 (s)	107.4	5.59 (s)
4c	152.6		152.60		152.6	
4d	131.9		131.90		131.9	
4e	151.2		151.20		151.2	
4f	104.6	5.19 (s)	104.70	5.19 (s)	104.6	5.19 (s)
5						

FIG. 4

C5	167.8		169.10		169.1	
X5	53.7	4.43 (d, 5.7)	53.70	4.44 (br d, 4.6)	53.7	4.44 (d, 4.1)
W5		8.68 (br s)		8.66 (br s)		8.67 (s)
5a	126.2		126.20		126.2	
5b	135.7	7.16 (s)	135.60	7.19 (s)	135.6	7.19 (s)
5c	121.6		121.90		121.9	
5d	155.1		155.10		155.1	
5d-OH		9.17 (s)		8.98 (s)		8.98 (s)
5e	116.2	6.71 (d, 8.6)	116.30	6.67 (d, 8.4)	116.4	6.70 (d, 8.4)
5f	125.5	6.77 (dd, 8.4, 1.6)	125.30	6.75 (d, 8.5)	125.3	6.75 (d, 8.5)
6						
C6	167.8		167.90			
X6	61.8	4.18 (d, 11.3)	62.00	4.21 (d, 11.5)	62	4.20 (d, 11.4)
W6		6.68 (s)		6.67 (s)		6.67 (d, 11.8)
Z6	71.6	5.11 (s)	71.40	5.24 (s)	71.4	5.24 (s)
Z6-OH		5.95 (d, 6.4)		5.87 (s)		5.86 (d, 5.9)
6a	142.6		142.50		142.5	
6b	127.4	7.85 (s)	127.40	7.86 (s)	127.4	7.86 (s)
6c	127.2		126.30		126.3	
6d	148.2		148.10		148.1	
6e	123.4	7.35 (d, 8.3)	123.40	7.33 (d, 8.3)	123.4	7.34 (d, 8.3)
6f	127.4	7.46 (d, 8.3)	127.40	7.45 (d, 8.6)	127.4	7.45 (d, 8.4)
7						
C7	172.5		167.70		170.2	
X7	56.7	4.43 (d, 5.7)	57.50	4.38 (d, 5.7)	57.5	4.39 (d, 5.5)
W7		8.51 (br d, 4.8)		8.42 (s)		8.43 (s)
7a	136.1		137.70		137.6	
7b	118.0		118.00		118	
7c	156.6		156.30		156.3	
7c-OH		9.09 (s)		8.97 (s)		8.98 (s)
7d	102.4	6.40 (d, 2.0)	102.00	6.36 (s)	102.1	6.37 (s)
7e	157.2		157.10		157.1	
7e-OH		9.46 (s)		9.31 (s)		9.32 (s)
7f	105.7	6.24 (d, 2.0)	106.40	6.25 (d, 1.9)	106.4	6.25 (s)
glucose						
G1	101.3	5.24 (d, 5.08)	101.20	5.24 (o)	101.2	5.25 (d, 7.8)
G2	78.2	3.54 (t, 8.8)	78.20	3.55 (t, 8.9)	78.2	3.56 (o)
G3	77.0	3.46-3.42 (m)	77.00	3.33 (o)	77	3.46-3.43 (m)
G3-OH		5.37 (d, 5.6)		5.37 (s)		5.37 (d, 5.5)
G4	70.1	3.27 (o)	70.10	3.27 (o)	70.1	3.27 (o)
G4-OH		5.11 (s)		5.11 (s)		5.11 (s)
G5	76.7	3.27 (o)	76.70	3.27 (o)	76.7	3.27 (o)
G6	61.2	3.68 (dd, 10.2, 4.5) 3.53 (o)	61.20	3.72-3.67 (m) 3.56-3.52 (m)	61.2	3.68 (dd, 10.2, 3.7) 3.56 (o)
G6-OH		4.02 (t, 5.4)		4.03 (s)		4.03 (t, 5.3)

FIG. 4 cont'd

Vancosamine						
V1	96.8	5.24 (o)	96.8	5.24 (o)	96.8	5.24 (o)
V2	33.2	1.90 (br d, 12.3) (ax) 1.72 (d, 12.3) (eq)	33.2	1.90 (br d, 13.9) 1.73 (br d, 13.6)	33.2	1.90 (br d, 7.8) 1.73 (br d, 12.8)
V3	53.9		53.9		53.9	
V3-NH2		n/o		n/o		n/o
V4	70.7	3.18 (d, 6.5)	70.7	3.18 (s)	70.7	3.18 (d, 6.4)
V4-OH		3.47 (d, 6.5)		5.48 (s)		5.47 (d, 6.6)
V5	63.1	4.68 (q, 6.5)	63.10	4.68 (q, 6.3)	63.1	4.68 (q, 6.4)
V6	16.8	1.07 (d, 6.3)	16.80	1.07 (d, 6.3)	16.8	1.07 (d, 6.2)
V7	22.2	1.29 (s)	22.30	1.29 (o)	22.3	1.30 (s)
Linker						
L1 (CH_2-NH)			38.8	3.15-3.08 (m)	38.7	3.27 (o)
L2			29.3	1.48-1.44 (m)	68.9	3.51-3.48 (m)
L3			28.7	1.56-1.51 (m)	69.8	3.56 (s) (L3-L6)
L4			28.5	1.33-1.29 (m)	69.74	
L5			28.2	L2-L7	69.66	
L6			26.4		69.62	
L7			26.1		69.2	3.60 (t, 4.9)
L8-N_3			50.6	3.32 (t, 6.9)	50	3.39 (t, 5.1)
NH-L1				7.86-7.84 (m)		7.90 (t, 5.0)
Carbinyl						
	172.5		170.5		170.4	
	170.5		170.4		170.2	
	169.1		169.8		169.1	
	167.8		169.1		168.0	
	166.2		167.9		167.8	
			167.8		166.1	
			166.1			

FIG. 4 cont'd

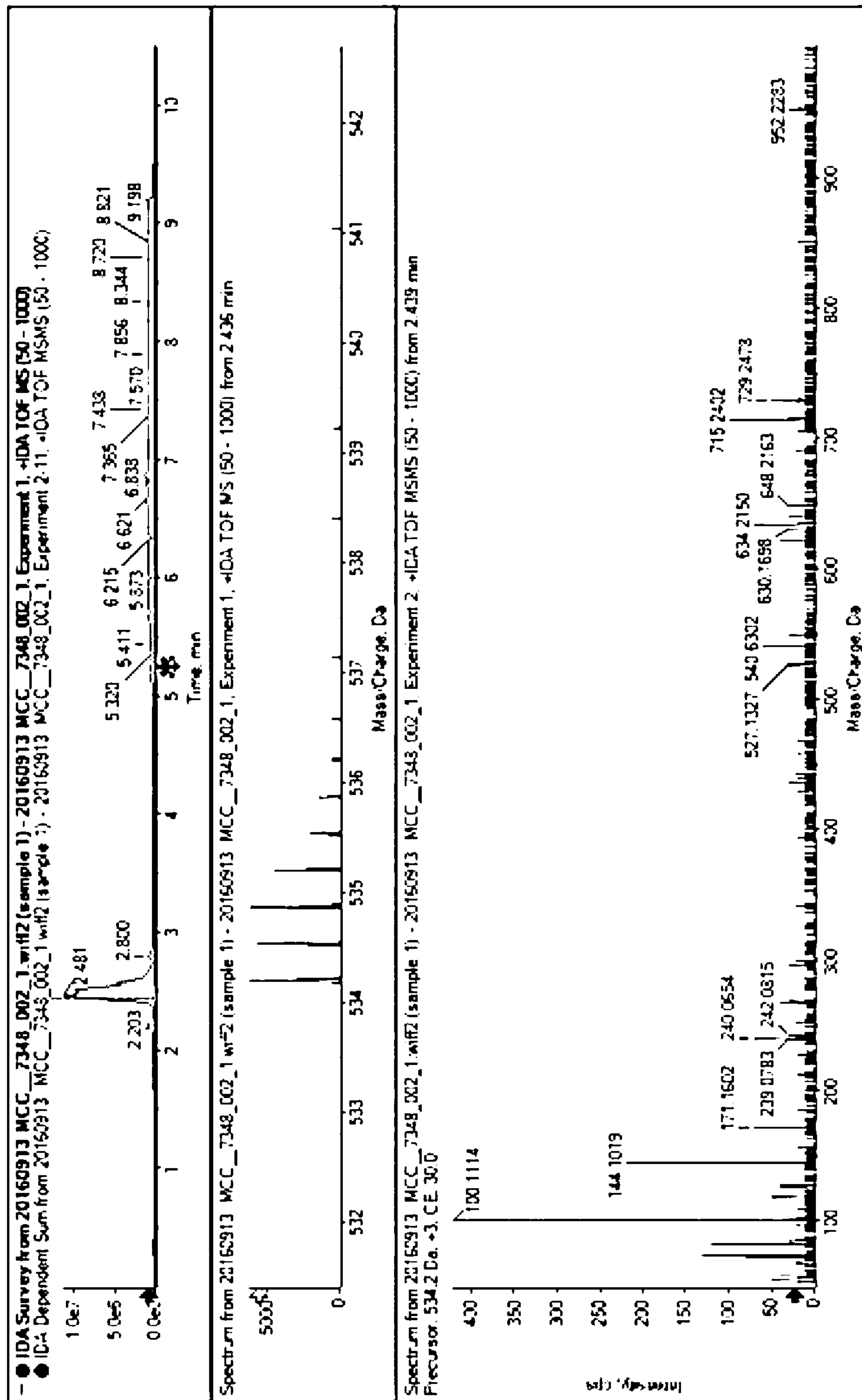


FIG. 5

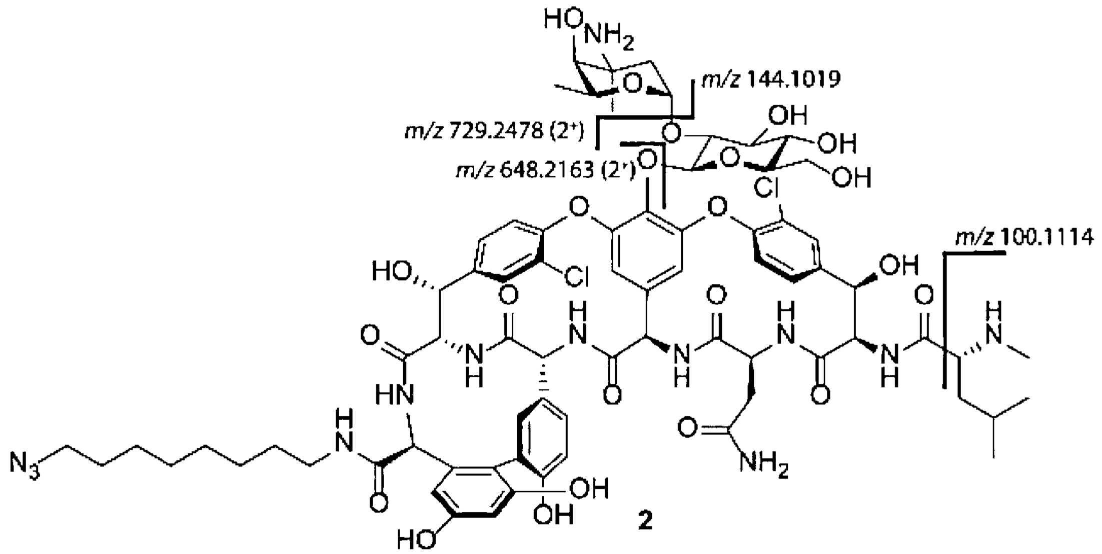


FIG. 5 cont'd

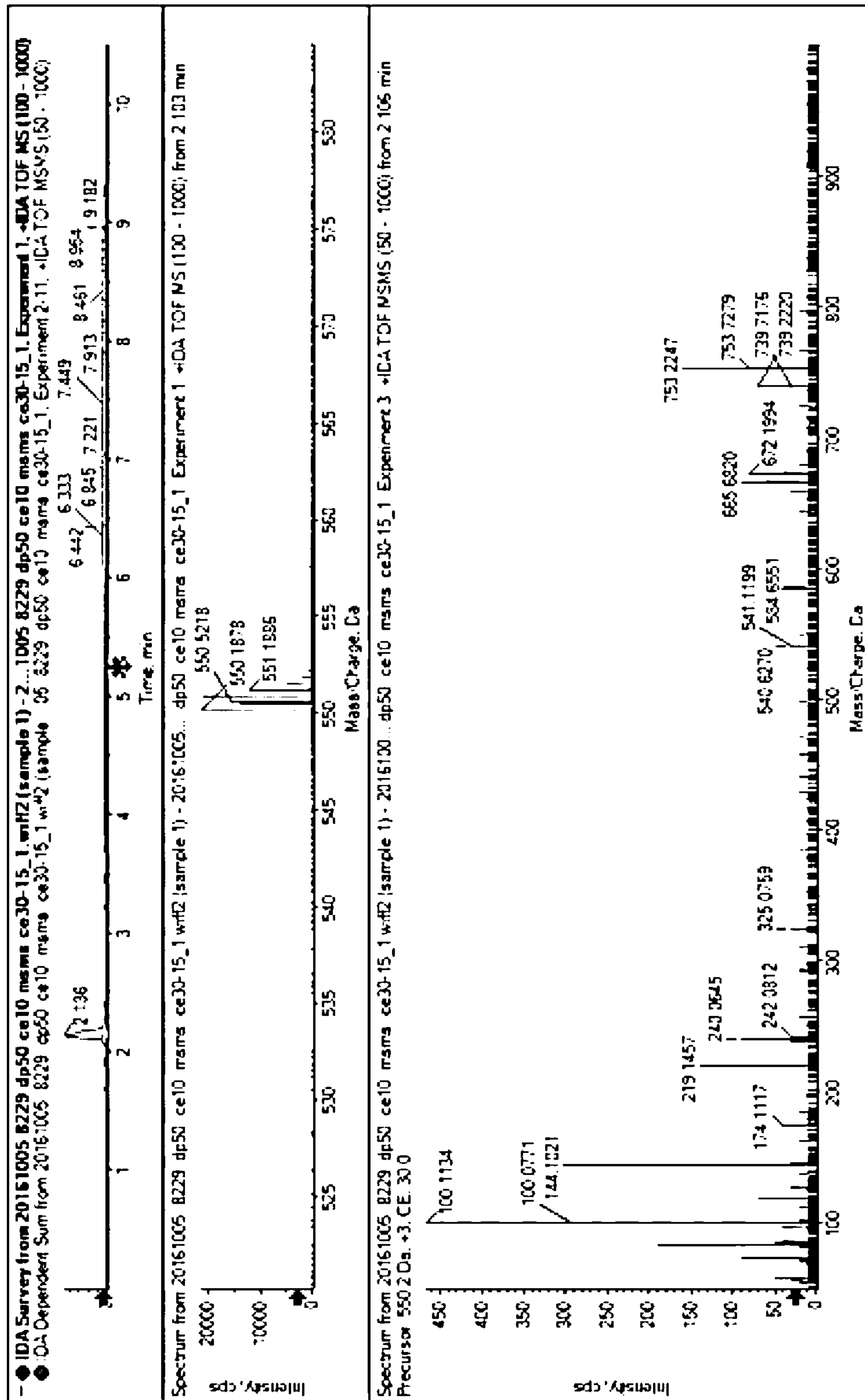


FIG. 6

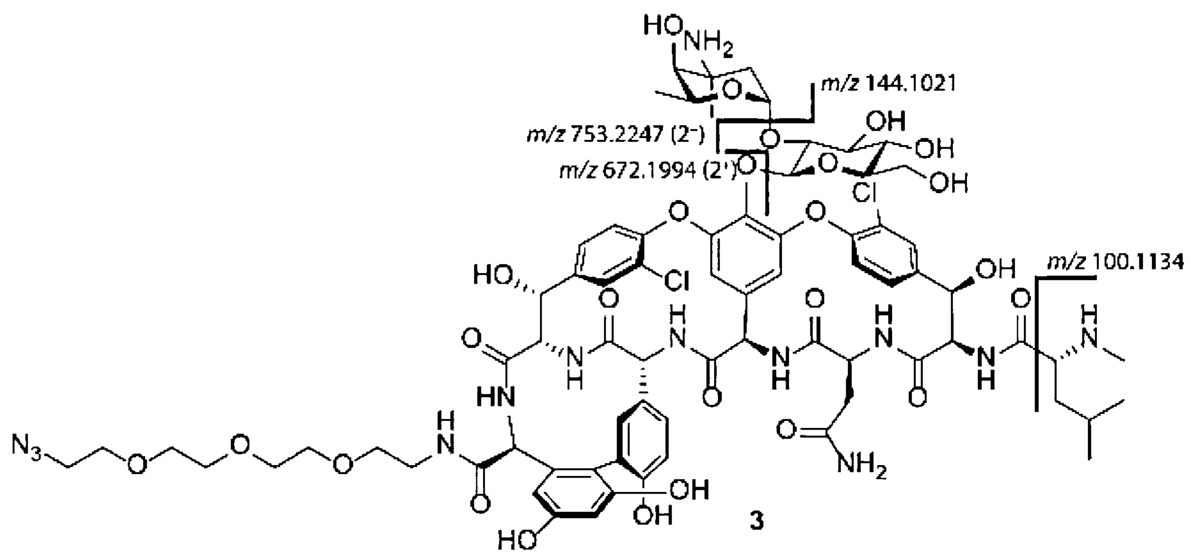


FIG. 6 cont'd

Position		12		13		14
	JMOD	^{1}H	JMOD	^{1}H	JMOD	^{1}H
1						
C1						
X1	59.6	3.93 (br s)	59.5	3.95 (br s)	59.6	3.95 (br s)
W1		n/o		n/o		n/o
1a	39.40	1.69-1.66 (m) 1.57.1.55 (m)	39.10	1.71-1.67 (m) 1.59-1.59 (m)	39.6 (o)	1.70-1.66 (m) 1.58-1.55 (m)
1b	23.70	1.65-1.62 (m)	23.70	1.65-1.61 (m)	23.7	1.65-1.60 (m)
1c	22.30	0.91 (d, 5.3)	22.30	0.91 (d, 6.1)	22.3	0.91 (d, 6.1)
1d	22.8	0.85 (d, 5.2)	22.8	0.86 (d, 6.2)	22.7	0.86 (d, 6.1)
1e	31.20	2.63 (s)	31.20	2.64 (s)	31.2	2.64 (s)
2						
C2						
X2	58.90	4.93 (br s)	58.90	4.94 (br s)	58.9	4.94 (br s)
W2		n/o		n/o		n/o
Z2	71.30	5.18 (o)	71.30	5.19 (s)	71.3	5.18 (o)
Z2-OH		5.99 (s)		5.98 (br s)		5.98 (s)
2a	140.0		139.8 0		139.8	
2b	128.6 0	7.47 (o)	128.6 0	7.48 (o)	128.6	7.49 (s)
2c	127.2 0		127.2 0		127.2	
2d	149.9 0		149.9 0		149.9	
2e	124.3 0	7.23 (s)	124.3 0	7.19 (d, 8.3)	124.3	7.19 (d, 8.3)
2f	127.3 0	7.55 (s)	127.3 0	7.55 (d, 8.2)	127.2	7.55 (d, 8.2)
3						
C3						
X3	51.00	4.21 (o)	51.00	4.24 (o)	51	
W3		n/o		6.57 (s)		
3a		2.57 (o) 2.15 (v br s)	canno t see peak from HSQC	2.59 (o) 2.16 (br s, 9.5)	Cann ot see from HSQC	2.57 (o) 2.16 (dd, 15.1, 6.2)
C3'						
C3'-NH2		7.06 (v br s)		7.06 (v br s)		7.06 (s)
4						
C4	169.1 0		169.1 0		169.1	
X4	55.00	5.76 (d, 7.0)	54.90	5.76 (d, 7.7)	54.9	5.76 (d, 7.8)
W4		n/o		n/o		n/o
4a	134.8 0		134.9 0		134.8	
4b	107.1 0	5.59 (s)	107.4 0	5.59 (s)	107.4	5.59 (s)
4c	152.5 0		152.6 0		152.6	
4d	131.9 0		131.9 0		131.9	
4e	151.2 0		151.2 0		151.2	
4f	104.5 0	5.18 (s)	104.7 0	5.19 (s)	104.6	5.18 (s)

FIG. 7

5						
C5					169.1	
X5	53.70	4.43 (br s)	53.70	4.44 (br s)	53.7	4.44 (d, 3.7)
W5		8.68 (v br s)		8.67 (br s)		8.67 (s)
5a	126.20		126.20			
5b	135.60	7.20 (s)	135.60	7.19 (s)	135.6	7.19 (s)
5c	121.90		121.90		121.9	
5d	155.10		155.10		155.1	
5d-OH		8.99 (s)		8.98 (s)		8.98 (s)
5e	116.30	6.69 (d, 8.0)	116.30	6.69 (d, 8.9)	116.4	6.69 (d, 8.2)
5f	125.20	6.75 (d, 8.4)	125.30	6.75 (d, 8.6)	125.3	6.75 (d, 8.61)
6						
C6						
X6	62.00	4.20 (d, 10.6)	62.00	4.20 (d, 11.2)	62	4.2 (d, 11.0)
W6		6.70 (s)		6.68 (o)		6.69 (o)
Z6	71.40	5.23 (s)	71.40	5.24 (s)	71.4	5.23 (s)
Z6-OH		5.87 (s)		5.87 (s)		5.86 (d, 5.9)
6a	142.60		142.50		142.5	
6b	127.40	7.86 (s)	127.50	7.86 (s)	127.4	7.86 (s)
6c	126.30		126.30		126.3	
6d	148.10		148.10		148.1	
6e	123.40	7.33 (d, 7.8)	123.40	7.33 (d, 8.3)	123.4	7.33 (d, 8.3)
6f	127.40	7.45 (d, 8.6)	127.50	7.45 (d, 8.7)	127.4	7.45 (d, 8.7)
7						
C7			167.90		170.4	
X7	57.50	4.37 (d, 5.1)	57.50	4.38 (d, 5.5)	57.5	4.39 (d, 5.5)
W7		8.4 (s)		8.42 (s)		8.43 (s)
7a	137.70		137.70		137.6	
7b	118.00		118.00		117.9	
7c	156.30		156.30		156.3	
7c-OH		8.98 (s)		8.97 (s)		8.97 (s)
7d	102.00	6.35 (s)	102.00	6.36 (s)	102.1	6.36 (s)
7e	157.00		157.10		157.1	
7e-OH		9.31 (s)		9.31 (s)		9.32 (s)
7f	106.40	6.24 (s)	106.40	6.25 (d, 1.8)	106.4	6.24 (s)
glucose						
G1	101.20	5.23 (o)	101.20	5.24 (o)	101.6	5.25 (d, 7.8)
G2	78.10	3.54 (t, 8.7)	78.20	3.56-3.52 (m)	78.2	3.56-3.52 (o)
G3	77.00	3.43 (o)	77.00	3.36 (o)	77	3.46 (o)
G3-OH		5.38 (d, 5.3)		5.36 (s)		5.36 (d, 4.5)
G4	70.10	3.27 (s)	70.11	3.27 (s)	70.1	3.27 (o)
G4-OH		5.12 (s)		5.11 (s)		5.11 (s)
G5	76.70	3.27 (s)	76.70	3.27 (s)	76.7	3.27 (o)
G6		3.72-3.67 (m) 3.56-3.53 (m)	61.20	3.69-3.67 (m) 3.56-3.52 (m)	61.2	3.68 (d, 10.2) 3.56-3.52 (o)
G6-OH		4.04 (t, 5.0)				4.03 (br t, 4.9)

FIG. 7 cont'd

Vancosamine						
V1	96.7	5.23 (o)	96.7	5.24 (o)	96.7	5.23 (o)
V2	33.1	1.90 (br s) 1.71 (o)	33.2	1.90 (br d, 9.9) 1.75 (o)	33.2	1.90 (br d, 7.8) 1.72 (d, 13.3)
V3	53.9		53.9		53.9	
V3-NH2		n/o		n/o		n/o
V4	70.7	3.18 (s)	70.7	3.18 (s)	70.7	3.18 (d, 6.0)
V4-OH		5.50 (d, 6.4)		5.47 (s)		5.47 (d, 6.4)
V5	63.10	4.69 (s)	63.10	4.68 (q, 6.4)	63.1	4.68 (q, 6.5)
V6	16.80	1.07 (d, 5.1)	16.80	1.07 (d, 6.3)	16.8	1.07 (d, 6.2)
V7	22.30	1.29 (s)	22.30	1.30 (s)	22.3	1.30 (s)
Linker						
L1 (CH_2-NH)	38.7	3.13-3.07 (m)	38.8	3.15-3.08 (m)	38.6	3.27 (o)
L2	29.1	1.43 (m)	29.2	1.45-1.43 (m)	69.62	
L3 – L6	28.6		28.6		69.57	
	28.3	1.23 (s) L3 – L6	28.3	1.26-1.20 (m) L3 – L6	69.48	3.49-3.44 (m) L2 – L7
	26.3		26.4		68.9	
	25.8		25.8		68.6	
L7	29.7	1.79-1.76 (m)	29.8	1.78-1.74 (m)		3.79 (t, 5.3)
L8-N_3	49.4	4.31 (t, 7.0)	49.3	4.31-4.29 (m)	49.4	4.49 (t, 5.1)
NH-L1		7.86 (o)		7.86 (o)		7.91 (t, 4.9)
NBD						
1Tz	142.4				142.4	
2Tz	123.3	8.12 (s)			123.8	8.09 (s)
A (Ar-H)	137.8	8.53 (d, 8.8)			137.8	8.53 (d, 8.9)
B (Ar-H)	99.9	6.52 (d, 9.0)			99.9	6.54 (d, 8.9)
Wc (NH)		9.89 (s)				9.88 (br t, 5.6)
D (CH_2)	38.6	4.75 (s)			38.5	4.76 (s)
QC	121.5				121.5	
	144.5				144.6	
DMACA						
1Tz			114.4			
2Tz			122.6	7.86 (o)		
2 (CH_2)			34.5	4.31-4.30 (m)		
Wd (NH)				8.72 (t, 5.5)		
1 (CH_2)			38.6	3.64 (s)		
D1 carbonyl			160.7			
D2			108.2			
D3			151.1	d2-d6		
D4			152.8			
D5			155.4			
D6 carbonyl			167.8			
CH_3-N			39.7	3.01 (s)		
A			126	7.52 (d, 9.0)		
B			108.9	6.69 (dd, 9.0, 2.3)		
C			97.5	6.55 (d, 2.5)		
D			109.5	6.0 (s)		
Carbonyl						
	170.5		170.4		170.4	
	170.4		169.8		170.2	
	169.8		169.1		169.1	
	169.1		167.9		168.0	
	167.9		167.8		167.7	
	166.1		167.7	D6 DMACA C=O	166.1	
			166.1			

FIG. 7 cont'd

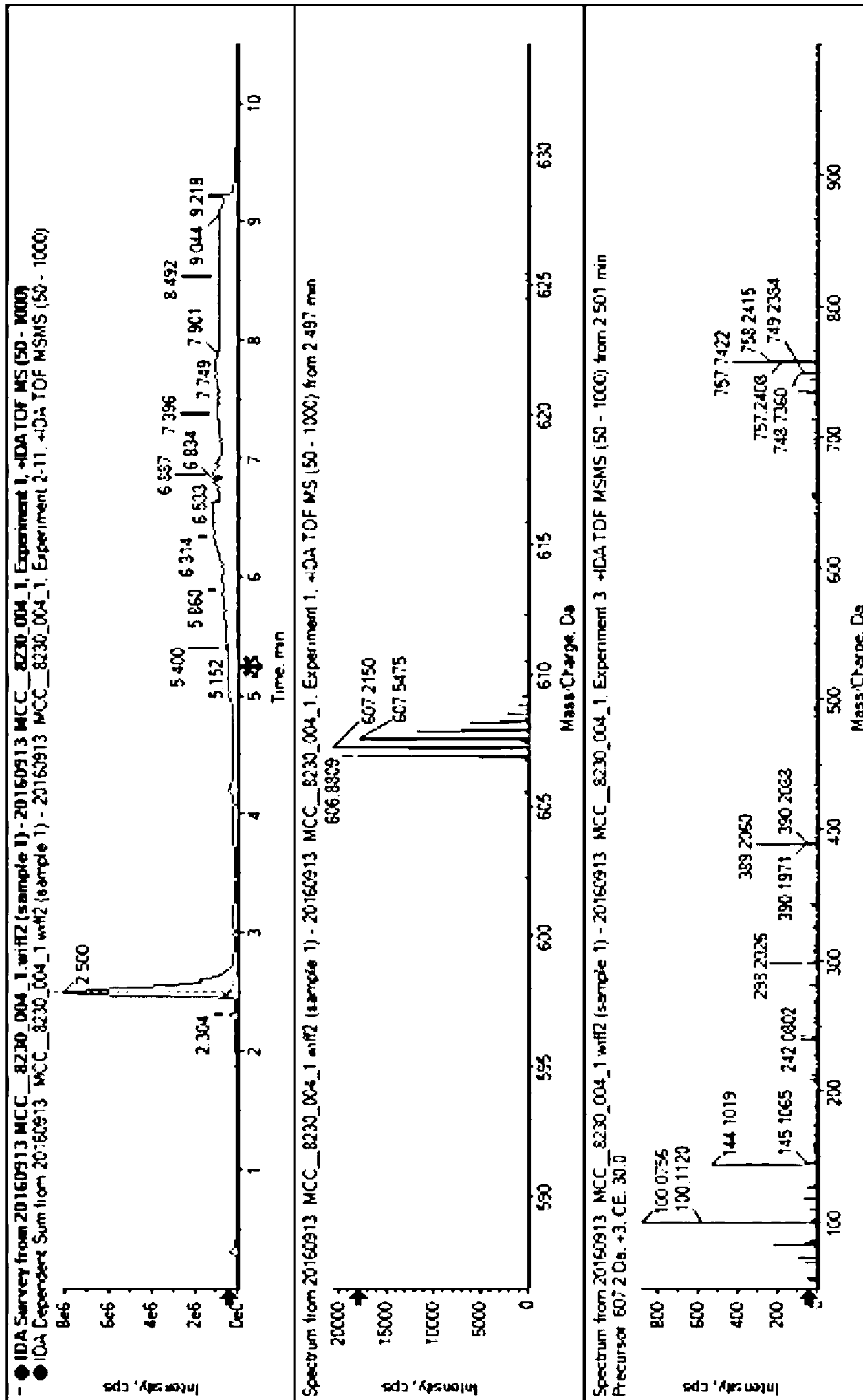


FIG. 8

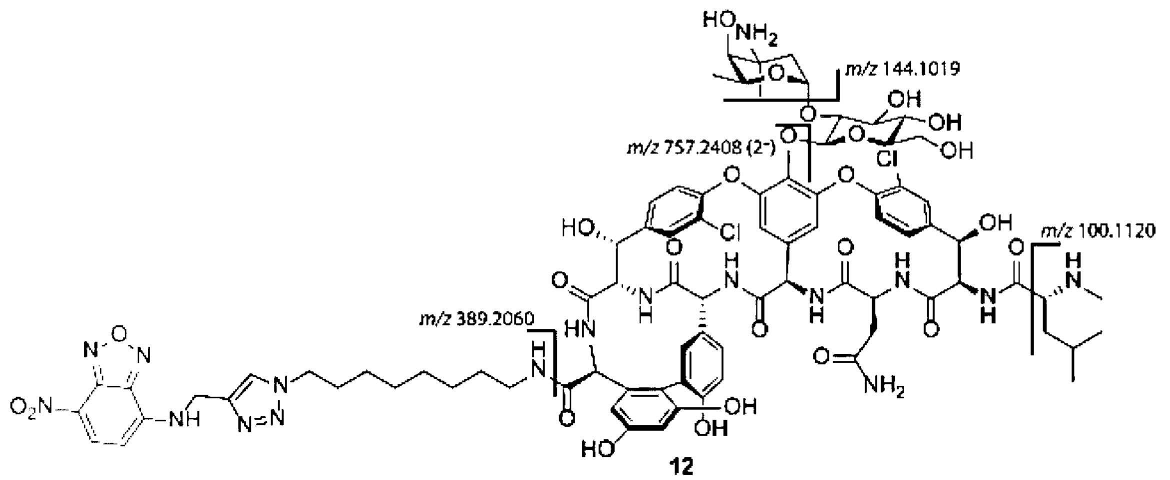


FIG. 8 cont'd

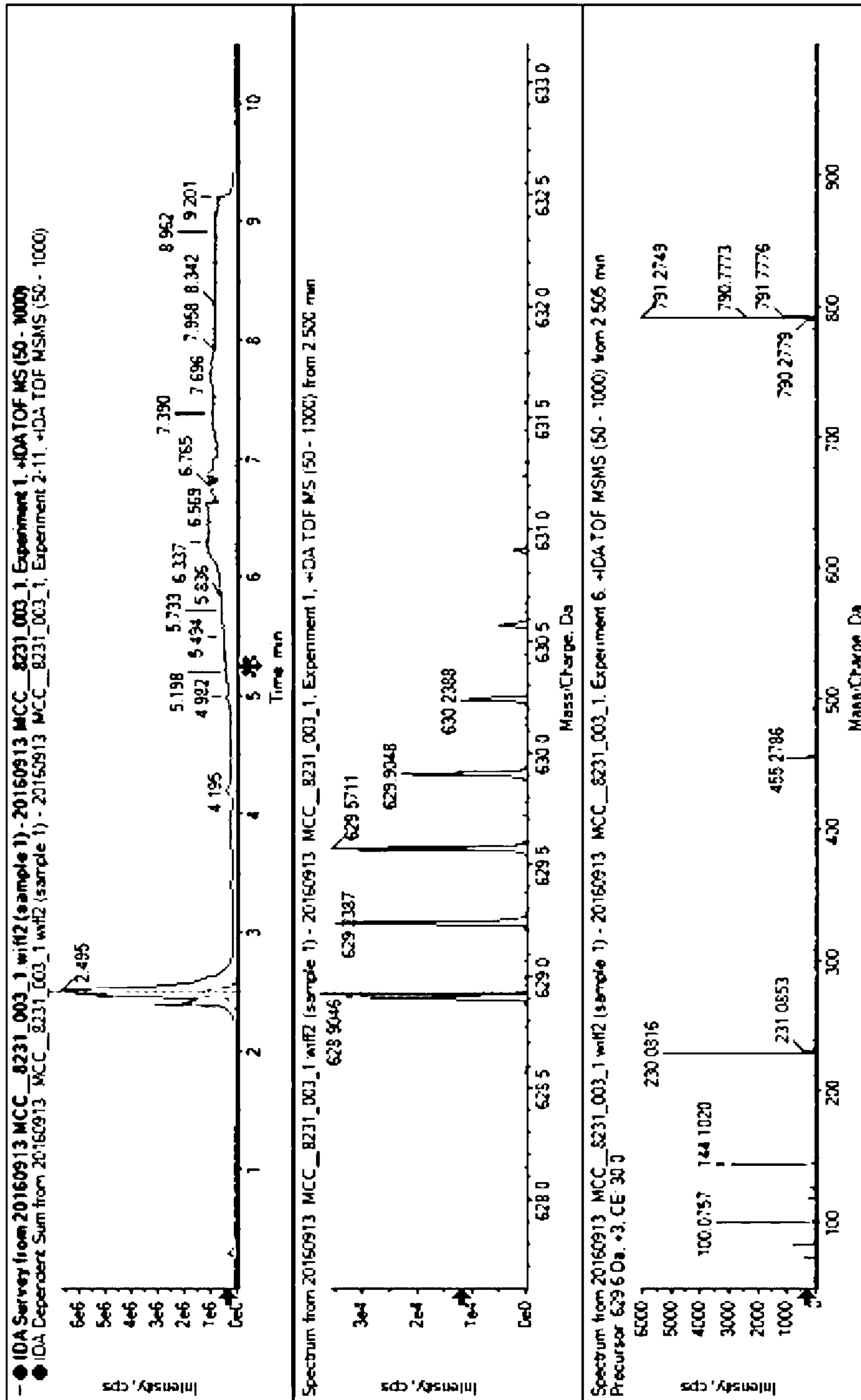


FIG. 9

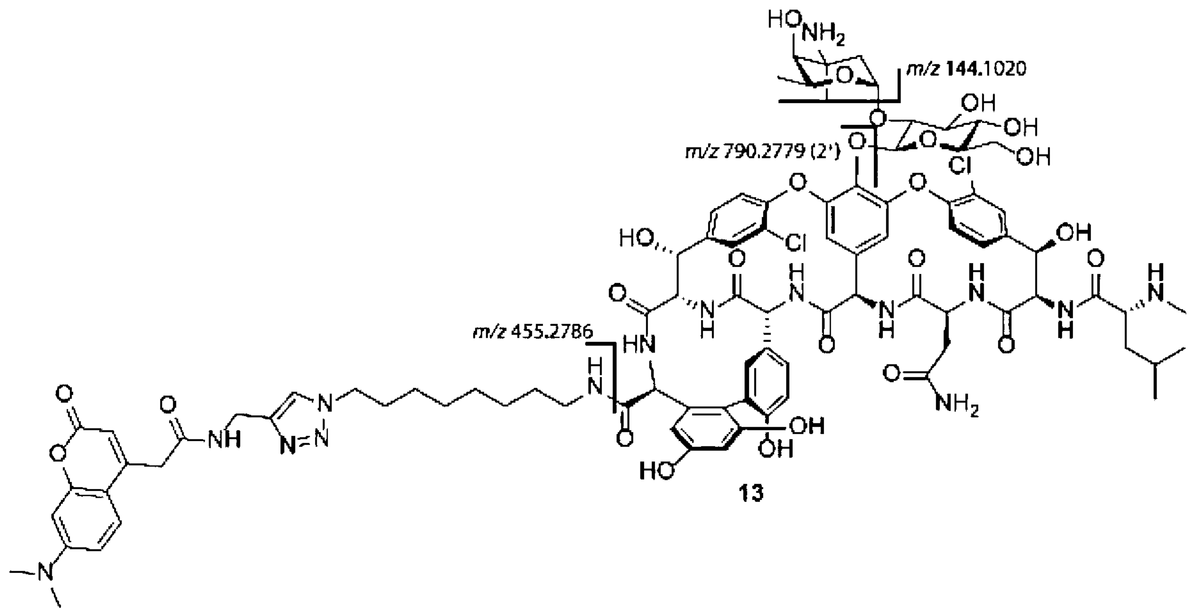


FIG. 9 cont'd

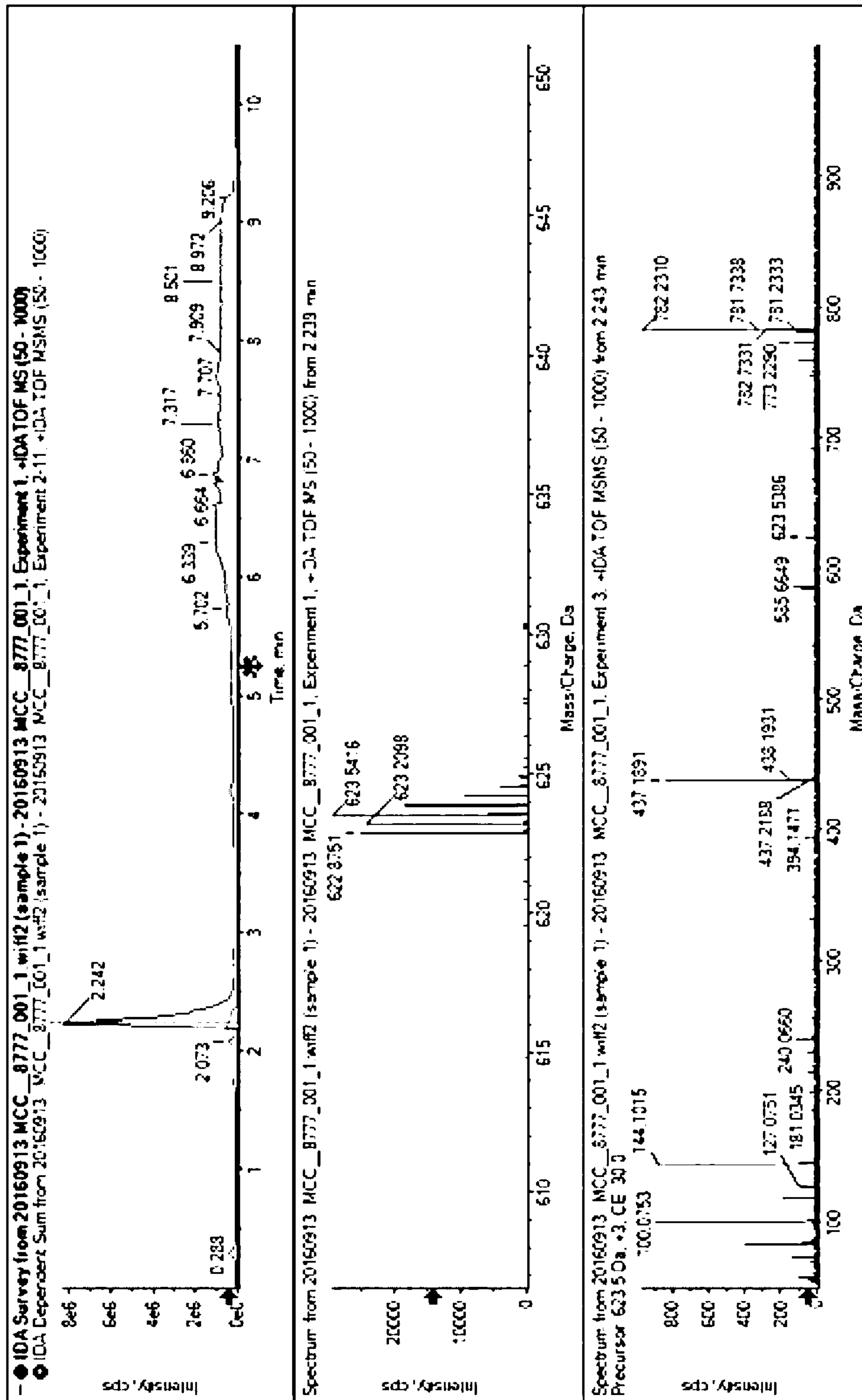


FIG. 10

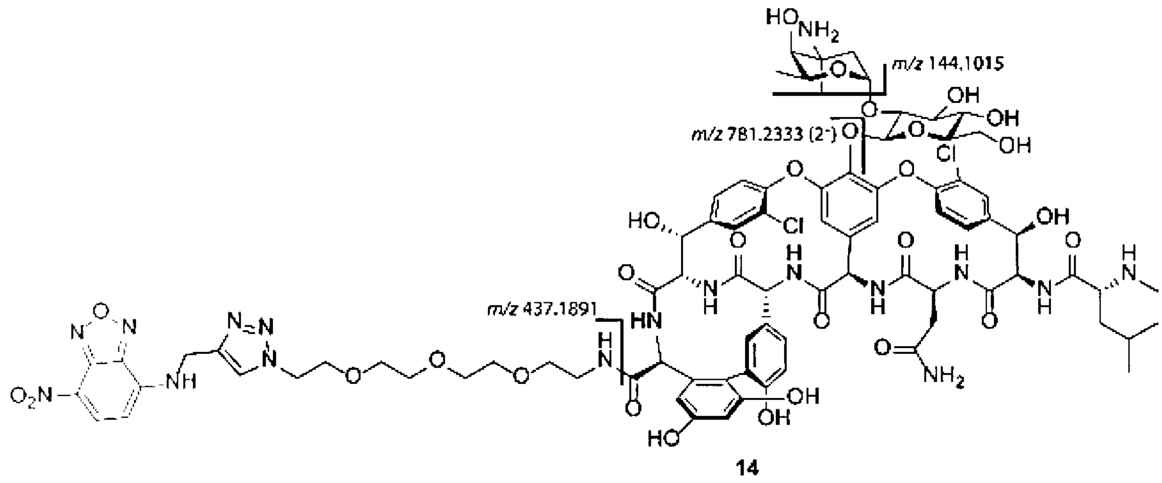


FIG. 10 cont'd

Compound	MIC (μg/mL)												
	S. aureus								*S. pneumoniae*	*E. faecalis*		*E. faecium*	
	ATCC 25923 Control	Clin isol MRSA	ATCC 43300 MRSA	NRS 17 GISA	NRS 1 GISA, MRSA	Clin isol MRSA, DRSA	VRS 3b NARSA	VRS 10 NARSA	ATCC 700677 MDR	ATCC 29212 Control	Clin isol VanA	ATCC 35667 Control	ATCC 51559 MDR-VanA
5.01 Vancomycin	2	2	1	8	8	2	64	>64	2	4	32	0.5	>64
5.02 Van-3C-N_3	NA	2	2	4	4	2	>64	>64	2	2	>64	1	>64
5.03 Van-8C-N_3	NA	0.5	0.5	2	2	0.5	16	64	0.5	0.5	32	0.25	32
5.04 Van-3PEG-N_3	NA	4	2	8	8	4	>64	>64	4	4	>64	1	>64
5.15 Van-8C-Tz-NBD	0.5	1	0.5	2	2	0.5	16	16	0.5	0.5	16	[illegible]	32
5.16 Van-8C-Tz-DMACA	0.5	1	0.5	2	4	1	32	32	1	0.5	16	0.125	32
5.17 Van-3PEG-Tz-NBD	2	4	2	8	8	2	>64	>64	2	4	>64	0.5	>64

FIG. 11

Compound	MIC (µg/mL)											
	S. aureus							*S. pneumoniae*	*E. faecalis*		*E. faecium*	
	Clin isol MRSA	ATCC 43300 MRSA	NRS 17 GISA	NRS 1 GISA, MRSA	Clin isol MRSA, DRSA	VRS 3b NARSA	VRS 10 NARSA	ATCC 700677 MDR	ATCC 29212 Control	Clin isol VanA	ATCC 35667 Control	ATCC 51559 MDR-VanA
1 Vancomycin	2	1	4	8	2	64	32	1	1	32	0.25	>64
2 Van-8C-N_3	0.5	0.5	2	2	0.5	16	64	0.5	0.5	32	0.25	32
3 Van-3PEG-N_3	4	2	8	8	4	>64	>64	4	4	>64	1	>64
12 Van-8C-Tz-NBD	1	0.5	2	2	0.5	16	16	0.5	0.5	16	0.06	32
13 Van-8C-Tz-DMACA	1	0.5	2	4	1	32	32	1	0.5	16	0.125	32
14 Van-3PEG-Tz-NBD	4	2	8	8	2	>64	>64	2	4	>64	0.5	>64

FIG. 11 cont'd

FIG. 12

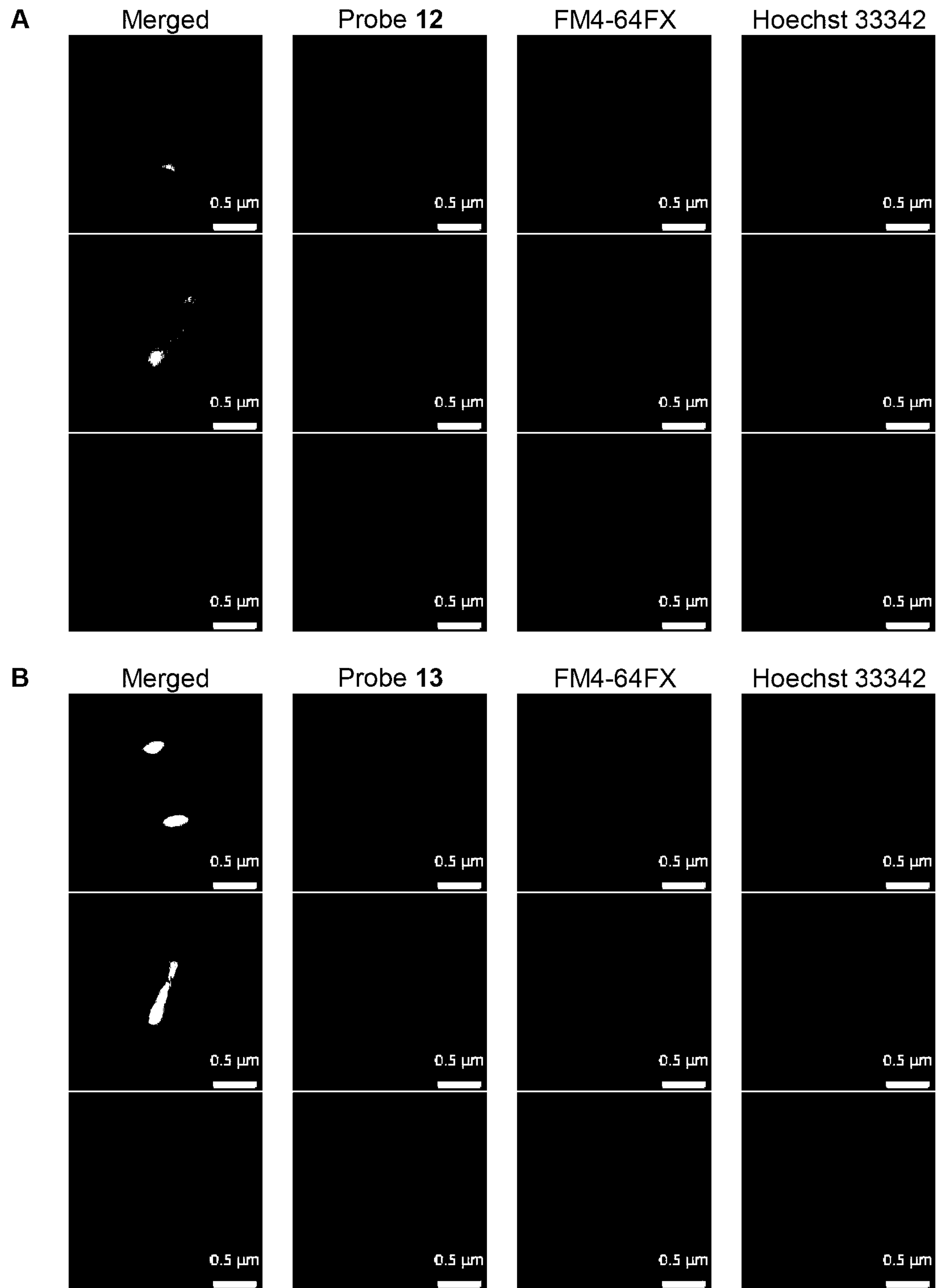


FIG. 13

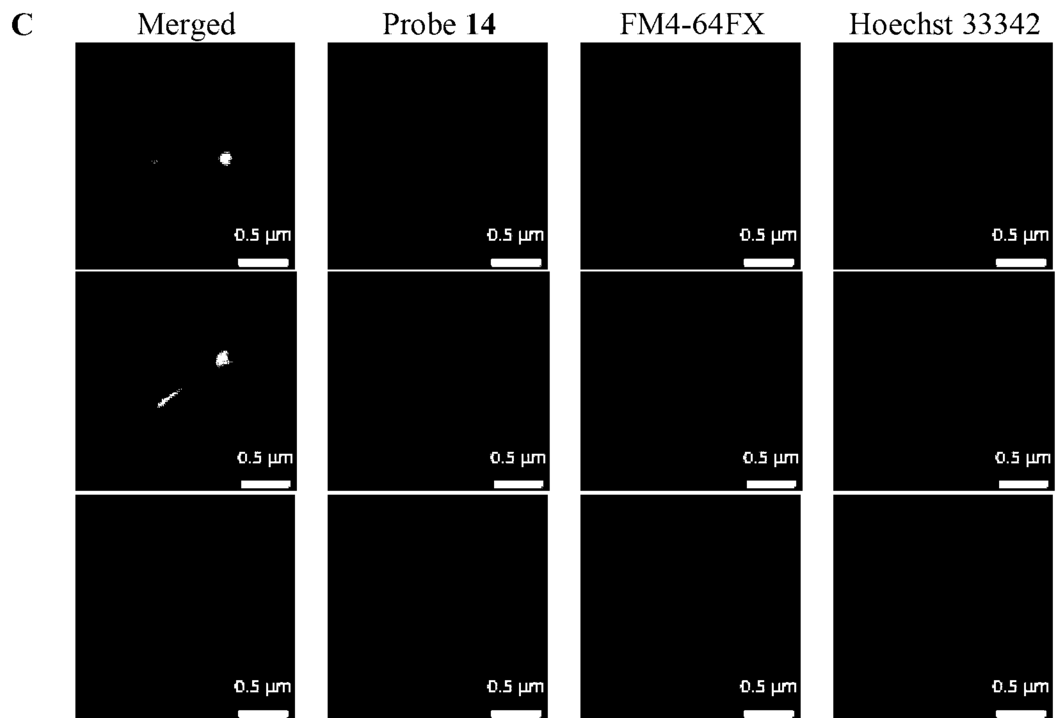


FIG. 13 cont'd

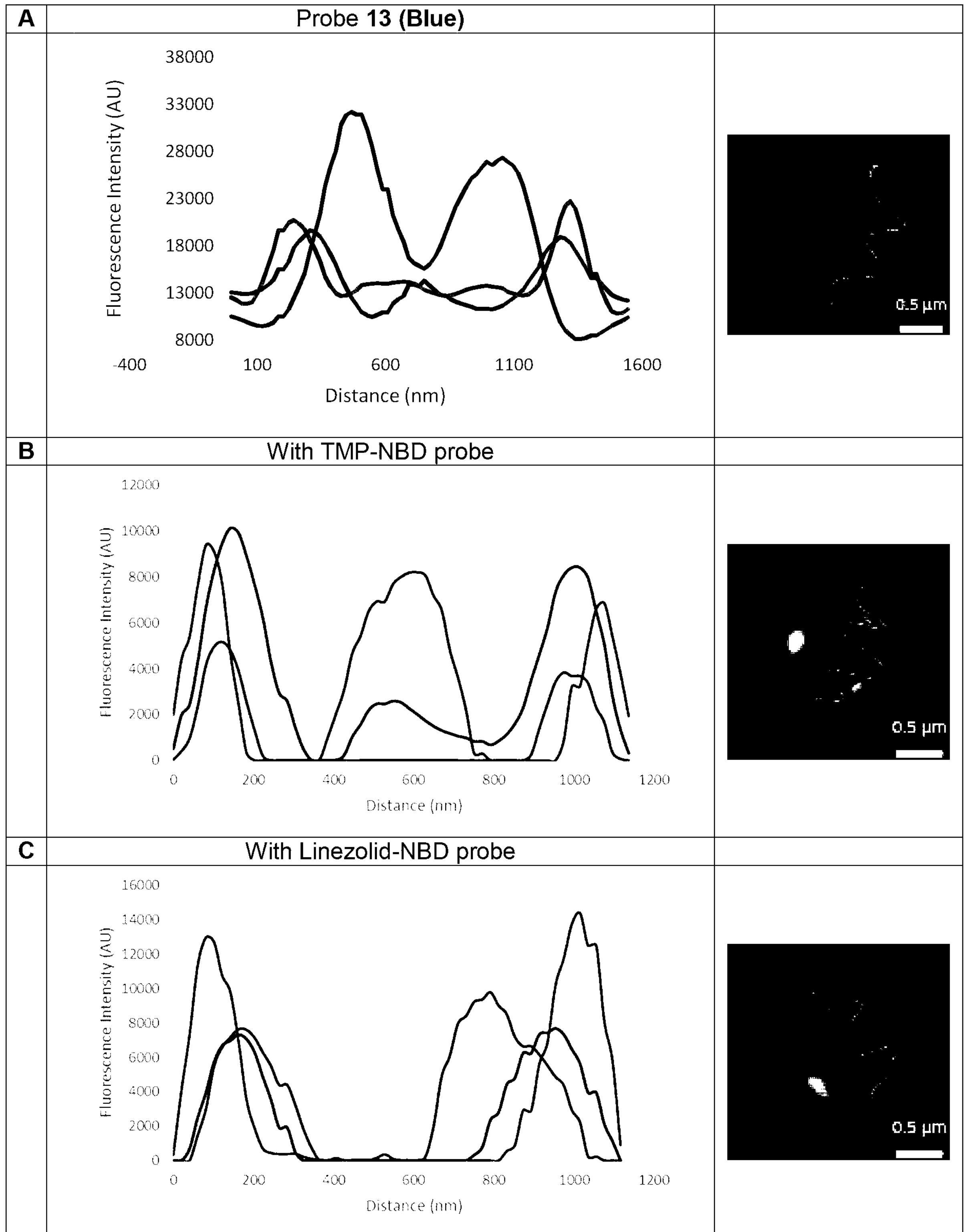
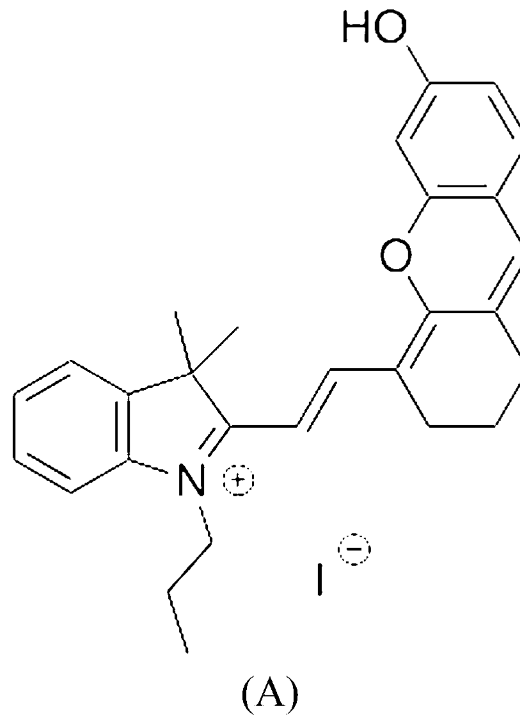
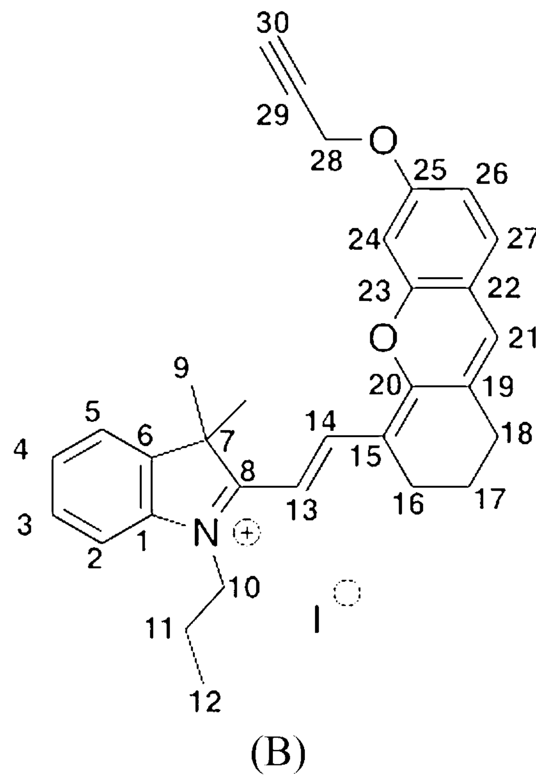


FIG. 14

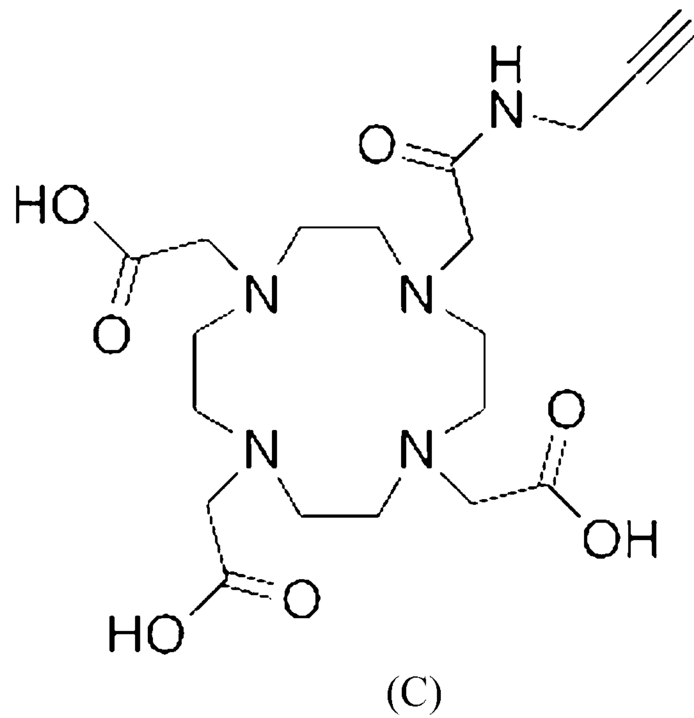


(A)



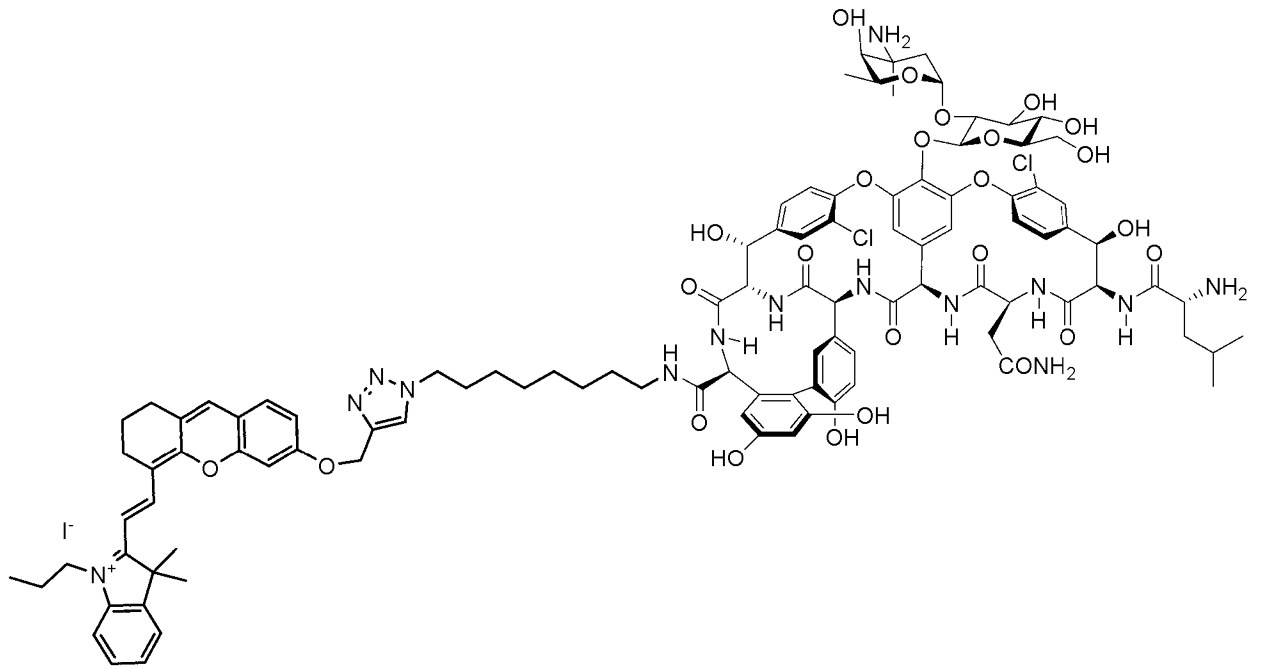
(B)

FIG. 15

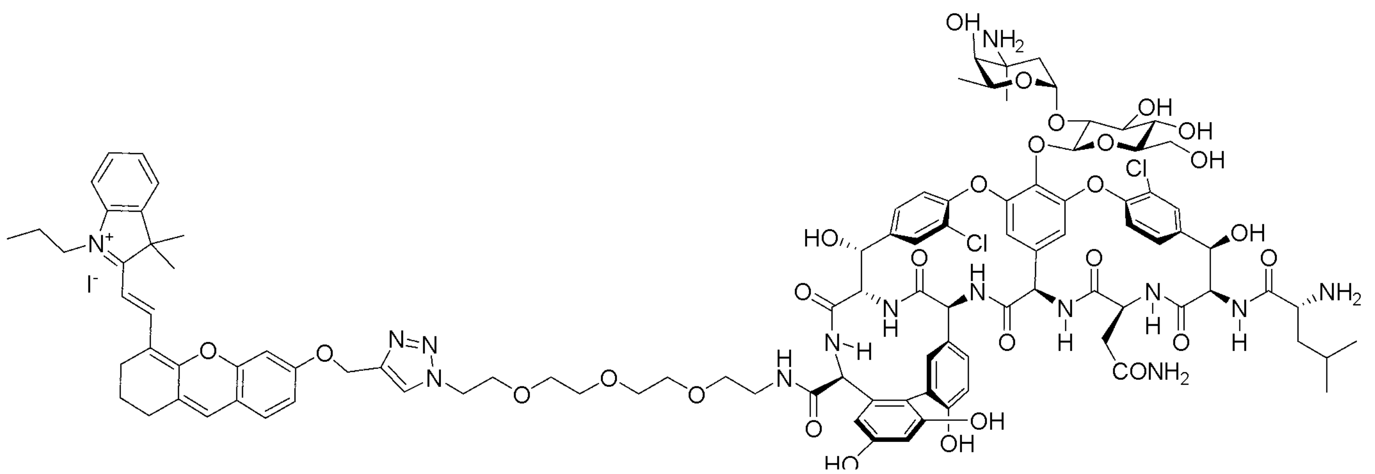


(C)

FIG. 15 cont'd

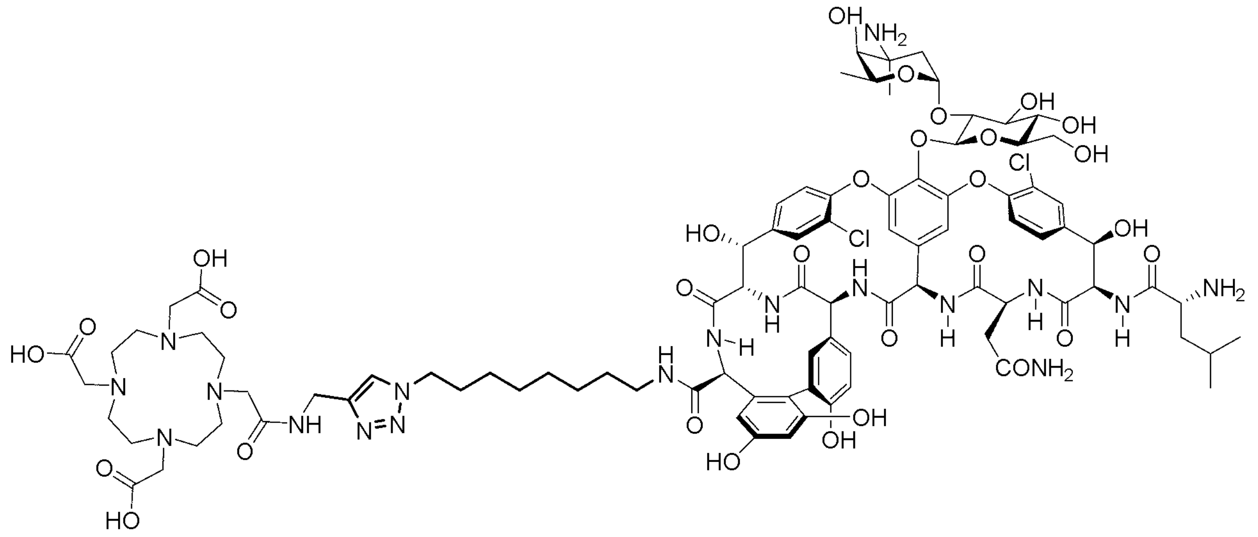


(A)

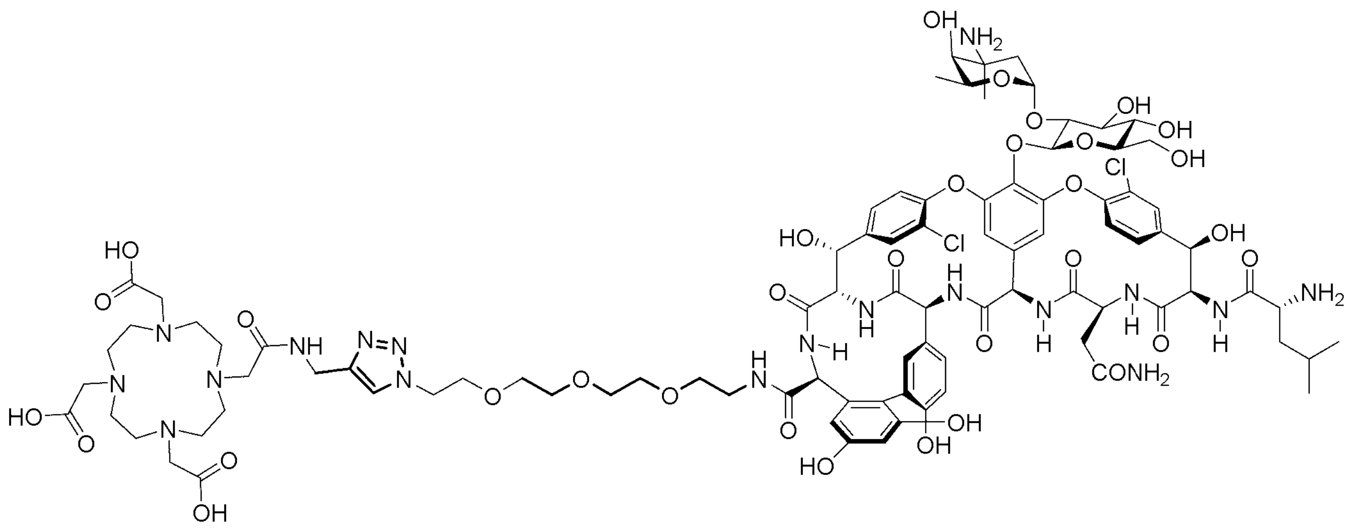


(B)

FIG. 16

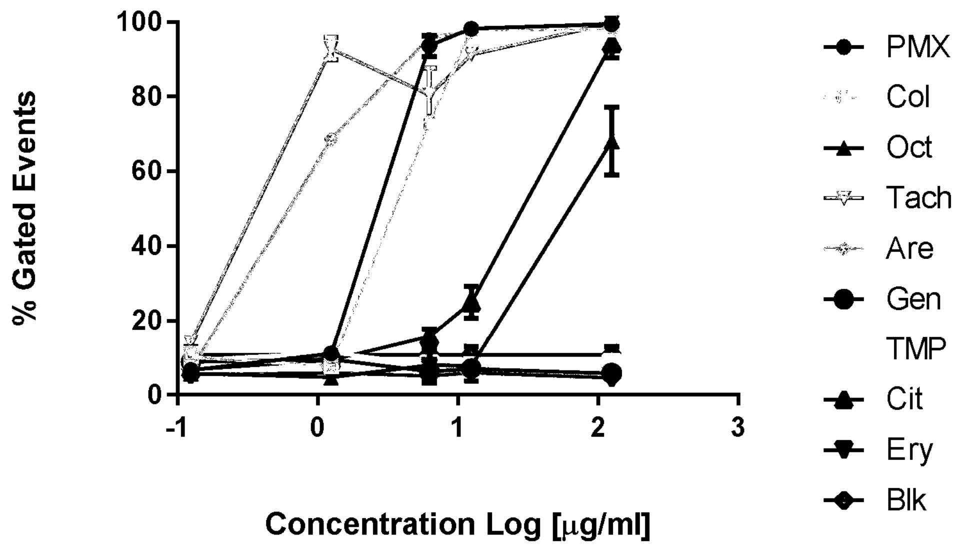


(A)



(B)

FIG. 17



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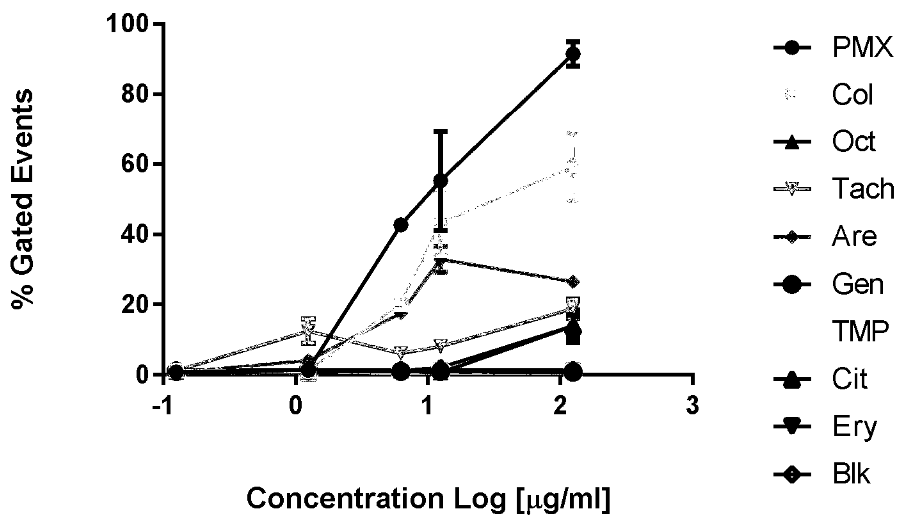
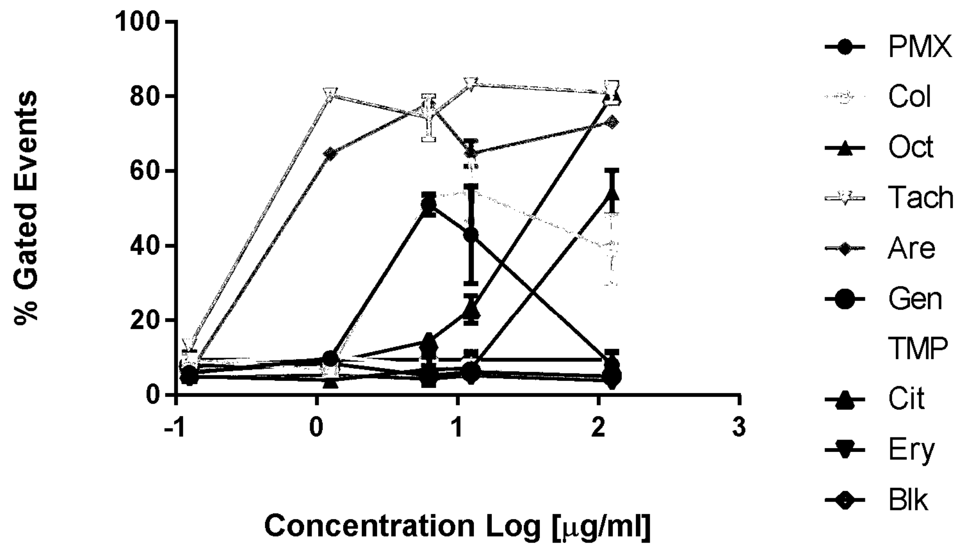


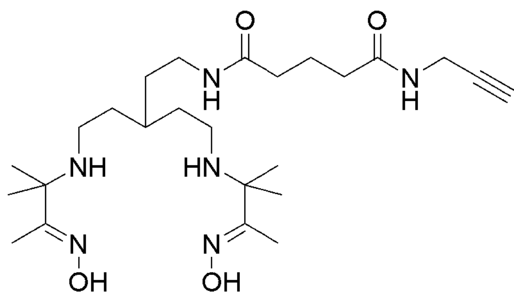
(B)

FIG. 18

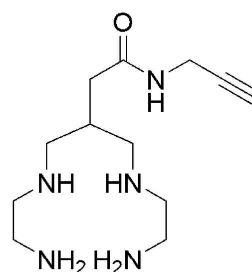


(C)

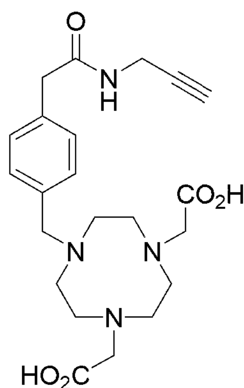
FIG. 18 cont'd



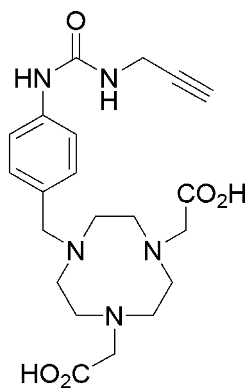
bisamineoxime
e.g for SPECT as ^{99m}Tc chelator



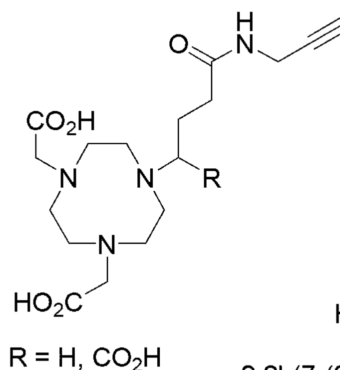
tetraamine
e.g for SPECT as ^{99m}Tc chelator



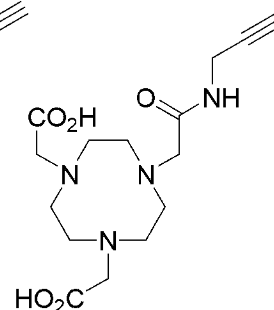
2,2'-(7-(4-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)benzyl)-1,4,7-triazonane-1,4-diyl)diacetic acid



2,2'-(7-(4-(3-(prop-2-yn-1-yl)ureido)benzyl)-1,4,7-triazonane-1,4-diyl)diacetic acid



R = H, CO_2H



2,2'-(7-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-1,4,7-triazonane-1,4-diyl)diacetic acid

NODA / NOTA analogs
e.g for PET as ^{68}Ga / ^{64}Cu / Al^{18}F chelator

FIG. 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2017/051365

A. CLASSIFICATION OF SUBJECT MATTER

A61K 38/14 (2006.01) A61K 38/12 (2006.01) A61K 47/50 (2017.01) A61K 47/60 (2017.01) A61K 49/00 (2006.01) C07K 1/04 (2006.01) C07K 1/13 (2006.01) C07K 9/00 (2006.01) G01N 21/64 (2006.01) G01N 33/68 (2006.01) A61P 31/04 (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)

EPOQUE, STN, Internet; Databases & webpages: PATENW = English language databases; Epodoc, WPIAP, WPI, Medline, CAplus, Biosis, Embase, Google.com, Google Scholar, PubMed, Espacenet, PatentScope, AusPat; Keywords: nano- and micro-particle, -sphere, -powder, -cluster, -crystal, micron, nanometre, glycosylated, glycopeptide, antibiotic, vancomycin, VAN, teicoplanin, oritavancin, telavancin, chloroeremomycin, balhimycin, ramoplanin, decaplanin, bleomycin, superparamagnetic, SPION, magnetic, separation particle, link, linker, construct, conjugate, poly ethylene glycol, pegylate, PEG-3, PEG-4, alkyl, carbon, linear, chain, methane, methyl, ethane, ethyl, propane, propyl, butane, butyl, pentane, pentyl, hexane, hexyl, heptane, heptyl, octane, octyl, nonane, nonyl, decane, decyl, azide, azido, N3, triazole, Click chemistry, reaction, visual, light emitting, infrared, component, agent, fluorescent, NBD, DMACA, dansyl, BODIPY, HXPI, PXPI, quantum dot & like terms. IPC/CPCs: A61K47/6923, A61K47/50/low. Inventor and Name Applicant searches.

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	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
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- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 March 2018	22 March 2018
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Ross Heisey AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262833185

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/187954 A1 (THE GENERAL HOSPITAL CORPORATION) 19 December 2013 Abstract; page 13, lines 11-16; page 22, lines 6-29; page 23, line 28 - page 25, line 8; page 27, line 28 - page 37, line 12; page 40, line 6 - page 47, line 16; claims 21-24, 75, 79; Figures 2, 19	1-8, 10-39, 41-44, 46-50, 52, 55
X	WO 2016/041022 A1 (THE UNIVERSITY OF QUEENSLAND) 24 March 2016 Abstract; para. 0007, 0012, 0034-0037, 0046, 0063, 0067, 0072, 0184-0188, 0194-0200; Figures 1-4, 11-12; claims 24-26	1-11, 14, 16-20, 24-39, 41-44, 46-47, 49-50, 52, 55
X	US 2012/0172289 A1 (XING et al.) 05 July 2012 Abstract; Figures 1-2; para. 0002, 0059, 0065-0067, 0077; Compounds II & III; Examples 1-8	1-8, 13, 16-17, 24-26, 36-44, 46, 49, 51-55
X	VAN OOSTEN, M., et al., "Real-time *in vivo* imaging of invasive- and biomaterial-associated bacterial infections using fluorescently labelled vancomycin", NATURE COMMUNICATIONS, 2013, 4:2584, DOI:10.1038/ncomms3584 Whole document, in particular, Abstract; Figures 1, 3; Methods at pages 6-7	1-7, 16-17, 21-22, 24-27, 36-39, 41-42, 51-52, 55
X	TIYANONT, K, et al., "Imaging peptidoglycan biosynthesis in *Bacillus subtilis* with fluorescent antibiotics", PNAS, 2006, vol. 103(29), pages 11033-11038 Whole document, in particular, Abstract; Figures 1-4; Table 1 at page 11034	1-9, 16-17, 24-25, 27, 36-39, 43-44, 46, 49, 52, 55
X	QI, G., et al., "Vancomycin-Modified Mesoporous Silica Nanoparticles for Selective Recognition and Killing of Pathogenic Gram-Positive Bacteria Over Macrophage-Like Cells", ACS Applied Materials & Interfaces, 2013, vol. 5, pages 10874-10881 Whole document, in particular, Abstract; Synthesis chapter 2 at pages 10875-10876; Figure 2 at page 10876; Figures 8-9 at page 10880	1-8, 16-17, 24-27, 36-44, 46, 49-55
X	WO 2015/117196 A1 (THE UNIVERSITY OF QUEENSLAND) 13 August 2015 Abstract; Examples 2-6, 8-9; Table 2; Figures 1-3; claims 22-24	43-44, 46, 48-55
X	WO 2001/081373 A2 (MERCK & CO., INC.) 01 November 2001 Abstract; para. page 3, lines 10-18; Examples 4-6 at pages 38-42; Example 22 at pages 59-60; Example 25 at page 63; claims 13, 17, 21-22, 23, 43-44	43-44, 46-55
X	US 6518242 B1 (CHEN et al.) 11 February 2003 Abstract; col. 1, lines 12-33; col. 19, lines 40-64; Example 7, compounds 19, 22, 52, 61; Example 9, compounds 12-14, 21-22, 26-27, 31, 44; Example 13, compounds 2-3, 29-31	43-44, 46, 48-49, 51-55
X	US 2006/0105941 A1 (SCHIFFMAN et al.) 18 May 2006 Abstract; para. 0033, 0041, 0049, 0079; Compounds 5, 6; claim 11	43-44, 46-47, 49, 51-55
X	GB 2449156 A (LEAD Therapeutics, Inc) 12 November 2008 Abstract; para. 0020, 0023; compounds 17-24, 45, 64-65, 68, 73, 77-78; Table 1; claim 27	43-44, 46, 48-50, 51-55
X	WO 2015/116537 A9 (ACADEMIA SINICA, et al.) 06 August 2015 Abstract; Compounds 7, 11; Figure 1; Example 2; claim 24	43-44, 46, 48-49, 51-55
P,X	HASSAN, M.M., et al., "Surface Ligand Density of Antibiotic-Nanoparticle Conjugates Enhances Target Avidity and Membrane Permeabilization of Vancomycin-Resistant Bacteria", Bioconjugate Chemistry, 2017, vol. 28, pages 353-361. Published: 13 December 2016 Whole document, in particular, Abstract; Figures 1, 3; Experimental	43-44, 46-47, 49-55

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2017/161296 A1 (NORTHWESTERN UNIVERSITY, et al.) 21 September 2017 Abstract; Figs 1, 15; page 1, line 22 - page 2, line 4; page 12, lines 3-6; page 13, line 25 - page 14, line 3; Example 2; claims 3, 7-12	43-44, 46-55
E	WO 2017/218796 A1 (OTT et al.) 21 December 2017 Abstract; page 15, line 8, lines 8-22; page 60, line 27 - page 64, line 16; Figures 22-24; claims 21-23, 30	43-44, 46-55

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2017/051365**

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 2013/187954 A1	19 December 2013	WO 2013187954 A1	19 Dec 2013
		US 2015125884 A1	07 May 2015
WO 2016/041022 A1	24 March 2016	WO 2016041022 A1	24 Mar 2016
US 2012/0172289 A1	05 July 2012	US 2012172289 A1	05 Jul 2012
WO 2015/117196 A1	13 August 2015	WO 2015117196 A1	13 Aug 2015
		AU 2015213478 A1	15 Sep 2016
		AU 2015213478 A2	15 Sep 2016
		CA 2939145 A1	13 Aug 2015
		CN 107027309 A	08 Aug 2017
		EP 3105244 A1	21 Dec 2016
		JP 2017507174 A	16 Mar 2017
		MX 2016010385 A	12 May 2017
		US 2017204138 A1	20 Jul 2017
WO 2001/081373 A2	01 November 2001	WO 0181373 A2	01 Nov 2001
		AU 5716301 A	07 Nov 2001
		US 2002045574 A1	18 Apr 2002
US 6518242 B1	11 February 2003	US 6518242 B1	11 Feb 2003
		AR 019817 A1	20 Mar 2002
		AU 3307399 A	06 Sep 1999
		CA 2318394 A1	26 Aug 1999
		EP 1060189 A1	20 Dec 2000
		FR 2778184 A1	05 Nov 1999
		IT TO990134 A1	20 Aug 1999
		IT 1307018 B1	23 Oct 2001
		US 2005171010 A1	04 Aug 2005
		US 7067620 B2	27 Jun 2006
		WO 9942476 A1	26 Aug 1999
		ZA 9901412 B	22 Aug 2000

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2017/051365**

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**
US 2006/0105941 A1	18 May 2006	US 2006105941 A1	18 May 2006
		WO 2006055359 A1	26 May 2006
GB 2449156 A	12 November 2008	GB 2449156 A	12 Nov 2008
		GB 2449156 B	28 Apr 2010
		AR 066345 A1	12 Aug 2009
		HK 1125114 A1	10 Sep 2010
		TW 200848062 A	16 Dec 2008
		WO 2008140973 A1	20 Nov 2008
WO 2015/116537 A9	06 August 2015	WO 2015116537 A1	06 Aug 2015
		CN 106132980 A	16 Nov 2016
		EP 3099703 A1	07 Dec 2016
		TW 201540729 A	01 Nov 2015
		TW I561532 B	11 Dec 2016
		US 2016347795 A1	01 Dec 2016
WO 2017/161296 A1	21 September 2017	WO 2017161296 A1	21 Sep 2017
WO 2017/218796 A1	21 December 2017	WO 2017218796 A1	21 Dec 2017
		US 2017362266 A1	21 Dec 2017

End of Annex

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