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(54) Title: ANTIBACTERIAL AGENT

(57) **Abstract:** The invention provides a compound which is a peptide derivative of formula (1) as defined in the claims or a salt thereof: R^G is H, $-CH_2-R^{GA}$ or C_{1-12} hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-OR'$, $-NO_2$, $-CN$, halogen and 5-6 membered heterocyclyl; or R^G and R^{N1} together form a 5-6 membered heterocyclic ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen; R^{GA} is C_{1-11} hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-NR''R''$, $-OR'$, $-NO_2$, $-CN$, halogen and 5-6 membered heterocyclyl; R^S is C_{1-16} hydrocarbyl, a 5-10 membered heterocyclyl or heteroaryl group, $-(C_{1-4} \text{ alkylene})-R^{SA}$ or $-(C_{1-4} \text{ alkylene})-L-R^{SA}$; R^{SA} is C_{1-16} hydrocarbyl, or a 5-10 membered heterocyclyl or heteroaryl group; L is $-SO_2-$; and wherein R^S and R^{SA} are optionally substituted with a group R^b and/or from 1 to 5 substituents selected from R^a , $-NR''R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR''R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $-SR'$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2OR'$, $-P(O)(OR')(OR'')$, $=O$, $-SiR''R''R''$ and halogen; or R^a and an R^{C1} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen; R^b is phenyl, benzyl, C_{3-6} cycloalkyl or 5-6 membered heterocyclyl; Q is $-C(O)-N(R^{N2})-$, $-C(O)-C(R''R'')$, $-C(O)-O-$, $-N(R^{N2})-C(O)-$, $-C(R''R'')$, $-C(O)-$, $-O-C(O)-$, $-C(R''R'')$, $-C(R''R'')$, $-C(R''R'')$, $-O-C(R''R'')$, $-C(R''R'')$, $-N(R^{N2})-$, $-N(R^{N2})-C(R''R'')$ or $-C(R')=C(R'')$; Z is a group selected from $-C(O)-O-$, R^O and $-CN_4R'$ (tetrazolyl); R^O is H or C_{1-12} hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-NR''R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR''R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $-SR'$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2OR'$, $-P(O)(OR')(OR'')$, $=O$, halogen and 5-6 membered heterocyclyl; or R^O and an R^{C2} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen; each R^{N1} and R^{N2} is independently H or C_{1-4} alkyl; each R^{C1} , R^{C2} , R' , R'' , R''' and R'''' is independently H or C_{1-4} alkyl and is optionally substituted with 1 to 3 substituents selected from halogen, $-OH$ and $-O-C_{1-4}$ alkyl; and each R^a is independently C_{1-4} alkyl which is optionally substituted with 1 to 3 halogen atoms.

ANTIBACTERIAL AGENT

FIELD OF THE INVENTION

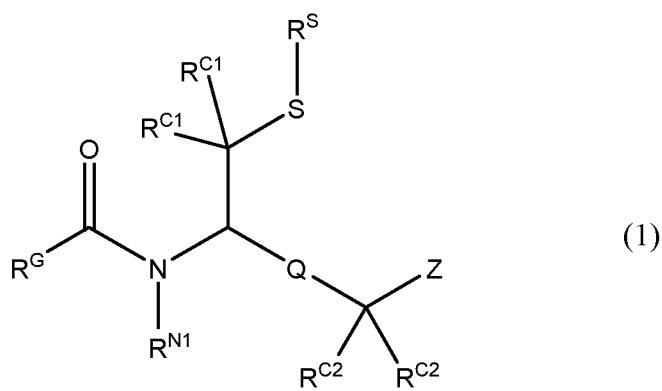
The present invention relates to compounds useful as antibacterial agents. The use of such compounds in treating or preventing bacterial infection is also described.

BACKGROUND OF THE INVENTION

The potassium efflux system, Kef, protects bacteria against the detrimental effects of electrophilic compounds via acidification of the bacterial cytoplasm. Glutathione (GSH) and glutathione-S-conjugate (GS-X) compounds are known to affect Kef. In particular, GSH inhibits Kef, whereas some GS-X compounds activate Kef (Healy *et al*, Understanding the Structural Requirements for Activators of the Kef Bacterial Potassium Efflux System, Biochemistry, 2014 Apr 1; 53(12):1982-92). Activation of the Kef system results in K⁺ efflux and concomitant H⁺ influx. Consequently, Kef activation results in a decrease in cellular pH, protonating nucleophiles in DNA and proteins and minimising their reactivity with electrophilic species. Kef activation is thus known to interfere with normal bacterial functioning and therefore has the potential as a therapeutic target for preventing or treating bacterial infections.

SUMMARY OF THE INVENTION

The invention provides a compound which is a peptide derivative of formula (1) or a salt thereof:



wherein:

R^G is H, $-CH_2-R^{GA}$ or C_{1-12} hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-OR'$, $-NO_2$, $-CN$, halogen and 5-6 membered heterocyclyl; or R^G and R^{N1} together form a 5-6 membered heterocyclic ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen;

R^{GA} is C_{1-11} hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, halogen and 5-6 membered heterocyclyl;

R^S is C_{1-16} hydrocarbyl, a 5-10 membered heterocyclyl or heteroaryl group, $-(C_{1-4} \text{ alkylene})-R^{SA}$ or $-(C_{1-4} \text{ alkylene})-L-R^{SA}$; R^{SA} is C_{1-16} hydrocarbyl, or a 5-10 membered heterocyclyl or heteroaryl group; L is $-SO_2-$; and wherein R^S and R^{SA} are optionally substituted with a group R^b and/or from 1 to 5 substituents selected from R^a , $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $-SR'$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2OR'$, $-P(O)(OR')(OR'')$, $=O$, $-SiR'R''R'''$ and halogen; or R^S and an R^{C1} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen;

R^b is phenyl, benzyl, C_{3-6} cycloalkyl or 5-6 membered heterocyclyl;

Q is $-C(O)-N(R^{N2})-$, $-C(O)-C(R'R'')-$, $-C(O)-O-$, $-N(R^{N2})-C(O)-$, $-C(R'R'')-$, $-C(O)-$, $-O-C(O)-$, $-C(R'R'')-C(R'''R''''-)$, $-C(R'R'')-O-$, $-O-C(R'R'')-$, $-C(R'R'')-N(R^{N2})-$, $-N(R^{N2})-C(R'R'')-$ or $-C(R')=C(R'')-$;

Z is a group selected from $-C(O)-O-R^O$ and $-CN_4R'$ (tetrazolyl);

R^O is H or C_{1-12} hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $-SR'$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2OR'$, $-P(O)(OR')(OR'')$, $=O$, halogen and 5-6 membered heterocyclyl; or R^O and an R^{C2} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen;

each R^{N1} and R^{N2} is independently H or C_{1-4} alkyl;

each R^{C1} , R^{C2} , R' , R'' , R''' and R'''' is independently H or C_{1-4} alkyl and is optionally substituted with 1 to 3 substituents selected from halogen, $-OH$ and $-O-C_{1-4}$ alkyl; and

each R^a is independently C_{1-4} alkyl which is optionally substituted with 1 to 3 halogen atoms.

The inventors have found surprisingly that the glutamate residue of GS-X compounds makes a negligible contribution to the affinity of the compounds to Kef. Thus, truncation or replacement of the glutamate residue in GS-X compounds has allowed the development of a new class of antibacterial compounds. These compounds not only demonstrate good binding

to Kef, but have also been shown to cause activation of the Kef system. Moreover, the compounds have reduced polar groups and a shorter peptide chain, and therefore are expected to demonstrate better ability to cross cell membranes than the known GSH. The compounds are thus useful in the treatment and prevention of bacterial infections in animals as well as plants. These compounds can effectively inhibit or damage bacteria through activation of the Kef system and resulting K⁺ efflux.

The invention also provides a pharmaceutical composition comprising (a) a compound which is a peptide derivative or a salt thereof as defined herein, wherein the salt is a pharmaceutically acceptable salt, and (b) one or more pharmaceutically acceptable excipients or diluents.

The invention also provides a compound which is a peptide derivative or a salt thereof as defined herein, for use in the treatment or prevention of an infection caused by bacteria, wherein the salt is a pharmaceutically acceptable salt.

The invention also provides a method of treating or preventing infection caused by bacteria in a subject, wherein the infection is as defined herein, which method comprises administering an effective amount of a peptide derivative or a salt thereof as defined herein to the subject, wherein the salt is a pharmaceutically acceptable salt.

The invention also provides a combination comprising (a) a compound which is a peptide derivative or a salt thereof as defined herein, wherein the salt is a pharmaceutically acceptable salt, and (b) a further active agent which is an antibiotic compound or a pharmaceutically acceptable salt thereof.

The invention also provides a combination for use in the treatment or prevention of an infection caused by bacteria as defined herein, which combination comprises (a) a compound which is a peptide derivative or a salt thereof as defined herein, wherein the salt is a pharmaceutically acceptable salt, and (b) a further active agent which is an antibiotic compound.

The invention also provides a kit comprising (a) a compound which is a peptide derivative or a salt thereof as defined herein, wherein the salt is a pharmaceutically acceptable salt, and (b) a further antibiotic compound or a pharmaceutically acceptable salt thereof.

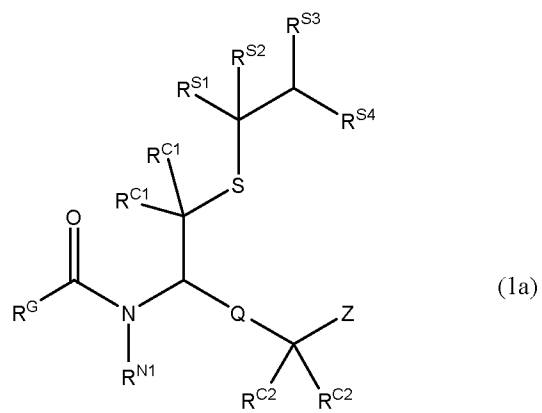
The invention also provides a compound which is a peptide derivative or a salt thereof as defined herein for use in a method of activating potassium efflux in bacteria, wherein the bacteria are as defined herein.

The invention also provides a method for preventing or treating infection of a plant caused by bacteria, which method comprises treating the plant, the seed from which the plant grows, or the place at which the plant grows, with a composition comprising a compound as defined herein.

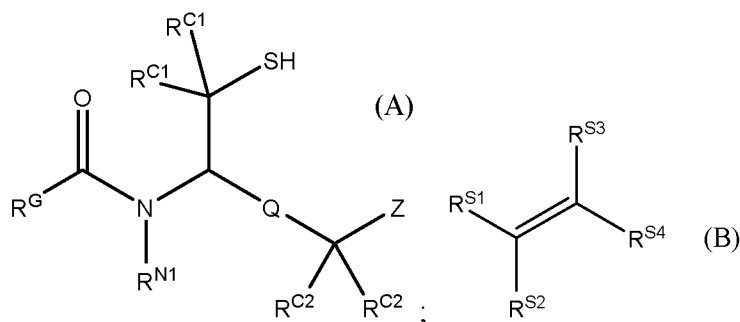
The invention also provides an in vitro method for killing or inhibiting the growth of bacteria, which method comprises applying a composition comprising a compound which is a peptide derivative or salt thereof as defined herein to a surface, to an article or to the skin. In the case of application to the skin, the method is typically not a method of treatment of the human or animal body, but merely a method for killing or inhibiting bacteria present on the skin.

The invention also provides a soap product, washing product or cleaning product comprising a compound which is a peptide derivative or salt thereof as defined herein.

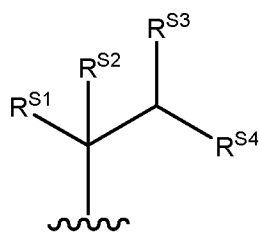
The invention also provides a process for producing a compound which is a peptide derivative of formula (1a) or a salt thereof:



which process comprises reacting a thiol compound of formula (A) with an alkene of formula (B) in the presence of an initiator,



wherein R^G , R^{N1} , R^{C1} , Q , R^{C2} and Z are as defined herein and the group



corresponds to R^S as defined herein.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1 to 3 show the results of competition fluorescence assays for compounds according to the invention and comparative compounds.

Figures 4 to 10 show Kd curves for various compounds obtained by CPMG NMR.

Figure 11 shows the results of a Kirby-Bauer disk diffusion assay performed for a compound of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

A hydrocarbonyl group is a group comprising only carbon atoms and hydrogen atoms. Thus, hydrocarbonyl groups are those derived by the removal of a hydrogen atom from a hydrocarbon compound. Examples of hydrocarbonyl groups include alkyl groups, cycloalkyl groups, alkenyl groups, cycloalkenyl groups, alkynyl groups, aryl groups, aryl groups substituted with one or more alkyl groups, alkyl groups substituted with one or more aryl groups and alkyl groups substituted with aryl groups which are themselves substituted with one or more alkyl groups. A hydrocarbonyl group may be defined by the number of carbon atoms it contains. For instance, a C_{1-16} hydrocarbonyl group comprises from 1 to 16 carbon atoms. Examples of a C_{1-16} hydrocarbonyl group include C_{1-16} alkyl, phenyl, C_{3-6} cycloalkyl, and C_{1-10} alkyl substituted

with a phenyl or C₃₋₆ cycloalkyl group, wherein each of these groups may be substituted with 1 to 3 C₁₋₃ alkyl groups. C₁₋₁₆ hydrocarbyl is typically C₁₋₁₂ hydrocarbyl or C₁₋₁₁ hydrocarbyl. Typically, C₁₋₁₂ hydrocarbyl or C₁₋₁₁ hydrocarbyl is C₁₋₈ alkyl, or a C₃₋₆ cycloalkyl, phenyl, benzyl or ethyl-phenyl group, each of which may optionally be substituted with 1 to 3 C₁₋₄ alkyl groups.

An alkyl group is a linear or branched chain saturated hydrocarbon group. An alkyl group may be a C₁₋₁₆ alkyl group, a C₁₋₈ alkyl group, or a C₁₋₄ alkyl group. Examples of a C₁₋₁₆ alkyl group are methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, neopentyl, hexyl, heptyl and octyl.

A cycloalkyl group is typically a C₃₋₆ cycloalkyl group. Examples of a C₃₋₆ cycloalkyl group include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. A cycloalkyl group is unsubstituted or substituted. Typically, it is substituted with one or two C₁₋₄ alkyl groups, e.g. 1 or 2 methyl groups, or is unsubstituted. Preferably, a cycloalkyl group is unsubstituted.

An alkenyl group is a linear or branched chain hydrocarbon radical comprising one or more double bonds. An alkenyl group may be a C₂₋₁₀ alkenyl group. Examples of C₂₋₁₀ alkenyl groups include those related to C₂₋₁₀ alkyl groups by the insertion of one or more double bonds, e.g. ethenyl (–CH=CH₂), propenyl (–CH₂–CH=CH₂ or –CH=CH–CH₃) and butenyl (–CH₂–CH₂–CH=CH₂ or –CH₂–CH=CH–CH₃ or –CH=CH–CH₂–CH₃). Alkenyl groups typically comprise one or two double bonds.

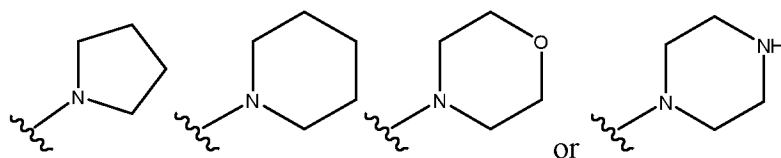
An alkynyl group is a linear or branched chain hydrocarbon radical comprising one or more triple bonds. An alkynyl group may be a C₂₋₁₀ alkynyl group. Examples of C₂₋₁₀ alkynyl groups include those related to C₂₋₁₀ alkyl groups by the insertion of one or more triple bonds, e.g. ethynyl (–C≡CH), propynyl (–CH₂–C≡CH or –C≡C–CH₃) and butynyl (–CH₂–CH₂–C≡CH or –CH₂–C≡C–CH₃ or –C≡C–CH₂–CH₃). Alkynyl groups typically comprise one or two triple bonds.

An alkyl, alkenyl or alkynyl group may be unsubstituted or substituted with one or more, e.g. from 1 to 3, substituents. Typical substituents are selected from –NR'R'', –OR', halogen, 5-6 membered heterocyclyl, –C(O)R', –C(O)OR', –C(O)NR'R'', –O–C(O)R', –N(R')–C(O)R'', –SR', –S(O)R', –S(O)₂R', –S(O)₂OR', –P(O)(OR')(OR''), =O and –SiR'R''R''', wherein each R', R'' and R''' is independently H or C₁₋₄ alkyl and is optionally substituted with 1 to 3 substituents selected from halogen, –OH and –O–C₁₋₄ alkyl. Preferred substituents are

selected from $-NR'R''$, $-OR'$, halogen and 5-6 membered heterocyclyl, wherein each R' and R'' is independently H or C_{1-4} alkyl, most preferably from $-NR'R''$, $-OR'$, halogen and morpholinyl, wherein each R' and R'' is independently H or C_{1-4} alkyl. Typically, an alkyl, alkenyl or alkynyl group is unsubstituted or substituted with from 1 to 3 halogen atoms, e.g. it is unsubstituted.

An aryl group is a monocyclic, bicyclic or polycyclic aromatic ring which contains from 6 to 14 carbon atoms, typically from 6 to 10 carbon atoms, in the ring portion. Examples include phenyl, naphthyl, indenyl, indanyl, anthracenyl and pyrenyl groups. Often an aryl group is phenyl.

A heterocyclyl group is a monovalent moiety obtained by removing a hydrogen atom from a ring atom of a heterocyclic compound. Preferably, a heterocyclyl group has from 5 to 10 ring atoms (5-10 membered heterocyclyl), more preferably 5 or 6 ring atoms (5-6 membered heterocyclyl), including at least one (e.g. one, two, three or four, preferably one or two) heteroatoms selected from N, O and S. A 5-10 membered heterocyclyl group may for instance be a group derived from pyrrolidine, imidazolidine, tetrahydrofuran, tetrahydropyran, piperidine, piperazine, morpholine, azepane, oxepane and indoline. 5-6 membered heterocyclyl may be derived from pyrrolidine, imidazolidine, tetrahydrofuran, tetrahydropyran, piperidine, piperazine, and morpholine, i.e. pyrrolidinyl, imidazolidinyl, tetrahydrofuranyl, tetrahydropyranyl, piperidinyl, piperazinyl and morpholinyl. The heterocyclyl group may be linked to the rest of the molecule via any of its ring atoms, typically a ring C or N atom. Typically, 5-6 membered heterocyclyl is



A heteroaryl group is a monocyclic or bicyclic heteroaromatic ring which typically contains from six to ten atoms in the ring portion including one or more heteroatoms. A heteroaryl group is generally a 5- or 6-membered ring, containing at least one heteroatom selected from O, S and N. It may contain, for example, one, two, three or four, e.g. one or two, heteroatoms. Examples of heteroaryl groups include pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, furanyl, thienyl, pyrazolidinyl, pyrrolyl, tetrazolyl, oxazolyl, oxadiazolyl,

isoxazolyl, thiadiazolyl, thiazolyl, isothiazolyl, imidazolyl, pyrazolyl, quinolyl and isoquinolyl. Typically, a heteroaryl group is pyridyl, pyrrolyl, tetrazolyl or oxazolyl.

An aryl, heteroaryl or heterocyclyl group may be unsubstituted or substituted. A substituted group typically carries from 1 to 5, e.g. 1, 2 or 3, substituents. Substituents are typically selected from R^a , $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $-SR'$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2OR'$, $-P(O)(OR')(OR'')$, $=O$, $-SiR'R''R'''$ and halogen, wherein R^a , R' , R'' and R''' are independently H or C_{1-4} alkyl and are optionally substituted with 1 to 3 substituents selected from halogen, $-OH$ and $-O-C_{1-4}$ alkyl. Preferred substituents on an aryl, heteroaryl or heterocyclyl group are C_{1-4} alkyl, $-CF_3$, $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $=O$ and halogen, wherein R' and R'' are independently H or C_{1-4} alkyl.

Halogen is chlorine, fluorine, bromine or iodine (a chloro group, a fluoro group, a bromo group or an iodo group). Halogen is typically chlorine or fluorine.

A C_{1-4} alkoxy group is a group of formula $-OR$ wherein R is a C_{1-4} alkyl group. Examples of C_{1-4} alkoxy include $-OMe$, $-OEt$, $-O^iPr$, $-O^tBu$ and $-O^iBu$.

An alkylene group is a bivalent group derived by removing two hydrogen atoms from a straight chain or branched alkane. Examples of a C_{1-4} alkylene group are $-CH_2-$, $-CH(CH_3)-$, $-C(CH_3)_2-$, $-CH_2CH_2-$, $-CH(CH_3)CH_2-$, $-CH_2CH(CH_3)-$, $-CH_2C(CH_3)_2-$, $-C(CH_3)_2CH_2-$, $-CH_2CH_2CH_2-$, $-CH(CH_3)CH_2CH_2-$, $-CH_2CH(CH_3)CH_2-$, $-CH_2CH_2CH(CH_3)-$ and $-CH_2CH_2CH_2CH_2-$. Typically, a C_{1-4} alkylene group is $-CH_2-$, $-CH_2CH_2-$, $-CH_2CH_2CH_2-$ or $-CH_2CH_2CH_2CH_2-$ and a C_{1-3} alkylene group is $-CH_2-$, $-CH_2CH_2-$ or $-CH_2CH_2CH_2-$.

Unless otherwise specified, included in groups and substituents defined herein are the well known ionic, salt, solvate, and protected forms of these groups and substituents. For example, a reference to carboxylic acid or carboxyl group ($-COOH$) also includes the anionic (carboxylate) form ($-COO^-$), a salt or solvate thereof. Similarly, a reference to an amino group includes the protonated form ($-N^+HR^1R^2$), a salt or solvate of the amino group, for example, a hydrochloride salt. Similarly, a reference to a hydroxyl group also includes the anionic form ($-O^-$), a salt or solvate thereof.

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diastereomeric, epimeric, atropic, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α - and β -forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Substantially optically pure forms are also included. Methods for the preparation (e.g., asymmetric synthesis) and separation (e.g., fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting known methods, in a known manner.

The term "salt", as used herein, refers to salt comprising a cationic or ionic form of the compound together with a counter ion. Such salts are typically acid addition salts. Often, the salt is a pharmaceutically acceptable salt. A pharmaceutically acceptable salt is a salt formed with a pharmaceutically acceptable acid or base. Pharmaceutically acceptable acids include both inorganic acids such as hydrochloric, sulphuric, phosphoric, diphosphoric, hydrobromic or nitric acid and organic acids such as citric, fumaric, maleic, malic, ascorbic, succinic, tartaric, benzoic, acetic, methanesulphonic, ethanesulphonic, benzenesulphonic or p-toluenesulphonic acid. Pharmaceutically acceptable bases include alkali metal (e.g. sodium or potassium) and alkali earth metal (e.g. calcium or magnesium) hydroxides and organic bases such as alkyl amines, aralkyl amines and heterocyclic amines.

Compound

The invention provides a compound which is a peptide derivative of formula (1) or a salt thereof as defined above.

Q is typically $-\text{C}(\text{O})-\text{N}(\text{R}^{\text{N}2})-$, $-\text{C}(\text{O})-\text{C}(\text{R}'\text{R}'')$, $-\text{C}(\text{O})-\text{O}-$, $-\text{N}(\text{R}^{\text{N}2})-\text{C}(\text{O})-$, $-\text{C}(\text{R}'\text{R}'')$, $-\text{C}(\text{O})-$ or $-\text{O}-\text{C}(\text{O})-$.

More typically, **Q** is $-\text{C}(\text{O})-\text{N}(\text{R}^{\text{N}2})-$, $-\text{C}(\text{O})-\text{CH}_2-$ or $-\text{C}(\text{O})-\text{O}-$.

Preferably, **Q** is $-\text{C}(\text{O})-\text{N}(\text{R}^{\text{N}2})-$, most preferably $-\text{C}(\text{O})-\text{NH}-$.

Typically, **Z** is $-\text{C}(\text{O})-\text{O}-\text{R}^{\text{O}}$ and **R^O** is H, C₁₋₄ alkyl, phenyl or benzyl, and **R^O** is optionally substituted with 1 to 4 substituents selected from R^a, $-\text{NR}'\text{R}''$, $-\text{OR}'$, $-\text{C}(\text{O})\text{R}'$, $-\text{C}(\text{O})\text{OR}'$, $-\text{O}-\text{C}(\text{O})\text{R}'$, $=\text{O}$ and halogen.

Preferably, **Z** is $-\text{C}(\text{O})-\text{O}-\text{R}^{\text{O}}$ and **R^O** is H, C₁₋₄ alkyl, phenyl or benzyl, wherein the phenyl and benzyl groups are optionally substituted with 1 to 4 substituents selected from R^a, $-\text{OR}'$ and halogen. Preferred substituents are C₁₋₄ alkyl, $-\text{CF}_3$, $-\text{O}(\text{C}_{1-4})\text{alkyl}$ and halogen, in particular methyl and halogen. Typically, **R^O** is unsubstituted.

Preferably, **R^O** is H or C₁₋₄ alkyl optionally substituted with $-\text{O}-\text{C}_{1-4}\text{ alkyl}$. For example, **R^O** may be H or methyl, e.g. H.

Alternatively, **Z** may be $-\text{CN}_4\text{H}$ (tetrazolyl).

Often, each R^a is independently methyl or ethyl which is optionally substituted with 1 to 3 fluorine atoms.

Typically, in formula (1) or (2), each R^{N1} and R^{N2} is independently H or methyl. Preferably, R^{N1} and R^{N2} are each H. R^{N1} and R^{N2} may be the same or different.

Typically, in formula (1) or (2), each R^{C1} is the same or different and is independently H or methyl. Typically, in formula (1) or (2), each R^{C2} is the same or different and is independently H or methyl. Preferably, R^{C1} and R^{C2} are all H.

Typically, each R', R'', R''' and R'''' is independently H or C₁₋₄ alkyl, preferably H.

Typically, **R^G** is H, $-\text{CH}_2-\text{R}^{\text{GA}}$ or C₁₋₁₂ hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-\text{OR}'$, halogen and 5-6 membered heterocyclyl; and **R^{GA}** is C₁₋₁₁ hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-\text{NR}'\text{R}''$, $-\text{OR}'$, halogen and 5-6 membered heterocyclyl.

Alternatively, **R^G** may be H, $-\text{CH}_2-\text{R}^{\text{GA}}$ or a C₁₋₈ alkyl group which is optionally substituted with 1 to 4 substituents selected from $-\text{OR}'$, halogen and 5-6 membered heterocyclyl; or **R^G** and R^{N1} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a, $-\text{OR}'$ and halogen. Preferably, **R^G** is H, $-\text{CH}_2-\text{R}^{\text{GA}}$ or a

C₁₋₈ alkyl group which is optionally substituted with 1 to 4 substituents selected from -OR', halogen and 5-6 membered heterocyclyl. **R^{GA}** is typically a C₁₋₇ alkyl group which is optionally substituted with 1 to 4 substituents selected from -NR'R'', -OR', halogen and 5-6 membered heterocyclyl.

For instance, **R^G** may be C₁₋₈ alkyl, phenyl or benzyl, each of which is optionally substituted with 1 to 4 substituents selected from halogen, -OH and -O-C₁₋₄ alkyl.

More typically, **R^G** is C₁₋₈ alkyl optionally substituted with 1 to 4 substituents selected from halogen, -OH and -O-C₁₋₄ alkyl.

Preferably, **R^G** is methyl or ethyl.

Typically, **R^O** is H, C₁₋₈ alkyl, phenyl or benzyl, each of which is optionally substituted with 1 to 4 substituents selected from -NR'R'', -OR', -C(O)R', -C(O)OR', -C(O)NR'R'', -O-C(O)R', -N(R')-C(O)R'', =O, halogen and 5-6 membered heterocyclyl.

More typically, **R^O** is H or C₁₋₄ alkyl optionally substituted with -O-C₁₋₄ alkyl.

Preferably, **R^O** is H, methyl or ethyl.

For instance, **R^G** may be C₁₋₈ alkyl optionally substituted with 1 to 4 substituents selected from halogen, -OH and -O-C₁₋₄ alkyl and **R^O** may be H or C₁₋₄ alkyl optionally substituted with -O-C₁₋₄ alkyl.

Typically, **R^S** is C₁₋₁₆ hydrocarbyl, a 5-10 membered heterocyclyl or heteroaryl group, -(C₁₋₄ alkylene)-**R^{SA}** or -(C₁₋₄ alkylene)-L-**R^{SA}**; **R^{SA}** is C₁₋₁₆ hydrocarbyl, or a 5-10 membered heterocyclyl or heteroaryl group; L is -SO₂-; wherein **R^S** and **R^{SA}** are optionally substituted with a group **R^b** and/or from 1 to 5 substituents selected from **R^a**, -NR'R'', -OR', -NO₂, -CN, -C(O)R', -C(O)OR', -C(O)NR'R'', -O-C(O)R', -N(R')-C(O)R'', -SR', =O and halogen; or **R^S** and an **R^{C1}** together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from **R^a**, -OR' and halogen; wherein **R_a**, **R'** and **R''** are as defined herein, and **R^b** is phenyl, benzyl, C₃₋₆ cycloalkyl or 5-6 membered heterocyclyl.

Typically, where R^S is as defined above, each R' and R'' is independently H or C_{1-4} alkyl which is optionally substituted with 1 to 3 substituents selected from halogen, $-OH$ and $-O-$ C_{1-4} alkyl; and each R^a is independently C_{1-4} alkyl which is optionally substituted with 1 to 3 halogen atoms. Preferably, where R^S is as defined above, R^a is C_{1-4} alkyl or $-CF_3$ and R' and R'' are each independently H or C_{1-4} alkyl.

Typical substituents on R^S are a group R^b and/or from 1 to 5 substituents selected from C_{1-4} alkyl, $-CF_3$, $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $=O$ and halogen. Preferably, R^S is substituted with 0 or 1 groups R^b and 0, 1, 2 or 3 substituents selected from C_{1-4} alkyl, $-CF_3$, $-OC_{1-4}alkyl$, $-NO_2$, $-CN$, $=O$ and halogen; wherein R^b is phenyl, benzyl or C_{3-6} cycloalkyl.

More typically, R^S is C_{1-10} alkyl, $-(C_{1-3} alkylene)-R^{SA}$, $-(C_{1-3} alkylene)-L-R^{SA}$, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-6} cycloalkyl, 5- to 10 membered heterocyclyl, C_{6-10} aryl or 5- to 10-membered heteroaryl, wherein L is SO_2 , R^{SA} is C_{3-6} cycloalkyl, 5- to 10 membered heterocyclyl, C_{6-10} aryl or 5- to 10-membered heteroaryl and R^S and R^{SA} are optionally substituted with a group R^b and/or from 1 to 5 substituents selected from C_{1-4} alkyl, $-CF_3$, $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $=O$ and halogen; wherein each R' and R'' is independently H or C_{1-4} alkyl; and R^b is phenyl, benzyl or C_{3-6} cycloalkyl.

More typically, R^S is C_{1-10} alkyl, $-(C_{1-3} alkylene)-R^{SA}$, $-(C_{1-3} alkylene)-L-R^{SA}$, C_{3-6} cycloalkyl, 5-6 membered heterocyclyl or phenyl, wherein L is SO_2 , R^{SA} is C_{3-6} cycloalkyl, 5-6 membered heterocyclyl or phenyl and R^S and R^{SA} are optionally substituted with a group R^b and/or from 1 to 3 substituents selected from C_{1-4} alkyl, $-CF_3$, $-OC_{1-4}alkyl$, $-NO_2$, $-CN$, $=O$ and halogen; wherein R^b is phenyl, benzyl or C_{3-6} cycloalkyl.

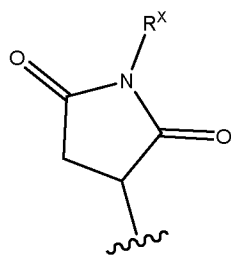
For instance, R^S may be:

(i) C_{1-10} alkyl, $-(C_{1-2} alkylene)-(C_{3-6} cycloalkyl)$ or C_{3-6} cycloalkyl, wherein the cycloalkyl ring is optionally substituted with from 1 to 3 methyl groups;

(ii) 5-6 membered heterocyclyl or $-CH_2CH_2-(5-6 membered heterocyclyl)$ wherein the heterocyclyl group in each case is optionally substituted with phenyl, benzyl or C_{3-6} cycloalkyl and/or with from 1 to 3 substituents selected from C_{1-4} alkyl, $-NO_2$, halogen, C_{1-4} alkoxy, and $=O$; or

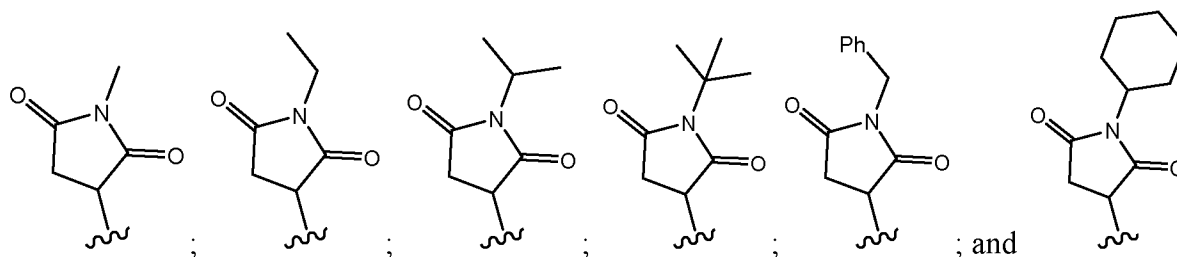
(iii) $-\text{CH}_2\text{CH}_2-\text{Ph}-(\text{Y})_n$ or $-\text{CH}_2\text{CH}_2-\text{SO}_2-\text{Ph}-(\text{Y})_n$, wherein n is 0 or 1 and Y is $-\text{NO}_2$, $-\text{CF}_3$, $-\text{F}$ or $-\text{OCH}_3$.

In some embodiments, R^{S} is a group of formula:



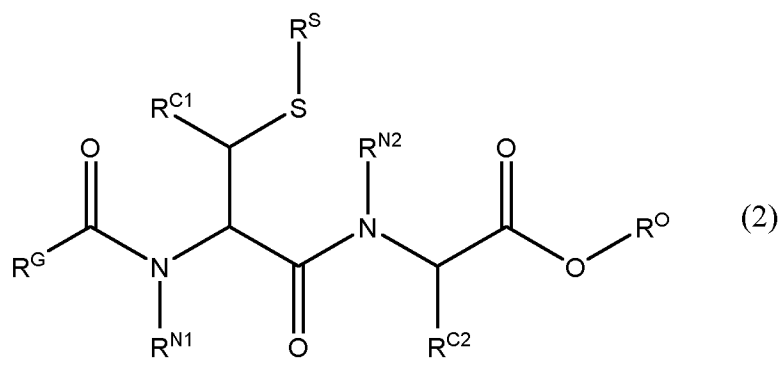
where R^{X} is H, phenyl, benzyl, C_{3-6} cycloalkyl or C_{1-4} alkyl.

For instance, R^{S} may be a group selected from the following groups:



Typically, each $\text{R}^{\text{C}1}$ and $\text{R}^{\text{C}2}$ is independently H or C_{1-4} alkyl, preferably H.

Typically, the compound is a peptide derivative of formula (2) or a salt thereof:



wherein:

R^{G} is H, $-\text{CH}_2-\text{R}^{\text{GA}}$ or a C_{1-8} alkyl group which is optionally substituted with 1 to 4 substituents selected from $-\text{OR}'$, halogen and 5-6 membered heterocyclyl; or R^{G} and $\text{R}^{\text{N}1}$ together form a 5-6 membered heterocyclic ring which is optionally substituted with 1 to 3 substituents selected from R^{a} , $-\text{OR}'$ and halogen;

R^{GA} is C_{1-7} alkyl group which is optionally substituted with 1 to 4 substituents selected from $-\text{NR}'\text{R}''$, $-\text{OR}'$, halogen and 5-6 membered heterocyclyl;

R^S is C_{1-16} hydrocarbyl, a 5-10 membered heterocyclyl or heteroaryl group, $-(C_{1-4}$ alkylene)- R^{SA} or $-(C_{1-4}$ alkylene)-L- R^{SA} ; R^{SA} is C_{1-16} hydrocarbyl, or a 5-10 membered heterocyclyl or heteroaryl group; L is $-SO_2-$; and wherein R^S and R^{SA} are optionally substituted with a group R^b and/or from 1 to 5 substituents selected from R^a , $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $-SR'$, $=O$ and halogen; or R^S and an R^{C1} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen;

R^b is phenyl, benzyl, C_{3-6} cycloalkyl or 5-6 membered heterocyclyl;

R^O is H, C_{1-4} alkyl, phenyl or benzyl, wherein the phenyl and benzyl groups are optionally substituted with 1 to 4 substituents selected from R^a , $-OR'$ and halogen;

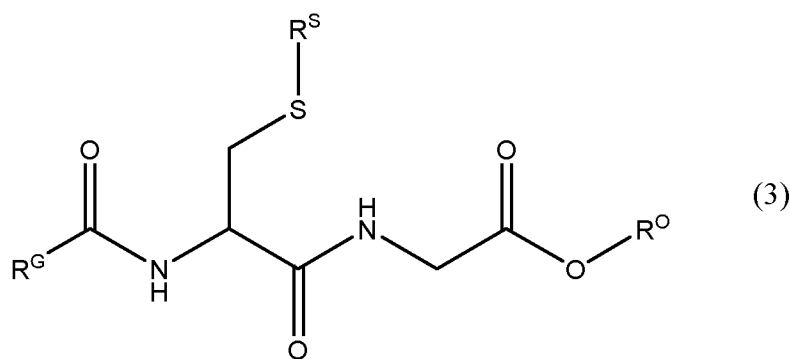
R^{N1} and R^{N2} are each independently H or C_{1-4} alkyl;

R^{C1} and R^{C2} are each independently H or C_{1-4} alkyl;

each R' and R'' is independently H or C_{1-4} alkyl which is optionally substituted with 1 to 3 substituents selected from halogen, $-OH$ and $-O-C_{1-4}$ alkyl; and

each R^a is independently C_{1-4} alkyl which is optionally substituted with 1 to 3 halogen atoms.

More typically, the compound is a peptide derivative of formula (3) or a salt thereof:



wherein:

R^G is H, $-CH_2-R^{GA}$ or a C_{1-8} alkyl group which is optionally substituted with 1 to 4 substituents selected from $-OR'$, halogen and 5-6 membered heterocyclyl;

R^{GA} is C_{1-7} alkyl group which is optionally substituted with 1 to 4 substituents selected from $-NR'R''$, $-OR'$, halogen and 5-6 membered heterocyclyl;

R^S is C_{1-16} hydrocarbyl, a 5-10 membered heterocyclyl or heteroaryl group, $-(C_{1-4}$ alkylene)- R^{SA} or $-(C_{1-4}$ alkylene)-L- R^{SA} ; R^{SA} is C_{1-16} hydrocarbyl, or a 5-10 membered heterocyclyl or heteroaryl group; L is $-SO_2-$; and wherein R^S and R^{SA} are optionally

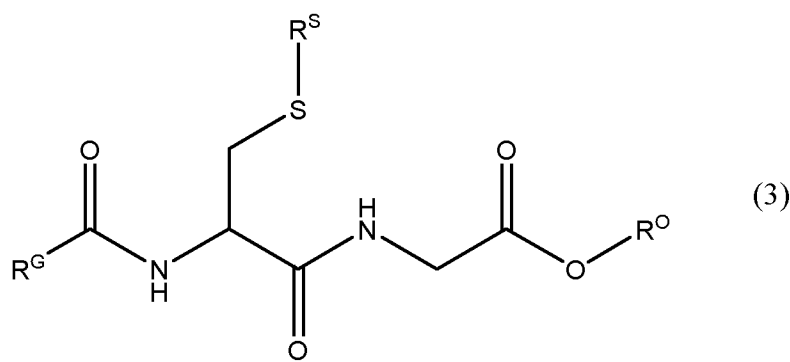
substituted with a group R^b and/or from 1 to 5 substituents selected from C_{1-4} alkyl, $-CF_3$, $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $=O$ and halogen;

R^b is phenyl, benzyl, C_{3-6} cycloalkyl or 5-6 membered heterocyclyl;

R^O is H or C_{1-4} alkyl optionally substituted with $-O-C_{1-4}$ alkyl; and

each R' and R'' is independently H or C_{1-4} alkyl.

Thus, the compound of the invention may be a peptide derivative of formula (3) or a salt thereof:



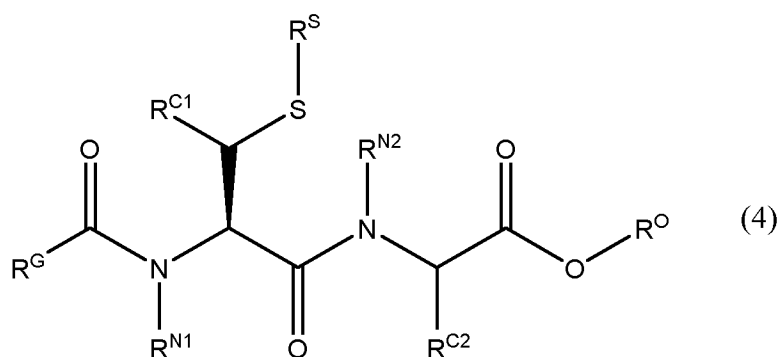
wherein:

R^G is C_{1-8} alkyl optionally substituted with 1 to 4 substituents selected from halogen, $-OH$ and $-O-C_{1-4}$ alkyl.

R^O is H, C_{1-8} alkyl, phenyl or benzyl, each of which is optionally substituted with 1 to 4 substituents selected from $-NR'R''$, $-OR'$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $=O$, halogen and 5-6 membered heterocyclyl;

R^S is (i) 5-6 membered heterocyclyl or $-CH_2CH_2-(5-6 \text{ membered heterocyclyl})$ wherein the heterocyclyl group in each case is optionally substituted with phenyl, benzyl or C_{3-6} cycloalkyl and/or with from 1 to 3 substituents selected from C_{1-4} alkyl, $-NO_2$, halogen, C_{1-4} alkoxy, and $=O$; or (ii) $-CH_2CH_2-Ph-(Y)_n$ or $-CH_2CH_2-SO_2-Ph-(Y)_n$, wherein n is 0 or 1 and Y is $-NO_2$, $-CF_3$, $-F$ or $-OCH_3$.

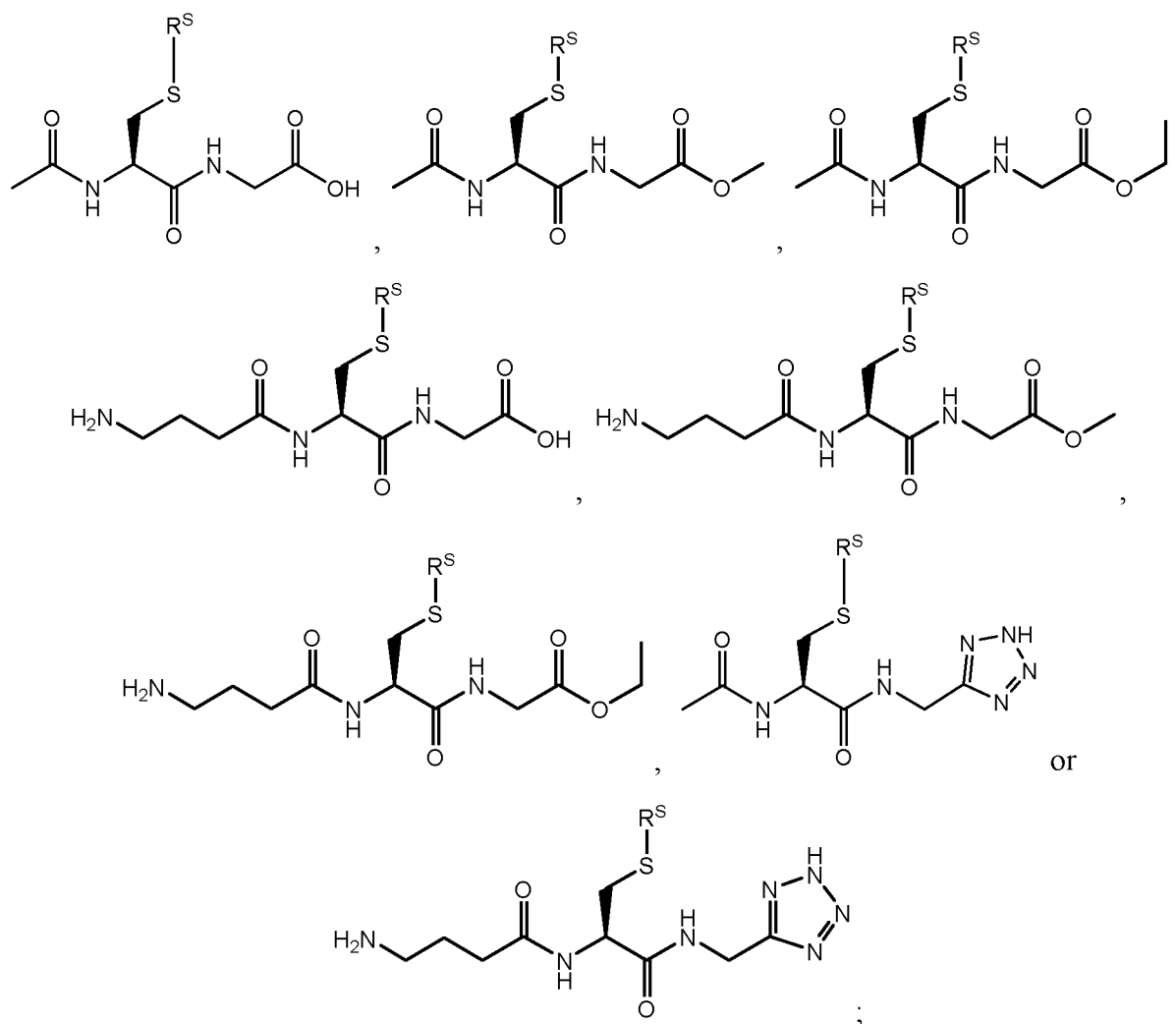
Preferably, the compound is a peptide derivative of formula (4) or a salt thereof:



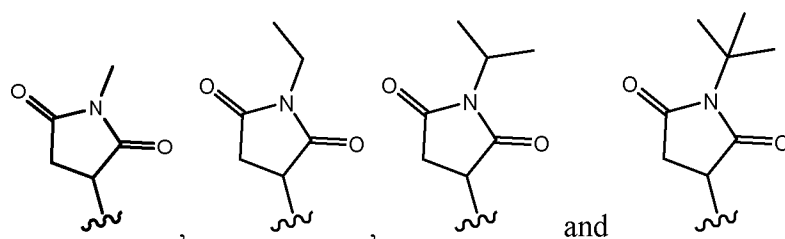
wherein R^G , R^{N1} , R^S , R^{C1} , R^{N2} , R^{C2} and R^O are as defined herein.

Preferably in a compound of the invention R^G is methyl and R^O is H or methyl.

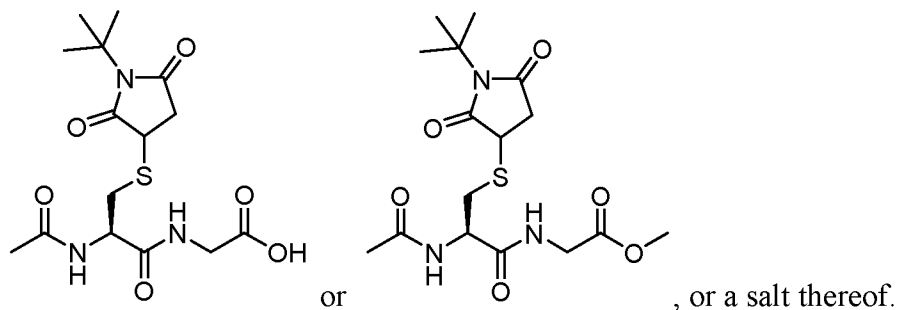
The compound of the invention may for instance be a compound of formula



or a salt thereof, and where R^S is a group selected from



Preferably, the compound is



If the compound of the invention is a salt of the peptide derivative, it is typically an acid addition salt.

Compositions and products

The invention provides a pharmaceutical composition comprising (a) a compound which is a peptide derivative or a salt thereof as defined herein, wherein the salt is a pharmaceutically acceptable salt, and (b) one or more pharmaceutically acceptable excipients or diluents.

The compounds of the invention may be administered as pharmaceutical compositions in a variety of dosage forms. Thus, they can be administered orally, for example as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules. The compounds of the invention may also be administered parenterally, whether subcutaneously, intravenously, intramuscularly, intrasternally, transdermally or by infusion techniques. The compounds may also be administered as suppositories.

The one or more pharmaceutically acceptable excipients or diluents may be any suitable excipients or diluents. For example, a pharmaceutical composition which is a solid oral form may contain, together with the active compound, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents; e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating agents, e.g. starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non toxic and pharmacologically inactive substances used in pharmaceutical formulations. Such pharmaceutical preparations may be manufactured in known manner, for example, by means of mixing, granulating, tableting, sugar coating, or film coating processes.

A pharmaceutical composition which is a liquid dispersion for oral administration may be a syrup, emulsion and suspension. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol.

Suspensions and emulsions may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspension or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

Solutions for injection or infusion may contain as carrier, for example, sterile water or preferably they may be in the form of sterile, aqueous, isotonic saline solutions.

The compounds of the invention can be used in combination with further active agents, and in particular in combination with one or more antibiotic compounds. For the avoidance of doubt, antibiotic compounds may be provided in the form of pharmaceutically acceptable salts.

The invention therefore provides a combination comprising (a) a compound which is a peptide derivative or a salt thereof as defined herein, wherein the salt is a pharmaceutically acceptable salt, and (b) a further active agent which is an antibiotic compound.

The further active agent may be any suitable antibiotic compound. The antibiotic compound may be a compound of the aminoglycoside class, ansamycin class, carbacephem class, carbapenem class, cephalosporin class, glycopeptide class, lincosamide class, lipopeptide class, macrolide class, monobactam class, nitrofurans class, oxazolidinone class, penicillin class, polypeptide class, quinolone class, sulfonamide class or tetracyclines class. For instance, the further antibiotic may be phenoxymethylpenicillin, flucloxacillin, amoxicillin, cefaclor, cefadroxil, cefalexin, tetracycline, doxycycline, lymecycline, gentamicin, tobramycin, erythromycin, azithromycin, clarithromycin, clindamycin, co-trimoxazole, metronidazole, tinidazole, ciprofloxacin, levofloxacin or norfloxacin.

The combination typically further comprises (c) one or more pharmaceutically acceptable excipients or diluents. The one or more pharmaceutically acceptable excipients or diluents may be as defined above.

The combination of the invention may for instance be a fixed combination. In a fixed combination, components (a) and (b) are present in the same composition. The fixed combination can be used for simultaneous administration of two compounds. The fixed combination is typically a solid composition (e.g. a tablet) or a solution (e.g. an injectable composition). The two components in a fixed combination are typically intermixed.

Alternatively, the combination of the invention may be a free combination. In a free combination, the active components (a) and (b) are typically separate from each other and packaged in one unit for simultaneous, substantially simultaneous, separate or sequential administration.

The invention also provides a kit comprising (a) a compound which is a peptide derivative or a salt thereof as herein, wherein the salt is a pharmaceutically acceptable salt, and (b) a further antibiotic compound. The kit may further comprise instructions for using or administering components (a) and (b), for instance in a method of treating or preventing an infection caused by bacteria.

The invention provides a soap product, cleaning product or washing product comprising a compound as described herein. A soap product is a composition or article which is suitable for washing the skin. The soap product may be a liquid soap product (for instance a hand wash, body wash, shampoo or face wash) or a solid soap product (for instance a bar of soap or a disposable wipe). A soap product according to the invention typically further comprises a soap, which is usually a surfactant. Examples of soaps include salts of fatty acids (i.e. linear C₆₋₁₈ alkyl or alkenyl carboxylic acids), for instance a sodium or potassium salt of lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid or linolenic acid, and salts of long chain sulfonic acids (i.e. linear C₆₋₁₈ alkyl or alkenyl sulfonic acids), for instance sodium laureth sulfate.

The soap product may for instance be a liquid soap product comprising one or more solvents, for instance selected from water and alcohols. Often, the soap product is a liquid soap product which further comprises ethanol.

A cleaning product includes household cleaning products such as surface cleaning products, fabric cleaners, washing powders, and cleaning products suitable for use in a medical environment, for example to clean or disinfect surfaces in a hospital or equipment found in a clinical environment such as medical equipment.

Treatment or prevention of bacterial infections

The compounds of the invention can kill or inhibit the growth of bacteria. The invention therefore provides a compound which is a peptide derivative or a salt thereof as defined herein, for use in the treatment or prevention of an infection caused by bacteria, wherein the salt is a pharmaceutically acceptable salt.

The compounds of the invention can treat a number of different bacterial infections. The infection may be caused by bacteria which are gram-negative or gram-positive. Typically, the bacterial infection is caused by gram-negative bacteria. The infection may be caused by bacteria of genus *Shewanella*, *Acinetobacter*, *Bacillus*, *Bartonella*, *Bordetella*, *Borrelia*, *Brucella*, *Campylobacter*, *Chlamydia*, *Chlamydophila*, *Clostridium*, *Corynebacterium*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Francisella*, *Haemophilus*, *Helicobacter*, *Klebsiella*, *Legionella*, *Leptospira*, *Listeria*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Pseudomonas*, *Rickettsia*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Treponema*, *Vibrio* or *Yersinia*. For example, the bacterial infection may be caused by bacteria of genus *Shewanella* or *Escherichia*.

Gram-negative bacteria include bacteria of the genus *Shewanella*, *Escherichia*, *Salmonella*, *Shigella*, *Pseudomonas*, *Moraxella*, *Helicobacter*, *Stenotrophomonas*, *Bdellovibrio* and *Legionella*. In particular, the compound of the invention may be used to treat or prevent a bacterial infection caused by *Staphylococcus aureus*, *Escherichia coli*, *Shewanella denitrificans*, *Hemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*, *Helicobacter pylori*, *Salmonella enteritidis*, *Salmonella typhi* or *Acinetobacter baumannii*.

The invention also provides a method of treating or preventing infection caused by bacteria as defined above in a subject, which method comprises administering an effective amount of a peptide derivative or a salt thereof as defined herein to the subject, wherein the salt is a pharmaceutically acceptable salt. The invention also provides the use of a peptide derivative or a salt thereof as defined herein in the manufacture of a medicament for use in the treatment or prevention of infection caused by bacteria as defined above in a subject.

In the invention, the subject is typically a mammal, more typically a human. The bacterial infection may be of any part of the body, for instance part of the skin, the lungs or the gut.

A therapeutically effective amount of a compound of the invention is administered to a patient. A typical dose is from 0.001 to 1000 mg, according to the activity of the specific compound, the age, weight and conditions of the subject to be treated, the type and severity of the disease and the frequency and route of administration. Preferably, daily dosage levels are from 0.001 mg to 4000 mg.

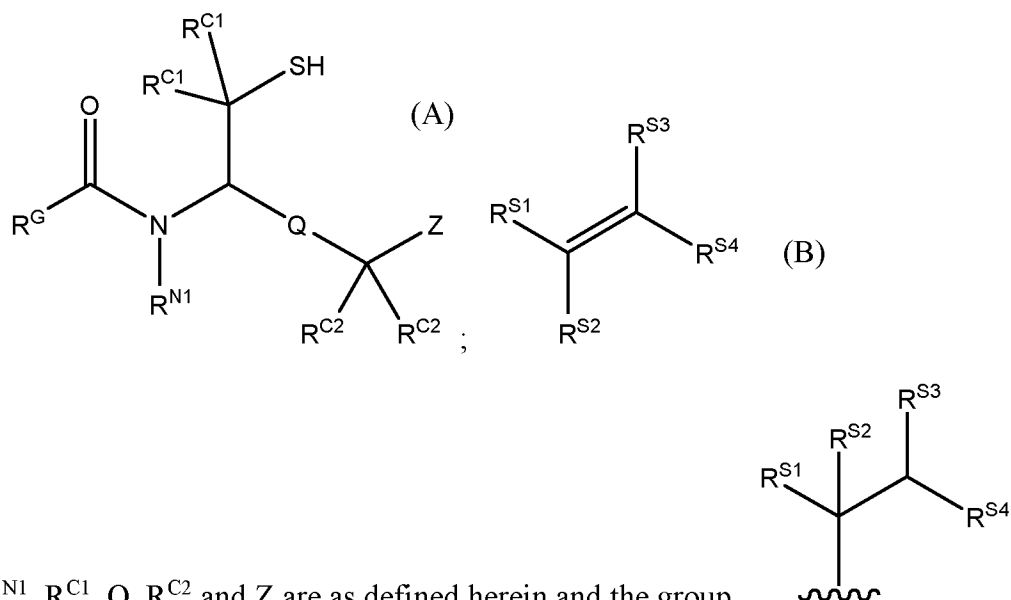
As discussed above, the compounds of the invention may be used alone, or alternatively they may be used in combination with a second active agent, typically an antibiotic compound. The combination may be for use in the treatment or prevention of an infection caused by bacteria as defined above, which combination comprises (a) a compound which is a peptide derivative or a salt thereof as defined herein, wherein the salt is a pharmaceutically acceptable salt, and (b) a further active agent which is an antibiotic compound or a pharmaceutically acceptable salt thereof.

The invention provides a compound which is a peptide derivative or a salt thereof as defined herein, for use in the treatment or prevention of an infection caused by bacteria as defined herein combination with a further active agent which is an antibiotic compound, wherein the salt is a pharmaceutically acceptable salt. A method of treating or preventing infection caused by bacteria as defined herein in a subject is also described, which method comprises administering an effective amount of a peptide derivative or a salt thereof as defined herein to the subject in combination with a further active agent which is an antibiotic compound, wherein the salt is a pharmaceutically acceptable salt. The invention also provides the use of a peptide derivative or a salt thereof as defined herein in the manufacture of a medicament for use in the treatment or prevention of infection caused by bacteria as defined herein in a subject, in combination with a further active agent which is an antibiotic compound.

In the uses of the combination according to the invention, the compound of the invention and the further active agent may be used simultaneously or sequentially.

Methods of controlling bacteria

The invention also provides a method for preventing or treating infection of a plant caused by bacteria, which method comprises treating the plant, the seed from which the plant grows, or the place at which the plant grows, with a composition comprising a compound as defined herein.



wherein R^G , R^{N1} , R^{C1} , Q , R^{C2} and Z are as defined herein and the group corresponds to R^S as defined herein.

Typically:

R^{S1} , R^{S2} , R^{S4} are independently H or C_{1-4} alkyl and

R^{S3} is a C_{1-16} hydrocarbyl, a 5-10 membered heterocyclyl or heteroaryl group, $-(C_{1-2}$ alkylene)- R^{SA} or $-(C_{1-2}$ alkylene)- $L-R^{SA}$; R^{SA} is C_{1-16} hydrocarbyl, or a 5-10 membered heterocyclyl or heteroaryl group; L is $-SO_2-$; and wherein R^{S3} and R^{SA} are optionally substituted with a group R^b and/or from 1 to 5 substituents selected from R^a , $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $-SR'$, $=O$ and halogen; or

wherein R^{S1} and R^{S3} together form a 5-10 membered heterocyclyl group or a C_{3-6} cycloalkyl group optionally substituted with a group R^b and/or from 1 to 5 substituents selected from R^a , $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $-SR'$, $=O$ and halogen;

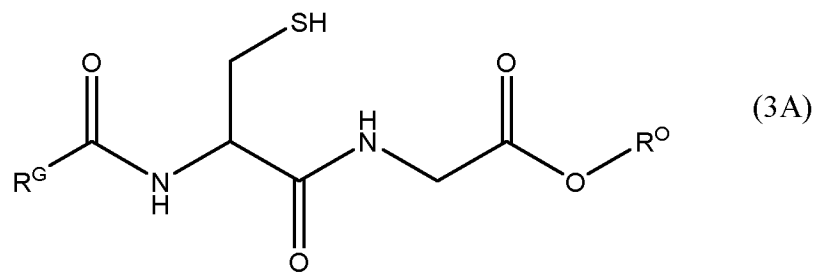
R^b is phenyl, benzyl, C_{3-6} cycloalkyl or 5-6 membered heterocyclyl;

each R' and R'' is independently H or C_{1-4} alkyl which is optionally substituted with 1 to 3 substituents selected from halogen, $-OH$ and $-O-C_{1-4}$ alkyl; and

each R^a is independently C_{1-4} alkyl which is optionally substituted with 1 to 3 halogen atoms.

For instance, R^{S1} , R^{S2} , R^{S4} may be H and R^{S3} may be phenyl or $-SO_2$ -phenyl and R^{S3} is optionally substituted with a group selected from halogen, $-NO_2$, $-OMe$ and $-CF_3$.

Typically, compound (A) is a compound of formula (3A) or a salt thereof:



wherein:

R^G is H, $-CH_2-R^{GA}$ or a C_{1-8} alkyl group which is optionally substituted with 1 to 4 substituents selected from $-OR'$, halogen and 5-6 membered heterocyclyl;

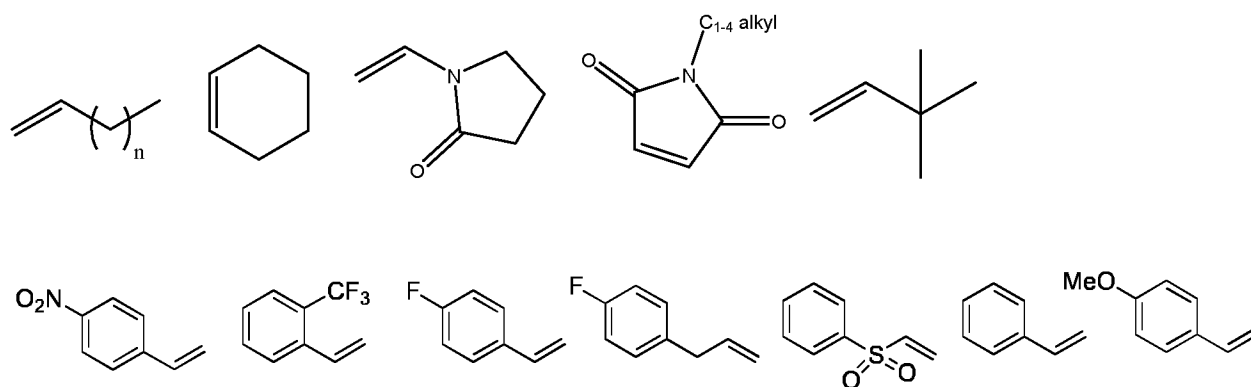
R^{GA} is C_{1-7} alkyl group which is optionally substituted with 1 to 4 substituents selected from $-NR'R''$, $-OR'$, halogen and 5-6 membered heterocyclyl;

R^O is H or C_{1-4} alkyl optionally substituted with $-O-C_{1-4}$ alkyl; and

each R' and R'' is independently H or C_{1-4} alkyl.

Typically R^G may be C_{1-8} alkyl optionally substituted with 1 to 4 substituents selected from halogen, $-OH$ and $-O-C_{1-4}$ alkyl and R^O may be H or C_{1-4} alkyl optionally substituted with $-O-C_{1-4}$ alkyl.

Typically, compound (B) is a compound selected from the formulae:



where n is from 0 to 11.

The initiator may be a chemical initiator (for instance a free radical) or a physical initiator (for instance UV light or heat). Typically, the initiator is UV light. The reaction may use UV light together with a chemical initiator (e.g. 2,2-dimethoxyphenyl acetophenone).

The temperature of the reaction is typically from 10 to 40°C.

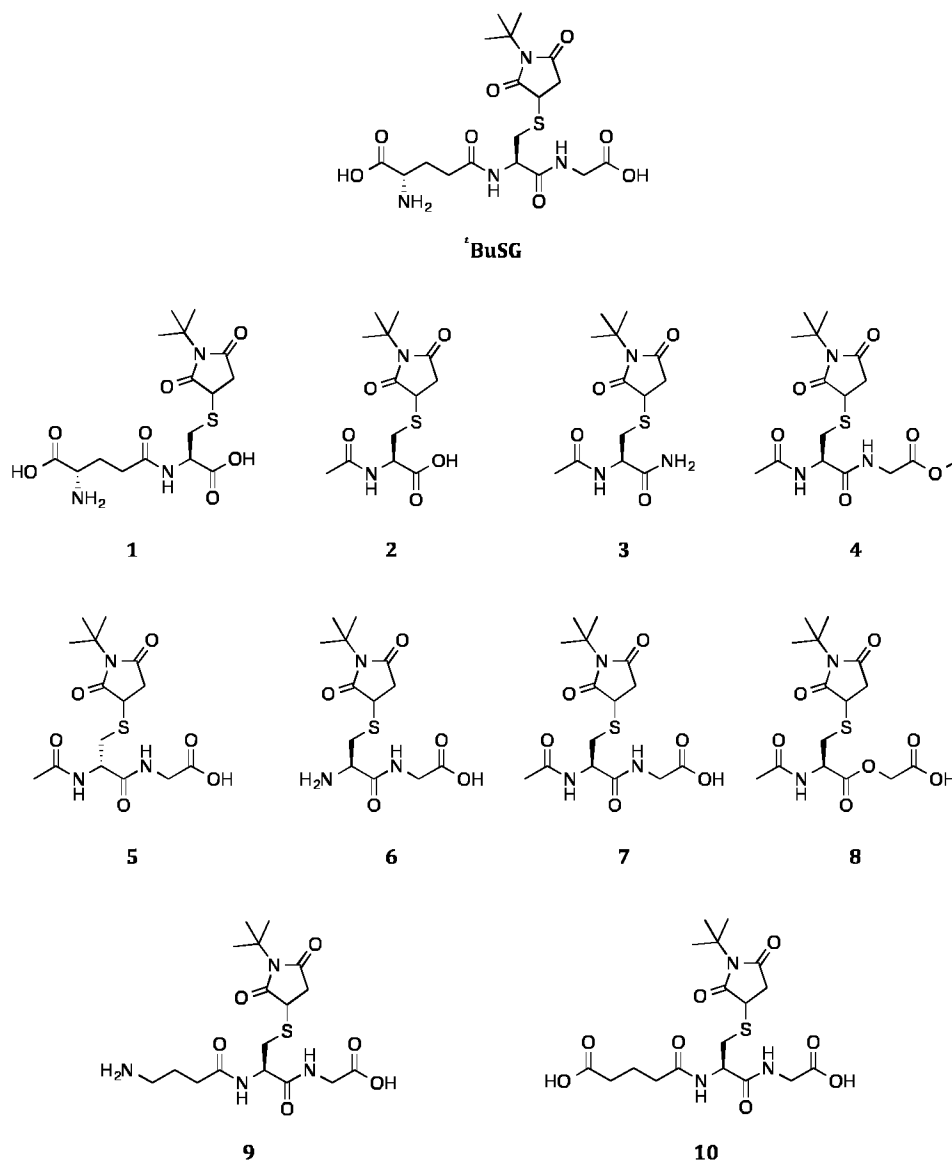
Typically, the alkene (compound (B)) and the thiol (compound (A)) are stirred at from 10 to 40°C in a solvent (for instance THF/H₂O (1:2, 3 mL)) in the presence of light (e.g. at 365 nm) for from 1 to 10 h. After this time the reaction may be filtered, and the solid washed with ethanol and water.

The following Examples illustrate the invention. They do not however, limit the invention in any way. In this regard, it is important to understand that the particular assay used in the Examples section is designed only to provide an indication of activity. There are many assays available to determine such activity, and a negative result in any one particular assay is therefore not determinative.

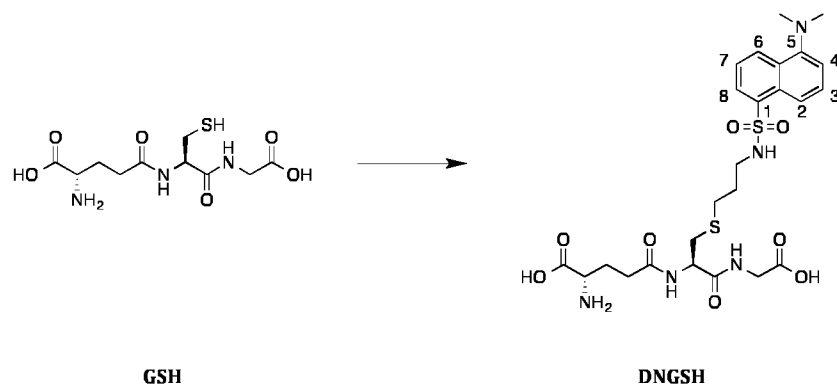
EXAMPLES

Example – synthesis procedure

The following compounds were synthesised by the procedures described below.

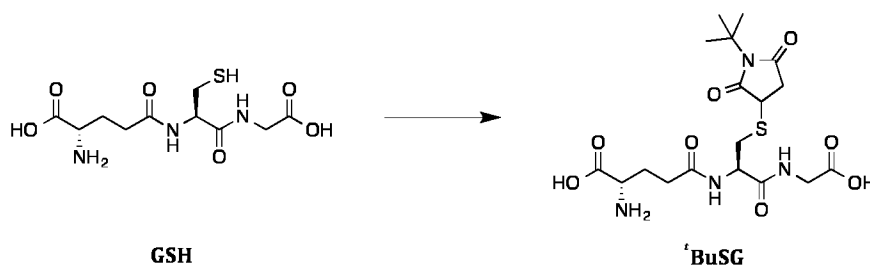


Synthesis of DNGSH



N-Allyl-5-(dimethylamino)naphthalene-1-sulfonamide (0.10 g, 0.34 mmol, 1.0 eq.), L-glutathione (0.42 g, 1.36 mmol, 4.0 eq.), TCEP·HCl (0.19 mg, 0.68 mmol, 2.0 eq.) and 2,2-dimethoxyphenyl acetophenone (0.02 g, 0.07 mmol, 0.2 eq.) were stirred at RT in THF/H₂O (1:2, 3 mL) in the presence of light (365 nm, 4 x 15 W) for 5 h. After this time, the reaction was extracted with CH₂Cl₂. The aqueous layer was lyophilized and the crude material purified by RP C-18 silica gel column chromatography (MeOH : H₂O 0:100 → 50:50), yielding **DNGSH** (0.02 g, 11%) as a hygroscopic yellow solid: *R*_f 0.4 (50:50 MeOH : H₂O (RP)); $[\alpha]_D^{20}$ -19.1 (c 0.25, water) (Lit. Value $[\alpha]_D^{20}$ -19.2 (c 0.25, water))²; ¹H NMR (400 MHz; D₂O): δ/ppm 8.55 (d, 1H, *J* 7.8 Hz, Ar C⁸H), 8.44 (d, 1H, *J* 8.7 Hz, Ar C⁶H), 8.32 (d, 1H, *J* 7.4 Hz, Ar C³H), 7.83-7.74 (m, 3H, Ar C²H & Ar C⁴H & Ar C⁷H), 4.26 (dd, 1H, *J* 8.8 Hz, *J* 5.0 Hz, Cys-α-CH), 3.76-3.68 (m, 3H, Gly-CH₂, Glu-α-CH), 3.21 (s, 6H, N(CH₃)₂), 3.00 (t, 2H, *J* 6.8 Hz, Linker-CH₂), 2.54 (dd, 1H, *J* 14.2 Hz, *J* 5.0 Hz, Cys-β-CH₂), 2.49-2.38 (m, 3H, Cys-β-CH₂, Glu-β-CH₂), 2.21-2.13 (m, 2H, 6.8 Hz, Linker-CH₂), 2.12-2.03 (m, 2H, Glu-γ-CH₂), 1.45 (qn, 2H, *J* 6.8 Hz, Linker-CH₂), *m/z* (ES⁺) 598 ([M+H]⁺, 100%). The data are in good agreement with the literature values.²

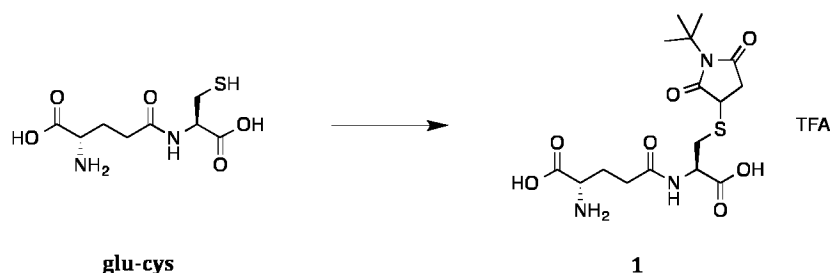
Synthesis of **^tBuSG**:



L-Glutathione (0.2 g, 0.65 mmol, 1.0 eq.) and NaOH (0.03 g, 0.65 mmol, 1.0 eq.) were stirred in water (5 mL), to this *N*-tert-butyl-maleimide (0.01 g, 0.09 mL, 0.65 mmol, 1.0 eq.) was added and the resulting solution stirred at RT for 20 minutes. After this time, the reaction solution was lyophilized, purified by RP C-18 silica gel chromatography, eluting with water to afford *S*-*N*-tert-butylsuccinimido glutathione **^tBuSG** (mixture of diastereoisomers) as a colourless hygroscopic solid (0.26 g, 87%): *R*_f 0.7 (water (RP)); $[\alpha]_D^{20}$ -13.3 (c 1.0, water) (Lit. Value $[\alpha]_D^{20}$ -12.3 (c 1.0, water))²; ¹H NMR (400 MHz; D₂O): δ/ppm 4.68-4.60 (m, 1H, Cys-α-CH), 3.91-3.81 (m, 1H, Succinimide-CH), 3.81-3.67 (m, 3H, Glu-α-CH, Gly-CH₂), 3.32 (dd, 0.5H, *J* 14.2 Hz, *J* 4.9 Hz, Cys-β-CH₂), 3.26-3.04 (m, 2H, Cys-β-CH₂, Succinimide-CH₂), 2.96 (dd, 0.5H, *J* 14.2 Hz, *J* 9.2 Hz, Cys-β-CH₂), 2.60-2.43 (m, 3H, Glu-γ-CH₂, Succinimide-CH₂), 2.20-2.07 (m, 2H, Glu-β-CH₂), 1.51 (s, 4.5H, NC(CH₃)₃), 1.51 (s, 4.5H,

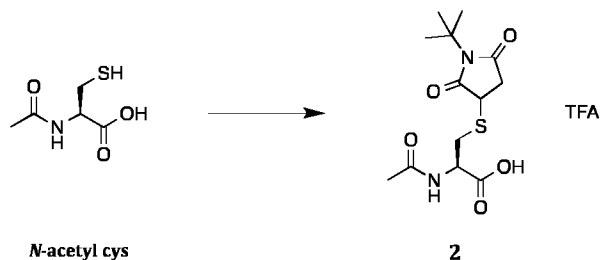
$\text{NC}(\text{CH}_3)_3$; m/z (ES^-) 459 ($[\text{M}-\text{H}]^-$, 100%). The data are in good agreement with literature values²

Synthesis of compound **1**:

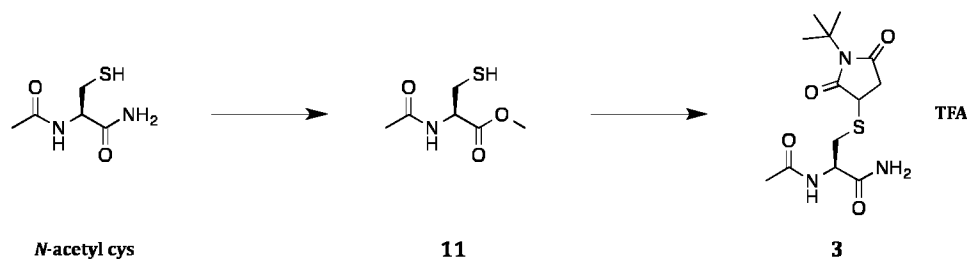


Glu-Cys (15.3 mg, 0.06 mmol, 1.0 eq.) and NaOH (2.4 mg, 0.06 mmol, 1.0 eq.) were stirred in water (0.4 mL), to this *N-tert*-butyl-maleimide (9.4 mg, 8.8 μL , 0.06 mmol, 1.0 eq.) was added and the resulting solution stirred at RT for 4.5 h. After this time, the reaction solution was lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H_2O : MeCN : TFA) to afford **1** (mixture of diastereoisomers) as a colourless solid (19.8 mg, 63%); R_f 0.8 (50:50 acetonitrile : water (RP)); $[\alpha]_D^{20}$ -6.4 (c 1.1, H_2O); $\nu_{\text{max}}/\text{cm}^{-1}$ (neat) 1739.3 (s), 1367.7 (w), 1215.6 (w); ^1H NMR (500 MHz; D_2O): δ/ppm 4.71-4.63 (m, 1H, Cys- α -CH), 4.04 (dd, 1H, J 7.0 Hz, J 6.5 Hz, Glu- α -CH), 3.92-3.85 (m, 1H, Succinimide-CH), 3.37 (dd, 0.5H, J 14.1 Hz, J 4.6 Hz, Cys- β -CH₂), 3.27 (dd, 0.5H, J 14.1 Hz, J 4.6 Hz, Cys- β -CH₂), 3.23-3.10 (m, 1.5H, Cys- β -CH₂, Succinimide-CH₂), 3.04 (dd, 0.5H, J 14.1 Hz, J 8.7 Hz, Cys- β -CH₂), 2.64-2.52 (m, 3H, Glu-Y-CH₂, Succinimide-CH₂), 2.31-2.15 (m, 2H, Glu- β -CH₂), 1.54 (s, 4.5H, $\text{NC}(\text{CH}_3)_3$), 1.53 (s, 4.5H, $\text{NC}(\text{CH}_3)_3$); ^{19}F NMR (decoupled) (376.6 MHz; D_2O): δ/ppm -75.7 (OCOCF_3); ^{13}C NMR (128.5 MHz; D_2O): δ/ppm 180.1 (Succinimide-CO), 180.0 (Succinimide-CO), 179.1 (Succinimide-CO), 179.0 (Succinimide-CO), 174.3 (Glu-CO), 174.3 (Glu-CO), 173.5 (Cys-COOH), 173.5 (Cys-COOH), 172.0 (Glu-COOH), 163.0 (q, J 35.3 Hz, OCOCF_3), 116.3 (q, J 291.5 Hz, OCOCF_3), 59.2 ($\text{NC}(\text{CH}_3)_3$), 59.2 ($\text{NC}(\text{CH}_3)_3$), 52.6 (Glu- α -CH), 52.2 (Cys- α -CH), 40.9 (Succinimide-CH), 39.9 (Succinimide-CH), 36.3 (Succinimide-CH₂), 35.8 (Succinimide-CH₂), 32.3 (Cys- β -CH₂), 31.8 (Cys- β -CH₂), 31.0 (Glu-Y-CH₂), 27.3 ($\text{NC}(\text{CH}_3)_3$), 25.7 (Glu- β -CH₂); HRMS m/z (ESI^+) (Found: $[\text{M}+\text{H}]^+$ 404.14836. $\text{C}_{16}\text{H}_{26}\text{O}_7\text{N}_3\text{S}$ requires M^+ 404.14860); m/z (ES^+) 404 ($[\text{M}+\text{H}]^+$, 100%); Analytical HPLC @ 220 nm (Acclaim® 120 C18 RP LC Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H_2O : MeCN : TFA) Ret. Time = 9.094 min, Purity: 98.57%.

Synthesis of compound **2**:



N-acetyl cysteine (20.0 mg, 0.12 mmol, 1.0 eq.) and NaOH (4.9 mg, 0.12 mmol, 1.0 eq.) were stirred in water (0.9 mL), to this *N*-tert-butyl-maleimide (18.7 mg, 17.7 μ L, 0.12 mmol, 1.0 eq.) was added and the resulting solution stirred at RT for 3.5 h. After this time, the reaction solution was lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) to afford **2** (mixture of diastereoisomers) as a pale yellow solid (35.6 mg, 67%); R_f 0.8 (50:50 acetonitrile : water (RP)); $[\alpha]_D^{20}$ -17.3 (c 1.0, MeOH); $\nu_{\max}/\text{cm}^{-1}$ (neat) 3329.1 (w), 2970.4 (w), 1737.9 (s), 1718.6 (s), 1697.4 (s), 1612.5 (m), 1558.5 (m), 1423.5 (w), 1365.6 (m), 1348.2 (m), 1315.5 (w), 1263.4 (m), 1228.7 (m), 1217.1 (s), 1205.5 (m), 1161.2 (m), 1116.8 (w), 1037.7 (w); ^1H NMR (500 MHz; D₂O): δ /ppm 4.70-4.63 (m, 1H, Cys- α -CH), 3.92-3.85 (m, 1H, Succinimide-CH), 3.36 (dd, 0.2H, J 14.1 Hz, J 4.6 Hz, Cys- β -CH₂), 3.29-3.10 (m, 2.6H, Cys- β -CH₂, Succinimide-CH₂), 3.04 (dd, 0.2H, J 14.1 Hz, J 8.6 Hz, Cys- β -CH₂), 2.61-2.51 (m, 1H, Succinimide-CH₂), 2.06 (s, 0.4H, CH₃), 2.05 (s, 2.6H, CH₃), 1.53 (s, 9H, NC(CH₃)₃); ^{19}F NMR (decoupled) (376.6 MHz; D₂O): δ /ppm -75.7 (OCOCF₃); ^{13}C NMR (128.5 MHz; D₂O): δ /ppm 180.2 (Succinimide-CO), 180.0 (Succinimide-CO), 179.1 (Succinimide-CO), 179.0 (Succinimide-CO), 174.2 (COCH₃), 174.2 (COCH₃), 173.5 (COOH), 162.9 (q, J 35.3 Hz, OCOCF₃), 116.3 (q, 291.5 Hz, OCOCF₃), 59.2 (NC(CH₃)₃), 52.6 (Cys- α -CH), 52.1 (Cys- α -CH), 40.9 (Succinimide-CH), 39.9 (Succinimide-CH), 36.2 (Succinimide-CH₂), 35.8 (Succinimide-CH₂), 32.4 (Cys- β -CH₂), 31.8 (Cys- β -CH₂), 27.3 (NC(CH₃)₃), 21.6 (CH₃), HRMS m/z (ESI⁺) (Found: $[\text{M}+\text{H}]^+$ 317.11603. C₁₃H₂₁O₅N₂S requires M^+ 317.11657); m/z (ES⁺) 317 ($[\text{M}+\text{H}]^+$, 100%); Analytical HPLC @ 220 nm (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) Ret. Time = 9.817 min, Purity: 97.46%; Chiral HPLC @ 220 nm (ChiralPak[®] AD-H column (5 μ m, 4.6 x 150 mm); Isocratic method: 75 : 25 Hexane : IPA, 0.3 mL / min) Ret. Time = 20.397 min, 99.99%.

Synthesis of compound **3**:Synthesis of **11**:

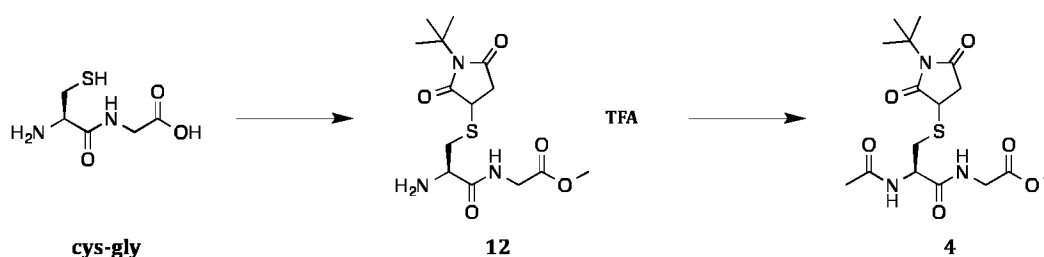
To a solution of *N*-acetyl-cysteine (1.00 g, 6.13 mmol, 1.0 eq.) stirring in methanol (22 mL) was added thionyl chloride (0.85 g, 0.52 mL, 7.11 mmol, 1.16 eq.) dropwise and the reaction mixture was stirred under argon at RT for 1.5 h. After this time, the reaction mixture was concentrated *in vacuo* and the resulting residue was partitioned between brine and ethyl acetate. The aqueous layer was extracted with ethyl acetate, and the organic layers were combined, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The resulting crude material was adsorbed onto Celite[®] and purified by silica gel chromatography, eluting with methanol and chloroform (1:99 $\rightarrow$ 10:90; methanol : chloroform) to yield *N*-acetyl-L-cysteine methyl ester **12** as a colourless solid (0.49 g, 45%); *R*_f 0.6 (5:95 MeOH : chloroform); mp 79-81 °C (MeOH : chloroform) (Lit. Value: 79-80 °C (ethyl acetate))³; [α]_D²⁰ +75.5 (c 1.0, chloroform) (Lit. Value [α]_D +71.0 (c 1.0, chloroform))⁴; ¹H NMR (400 MHz; CDCl₃): δ /ppm 6.68 (d, 1H, *J* 5.8 Hz, NH), 4.86-4.78 (m, 1H, Cys- α -CH), 3.72 (s, 3H, COOCH₃), 2.93 (dd, 2H, *J* 9.0 Hz, *J* 4.4 Hz, Cys- β -CH₂), 2.01 (s, 3H, COCH₃), 1.36 (t, 1H, *J* 8.8 Hz, SH); *m/z* (ES⁺) 178 ([M+H]⁺, 100%). The data are in good agreement with literature values.^{3,4}

Synthesis of **3**:

N-acetyl-L-cysteine methyl ester **12** (0.40 g, 2.26 mmol, 1.0 eq.) was stirred in a mixture of toluene (3 mL) and ammonium hydroxide (3 mL, 25% solution in water) under argon at RT for 19 h. After this time the reaction mixture was concentrated *in vacuo* at 60 °C to afford a mixture of *N*-acetyl-L-cysteinamide and oxidised *N*-acetyl-L-cysteinamide (0.37 g) as a colourless solid, which was used without further purification. 20.0 mg of the colourless solid containing *N*-acetyl-L-cysteinamide and oxidised *N*-acetyl-L-cysteinamide was stirred with triphenylphosphine, polymer-bound (79.0 mg, ~3 mmol/g triphenylphosphine loading - Sigma Aldrich) in DMF (0.5 mL) at RT for 4 h. After this time, the reaction mixture was

filtered and washed through with 0.5 mL DMF. *N-tert*-Butyl-maleimide (9.2 mg, 8.7 μ L) and triethylamine (8.4 μ L) were added to the filtrate, which was stirred at RT for 168 h. After this time, the reaction mixture was concentrated *in vacuo* and the crude reaction mixture was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) to afford **3** as a colourless solid (3.3 mg, 6%, over two steps): $[\alpha]_D^{20}$ -11.4 (c 0.33, water); $\nu_{\max}/\text{cm}^{-1}$ (neat); 3390.9 (w), 3315.6 (w), 2970.4 (w), 1737.9 (w), 1699.3 (s), 1635.6 (s), 1521.8 (m), 1458.2 (w), 1425.4 (m), 1398.4 (w), 1373.3 (m), 1346.3 (m), 1311.6 (w), 1280.7 (w), 1263.4 (m), 1228.7 (w), 1197.8 (m), 1166.9 (m), 1153.4 (m), 1114.9 (w), 1041.6 (w); ^1H NMR (500 MHz; D₂O): δ /ppm 4.61-4.54 (m, 1H, Cys- α -CH), 3.92-3.84 (m, 1H, Succinimide-CH), 3.36-3.10 (m, 2.5H, Cys- β -CH₂, Succinimide-CH₂), 3.00 (dd, 0.5H, J 14.1 Hz, J 8.8 Hz, Cys- β -CH₂), 2.58 (dd, 0.5H, J 4.7 Hz, J 4.3 Hz, Succinimide-CH₂), 2.54 (dd, 0.5H, J 4.7 Hz, J 4.3 Hz, Succinimide-CH₂), 2.07 (s, 1.5H, CH₃), 2.07 (s, 1.5H, CH₃), 1.54 (s, 9H, NC(CH₃)₃); ^{19}F NMR (decoupled) (376.6 MHz; D₂O): δ /ppm -75.6 (OCOCF₃); ^{13}C NMR (128.5 MHz; D₂O): δ /ppm 180.2 (Succinimide-CO), 180.2 (Succinimide-CO), 179.1 (Succinimide-CO), 179.0 (Succinimide-CO), 174.6 (CONH₂), 174.6 (CONH₂), 174.4 (COCH₃), 174.3 (COCH₃), 59.2 (NC(CH₃)₃), 59.1 (NC(CH₃)₃), 53.0 (Cys- α -CH), 52.6 (Cys- α -CH), 40.9 (Succinimide-CH), 40.0 (Succinimide-CH), 36.2 (Succinimide-CH₂), 35.9 (Succinimide-CH₂), 32.7 (Cys- β -CH₂), 32.3 (Cys- β -CH₂), 27.3 (NC(CH₃)₃), 21.7 (CH₃); HRMS m/z (ESI⁺) (Found: [M+H]⁺ 316.13229. C₁₃H₂₂N₃O₄S requires M⁺ 316.13255); m/z (ES⁺) 316 ([M+H]⁺, 100%); Analytical HPLC @ 220 nm (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) Ret. Time = 9.061 min, Purity: 96.00%; Chiral HPLC @ 220 nm (ChiralPak[®] AD-H column (5 μ m, 4.6 x 150 mm); Isocratic method: 75 : 25 Hexane : IPA, 0.3 mL / min) Diastereomer 1: Ret. Time = 24.185 min, 45.97%; Diastereomer 2: Ret. Time = 32.510 min, 53.84%.

Synthesis of compound **4**:



Synthesis of **12**:

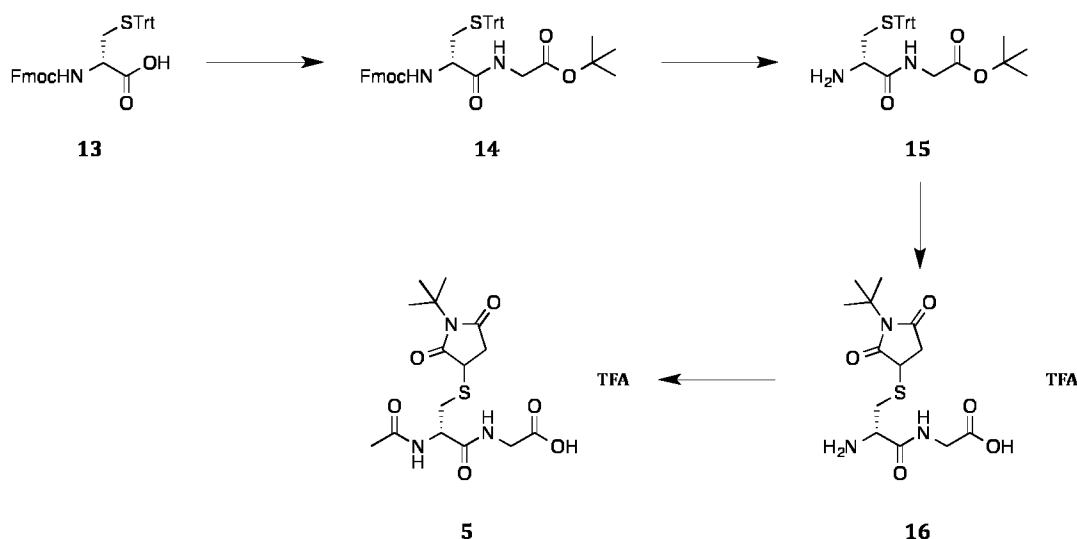
To a solution of cys-gly (24.6 mg, 0.14 mmol, 1.0 eq.) stirring in methanol (1.1 mL) was added thionyl chloride (19.1 mg, 11.6 μ L, 0.16 mmol, 1.16 eq.) and the reaction mixture was stirred at RT for 2.5 h. After this time, the reaction mixture was concentrated *in vacuo* and the resulting residue was lyophilized to afford cys-gly methyl ester as a colourless solid (26.5 mg), which was used without further purification. To cys-gly methyl ester (26.5 mg) stirring in water (1.1 mL) was added *N-tert*-butyl-maleimide (21.1 mg, 20.0 μ L, 0.14 mmol, 1.0 eq. with respect to the previous step) and the resulting solution was stirred at RT for 8.5 h. After this time, the reaction solution was lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 $\rightarrow$ 43:57:0.1; H₂O : MeCN : TFA) to afford **12** (mixture of diastereoisomers) as a colourless solid (35.9 mg, 57%); $[\alpha]_D^{20}$ +20.4 (c 1.0, chloroform); $\nu_{\max}/\text{cm}^{-1}$ (neat) 2980.0 (w), 1747.5 (w), 1664.6 (s), 1460.1 (w), 1417.7 (w), 1367.5 (w), 1344.4 (m), 1265.3 (m), 1197.8 (s), 1165.0 (s), 1128.4 (s), 1028.1 (w); ^1H NMR (500 MHz; D₂O): δ /ppm 4.43-4.34 (m, 1H, Cys- α -CH), 4.16-4.05 (m, 2H, Gly-CH₂), 3.96-3.90 (m, 1H, Succinimide-CH), 3.77 (s, 1.5H, COOCH₃), 3.76 (s, 1.5H, COOCH₃), 3.48 (dd, 0.5H, *J* 14.7 Hz, *J* 5.9 Hz, Cys- β -CH₂), 3.41-3.31 (m, 1H, Cys- β -CH₂), 3.24-3.12 (m, 1.5H, Cys- β -CH₂, Succinimide-CH₂), 2.58 (dd, 0.5H, *J* 5.2 Hz, *J* 4.6 Hz, Succinimide-CH₂), 2.55 (dd, 0.5H, *J* 5.2 Hz, *J* 4.6 Hz, Succinimide-CH₂), 1.54 (s, 9H, NC(CH₃)₃); ^{19}F NMR (decoupled) (376.6 MHz; D₂O): δ /ppm -75.6 (OCOCF₃); ^{13}C NMR (128.5 MHz; D₂O): δ /ppm 180.5 (Succinimide-CO), 180.2 (Succinimide-CO), 178.8 (Succinimide-CO), 178.8 (Succinimide-CO), 171.5 (COOCH₃), 171.5 (COOCH₃), 168.6 (Cys-CO), 168.6 (Cys-CO), 163.0 (q, *J* 35.1 Hz, OCOCF₃), 116.4 (q, *J* 291.5 Hz, OCOCF₃), 59.2 (NC(CH₃)₃), 52.9 (COOCH₃), 52.5 (Cys- α -CH), 52.2 (Cys- α -CH), 41.3 (Gly-CH₂), 41.2 (Gly-CH₂), 41.0 (Succinimide-CH), 40.1 (Succinimide-CH₂), 35.9 (Succinimide-CH₂), 35.6 (Succinimide-CH₂), 32.8 (Cys- β -CH₂), 32.2 (Cys- β -CH₂), 27.3 (NC(CH₃)₃); HRMS *m/z* (ESI⁺) (Found: [M+H]⁺ 346.14322. C₁₄H₂₄N₃O₅S requires M⁺ 346.14312); *m/z* (ES⁺) 346 ([M+H]⁺, 100%); Analytical HPLC @ 220 nm (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) Diastereomer 1: Ret. Time = 8.812 min, Purity: 37.16%; Diastereomer 2: Ret. Time = 8.940 min, Purity: 59.20%;

Synthesis of **4**:

To a solution of **12** (36.6 mg, 0.08 mmol, 1.0 eq.) and anhydrous triethylamine (17.0 mg, 23.4 μ L, 0.17 mmol 2.0 eq.) stirring in anhydrous THF (0.9 mL) was added acetyl chloride

(7.3 mg, 6.6 μ L, 0.09 mmol, 1.1 eq.). The reaction mixture was stirred under nitrogen at RT for 4 h. After this time the reaction mixture was diluted with water, lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5 $\rightarrow$ 5:95; H₂O : MeCN) to afford **4** (mixture of diastereoisomers) as a colourless hygroscopic solid (20.8 mg, 64%): $[\alpha]_D^{20}$ -14.5 (c 0.55, water); $\nu_{\max}/\text{cm}^{-1}$ (neat) 3297.8 (w), 2976.6 (w), 1749.4 (m), 1695.7 (s), 1650.1 (m), 1529.5 (m), 1457.9 (m), 1437.4 (m), 1411.4 (m), 1369.0 (m), 1342.2 (m), 1263.4 (m), 1204.2 (m), 1163.5 (m), 1039.9 (m); ^1H NMR (500 MHz; D₂O): δ /ppm 4.68-4.61 (m, 1H, Cys- α -CH), 4.04 (s, 2H, Gly-CH₂), 3.93-3.85 (m, 1H, Succinimide-CH), 3.75 (s, 3H, COOCH₃), 3.30 (dd, 0.5H, J 14.1 Hz, J 5.2 Hz, Cys- β -CH₂), 3.24-3.10 (m, 2H, Cys- β -CH₂, Succinimide-CH₂), 3.01 (dd, 0.5H, J 14.1 Hz, J 8.7 Hz, Cys- β -CH₂), 2.58 (dd, 0.5H, J 4.8 Hz, J 4.1 Hz, Succinimide-CH₂), 2.54 (dd, 0.5H, J 4.8 Hz, J 4.1 Hz, Succinimide-CH₂), 2.08 (s, 1.5H, COCH₃), 2.07 (s, 1.5H, COCH₃), 1.54 (s, 9H, NC(CH₃)₃); ^{13}C NMR (128.5 MHz; D₂O): δ /ppm 180.2 (Succinimide-CO), 180.1 (Succinimide-CO), 179.1 (Succinimide-CO), 179.0 (Succinimide-CO), 174.3 (COCH₃), 174.3 (COCH₃), 172.6 (Cys-CO), 172.5 (Cys-CO), 171.7 (COOCH₃), 171.7 (COOCH₃), 59.2 (NC(CH₃)₃), 59.1 (NC(CH₃)₃), 53.2 (Cys- α -CH), 52.8 (COOCH₃), 52.7 (Cys- α -CH), 41.2 (Gly-CH₂), 40.8 (Succinimide-CH), 40.0 (Succinimide-CH), 36.2 (Succinimide-CH₂), 35.9 (Succinimide-CH₂), 32.6 (Cys- β -CH₂), 32.3 (Cys- β -CH₂), 27.3 (NC(CH₃)₃), 21.7 (COCH₃); HRMS m/z (ESI⁺) (Found: $[\text{M}+\text{H}]^+$ 388.15286. C₁₆H₂₆N₃O₆S requires M⁺ 388.15368); m/z (ES⁺) 388 ($[\text{M}+\text{H}]^+$, 100%); Analytical HPLC @ 220 nm (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) Ret. Time = 9.677 min, Purity: 97.16%; Chiral HPLC @ 220 nm (ChiralPak[®] AD-H column (5 μ m, 4.6 x 150 mm); Isocratic method: 75 : 25 Hexane : IPA, 0.3 mL / min) Diastereomer 1: Ret. Time = 37.366 min, 38.54%; Diastereomer 2: Ret. Time = 40.515 min, 61.46%.

Synthesis of compound 5:



Synthesis of 14:

N-Fmoc-D-Cys(Trt)-OH (0.50 g, 0.85 mmol, 1.05 eq.), glycine *tert*-butyl ester hydrochloride (0.14 g, 0.81 mmol, 1.0 eq.) and HBTU (0.32 g, 0.85 mmol, 1.05 eq.) were stirred in anhydrous DMF (2 mL) for 5 minutes. After this time, DIPEA (0.21 g, 0.28 mL, 1.62 mmol, 2.0 eq.) was added and the reaction mixture was stirred under nitrogen at RT for 120 h. The reaction mixture was partitioned over ethyl acetate and 0.5 M LiCl and the organic layer was collected, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The resulting crude material was adsorbed onto Celite[®] and purified by silica gel chromatography, eluting with ethyl acetate, petroleum ether and triethylamine (10:89:1 → 80:19:1; ethyl acetate : petroleum ether : triethylamine) to yield *N*-Fmoc-S-trityl-D-cysteinyglycine *tert*-butyl ester (0.50 g, 84%) as a colourless solid: *R*_f 0.4 (30:70 ethyl acetate : petroleum ether); mp 85-88 °C (ethyl acetate : petroleum ether); [α]_D²⁰ -5.4 (c 1.0, chloroform); ν_{max}/cm⁻¹ (neat); 3296.4 (w), 3057.2 (w), 1728.2 (m), 1662.6 (m), 1595.1 (m), 1519.9 (m), 1489.1 (m), 1444.7 (m), 1367.5 (m), 1319.3 (m), 1217.1 (m), 1151.5 (m), 1082.1 (m), 1033.9 (m); ¹H NMR (500 MHz; CDCl₃): δ/ppm 7.82-7.18 (m, 23H, Ar CH), 6.34 (s, 1H, Gly-NH), 5.03 (d, 1H, *J* 7.3 Hz, Cys-NH), 4.44-4.36 (m, 2H, Fmoc-CH₂), 4.22 (t, 1H, *J* 7.3 Hz, *J* 6.8 Hz, Fmoc-CH), 3.93-3.77 (m, 3H, Cys-α-CH, Gly-CH₂), 2.75-2.61 (m, 2H, Cys-β-CH₂), 1.46 (s, 9H, OC(CH₃)₃); ¹³C NMR (128.5 MHz; CDCl₃): δ/ppm 170.1 (Cys-CO), 168.4 (COO^tBu), 156.1 (Fmoc-CONH), 144.5 (Ar C), 143.8 (Ar C), 141.4 (Ar C), 129.7 (Ar CH), 128.2 (Ar CH), 127.9 (Ar CH), 127.2 (Ar CH), 127.1 (Ar CH), 125.2 (Ar CH), 120.1 (Ar CH), 82.5 (OC(CH₃)₃), 67.5 (Fmoc-CH₂), 67.2 (SCPh₃) 54.0 (Cys-α-CH), 47.2 (Fmoc-CH), 42.2 (Gly-CH₂), 33.9 (Cys-β-CH₂), 28.1

(OC(CH₃)₃); HRMS m/z (ESI⁺) (Found: [M+Na]⁺ 721.26843. C₄₃H₄₂N₂NaO₅S requires M⁺ 721.27066); m/z (ES⁺) 117 (100%), 721 ([M+Na]⁺, 3%); Analytical HPLC @ 254 nm (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 → 5:95:0.1; H₂O : MeCN : TFA) Ret. Time = 16.693 min, Purity: 97.97%.

Synthesis of **15**:

N-Fmoc-*S*-trityl-D-cysteinylglycine *tert*-butyl ester **14** (0.4 g, 0.57 mmol, 1.0 eq.) was stirred under argon at RT in 20% piperidine in DMF (3.2 mL) for 4 h. After this time, the reaction mixture was concentrated *in vacuo* and the resulting residue was adsorbed onto Celite[®] and purified by silica gel chromatography, eluting with ethyl acetate, petroleum ether and triethylamine (12:87:1 → 99:0:1; ethyl acetate : petroleum ether : triethylamine) to yield *S*-trityl-D-cysteinylglycine *tert*-butyl ester **15** (0.13 g, 48%) as a yellow oil: *R*_f 0.3 (50:50 ethyl acetate : petroleum ether); [α]_D²⁰ -12.0 (c 0.59, chloroform); ν_{max}/cm⁻¹ (chloroform); 3373.5 (w), 2980.0 (m), 2929.9 (w), 1739.8 (m), 1672.3 (m), 1595.1 (m), 1514.1 (m), 1491.0 (m), 1444.7 (m), 1392.6 (m), 1367.5 (m), 1244.1 (m), 1155.4 (s), 1082.1 (m), 1033.9 (m); ¹H NMR (500 MHz; CDCl₃): δ/ppm 7.51 (t, 1H, *J* 5.3 Hz, Gly-NH), 7.48-7.15 (m, 15H, Ar CH), 3.90 (dd, 1H, *J* 18.4 Hz, *J* 5.3 Hz, Gly CH_AH_B), 3.81 (dd, 1H, *J* 18.4 Hz, *J* 5.3 Hz, Gly CH_AH_B), 3.06-2.96 (m, 1H, Cys-α-CH), 2.82-2.73 (m, 1H, Cys-β-CH₂), 2.55 (dd, 1H, *J* 12.8 Hz, *J* 8.8 Hz, Cys-β-CH₂), 1.45 (s, 9H, OC(CH₃)₃); ¹³C NMR (128.5 MHz; CDCl₃): δ/ppm 173.2 (Cys-CO), 169.0 (COOC(CH₃)₃), 144.7 (Ar C), 129.8 (Ar CH), 128.1 (Ar CH), 127.0 (Ar CH), 82.3 (OC(CH₃)₃), 67.2 (SCPh₃), 54.1 (Cys-α-CH), 41.9 (Gly-CH₂), 37.4 (Cys-β-CH₂), 28.2 (OC(CH₃)₃); HRMS m/z (ESI⁺) (Found: [M+H]⁺ 477.22004. C₂₈H₃₃N₂O₃S requires M⁺ 477.22064); m/z (ES⁺) 477 ([M+H]⁺, 100%); Analytical HPLC @ 254 nm (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 → 5:95:0.1; H₂O : MeCN : TFA) Ret. Time = 11.734 min, Purity: 95.46%.

Synthesis of **16**:

S-trityl-D-cysteinyl *tert*-butyl ester **15** (0.11 g, 0.23 mmol, 1.0 eq.) and triethylsilane (0.06 g, 0.08 mL, 0.5 mmol, 2.2 eq.) were stirred under argon at RT in 20% TFA in CH₂Cl₂ (5.8 mL) for 7 h. After this time the reaction solution was concentrated *in vacuo* and resulting residue was purified by RP C-18 silica gel column chromatography, eluting with water to afford D-cysteinylglycine trifluoroacetic acid salt as an off-white sticky foam (55.2 mg), which was used without further purification. D-cysteinylglycine trifluoroacetic acid salt (35.5 mg) was

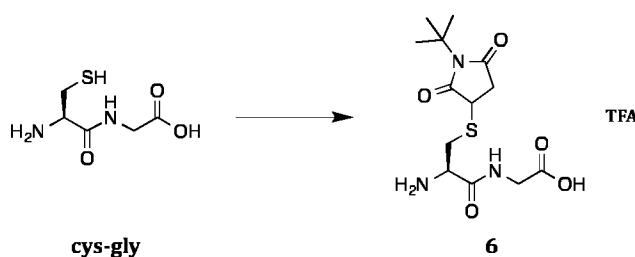
stirred in water (1.0 mL), to this *N*-*tert*-butyl-maleimide (21.0 mg, 19.8 μ L, 0.14 mmol) was added and the resulting solution stirred at RT for 16 h. After this time, the reaction solution was lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) to afford **16** (mixture of diastereoisomers) as a colourless solid (47.1 mg, 71% over 2 steps): $[\alpha]_D^{20}$ -26.4 (c 1.0, water); $\nu_{\max}/\text{cm}^{-1}$ (neat); 2970.4 (w), 2941.4 (w), 1737.9 (m), 1681.9 (s), 1460.1 (m), 1417.7 (m), 1348.2 (m), 1265.3 (m), 1228.7 (m), 1197.8 (s), 1166.9 (s), 1134.1 (s), 1024.2 (m); ^1H NMR (500 MHz; D₂O): δ /ppm 4.39 (dd, 0.5H, *J* 7.3 Hz, *J* 5.8 Hz, Cys- α -CH), 4.35 (dd, 0.5H, *J* 7.3 Hz, *J* 5.7 Hz, Cys- α -CH), 4.09 (s, 1H, Gly-CH₂), 4.08 (s, 1H, Gly-CH₂), 3.94-3.90 (m, 1H, Succinimide-CH), 3.48 (dd, 0.5H, *J* 14.8 Hz, *J* 5.9 Hz, Cys- β -CH₂), 3.41-3.30 (m, 1H, Cys- β -CH₂), 3.22-3.11 (m, 1.5H, Cys- β -CH₂, Succinimide-CH₂), 2.57 (dd, 0.5H, *J* 5.4 Hz, *J* 5.0 Hz, Succinimide-CH₂), 2.54 (dd, 0.5H, *J* 5.4 Hz, *J* 5.0 Hz, Succinimide-CH₂), 1.53 (s, 9H, NC(CH₃)₃); ^{19}F NMR (decoupled) (376.6 MHz; D₂O): δ /ppm -75.6 (OCOCF₃); ^{13}C NMR (128.5 MHz; D₂O): δ /ppm 180.4 (Succinimide-CO), 180.1 (Succinimide-CO), 178.8 (Succinimide-CO), 178.8 (Succinimide-CO), 172.7 (COOH), 172.7 (COOH), 168.5 (Cys-CO), 168.5 (Cys-CO), 162.9 (q, *J* 35.4 Hz, OCOCF₃), 116.3 (q, *J* 291.5 Hz, OCOCF₃), 59.2 (NC(CH₃)₃), 52.5 (Cys- α -CH), 52.3 (Cys- α -CH), 41.2 (Gly-CH₂), 41.2 (Gly-CH₂), 41.0 (Succinimide-CH), 40.1 (Succinimide-CH), 35.9 (Succinimide-CH₂), 35.6 (Succinimide-CH₂), 32.8 (Cys- β -CH₂), 32.2 (Cys- β -CH₂), 27.3 (NC(CH₃)₃); HRMS *m/z* (ESI⁺) (Found: [M+H]⁺ 332.12676. C₁₃H₂₂N₃O₅S requires M⁺ 332.12747); *m/z* (ES⁺) 332 ([M+H]⁺, 100%); Analytical HPLC @ 220 nm (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) Diastereomer 1: Ret. Time = 8.448 min, Purity: 35.99%; Diastereomer 2: Ret. Time = 8.541 min, Purity: 61.26%.

Synthesis of **5**:

To a solution of **16** (27.5 mg, 0.06 mmol, 1.0 eq.) and anhydrous triethylamine (12.5 mg, 17.2 μ L, 0.12 mmol, 2.0 eq.) stirring in anhydrous THF (0.6 mL) was added acetyl chloride (5.3 mg, 4.8 μ L, 0.07 mmol, 1.1 eq.). The reaction mixture was stirred at RT for 4 h. After this time the reaction mixture was diluted with water, lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) to afford **5** (mixture of diastereoisomers) as a colourless solid (13.9 mg, 46%): $[\alpha]_D^{20}$ +15.0 (c 1.0, water); $\nu_{\max}/\text{cm}^{-1}$ (neat); 3304.1 (w), 2980.0 (w), 1768.7 (w), 1693.5 (s), 1658.8 (m), 1531.5 (m), 1460.1 (m), 1402.3 (m), 1369.5 (m), 1342.5 (m),

1263.4 (m), 1232.5 (m), 1197.8 (m), 1163.1 (m), 1041.6 (m); ^1H NMR (500 MHz; D_2O): δ/ppm 4.68-4.61 (m, 1H, Cys- α -CH), 4.01 (s, 2H, Gly- CH_2), 3.92-3.84 (m, 1H, Succinimide-CH), 3.30 (dd, 0.5H, J 14.1 Hz, J 5.1 Hz, Cys- β - CH_2), 3.24-3.09 (m, 2H, Cys- β - CH_2 , Succinimide- CH_2), 2.99 (dd, 0.5H, J 14.1 Hz, J 8.7 Hz, Cys- β - CH_2), 2.57 (dd, 0.5H, J 4.4 Hz, J 3.9 Hz, Succinimide- CH_2), 2.53 (dd, 0.5H, J 4.4 Hz, J 3.9 Hz, Succinimide- CH_2), 2.07 (s, 1.5H, COCH_3), 2.06 (s, 1.5H, COCH_3), 1.53 (s, 9H, $\text{NC}(\text{CH}_3)_3$); ^{19}F NMR (decoupled) (376.6 MHz; D_2O): δ/ppm -75.7 (OCOCF_3); ^{13}C NMR (128.5 MHz; D_2O): δ/ppm 180.2 (Succinimide-CO), 180.2 (Succinimide-CO), 179.1 (Succinimide-CO), 179.0 (Succinimide-CO), 174.4 (COCH_3), 174.3 (COCH_3), 172.9 (COOH), 172.9 (COOH), 172.4 (Cys-CO), 172.4 (Cys-CO), 163.0 (q, J 35.1 Hz, OCOCF_3), 116.3 (q, J 290.9 Hz, OCOCF_3), 59.2 ($\text{NC}(\text{CH}_3)_3$), 59.1 ($\text{NC}(\text{CH}_3)_3$), 53.1 (Cys- α -CH), 52.7 (Cys- α -CH), 41.1 (Gly- CH_2), 40.8 (Succinimide-CH), 40.0 (Succinimide-CH), 36.2 (Succinimide- CH_2), 35.9 (Succinimide- CH_2), 32.7 (Cys- β - CH_2), 32.3 (Cys- β - CH_2), 27.3 ($\text{NC}(\text{CH}_3)_3$), 21.7 (COCH_3); HRMS m/z (ESI^+) (Found: $[\text{M}+\text{H}]^+$ 374.13761. $\text{C}_{15}\text{H}_{24}\text{N}_3\text{O}_6\text{S}$ requires M^+ 374.13803); m/z (ES^+) 374 ($[\text{M}+\text{H}]^+$, 100%); Analytical HPLC @ 220 nm (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H_2O : MeCN : TFA) Ret. Time = 9.237 min, Purity: 97.26%; Chiral HPLC @ 220 nm (ChiralPak[®] AD-H column (5 μm , 4.6 x 150 mm); Isocratic method: 75 : 25 Hexane : IPA, 0.3 mL / min) Diastereomer 1: Ret. Time = 40.368 min, 53.55%; Diastereomer 2: Ret. Time = 51.065 min, 44.86%.

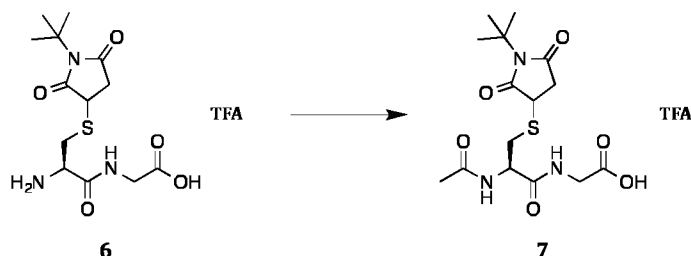
Synthesis of compound **6**:



Cys-Gly (15.0 mg, 0.08 mmol, 1.0 eq.) and NaOH (3.4 mg, 0.08 mmol, 1.0 eq.) were stirred in water (0.7 mL), to this *N*-*tert*-butyl-maleimide (12.9 mg, 12.2 μL , 0.08 mmol, 1.0 eq.) was added and the resulting solution stirred at RT for 1.5 h. After this time, the reaction solution was lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 $\rightarrow$ 81:19:0.1; H_2O : MeCN : TFA) to afford **6** (mixture of diastereoisomers) as a colourless solid (21.9 mg, 58%); R_f 0.3 (water (RP)); $[\alpha]_D^{20}$ +25.8 (c 0.65, H_2O); $\nu_{\text{max}}/\text{cm}^{-1}$ (neat); 2970.9 (w), 1675.7 (s), 1349.1 (m), 1264.9 (m),

1200.1 (s); ^1H NMR (500 MHz; D_2O): δ/ppm 4.39 (dd, 0.5H, J 7.5 Hz, J 5.8 Hz, Cys- α -CH), 4.35 (dd, 0.5H, J 7.5 Hz, J 5.8 Hz, Cys- α -CH), 4.08-3.96 (m, 2H, Gly- CH_2), 3.95-3.89 (m, 1H, Succinimide-CH), 3.49 (dd, 0.5H, J 14.7 Hz, J 5.8 Hz, Cys- β - CH_2), 3.41-3.30 (m, 1H, Cys- β - CH_2), 3.22-3.12 (m, 1.5H, Cys- β - CH_2 , Succinimide- CH_2), 2.58 (dd, 0.5H, J 5.6 Hz, J 5.2 Hz, Succinimide- CH_2), 2.55 (dd, 0.5H, J 5.6 Hz, J 5.2 Hz, Succinimide- CH_2), 1.54 (s, 9H, $\text{NC}(\text{CH}_3)_3$); ^{19}F NMR (decoupled) (376.6 MHz; D_2O): δ/ppm -75.6 (OCOCF_3); ^{13}C NMR (128.5 MHz; D_2O): δ/ppm 180.5 (Succinimide-CO), 180.2 (Succinimide-CO), 178.8 (Succinimide-CO), 178.8 (Succinimide-CO), 173.6 (COOH), 173.6 (COOH), 168.4 (Cys-CO), 168.3 (Cys-CO), 163.0 (q, J 35.6 Hz, OCOCF_3), 116.4 (q, J 290.9 Hz, OCOCF_3), 59.2 ($\text{NC}(\text{CH}_3)_3$), 52.6 (Cys- α -CH), 52.3 (Cys- α -CH), 41.8 (Gly- CH_2), 41.8 (Gly- CH_2), 41.0 (Succinimide-CH), 40.1 (Succinimide-CH), 35.9 (Succinimide- CH_2), 35.6 (Succinimide- CH_2), 32.8 (Cys- β - CH_2), 32.2 (Cys- β - CH_2), 27.3 ($\text{NC}(\text{CH}_3)_3$); HRMS m/z (ESI^+) (Found: $[\text{M}+\text{Na}]^+$ 354.1098. $\text{C}_{13}\text{H}_{21}\text{N}_3\text{NaO}_5\text{S}$ requires M^+ 354.1094); m/z (ES^+) 332 ($[\text{M}+\text{H}]^+$, 100%); Analytical HPLC (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H_2O : MeCN : TFA) Diastereomer 1: Ret. Time = 8.517 min, Purity: 40.55%; Diastereomer 2: Ret. Time = 8.605 min, Purity: 57.21%.

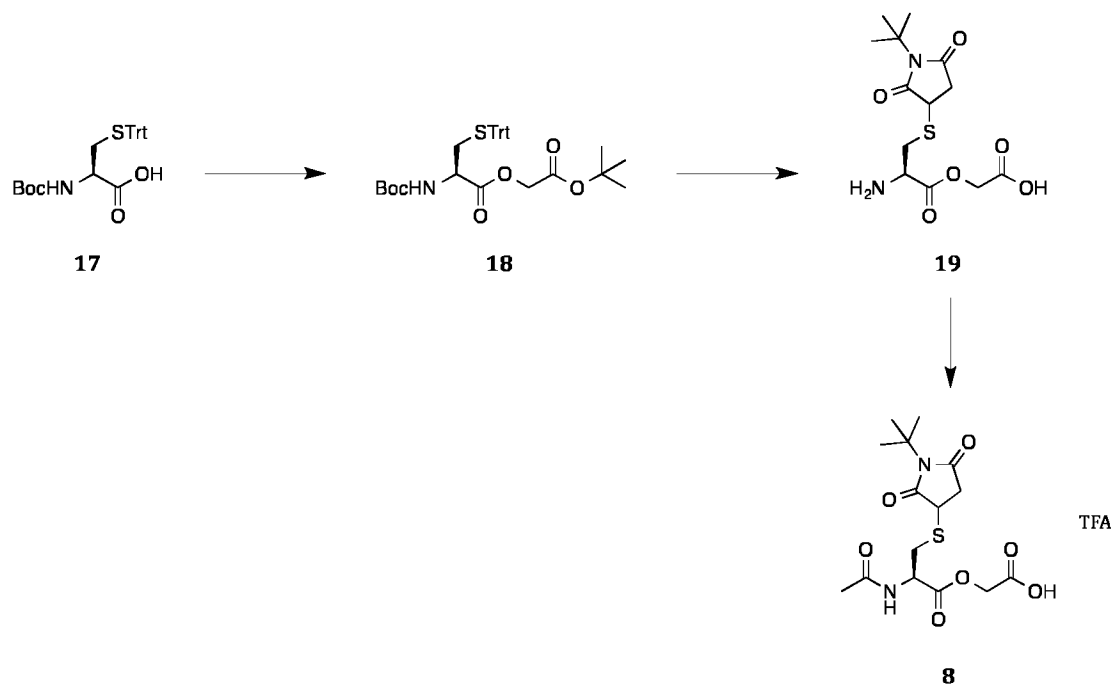
Synthesis of compound 7:



To a solution of **6** (15.0 mg, 0.03 mmol, 1.0 eq.) and anhydrous triethylamine (6.8 mg, 9.4 μL , 0.07 mmol, 2.0 eq.) stirring in anhydrous THF (0.3 mL) was added acetyl chloride (2.9 mg, 2.6 μL , 0.04 mmol, 1.1 eq.). The reaction mixture was stirred at RT for 6.5 h. After this time, the reaction mixture was diluted with water, lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H_2O : MeCN : TFA) to afford **7** (mixture of diastereoisomers) as a colourless solid (9.0 mg, 55%); R_f 1.0 (water (RP)); $[\alpha]_D^{20}$ -14.8 (c 0.46, H_2O); $\nu_{\text{max}}/\text{cm}^{-1}$ (neat) 2970.8 (w), 1737.8 (m), 1694.1 (s), 1532.9 (m), 1346.2 (m), 1263.7 (m), 1201.8 (m), 1163.7 (s); ^1H NMR (500 MHz; D_2O): δ/ppm 4.69-4.61 (m, 1H, Cys- α -CH), 4.01 (s, 2H, Gly- CH_2), 3.92-3.84 (m, 1H, Succinimide-CH), 3.31 (dd, 0.5H, J 14.0 Hz, J 5.1 Hz, Cys- β - CH_2) 3.24-3.10

(m, 2H, Cys- β -CH₂, Succinimide-CH₂), 3.00 (dd, 0.5H, *J* 14.0 Hz, *J* 8.7 Hz, Cys- β -CH₂), 2.57 (dd, 0.5H, *J* 4.3 Hz, *J* 3.6 Hz, Succinimide-CH₂), 2.54 (dd, 0.5H, *J* 4.3 Hz, *J* 3.6 Hz, Succinimide-CH₂), 2.08 (s, 1.5H, COCH₃), 2.07 (s, 1.5H, COCH₃), 1.54 (s, 4.5H, NC(CH₃)₃), 1.53 (s, 4.5H, NC(CH₃)₃); ¹⁹F NMR (decoupled) (376.6 MHz; D₂O): δ /ppm -75.6 (OCOCF₃); ¹³C NMR (128.5 MHz; D₂O): δ /ppm 180.2 (Succinimide-CO), 180.2 (Succinimide-CO), 179.1 (Succinimide-CO), 179.1 (Succinimide-CO), 174.4 (COCH₃), 174.3 (COCH₃), 172.9 (COOH), 172.4 (Cys-CO), 172.4 (Cys-CO), 163.0 (q, *J* 35.4 Hz, OCOCF₃), 116.4 (q, *J* 290.5 Hz, OCOCF₃), 59.2 (NC(CH₃)₃), 59.1 (NC(CH₃)₃), 53.1 (Cys- α -CH), 52.7 (Cys- α -CH), 41.1 (Gly-CH₂), 40.8 (Succinimide-CH), 40.0 (Succinimide-CH), 36.2 (Succinimide-CH₂), 35.9 (Succinimide-CH₂), 32.7 (Cys- β -CH₂), 32.3 (Cys- β -CH₂), 27.3 (NC(CH₃)₃), 21.7 (COCH₃); HRMS *m/z* (ESI⁻) (Found: [M-H]⁻ 372.12347. C₁₅H₂₂O₆N₃S requires M⁻ 372.12348); *m/z* (ES⁺) 374 ([M+H]⁺, 100%); Analytical HPLC @ 220 nm (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) Ret. Time = 9.519 min, Purity: 98.82%. Chiral HPLC @ 220 nm (ChiralPak[®] AD-H column (5 μ m, 4.6 x 150 mm); Isocratic method: 75 : 25 Hexane : IPA, 0.3 mL / min) Diastereomer 1: Ret. Time = 21.810 min, 32.99%; Diastereomer 2: Ret. Time = 23.640 min, 66.99%.

Synthesis of compound **8**:



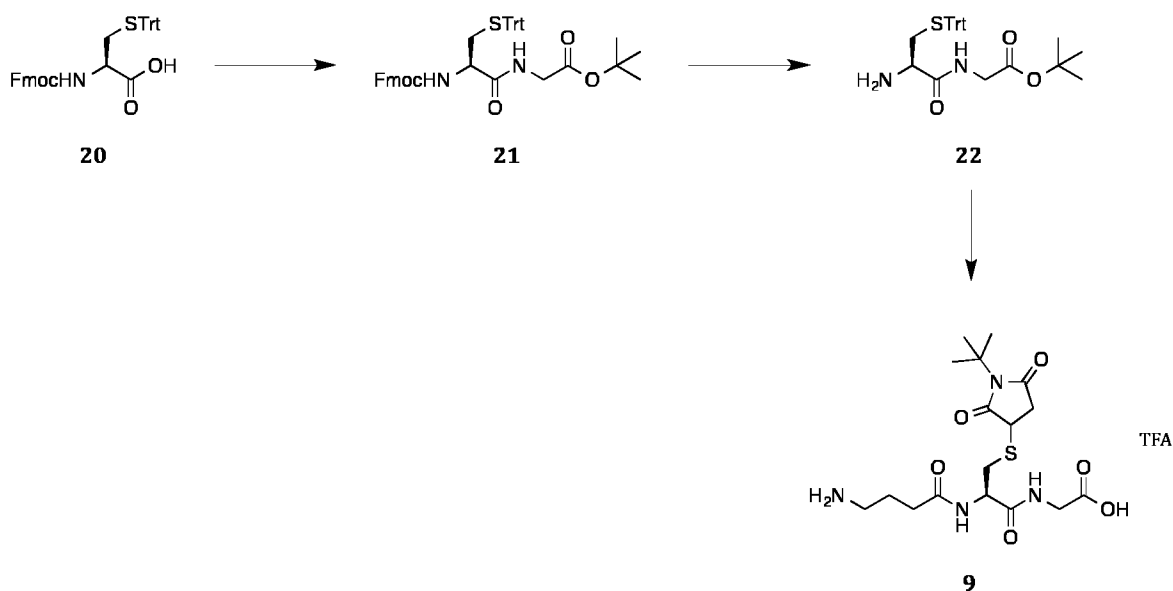
Boc-*L*-Cys(Trt)-OH (0.1 g, 0.22 mmol, 1.0 eq.) and *tert*-butyl-2-hydroxyacetate (55.6 mg, 0.42 mmol, 1.95 eq.) were stirred in anhydrous acetonitrile at 0 °C, to this *N,N'*-

dicyclohexylcarbodiimide (86.8 mg, 0.42 mmol, 1.95 eq.) was added and the resulting solution stirred under argon at 0 °C for a further 2 h before being stirred at RT for 15.5 h. After this time, the reaction mixture was filtered under reduced pressure, the filtrate was concentrated *in vacuo* and the resulting residue was adsorbed onto Celite® and purified by silica gel chromatography, eluting with ethyl acetate and petroleum ether (5:95 → 40:60; ethyl acetate : petroleum ether) to yield 2-(*tert*-butoxy)-2-oxoethyl *N*-(*tert*-butoxycarbonyl)-*S*-trityl-*L*-cysteinate as an impure oil (0.11 g), which was used without further purification. *N*-(*tert*-butoxycarbonyl)-*S*-trityl-*L*-cysteinate (0.11 g) and triethylsilane (0.08 g, 0.11 mL, 0.69 mmol) were stirred under argon at RT in 20% TFA in CH₂Cl₂ (6.5 mL) for 2 h. After this time the reaction solution was concentrated *in vacuo* and the resulting residue was purified by RP C-18 silica gel column chromatography, eluting with water to afford 2-((*L*-cysteinyl)oxy)acetic acid as an impure colourless solid (32.2 mg), which was used without further purification. To 2-((*L*-Cysteinyl)oxy)acetic acid (32.2 mg) stirring in water (3.6 mL) was added *N*-*tert*-butyl-maleimide (27.5 mg, 26.0 μL, 0.18 mmol) and the resulting solution was stirred at RT for 7 h. After this time, the reaction solution was lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 → 5:95:0.1; H₂O : MeCN : TFA) to afford (2*R*)-3-((1-(*tert*-butyl)-2,5-dioxopyrrolidin-3-yl)thio)-1-(carboxymethoxy)-1-oxopropan-2-aminium 2,2,2-trifluoroacetate (mixture of diastereoisomers) as a colourless solid (25.5 mg), which was used without further purification.

To a solution of (2*R*)-3-((1-(*tert*-butyl)-2,5-dioxopyrrolidin-3-yl)thio)-1-(carboxymethoxy)-1-oxopropan-2-aminium 2,2,2-trifluoroacetate (25.5 mg) and anhydrous triethylamine (11.5 mg, 15.9 μL, 0.11 mmol) stirring in anhydrous THF (0.5 mL) was added acetyl chloride (5.0 mg, 4.5 μL, 0.06 mmol). The reaction mixture was stirred at RT for 3.5 h. After this time, the reaction mixture was diluted with water, lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 → 5:95:0.1; H₂O : MeCN : TFA) to afford **8** (mixture of diastereoisomers) as a colourless solid (11.6 mg, 14%, over four steps): $[\alpha]_D^{20}$ -24.0 (*c* 0.75, H₂O); $\nu_{\max}/\text{cm}^{-1}$ (neat); 2980.0 (m), 1745.6 (m), 1693.5 (s), 1541.1 (m), 1460.1 (w), 1419.6 (m), 1371.4 (m), 1344.4 (m), 1263.4 (m), 1163.1 (s), 1053.1 (m); ¹H NMR (500 MHz; D₂O): δ /ppm 4.86-4.73 (m, 3H, Cys- α -CH, OCH₂CO₂H), 3.94-3.85 (m, 1H, Succinimide-CH), 3.42 (dd, 0.5H, *J* 14.2 Hz, *J* 4.9 Hz, Cys- β -CH₂), 3.31 (dd, 0.5H, *J* 14.2 Hz, *J* 4.9 Hz, Cys- β -CH₂), 3.25-3.10 (m, 1.5H, Cys- β -CH₂, Succinimide-CH₂), 3.06 (dd, 0.5H, *J* 14.2 Hz, *J* 8.9 Hz, Cys- β -CH₂), 2.58 (dd, 0.5H, *J* 4.4 Hz, *J* 3.2 Hz,

Succinimide-CH₂) 2.54 (dd, 0.5H, *J* 4.4 Hz, *J* 3.2 Hz, Succinimide-CH₂), 2.07 (s, 1.5H, COCH₃), 2.06 (s, 1.5H, COCH₃), 1.53 (s, 4.5H, NC(CH₃)₃), 1.53 (s, 4.5H, NC(CH₃)₃); ¹⁹F NMR (decoupled) (376.6 MHz; D₂O): δ/ppm -75.7 (OCOCF₃); ¹³C NMR (128.5 MHz; D₂O): δ/ppm 180.1 (Succinimide-CO), 180.0 (Succinimide-CO), 179.0 (Succinimide-CO), 179.0 (Succinimide-CO), 174.3 (COCH₃), 174.2 (COCH₃), 171.2 (Cys-CO), 171.2 (Cys-CO), 171.1 (CO₂H), 162.9 (q, *J* 35.3 Hz, OCOCF₃), 116.3 (q, *J* 290.7 Hz, OCOCF₃), 61.9 (OCH₂CO₂H), 59.2 (NC(CH₃)₃), 59.2 (NC(CH₃)₃), 52.5 (Cys-α-CH), 52.0 (Cys-α-CH), 40.9 (Succinimide-CH), 39.9 (Succinimide-CH), 36.2 (Succinimide-CH₂), 35.8 (Succinimide-CH₂), 32.2 (Cys-β-CH₂), 31.7 (Cys-β-CH₂), 27.3 (NC(CH₃)₃), 21.6 (COCH₃), 21.5 (COCH₃); HRMS *m/z* (ESI⁺) (Found: [M+H]⁺ 375.12147. C₁₅H₂₃O₇N₂S requires M⁺ 375.12205); *m/z* (ES⁺) 375 ([M+H]⁺, 100%); Analytical HPLC @ 220 nm (Acclaim[®] 120 C18 RP LC Column; 95:5:0.1 → 5:95:0.1; H₂O : MeCN : TFA) Ret. Time = 9.776 min, Purity: 95.47%; Chiral HPLC @ 220 nm (ChiralPak[®] AD-H column (5 μm, 4.6 x 150 mm); Isocratic method: 75 : 25 Hexane : IPA, 0.3 mL / min) Diastereomer 1: Ret. Time = 20.625 min, 50.48%; Diastereomer 2: Ret. Time = 29.359 min, 49.32%.

Synthesis of compound **9**:



Synthesis of compound **21**:

N-Fmoc-Cys(Trt)-OH **20** (0.75 g, 1.28 mmol, 1.05 eq.), glycine *tert*-butyl ester hydrochloride (0.20 g, 1.22 mmol, 1.0 eq.) and HBTU (0.49 g, 1.28 mmol, 1.05 eq.) were stirred in anhydrous DMF (3 mL) for 5 minutes. After this time, DIPEA (0.32 g, 0.42 mL, 2.43 mmol,

2.0 eq.) was added and the reaction mixture was stirred under argon at RT for 24 h. The reaction mixture was partitioned between ethyl acetate and 0.5 M LiCl and the organic layer was collected, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The resulting crude material was adsorbed onto Celite[®] and purified by silica gel chromatography, eluting with ethyl acetate, petroleum ether and triethylamine (10:89:1 → 80:19:1; ethyl acetate : petroleum ether : triethylamine) to yield *N*-Fmoc-*S*-trityl-L-cysteinylglycine *tert*-butyl ester **21** (0.85 g, 95%) as a colourless solid: *R*_f 0.8 (40:60 ethyl acetate : petroleum ether); mp 79-83 °C (ethyl acetate : petroleum ether); $[\alpha]_D^{20} +5.8$ (c 1.0, chloroform) (Lit. Value $[\alpha]_D^{25} +2.4$ (c 0.25, chloroform))⁵; ¹H NMR (400 MHz; CDCl₃): δ/ppm 7.73-7.08 (m, 23H, Ar CH), 6.25 (s, 1H, CONH), 4.92 (d, 1H, *J* 7.5 Hz, Cys-NH), 4.36-4.26 (m, 2H, Fmoc-CH₂), 4.13 (t, 1H, *J* 7.5 Hz, *J* 6.8 Hz, Fmoc-CH), 3.85-3.67 (m, 3H, Cys-α-CH, Gly-CH₂), 2.67-2.52 (m, 2H, Cys-β-CH₂), 1.37 (s, 9H, OC(CH₃)₃); *m/z* (ES⁺) 721 ([M+Na]⁺, 100%). The data are in good agreement with literature values.⁵

Synthesis of compound **22**:

N-Fmoc-*S*-trityl-L-cysteinylglycine *tert*-butyl ester **21** (0.75 g, 1.07 mmol, 1.0 eq.) was stirred under argon at RT in 20% piperidine in DMF (6 mL) for 5 h. After this time, the reaction mixture was concentrated *in vacuo* and the resulting residue was adsorbed onto Celite[®] and purified by silica gel chromatography, eluting with ethyl acetate, petroleum ether and triethylamine (12:87:1 → 99:0:1; ethyl acetate : petroleum ether : triethylamine) to yield *S*-trityl-L-cysteinylglycine *tert*-butyl ester **22** (0.38 g, 74%) as a yellow oil: *R*_f 0.2 (50:50 ethyl acetate : petroleum ether); $[\alpha]_D^{20} +13.2$ (c 1.0, chloroform) (Lit. Value $[\alpha]_D^{25} +9.2$ (c 0.5, chloroform))⁵; ¹H NMR (400 MHz; CDCl₃): δ/ppm 7.45 (t, 1H, *J* 5.3 Hz, Gly-NH), 7.40-7.02 (m, 15H, Ar CH), 3.77 (dd, 1H, *J* 18.3 Hz, *J* 5.4 Hz, Gly CH_AH_B), 3.69 (dd, 1H, *J* 18.3 Hz, *J* 5.4 Hz, Gly CH_AH_B), 2.91 (dd, 1H, *J* 8.8 Hz, *J* 3.8 Hz, Cys-α-CH), 2.65 (dd, 1H, *J* 12.8 Hz, *J* 3.8 Hz, Cys-β-CH₂), 2.45 (dd, 1H, *J* 12.8 Hz, *J* 8.8 Hz, Cys-β-CH₂), 1.33 (s, 9H, OC(CH₃)₃); *m/z* (ES⁺) 477 ([M+H]⁺, 100%). The data are in good agreement with literature values.⁵

Synthesis of compound **9**:

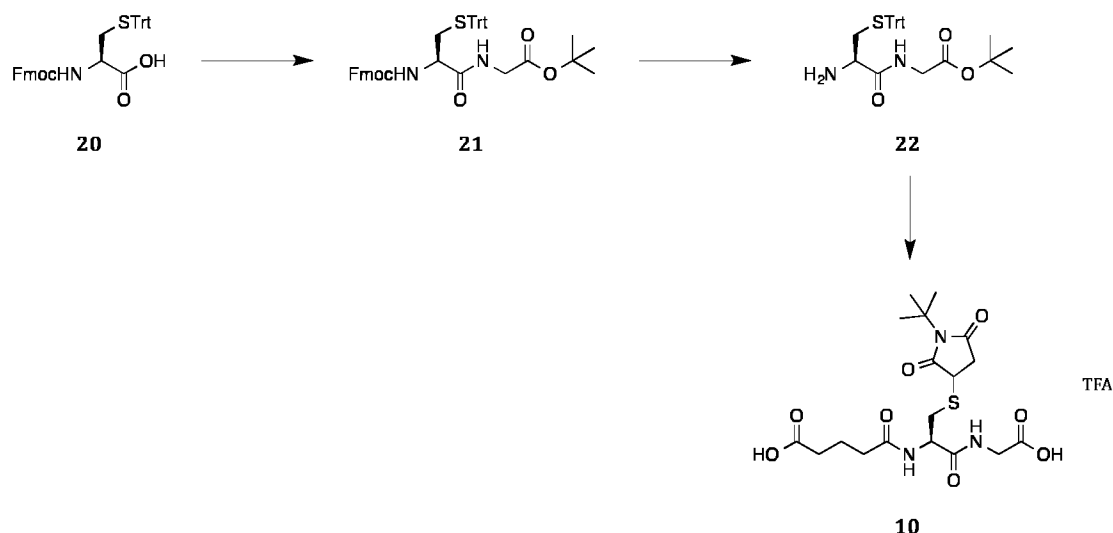
S-Trityl-L-cysteinylglycine *tert*-butyl ester **22** (89.3 mg, 0.19 mmol, 1.0 eq.), *N*-*tert*-butoxycarbonyl-L-aminobutyric acid (38.1 mg, 0.19 mmol, 1.0 eq.), EDCI·HCl (41.3 mg, 0.22 mmol, 1.15 eq.), HBTU (7.1 mg, 0.02 mmol, 0.1 eq.) and DIPEA (48.4 mg, 65.3 μL, 0.37 mmol, 2.0 eq.) were stirred at RT in anhydrous CH₂Cl₂ (9 mL) for 24 h. After this time, the reaction mixture was

concentrated *in vacuo* and the resulting residue was adsorbed onto Celite[®] and purified by silica gel chromatography, eluting with ethyl acetate, petroleum ether and triethylamine (12:87:1 → 99:0:1; ethyl acetate : petroleum ether : triethylamine) to yield *N*-*tert*-butyl-*tert*-butyl ester **23** (0.10 g) as a colourless oil, which was used without further purification.

N-Boc-Y-aminobutyric acid-*S*-trityl-L-cysteinylglycine *tert*-butyl ester **23** (95.0 mg) and triethylsilane (50.1 mg, 68.8 μ L, 0.43 mmol) were stirred in 20% TFA/CH₂Cl₂ (5 mL) at RT for 6.5 h. After this time, the reaction mixture was concentrated *in vacuo* and the resulting residue was purified by RP C-18 silica gel column chromatography, eluting with water to afford Y-aminobutyric acid-L-cysteinylglycine trifluoroacetic acid salt (33.3 mg) as a colourless residue, which was used without further purification. To Y-aminobutyric acid-L-cysteinylglycine trifluoroacetic acid salt (25.0 mg) stirring in water (0.5 mL) was added *N*-*tert*-butyl-maleimide (10.2 mg, 9.6 μ L, 0.07 mmol) and the resulting solution was stirred at RT for 19.5 h. After this time, the reaction solution was lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 → 5:95:0.1; H₂O : MeCN : TFA) to afford **9** (mixture of diastereoisomers) as a colourless solid (25.9 mg, 37% over 3 steps): $[\alpha]_D^{20}$ -14.5 (c 1.0, water); $\nu_{\max}/\text{cm}^{-1}$ (neat) 2939.5 (w), 1689.6 (m), 1653.0 (s), 1527.6 (m), 1460.1 (m), 1417.7 (m), 1346.3 (m), 1263.4 (m), 1230.6 (m), 1166.9 (s), 1130.3 (s); ¹H NMR (400 MHz; D₂O): δ /ppm 4.66-4.57 (m, 1H, Cys- α -CH), 3.97 (s, 2H, Gly-CH₂), 3.90-3.79 (m, 1H, Succinimide-CH), 3.28 (dd, 0.5H, *J* 14.2 Hz, *J* 5.1 Hz, Cys- β -CH₂), 3.22-3.04 (m, 2H, Cys- β -CH₂, Succinimide-CH₂), 3.04-2.89 (m, 2.5H, CH₂, Cys- β -CH₂), 2.54 (dd, 0.5H, *J* 8.1 Hz, *J* 4.3 Hz, Succinimide-CH₂), 2.49 (dd, 0.5H, *J* 8.1 Hz, *J* 4.3 Hz, Succinimide-CH₂), 2.46-2.38 (m, 2H, CH₂), 1.99-1.87 (m, 2H, CH₂), 1.49 (s, 4.5H, NC(CH₃)₃), 1.49 (s, 4.5H, NC(CH₃)₃); ¹⁹F NMR (decoupled) (376.6 MHz; D₂O): δ /ppm -75.6 (OCOCF₃); ¹³C NMR (128.5 MHz; D₂O): δ /ppm 180.5 (Succinimide-CO), 180.5 (Succinimide-CO), 179.4 (Succinimide-CO), 179.4 (Succinimide-CO), 175.4 (CH₂CH₂CONH), 175.3 (CH₂CH₂CONH), 173.2 (Gly-COOH), 173.2 (Gly-COOH), 172.8 (Cys-CO), 172.7 (Cys-CO), 163.3 (q, *J* 35.2 Hz, OCOCF₃), 116.7 (q, *J* 292.0 Hz, OCOCF₃), 59.6 (NC(CH₃)₃), 59.6 (NC(CH₃)₃), 53.4 (Cys- α -CH), 53.0 (Cys- α -CH), 41.5 (Gly-CH₂), 41.2 (Succinimide-CH), 40.4 (Succinimide-CH), 39.2 (CH₂), 36.6 (Succinimide-CH₂), 36.3 (Succinimide-CH₂), 33.0 (Cys- β -CH₂), 32.8 (Cys- β -CH₂), 32.5 (CH₂), 27.7 (NC(CH₃)₃), 23.1 (CH₂); HRMS *m/z* (ESI⁺) (Found: [M+H]⁺ 417.17911 C₁₇H₂₉N₄O₆S requires M⁺ 417.18023); *m/z* (ES⁺) 833 ([2M+H]⁺, 100%); Analytical HPLC @ 220 nm (Acclaim[®] 120 C18 RP LC

Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) Diastereomer 1: Ret. Time = 8.501 min, Purity: 28.42%; Diastereomer 2: Ret. Time = 8.553 min, Purity: 70.16%.

Synthesis of compound **10**:



Compounds **20-22** were synthesized in an equivalent manner to compound **9**.

Synthesis of compound **10**:

S-Trityl-L-cysteinyglycine *tert*-butyl ester (0.21 g, 0.44 mmol, 1.0 eq.) and triethylamine (0.06 g, 0.08 mL, 0.57 mmol, 1.3 eq.), were stirred at RT in anhydrous chloroform (2 mL) for 5 minutes. After this time, glutaric anhydride (0.06 g, 0.48 mmol, 1.1 eq.) was added and the reaction mixture was stirred at RT for 15.5 h. The reaction solution was concentrated *in vacuo* and the resulting residue was adsorbed onto Celite[®] and purified by silica gel chromatography, eluting with ethyl acetate, petroleum ether and formic acid (60:40:1) to yield slightly impure glutaric acid-*S*-trityl-L-cysteinyglycine *tert*-butyl ester (21.9 mg) as a colourless oil, which was used without further purification. Glutaric acid-*S*-trityl-L-cysteinyglycine *tert*-butyl ester (15.9 mg) and triethylsilane (94.1 mg, 0.13 mL, 0.81 mmol) were stirred at RT under argon in 20% TFA/CH₂Cl₂ (9.4 mL) at RT for 6.5 h. After this time, the reaction mixture was concentrated *in vacuo* and the resulting residue was purified by RP C-18 silica gel column chromatography, eluting with water to afford slightly impure glutaric acid-L-cysteinyglycine trifluoroacetic acid salt (23.7 mg) as a colourless solid, which was used without further purification.

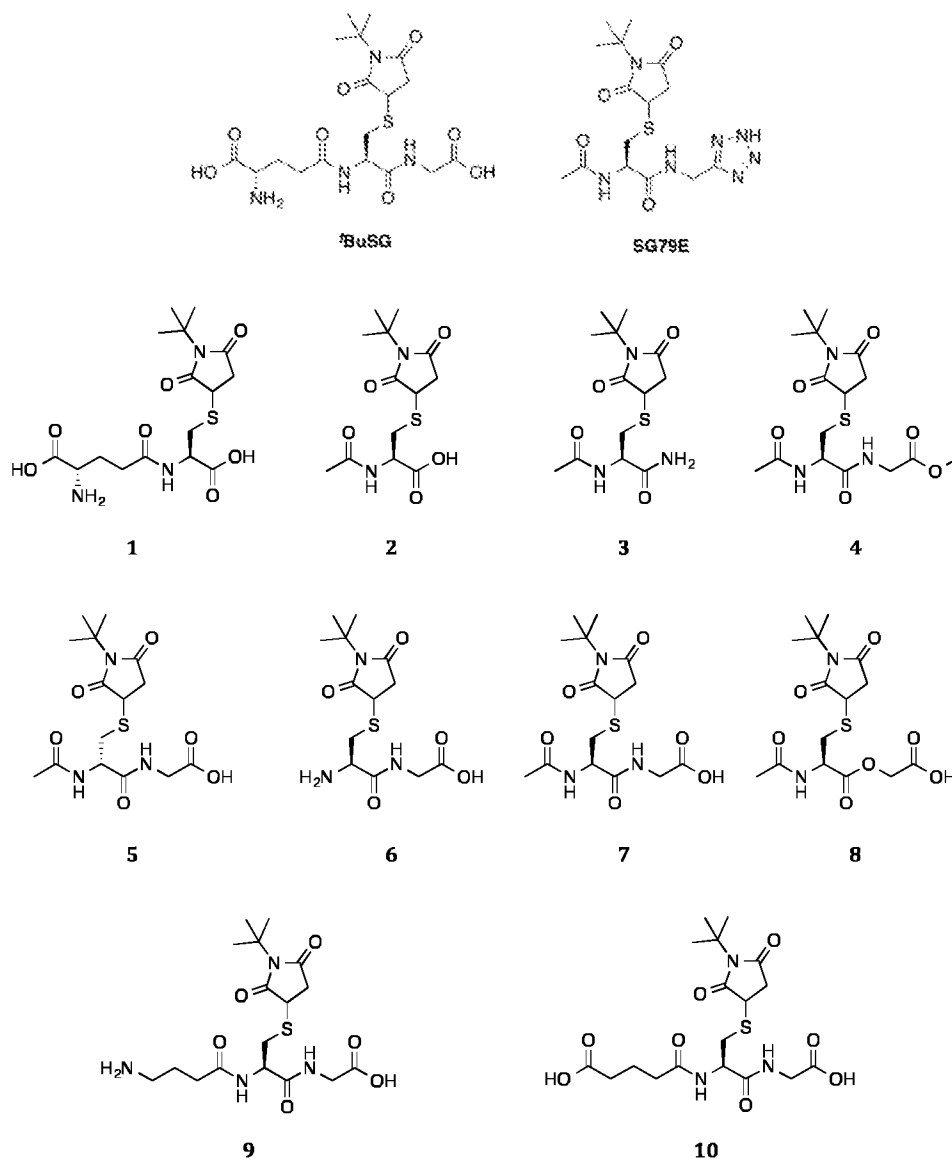
To glutaric acid-L-cysteinylglycine trifluoroacetic acid salt (22.4 mg) stirring in water (0.4 mL) was added *N-tert*-butyl-maleimide (8.4 mg, 8.0 μ L, 0.06 mmol) and the resulting solution was stirred at RT for 14 h. After this time, the reaction solution was lyophilized and the resulting solid was purified by semi-preparative HPLC (Agilent ZORBAX 300SB-C18 column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) to afford **10** (mixture of diastereoisomers) as a colourless solid (20.2 mg, 12% over 3 steps): $[\alpha]_D^{20}$ -20.0 (c 1.0, water); $\nu_{\max}/\text{cm}^{-1}$ (neat) 2970.4 (w), 2941.4 (w), 1737.9 (m), 1689.6 (s), 1529.6 (m), 1456.3 (m), 1365.6 (m), 1346.3 (m), 1263.4 (m), 1228.7 (m), 1217.1 (s), 1201.7 (s), 1159.2 (s), 1041.6 (m); ^1H NMR (500 MHz; D₂O): δ/ppm 4.70-4.63 (m, 1H, Cys- α -CH), 4.01 (s, 2H, Gly-CH₂), 3.91-3.83 (m, 1H, Succinimide-CH), 3.33 (dd, 0.5H, *J* 14.2 Hz, *J* 5.1 Hz, Cys- β -CH₂), 3.23 (dd, 0.5H, *J* 14.2 Hz, *J* 5.1 Hz, Cys- β -CH₂), 3.19-3.09 (m, 1.5H, Cys- β -CH₂, Succinimide-CH₂), 2.98 (dd, 0.5H, *J* 14.2 Hz, *J* 9.2 Hz, Cys- β -CH₂), 2.55 (dd, 0.5H, *J* 5.2 Hz, *J* 4.6 Hz, Succinimide-CH₂), 2.51 (dd, 0.5H, *J* 5.2 Hz, *J* 4.6 Hz, Succinimide-CH₂), 2.49-2.33 (m, 4H, CH₂), 1.96-1.86 (m, 2H, CH₂), 1.53 (s, 4.5H, NC(CH₃)₃), 1.52 (s, 4.5H, NC(CH₃)₃); ^{19}F NMR (decoupled) (376.6 MHz; D₂O): δ/ppm -75.7 (OCOCF₃); ^{13}C NMR (128.5 MHz; D₂O): δ/ppm 180.2 (Succinimide-CO), 180.1 (Succinimide-CO), 179.0 (Succinimide-CO), 179.0 (Succinimide-CO), 177.7 (CH₂CH₂COOH), 176.1 (CH₂CH₂CONH), 176.0 (CH₂CH₂CONH), 172.9 (Gly-COOH), 172.4 (Cys-CO), 172.4 (Cys-CO), 162.9 (q, *J* 35.5 Hz, OCOCF₃), 116.3 (q, *J* 292.0 Hz, OCOCF₃), 59.1 (NC(CH₃)₃), 59.1 (NC(CH₃)₃), 53.1 (Cys- α -CH), 52.5 (Cys- α -CH), 41.1 (Gly-CH₂), 41.1 (Gly-CH₂), 40.8 (Succinimide-CH), 39.9 (Succinimide-CH), 36.1 (Succinimide-CH₂), 35.9 (Succinimide-CH₂), 34.4 (CH₂), 32.8 (Cys- β -CH₂), 32.7 (CH₂), 32.5 (Cys- β -CH₂), 27.3 (NC(CH₃)₃), 20.4 (CH₂); HRMS *m/z* (ESI⁺) (Found: [M+Na]⁺ 468.13940. C₁₈H₂₇N₃NaO₈S requires M⁺ 468.14111); *m/z* (ES⁺) 468 ([M+Na]⁺, 100%); Analytical HPLC @ 220 nm (Acclaim® 120 C18 RP LC Column; 95:5:0.1 $\rightarrow$ 5:95:0.1; H₂O : MeCN : TFA) Ret. Time = 9.116 min, Purity: 97.61%.

References: 1. Mechanism of ligand-gated potassium efflux in bacterial pathogens. *PNAS* **107**, 19784–19789 (2010); 2. Healy, J. *et al.* Understanding the structural requirements for activators of the Kef bacterial potassium efflux system. *Biochemistry* **53**, 1982–1992 (2014); 3. Bernardes, G. J. L. *et al.* From Disulfide- to Thioether-Linked Glycoproteins. *Angewandte Chemie* **120**, 2276–2279 (2008); 4. Aroyan, C. E., Dermenci, A. & Miller, S. J. Development of a cysteine-catalyzed enantioselective Rauhut-Currier reaction. *J. Org. Chem.* **75**, 5784–5796 (2010); 5. Healy, J. Studies on the bacterial potassium efflux system KefC, and its ancillary protein KefF.

Example – competition fluorescence assay

Competition fluorescence assays were performed on a number compounds to determine binding to Kef relative to GS-X compounds. All measurements were performed by a Perkin Elmer LS-50B Spectrometer. Samples were run with the expressed C-terminal domain of *Schewanella denitrificans* Kef (6 μ M) and DNGSH (5 μ M) in 50 mM phosphate buffer pH 7.4 and 150 mM NaCl. The competing ligand was added to each sample at a final concentration of 1 mM. The samples were excited at 340 nm and the emission spectra measured from 375-670 nm. The data are represented as ratios of the fluorescence intensities before and after the addition of the tested compound at 525.5 nm (F_B/F_L) - 1.

The compounds tested are as follows.



Fluorescence competition assay results are shown in Figures 1 to 3. Significance of changes evaluated by a Student's *t*-test are shown in the Figures (where **** $p \leq 0.0001$, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$). It can be observed that the compounds of the invention competitively bind to Kef.

Example – CPMG NMR and K_D

K_D values were determined for certain compounds using ^1H CPMG NMR.

All the NMR experiments were performed on a Bruker Avance III 700 MHz spectrometer equipped with a TCI inverse cryoprobe at 298 K and processed and analysed by Bruker Topspin™ 3.2. The PROJECT-CPMG pulse sequence ($90^\circ_x - [\tau - 180^\circ_y - \tau - 90^\circ_y - \tau - 180^\circ_y - \tau]_n - \text{acq}$) as described by Aguilar, et al (Spin echo NMR spectra without J modulation. Chem. Commun. 2012, 48, 811–813) was used to remove the broad resonances of the protein. All spectra were processed with a Lorentzian line broadening of 0.3 Hz. The CPMG edited ^1H NMR experiments were recorded with a total filter time of 88 ms. Water suppression was achieved by presaturation. Bruker, MATCH (3 mm diameter) NMR tubes were used with a total sample volume of 160 μL . The solutions were buffered in 50 mM Na-phosphate, 150 mM NaCl, pH 7.4. For each sample the pulse tip-angle calibration was carried out using the single pulse nutation method (Wu, P. S. C.; Otting, G. Rapid pulse length determination in high-resolution NMR. J. Magn. Reson. 2005, 176, 115–119).

The titration, experiments were performed with a fixed concentration of the ligand (typically 10 μM) and a range of protein concentrations (typically 0-30 μM ; the concentration was increased until complete saturation of the ligand signal was achieved). The % decrease in signal intensity of the ligand was calculated as follows:

$$\% \text{ Decrease in signal intensity} = \frac{I_P - I_B}{I_B} \times 100$$

Where: I_B is the intensity of the ligand signal in the absence of protein and I_P is the intensity of the ligand signal in the presence of the protein at a given concentration.

The titration data (% decrease in ligand signal intensity plotted against protein concentration) was fitted using OriginPro 9.0 (Origin lab, Northampton, MA, USA) and the value of the K_D

was extracted using the equation described by Dalvit (Dalvit, C. Theoretical analysis of the competition ligand-based NMR experiments and selected applications to fragment screening and binding constant measurements. Concepts in Magnetic Resonance Part A. 2008 32A(5), 341–372):

$$y = A * (1 / (2 * C)) * ((B + x + C) - \sqrt{((B + x + C)^2 - (4 * x * C))})$$

Where: y is the % decrease in ligand signal intensity; A is the maximum loss in the signal intensity; C is the ligand concentration; B is the K_D , x is the concentration of the protein.

The results are shown in Figures 4 to 10.

Example – antibacterial activity of compounds

Antimicrobial susceptibility tests were carried out using a Kirby-Bauer disk diffusion assay. *Escherichia coli* strains (Table 1) or transformants were inoculated onto 2× TY agar plate [16 g/L Tryptone (Oxoid Ltd.), 10 g/L Yeast extract (Oxoid Ltd.), 5 g/L NaCl, 1.5% (w/v) Oxoid™ Bacto Agar (Thermo Fisher Scientific Inc.)] and incubated for 18-24 h at 37 °C for selection of well-isolated colonies. The full-length *Shewanella denitrificans* Kef channel (GenBank ID: ABE53663.1) encoded by the pTrcSdKefH₆ plasmid (J. Healy et al., Understanding the structural requirements for activators of the Kef bacterial potassium efflux system. Biochemistry. 53, 1982–1992 (2014)) was transformed into the *E. coli* MJF335 host (Table 1), in which its endogenous *E. coli* Kef systems were knocked out (S. Miller, R. M. Douglas, P. Carter, I. R. Booth, Mutations in the glutathione-gated KefC K⁺ efflux system of *Escherichia coli* that cause constitutive activation (1997)), as a model strain for testing in this study. For each isolate, three to five morphologically similar colonies were selected from the agar plate from the above step and transferred into a sterile Falcon™ 50-mL conical centrifuge tube (Thermo Fisher Scientific Inc.) containing 10 mL of sterile Mueller-Hinton broth (MHB; Sigma-Aldrich Co.) by using inoculation loop. The bacterial culture was allowed to grow aerobically to 0.5 McFarland turbidity standard (Clinical and Laboratory Standards Institute, M02-A11: Performance standards for antimicrobial disk susceptibility tests; approved standard — eleventh edition. 32, 1–76 (2012); Clinical and Laboratory Standards Institute, M100-S24: Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement (ed. 24, 2014)) at 37 °C with an agitation speed of 200 r.p.m. During this incubation period, test discs were prepared by impregnating 20 µL of different concentrations of test compounds onto Whatman® antibiotic assay discs of

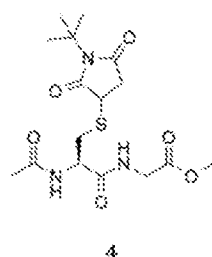
6 mm diameters (Sigma-Aldrich Co.). After the cell culture had been reached the cell density of 0.5 McFarland turbidity standard, the cell isolate was inoculated onto Muller-Hinton agar (MHA; Sigma-Aldrich Co.) plates by using sterile cotton swab. Subsequently the compound discs were aseptically placed onto the bacterial plates. After 24 h of incubation at 37 °C, inhibition zone diameters associated with the inhibition effect of the compounds were recorded by averaging both vertical and horizontal diameters of each zone. The whole experimental procedures were performed according to the standards set by the Clinical and Laboratory Standards Institute.

Table 1. *E. coli* strains used in this study.

<i>E. coli</i> strain	Genotype
Frag1	F ⁻ , <i>lacZ</i> 82(Am), λ^- , <i>rha-4</i> , <i>thiE1</i> , <i>gal-33</i>
Frag5	Frag1, $\Delta(kdpC-kdpA)$ 18
Frag56	Frag5, <i>gshA::Tn10</i> (Kan)
MJF335	Frag5, <i>lacI</i> , <i>kefB</i> , <i>kefC::Tn10</i> , <i>gshA::Tn10</i> (Kan)

See S. Miller, R. M. Douglas, P. Carter, I. R. Booth, Mutations in the glutathione-gated KefC K⁺ efflux system of Escherichia coli that cause constitutive activation (1997) and W. Epstein, B. S. Kim, Potassium transport loci in Escherichia coli K-12. J. Bacteriol. 108, 639–644 (1971).

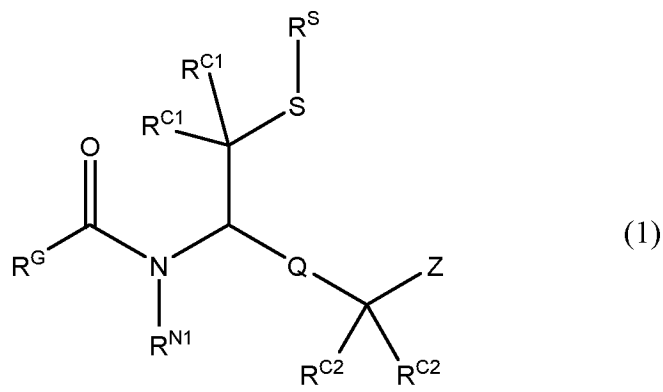
The assay was performed using compound **4** which was used at a number of concentrations.



The bacteria used was *E. coli* MJF335 (pTrcSdKefH6) which has the genotype Frag5, *lacI*, *kefB*, *kefC::Tn10*, *gshA::Tn10*(Kan). The disk diameter was 6 mm. The results of the assay are shown in Figure 11. A dose dependent increase in bacterial inhibition is clearly visible.

CLAIMS

1. A compound which is a peptide derivative of formula (1) or a salt thereof:



wherein:

R^G is H, $-CH_2-R^{GA}$ or C_{1-12} hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-OR'$, $-NO_2$, $-CN$, halogen and 5-6 membered heterocyclyl; or R^G and R^{N1} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen;

R^{GA} is C_{1-11} hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, halogen and 5-6 membered heterocyclyl;

R^S is C_{1-16} hydrocarbyl, a 5-10 membered heterocyclyl or heteroaryl group, $-(C_{1-4} \text{ alkylene})-R^{SA}$ or $-(C_{1-4} \text{ alkylene})-L-R^{SA}$; R^{SA} is C_{1-16} hydrocarbyl, or a 5-10 membered heterocyclyl or heteroaryl group; L is $-SO_2-$; and wherein R^S and R^{SA} are optionally substituted with a group R^b and/or from 1 to 5 substituents selected from R^a , $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $-SR'$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2OR'$, $-P(O)(OR')(OR'')$, $=O$, $-SiR'R''R'''$ and halogen; or R^S and an R^{C1} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen;

R^b is phenyl, benzyl, C_{3-6} cycloalkyl or 5-6 membered heterocyclyl;

Q is $-C(O)-N(R^{N2})-$, $-C(O)-C(R'R'')-$, $-C(O)-O-$, $-N(R^{N2})-C(O)-$, $-C(R'R'')-$, $-C(O)-$, $-O-C(O)-$, $-C(R'R'')-C(R''R''')-$, $-C(R'R'')-O-$, $-O-C(R'R'')-$, $-C(R'R'')-N(R^{N2})-$, $-N(R^{N2})-C(R'R'')-$ or $-C(R')=C(R'')-$;

Z is a group selected from $-C(O)-O-R^0$ and $-CN_4R'$ (tetrazolyl);

R^0 is H or C_{1-12} hydrocarbyl optionally substituted with 1 to 4 substituents selected from $-NR'R''$, $-OR'$, $-NO_2$, $-CN$, $-C(O)R'$, $-C(O)OR'$, $-C(O)NR'R''$, $-O-C(O)R'$, $-N(R')-C(O)R''$, $-SR'$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2OR'$, $-P(O)(OR')(OR'')$, $=O$, halogen and 5-6

membered heterocyclyl; or R^O and an R^{C2} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen; each R^{N1} and R^{N2} is independently H or C_{1-4} alkyl; each R^{C1} , R^{C2} , R' , R'' , R''' and R'''' is independently H or C_{1-4} alkyl and is optionally substituted with 1 to 3 substituents selected from halogen, $-OH$ and $-O-C_{1-4}$ alkyl; and each R^a is independently C_{1-4} alkyl which is optionally substituted with 1 to 3 halogen atoms.

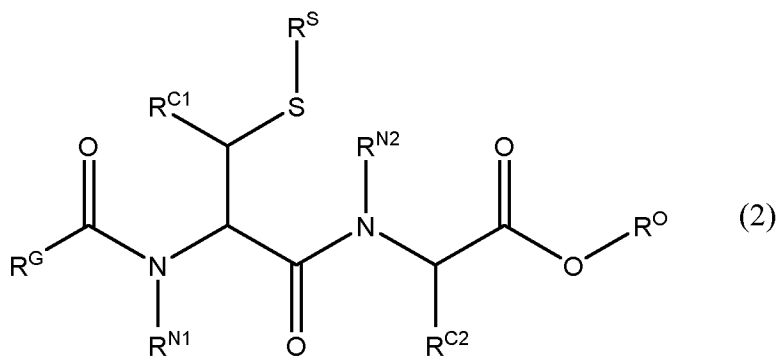
2. A compound according to claim 1, wherein **Q** is $-C(O)-N(R^{N2})-$.

3. A compound according to claim 1 or claim 2, wherein

Z is $-C(O)-O-R^O$ and **R^O** is H, C_{1-4} alkyl, phenyl or benzyl, wherein the phenyl and benzyl groups are optionally substituted with 1 to 4 substituents selected from R^a , $-OR'$ and halogen, or

Z is $-CN_4H$ (tetrazolyl).

4. A compound according to any one of the preceding claims, which compound is a peptide derivative of formula (2) or a salt thereof:



wherein:

R^G is H, $-CH_2-R^{GA}$ or a C_{1-8} alkyl group which is optionally substituted with 1 to 4 substituents selected from $-OR'$, halogen and 5-6 membered heterocyclyl; or **R^G** and R^{N1} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a , $-OR'$ and halogen;

R^{GA} is C_{1-7} alkyl group which is optionally substituted with 1 to 4 substituents selected from $-NR'R''$, $-OR'$, halogen and 5-6 membered heterocyclyl;

R^S is C_{1-16} hydrocarbyl, a 5-10 membered heterocyclyl or heteroaryl group,

–(C₁₋₄ alkylene)–R^{SA} or –(C₁₋₄ alkylene)–L–R^{SA}; **R^{SA}** is C₁₋₁₆ hydrocarbyl, or a 5-10 membered heterocyclyl or heteroaryl group; **L** is –SO₂–; and wherein R^S and R^{SA} are optionally substituted with a group R^b and/or from 1 to 5 substituents selected from R^a, –NR'R'', –OR', –NO₂, –CN, –C(O)R', –C(O)OR', –C(O)NR'R'', –O–C(O)R', –N(R')–C(O)R'', –SR', =O and halogen; or R^S and an R^{C1} together form a 5-6 membered heterocyclyl ring which is optionally substituted with 1 to 3 substituents selected from R^a, –OR' and halogen;

R^b is phenyl, benzyl, C₃₋₆ cycloalkyl or 5-6 membered heterocyclyl;

R^O is H, C₁₋₄ alkyl, phenyl or benzyl, wherein the phenyl and benzyl groups are optionally substituted with 1 to 4 substituents selected from R^a, –OR' and halogen;

R^{N1} and R^{N2} are each independently H or C₁₋₄ alkyl;

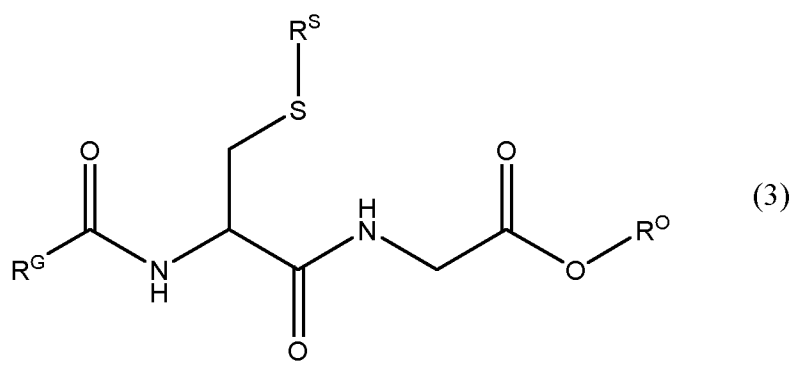
R^{C1} and R^{C2} are each independently H or C₁₋₄ alkyl;

each R' and R'' is independently H or C₁₋₄ alkyl which is optionally substituted with 1 to 3 substituents selected from halogen, –OH and –O–C₁₋₄ alkyl; and

each R^a is independently C₁₋₄ alkyl which is optionally substituted with 1 to 3 halogen atoms.

5. A compound according to any one of the preceding claims, wherein each R^{N1}, R^{N2}, R^{C1} and R^{C2} is independently H or methyl.

6. A compound according to any one of the preceding claims, which compound is a peptide derivative of formula (3) or a salt thereof:



wherein:

R^G is H, –CH₂–R^{GA} or a C₁₋₈ alkyl group which is optionally substituted with 1 to 4 substituents selected from –OR', halogen and 5-6 membered heterocyclyl;

R^{GA} is C₁₋₇ alkyl group which is optionally substituted with 1 to 4 substituents selected from –NR'R'', –OR', halogen and 5-6 membered heterocyclyl;

R^S is C₁₋₁₆ hydrocarbyl, a 5-10 membered heterocyclyl or heteroaryl group,

–(C₁₋₄ alkylene)–R^{SA} or –(C₁₋₄ alkylene)–L–R^{SA}; **R^{SA}** is C₁₋₁₆ hydrocarbyl, or a 5-10 membered heterocyclyl or heteroaryl group; **L** is –SO₂–; and wherein **R^S** and **R^{SA}** are optionally substituted with a group **R^b** and/or from 1 to 5 substituents selected from C₁₋₄ alkyl, –CF₃, –NR'R'', –OR', –NO₂, –CN, –C(O)R', –C(O)OR', –C(O)NR'R'', =O and halogen;

R^b is phenyl, benzyl, C₃₋₆ cycloalkyl or 5-6 membered heterocyclyl;

R^O is H or C₁₋₄ alkyl optionally substituted with –O–C₁₋₄ alkyl; and

each **R'** and **R''** is independently H or C₁₋₄ alkyl.

7. A compound according to any one of the preceding claims, wherein **R^G** is C₁₋₈ alkyl optionally substituted with 1 to 4 substituents selected from halogen, –OH and –O–C₁₋₄ alkyl, preferably wherein **R^G** is methyl or ethyl.

8. A compound according to any one of the preceding claims, wherein **R^O** is H or C₁₋₄ alkyl optionally substituted with –O–C₁₋₄ alkyl, preferably wherein **R^O** is H, methyl or ethyl.

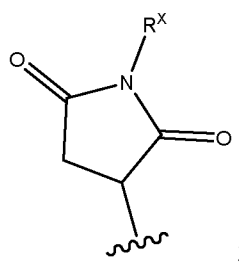
9. A compound according to any one of the preceding claims, wherein **R^S** is C₁₋₁₀ alkyl, –(C₁₋₃ alkylene)–R^{SA}, –(C₁₋₃ alkylene)–L–R^{SA}, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₆ cycloalkyl, 5- to 10 membered heterocyclyl, C₆₋₁₀ aryl or 5- to 10-membered heteroaryl, wherein **L** is SO₂, **R^{SA}** is C₃₋₆ cycloalkyl, 5- to 10 membered heterocyclyl, C₆₋₁₀ aryl or 5- to 10-membered heteroaryl and **R^S** and **R^{SA}** are optionally substituted with a group **R^b** and/or from 1 to 5 substituents selected from C₁₋₄ alkyl, –CF₃, –NR'R'', –OR', –NO₂, –CN, –C(O)R', –C(O)OR', –C(O)NR'R'', =O and halogen; wherein each **R'** and **R''** is independently H or C₁₋₄ alkyl; and **R^b** is phenyl, benzyl or C₃₋₆ cycloalkyl.

10. A compound according to any one of the preceding claims, wherein **R^S** is C₁₋₁₀ alkyl, –(C₁₋₃ alkylene)–R^{SA}, –(C₁₋₃ alkylene)–L–R^{SA}, C₃₋₆ cycloalkyl, 5-6 membered heterocyclyl or phenyl, wherein **L** is SO₂, **R^{SA}** is C₃₋₆ cycloalkyl, 5-6 membered heterocyclyl or phenyl and **R^S** and **R^{SA}** are optionally substituted with a group **R^b** and/or from 1 to 3 substituents selected from C₁₋₄ alkyl, –CF₃, –OC₁₋₄alkyl, –NO₂, –CN, =O and halogen; wherein **R^b** is phenyl, benzyl or C₃₋₆ cycloalkyl.

11. A compound according to any one of the preceding claims, wherein each **R'**, **R''**, **R'''** and **R''''** is independently H or C₁₋₄ alkyl, preferably H.

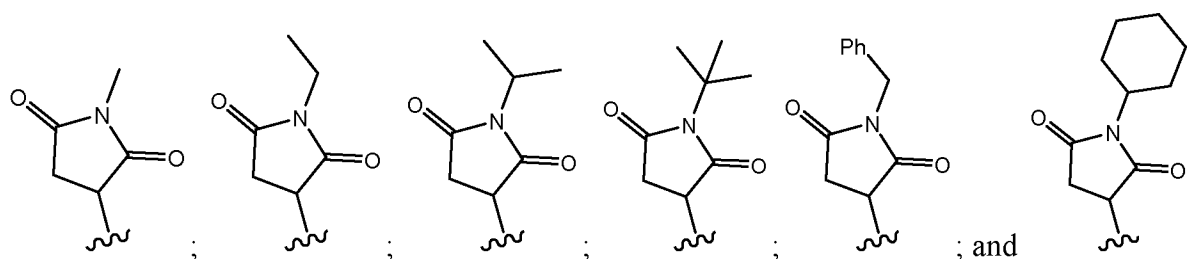
12. A compound according to any one of the preceding claims, wherein R^S is
- (i) C_{1-10} alkyl, $-(C_{1-2}$ alkylene)-(C_{3-6} cycloalkyl) or C_{3-6} cycloalkyl, wherein the cycloalkyl ring is optionally substituted with from 1 to 3 methyl groups;
 - (ii) 5-6 membered heterocyclyl or $-\text{CH}_2\text{CH}_2-(5-6 \text{ membered heterocyclyl})$ wherein the heterocyclyl group in each case is optionally substituted with phenyl, benzyl or C_{3-6} cycloalkyl and/or with from 1 to 3 substituents selected from C_{1-4} alkyl, $-\text{NO}_2$, halogen, C_{1-4} alkoxy, and $=\text{O}$; or
 - (iii) $-\text{CH}_2\text{CH}_2-\text{Ph}-(Y)_n$ or $-\text{CH}_2\text{CH}_2-\text{SO}_2-\text{Ph}-(Y)_n$, wherein n is 0 or 1 and Y is $-\text{NO}_2$, $-\text{CF}_3$, $-\text{F}$ or $-\text{OCH}_3$.

13. A compound according to any one of the preceding claims, wherein R^S is a group of formula:

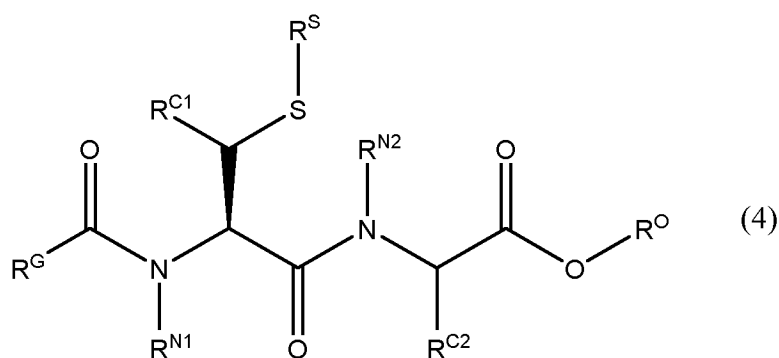


where R^X is H, phenyl, benzyl, C_{3-6} cycloalkyl or C_{1-4} alkyl.

14. A compound according to any one of the preceding claims, wherein R^S is a group selected from the following groups:



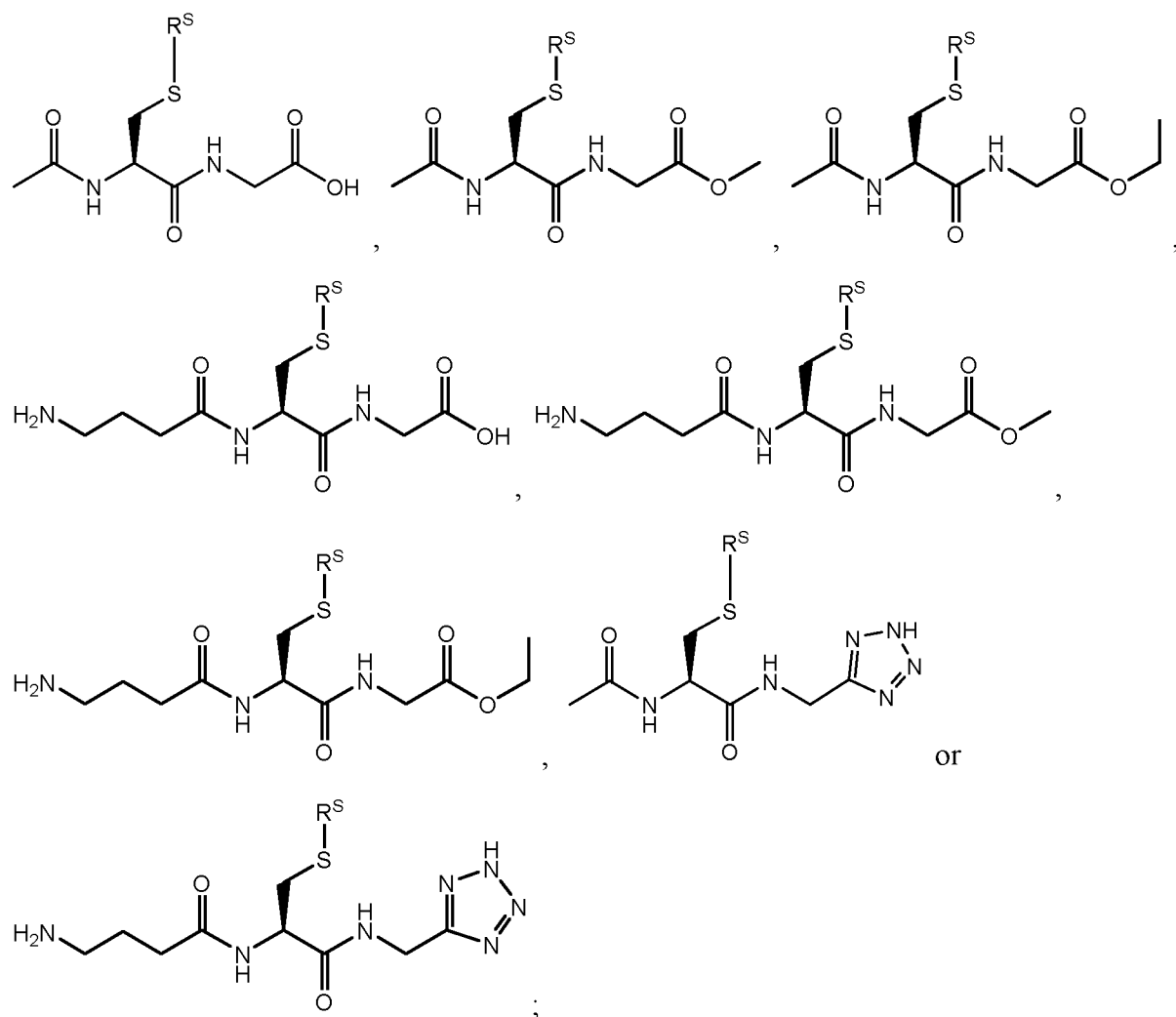
15. A compound according to any one of claims 1 to 4, which compound is a peptide derivative of formula (4) or a salt thereof:



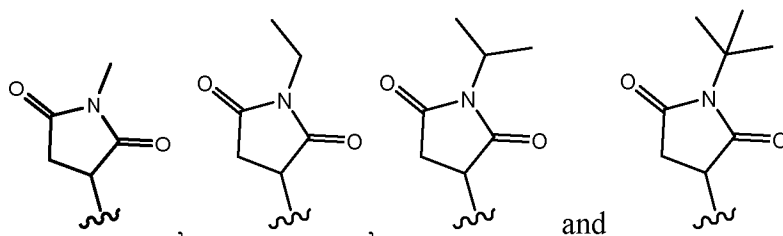
wherein $\mathbf{R}^G$, $\mathbf{R}^{N1}$, $\mathbf{R}^S$, $\mathbf{R}^{C1}$, $\mathbf{R}^{N2}$, $\mathbf{R}^{C2}$ and $\mathbf{R}^O$ are as defined in any one of the preceding claims.

16. A compound according to any one of the preceding claims, wherein $\mathbf{R}^G$ is methyl and $\mathbf{R}^O$ is H or methyl.

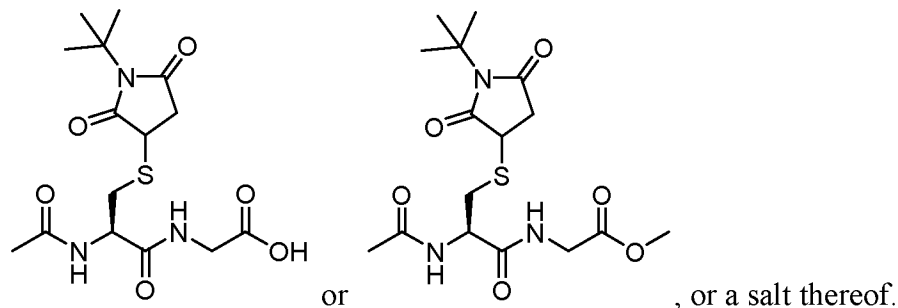
17. A compound according to claim 1, which compound is



or a salt thereof, and where $\mathbf{R}^S$ is a group selected from



18. A compound according to any one of the preceding claims, which compound is



19. A pharmaceutical composition comprising (a) a compound which is a peptide derivative or a salt thereof as defined in any one of claims 1 to 18, wherein the salt is a pharmaceutically acceptable salt, and (b) one or more pharmaceutically acceptable excipients or diluents.

20. A compound which is a peptide derivative or a salt thereof as defined in any one of claims 1 to 18, for use in a method of treatment or prevention of bacterial infection, wherein the salt is a pharmaceutically acceptable salt.

21. A compound for use according to claim 20, wherein the bacterial infection is caused by bacteria of genus *Shewanella*, *Acinetobacter*, *Bacillus*, *Bartonella*, *Bordetella*, *Borrelia*, *Brucella*, *Campylobacter*, *Chlamydia*, *Chlamydophila*, *Clostridium*, *Corynebacterium*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Francisella*, *Haemophilus*, *Helicobacter*, *Klebsiella*, *Legionella*, *Leptospira*, *Listeria*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Pseudomonas*, *Rickettsia*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Treponema*, *Vibrio* or *Yersinia*. For example, the bacterial infection may be caused by bacteria of genus *Shewanella* or *Escherichia*.

22. A compound for use according to claim 20 or 21, wherein the bacterial infection is caused by *Staphylococcus aureus*, *Escherichia coli*, *Shewanella denitrificans*, *Hemophilus*

influenzae, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*, *Helicobacter pylori*, *Salmonella enteritidis*, *Salmonella typhi* or *Acinetobacter baumannii*.

23. A method of treating or preventing bacterial infection in a subject, wherein the infection is as defined in any one of claims 20 to 22, which method comprises administering an effective amount of a peptide derivative or a salt thereof as defined in any one of claims 1 to 18 to the subject, wherein the salt is a pharmaceutically acceptable salt.

24. Use of a peptide derivative or a salt thereof as defined in any one of claims 1 to 18 in the manufacture of a medicament for use in a method of treatment or prevention of bacterial infection, wherein the infection is as defined in any one of claims 20 to 22, and wherein the salt is a pharmaceutically acceptable salt.

25. A combination comprising (a) a compound which is a peptide derivative or a salt thereof as defined in any one of claims 1 to 18, wherein the salt is a pharmaceutically acceptable salt, and (b) a further active agent which is an antibiotic compound.

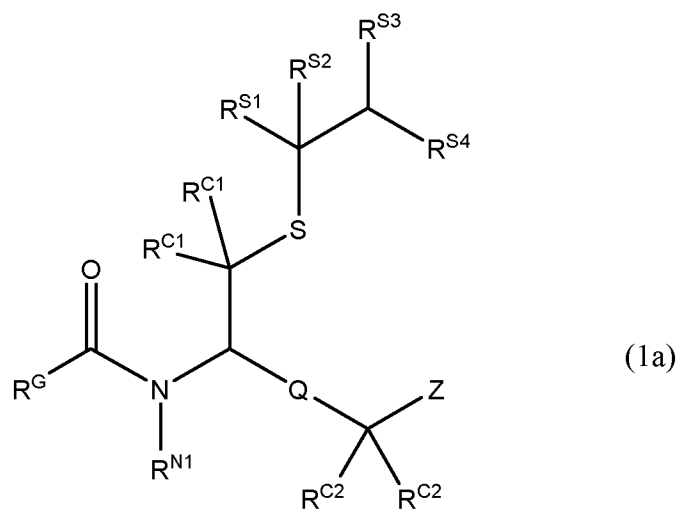
26. A compound for use according to any one of claims 20 to 22, wherein the method comprises administering the compound in combination with a further active agent which is an antibiotic compound.

27. A method of treating or preventing infection according to claim 23, wherein the method comprises administering the peptide derivative or salt thereof in combination with a further active agent which is an antibiotic compound.

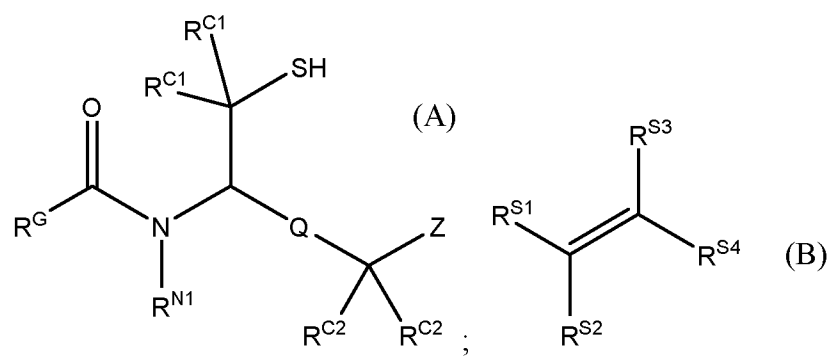
28. A kit comprising (a) a compound which is a peptide derivative or a salt thereof as defined in any one of claims 1 to 18, wherein the salt is a pharmaceutically acceptable salt, and (b) a further active agent which is an antibiotic compound.

29. Use according to claim 24, wherein the method comprises administering the compound in combination with a further active agent which is an antibiotic compound.

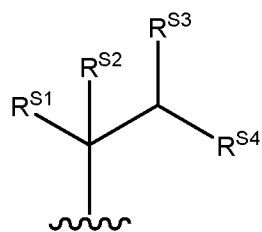
30. A compound which is a peptide derivative or a salt thereof as defined in any one of claims 1 to 18 for use in a method of activating potassium efflux in bacteria, wherein the bacteria are as defined in any one of claims 20 to 22.
31. A method for preventing or treating bacterial infection of a plant, which method comprises treating the plant, the seed from which the plant grows, or the place at which the plant grows, with a composition comprising a compound as defined in any one of claims 1 to 18.
32. A method according to claim 31, wherein the infection is caused by bacteria of the genus *Shewanella*, *Erwinia*, *Agrobacterium*, *Phytoplasma*, *Burkholderia*, *Proteobacteria*, *Xanthomonas* or *Pseudomonas*.
33. An in vitro method for killing or inhibiting the growth of bacteria, which method comprises applying a composition comprising a compound which is a peptide derivative or salt thereof as defined in any one of claims 1 to 18 to a surface, to an article or to the skin.
33. A soap product, washing product or cleaning product comprising a compound which is a peptide derivative or salt thereof as defined in any one of claims 1 to 18.
34. A process for producing a compound which is a peptide derivative of formula (1a) or a salt thereof:



which process comprises reacting a thiol compound of formula (A) with an alkene of formula (B) in the presence of an initiator,



wherein R^G , R^{N1} , R^{C1} , Q , R^{C2} and Z are as defined in any one of claims 1 to 18 and the group



is a group R^S as defined in any one of claims 1 to 18.

1/10

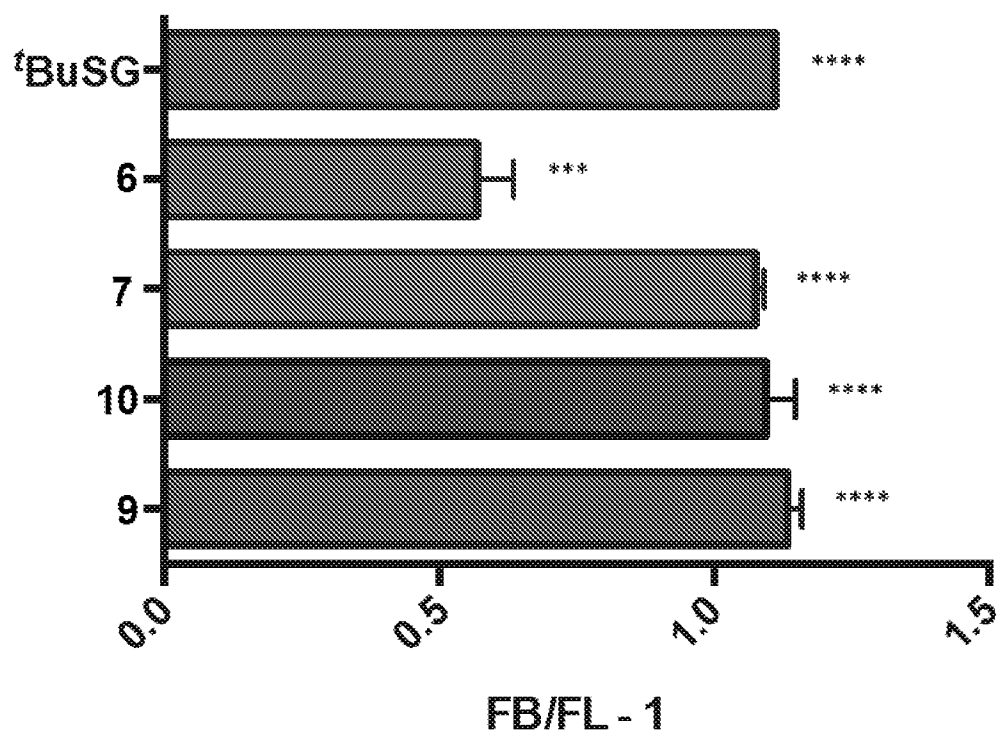


Figure 1

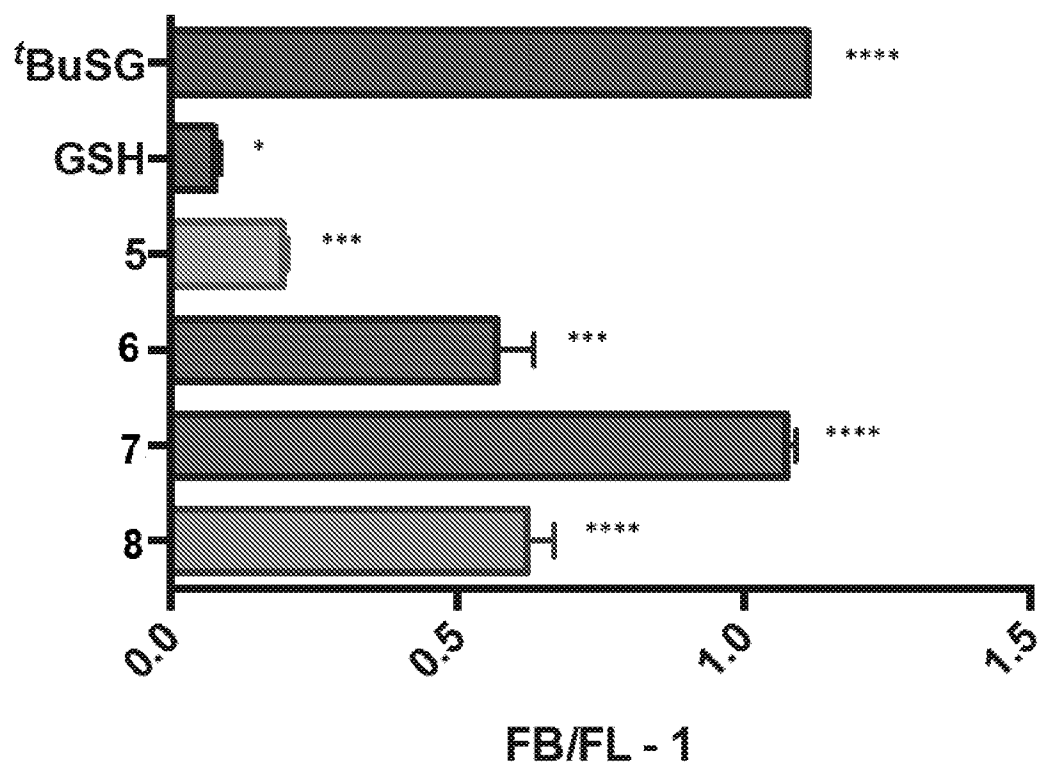


Figure 2

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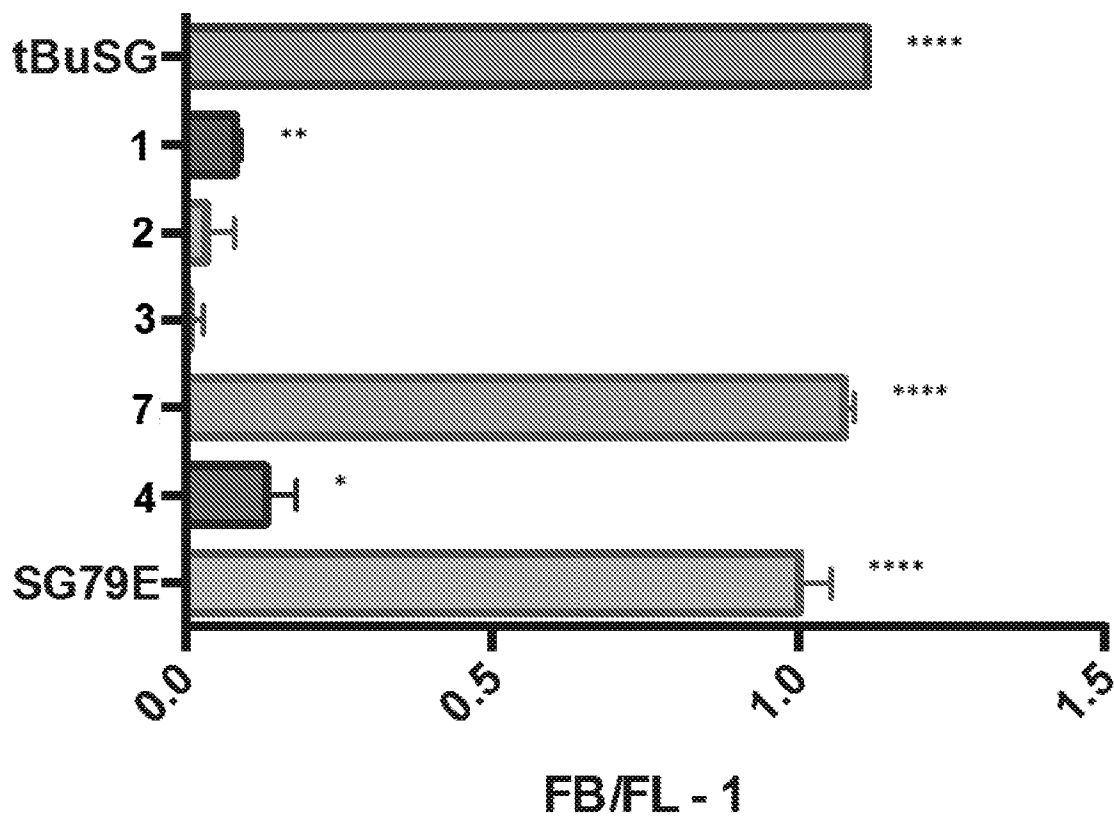
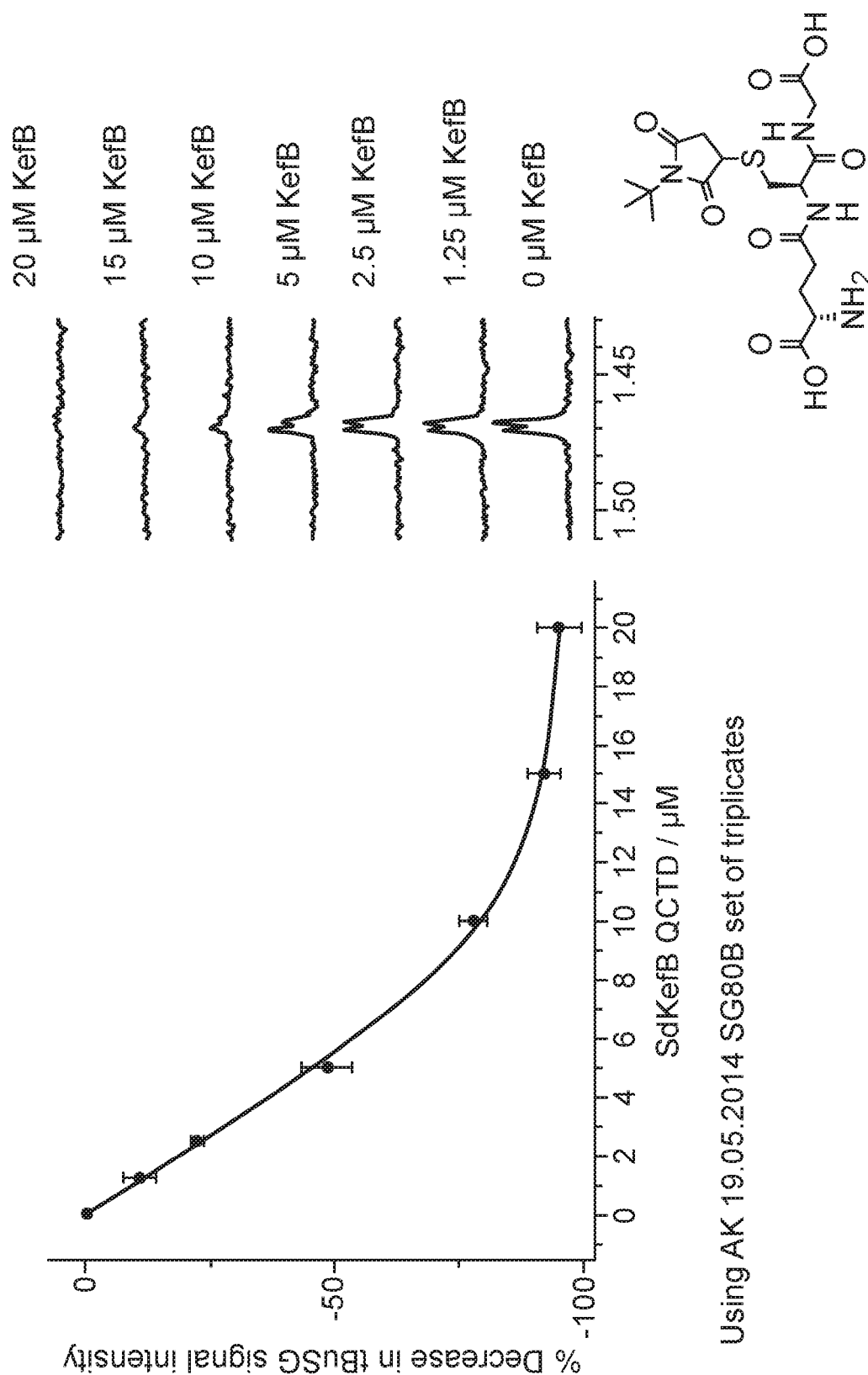


Figure 3

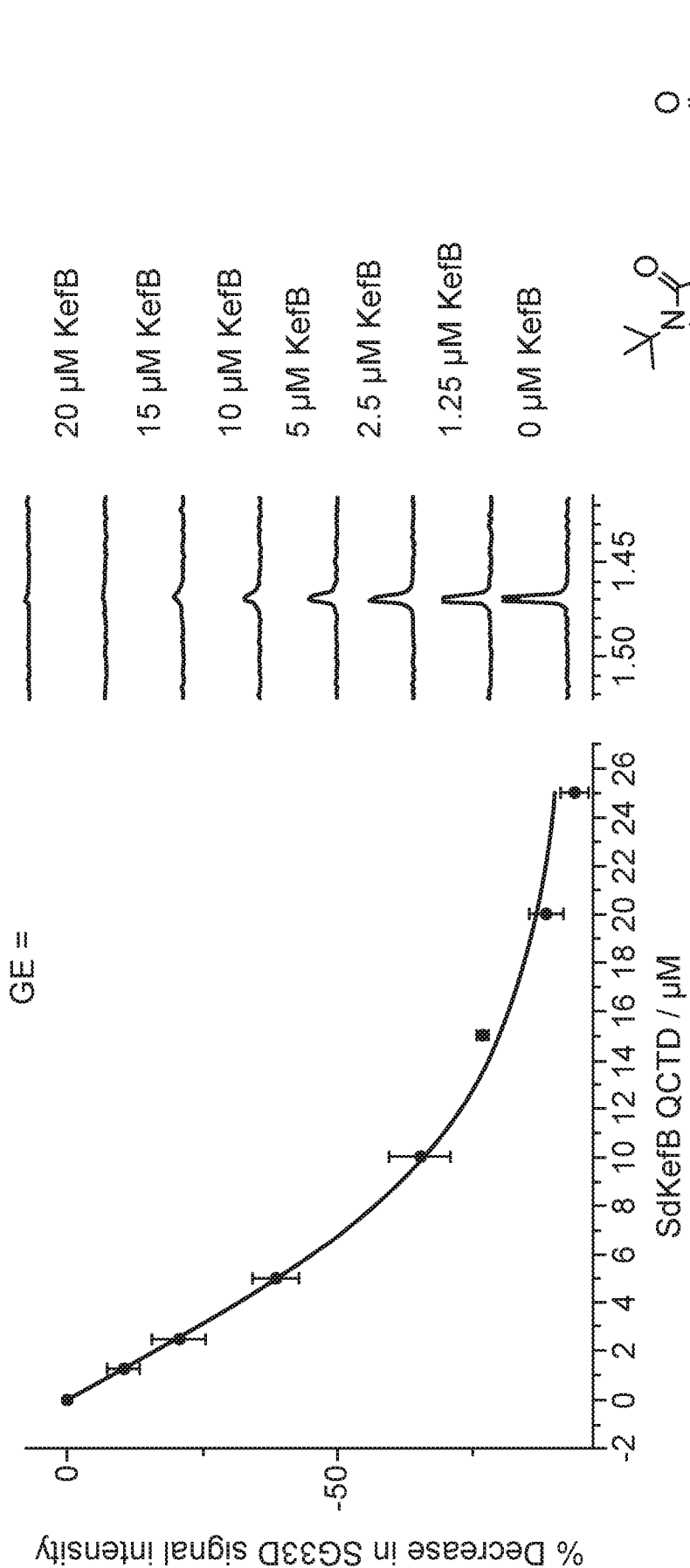
3/10

Fig. 4

Kd curve for tBuSG: 540 ± 33 nM

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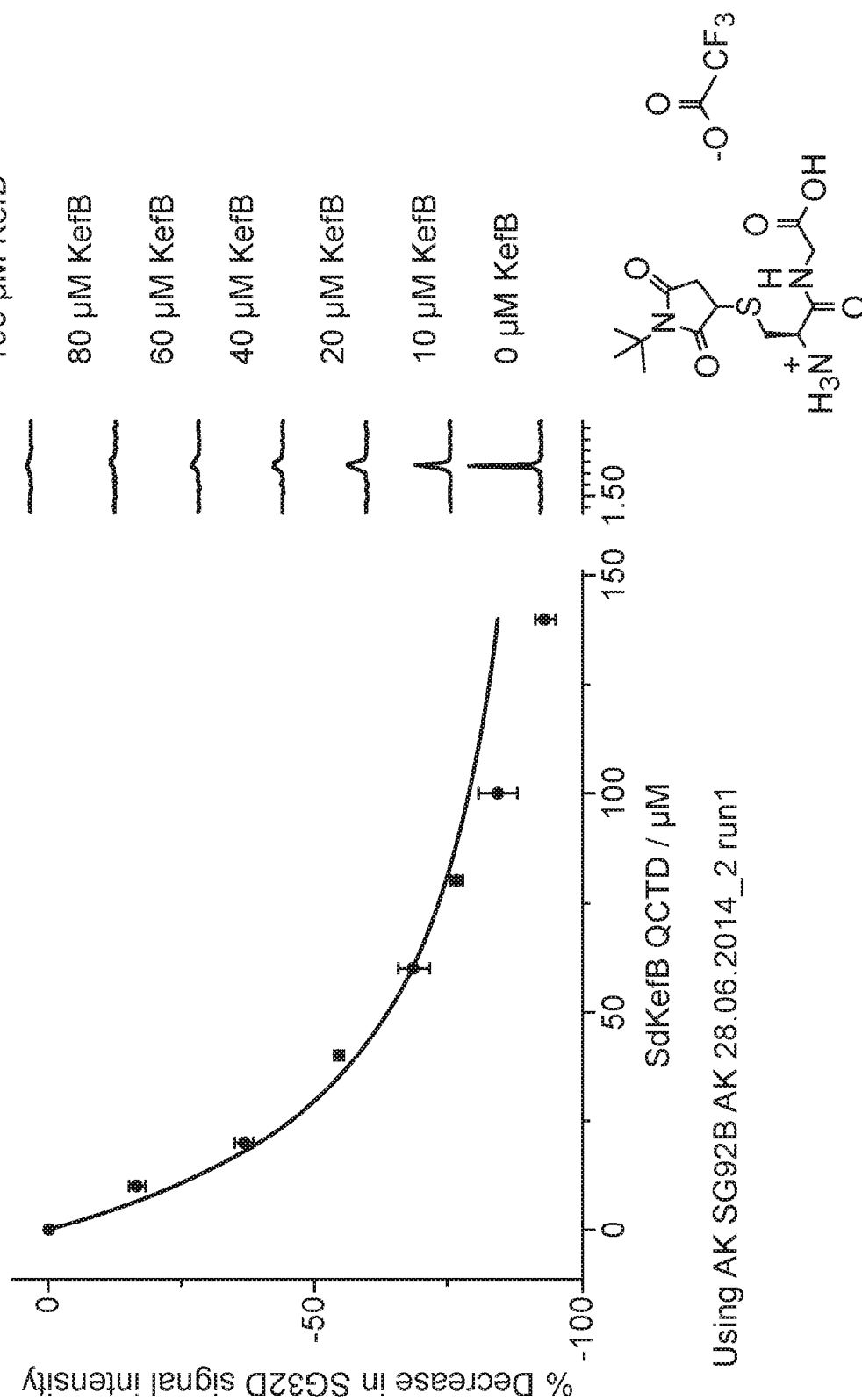
Fig. 5

Kd curve for SG33D: $1.8 \pm 0.2 \mu\text{M}$ 

Using SG76B AK.09.04.2014 Kd run 1

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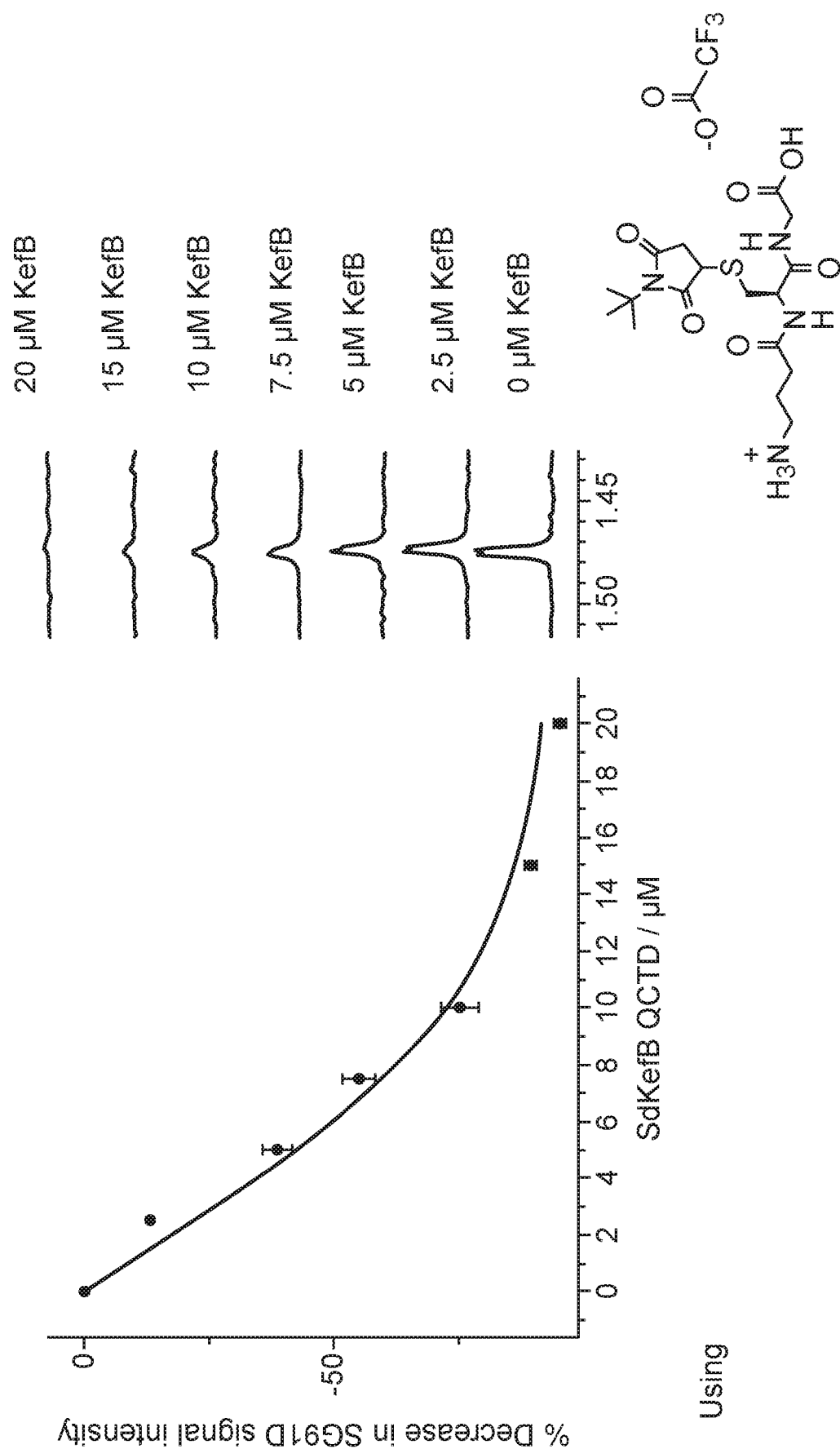
Fig. 6

Kd curve for SG32D: $25 \pm 0.8 \mu\text{M}$ 

Using AK SG92B AK 28.06.2014_2 run1

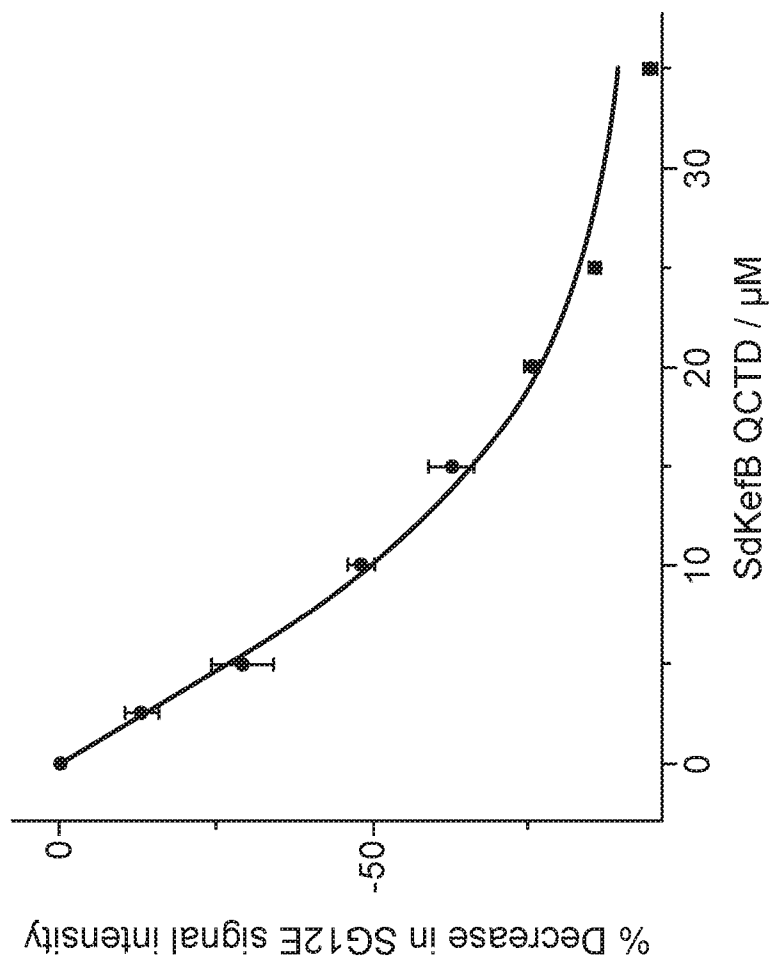
6/10

Fig. 7

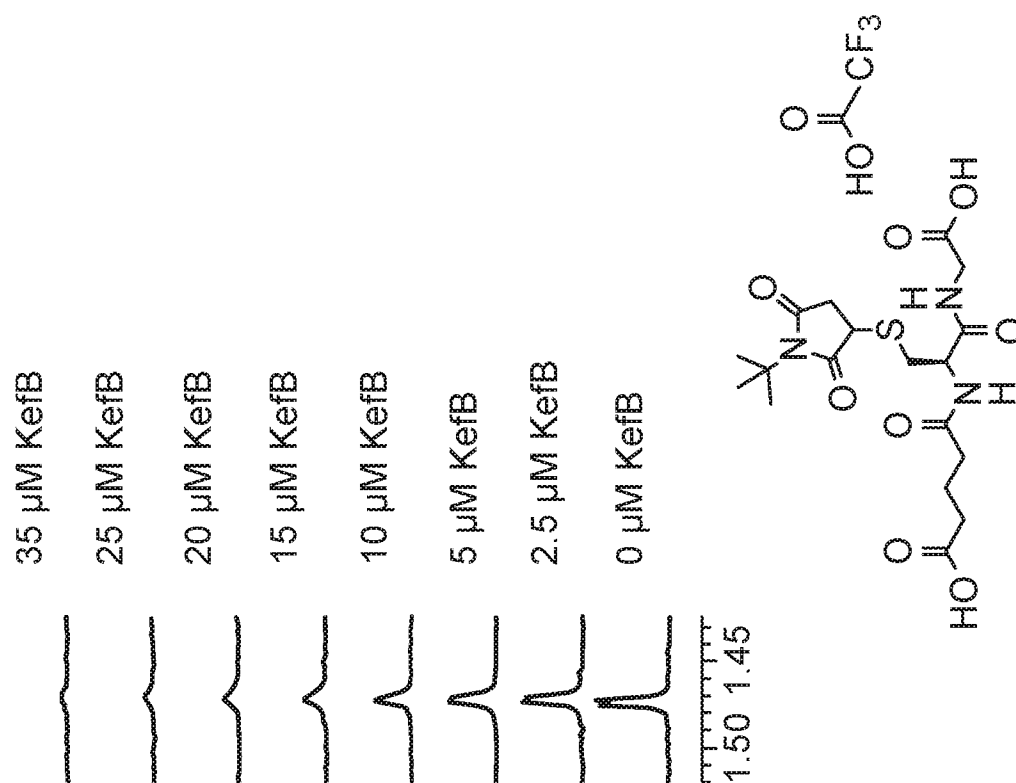
K_d curve for SG91D: $1.0 \pm 0.1 \mu\text{M}$ 

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Fig. 8

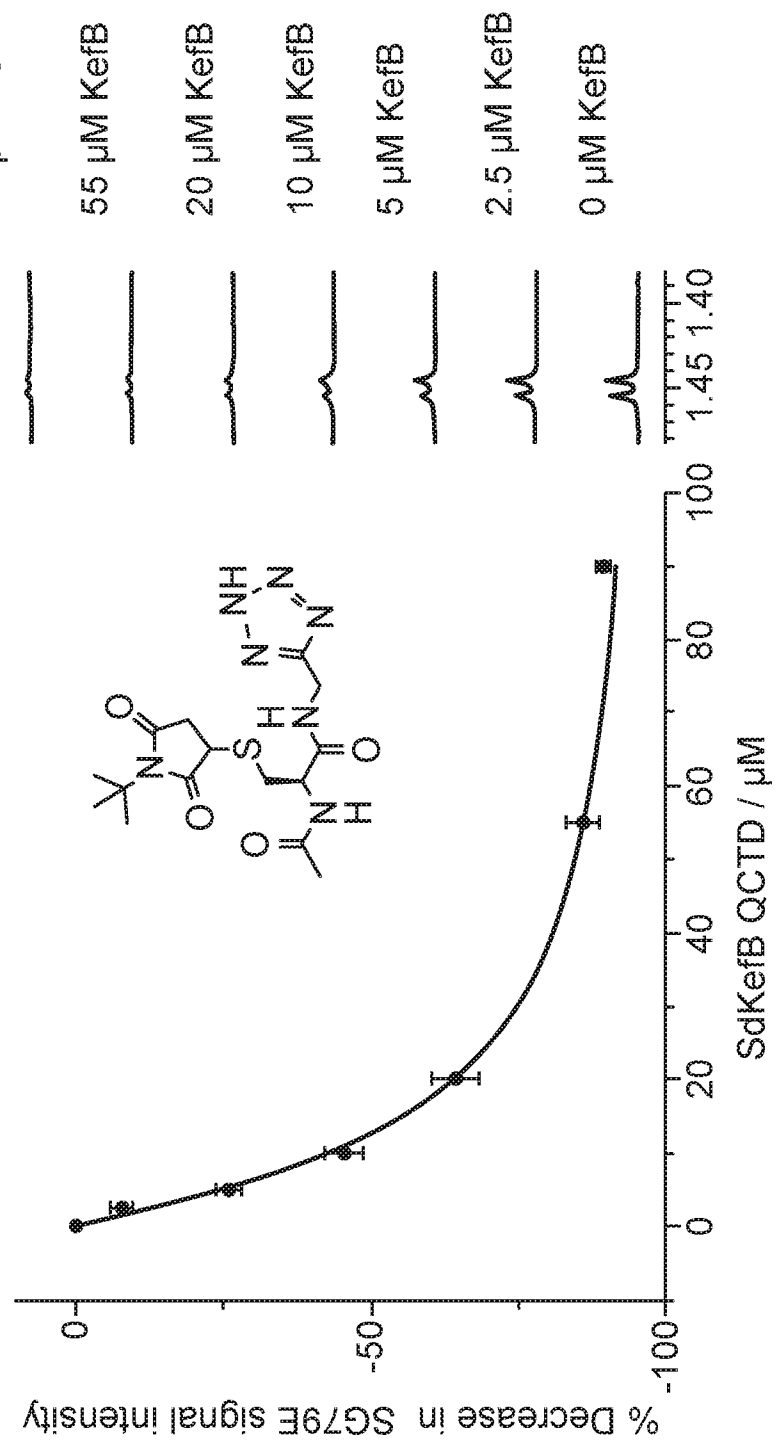
Kd curve for SG12E: $2.6 \pm 0.3 \mu\text{M}$ 

Using SG104B AK.18.08.2014 Kd run 1



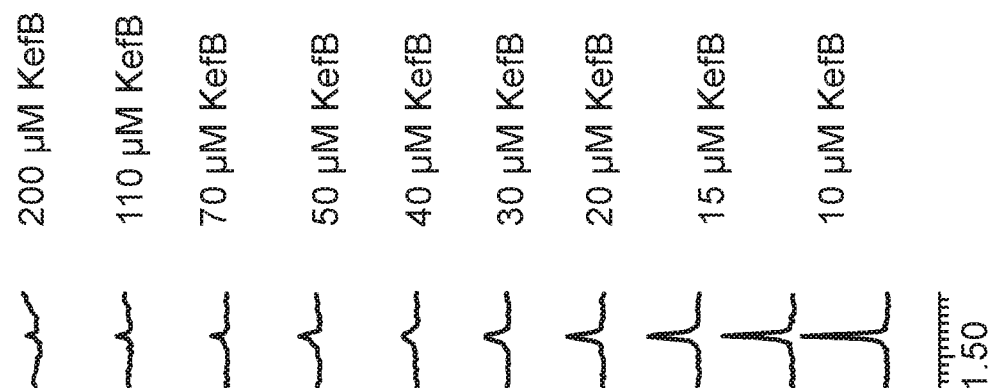
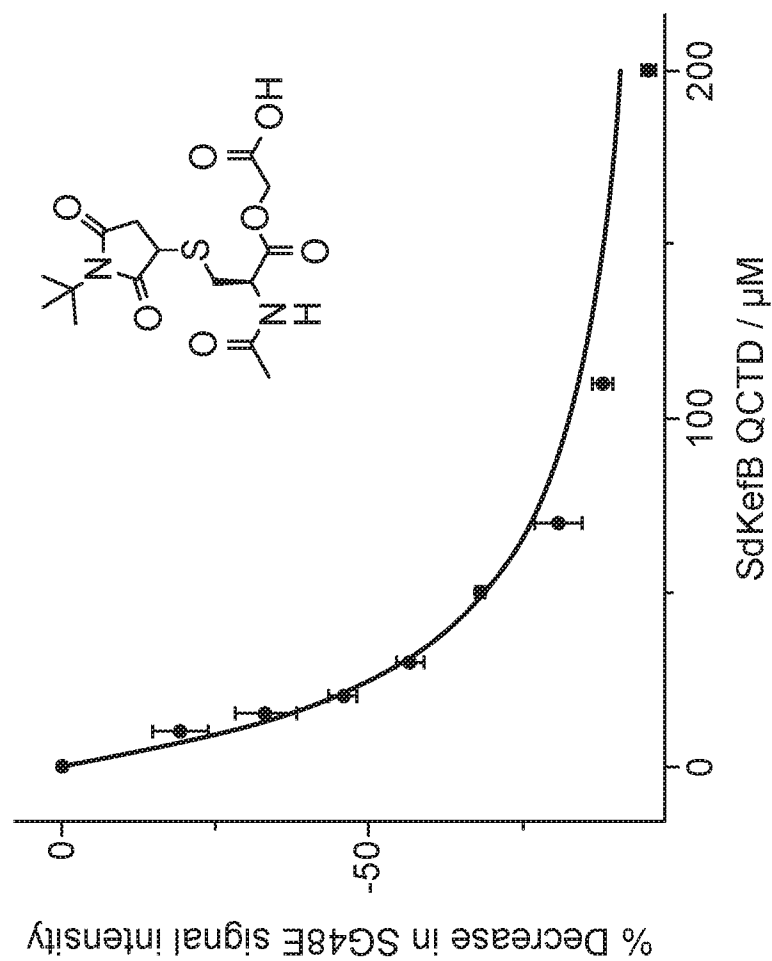
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Fig. 9

Kd curve for SG79E $7.6 \pm 0.5 \mu\text{M}$ 

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Fig. 10

K_d curve for SG48E $20 \pm 0.8 \mu\text{M}$ 

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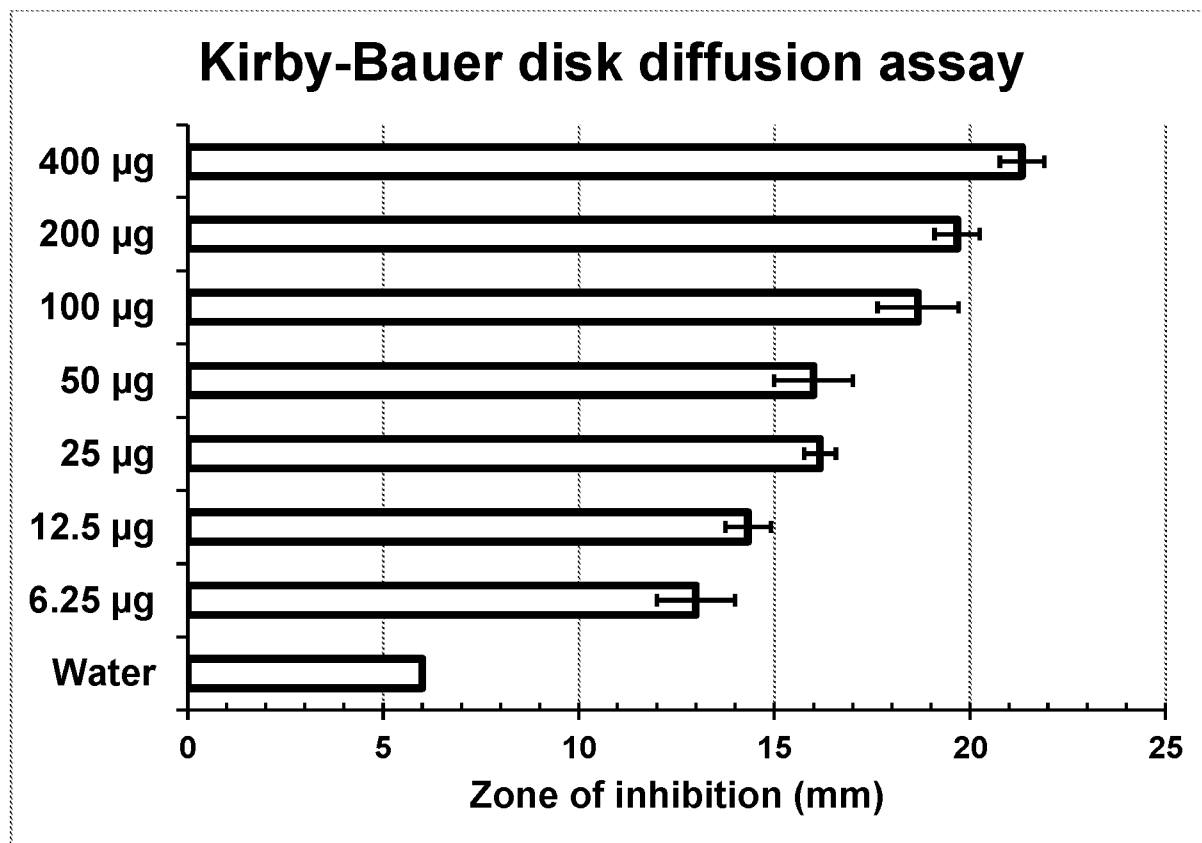


Figure 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/051420

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K5/062 C07D257/04 A61P31/04
ADD.

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)
C07K C07D

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)

EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/113634 A1 (UNIV KANSAS [US]) 24 July 2014 (2014-07-24) page 7, line 1 - line 12; claims 1, 29-32 -----	1-5,7,8, 11,15,19
A	JESSICA HEALY ET AL: "Understanding the Structural Requirements for Activators of the Kef Bacterial Potassium Efflux System", BIOCHEMISTRY, vol. 53, no. 12, 1 April 2014 (2014-04-01) , pages 1982-1992, XP055286326, US ISSN: 0006-2960, DOI: 10.1021/bi5001118 Scheme 1;; page 1989, column 2, line 28 - page 1991, line 51; figure 8 -----	1-34



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step 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

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Date of the actual completion of the international search

8 July 2016

Date of mailing of the international search report

15/07/2016

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Jetter, Sonya

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2016/051420

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014113634 A1	24-07-2014	US 2015337009 A1 WO 2014113634 A1	26-11-2015 24-07-2014
