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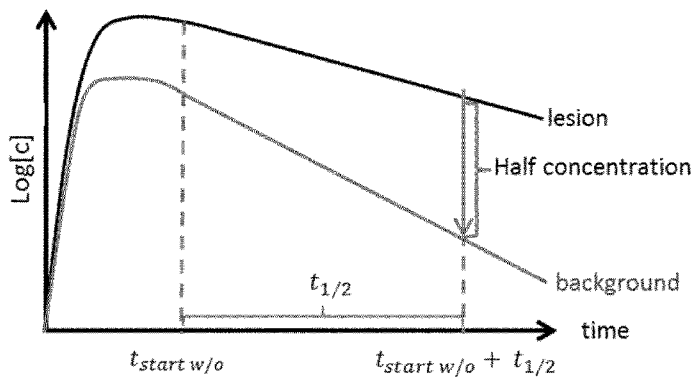
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[Continued on next page]

(54) Title: INFUSION IMAGING METHOD

Figure 1: Schematic of the Logarithm of the concentration [c] of Compound 3 in tissue versus time in the lesion and background. $t_{1/2}$ is the half-life of Compound 3 in background tissue. $t_{start\ w/o}$ is the time of the start of the exponential washout phase. $t_{start\ w/o} + t_{1/2}$ is the time of the start of the exponential washout plus the half-life of Compound 3 in background tissue.



(57) Abstract: The present invention relates to a method of optical imaging of a mammalian subject *in vivo*, wherein the imaging agent has been previously administered *via* intravenous infusion. The imaging agent comprises a peptide which targets c-Met labelled with an optical reporter group suitable for imaging in the red to near-infrared region. Also disclosed are *in vivo* imaging methods, especially of use in the diagnosis of colorectal cancer (CRC).



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Infusion Imaging Method.

Field of the Invention.

The present invention relates to a method of optical imaging of a mammalian subject *in vivo*, wherein the imaging agent has been previously administered *via* intravenous infusion. The imaging agent comprises a peptide which targets c-Met labelled with an optical reporter group suitable for imaging in the red to near-infrared region. Also disclosed are *in vivo* imaging methods, especially of use in the diagnosis of colorectal cancer (CRC).

Background to the Invention.

WO 2005/030266 discloses that there is a medical need for early diagnosis of colorectal cancer (CRC). WO 2005/030266 discloses optical imaging contrast agents which have affinity for a biological target abnormally expressed in CRC. The biological target is selected from: COX-2, beta-catenin, E-cadherin, P-cadherin, various kinases, Her-2, matrix metalloproteinases (MMPs), cyclins, P53, thymidylate synthase, VEGF receptors, EGF receptors, K-ras, adenomatous polyposis coli protein, cathepsin B, uPAR, c-Met, mucins and gastrin receptors. Preferred such targets (p.7 lines 11-12) are said to be: c-Met, MMP-14, COX-2, beta-catenin and Cathepsin B. The vector of WO 2005/030266 can be: a peptide, peptoid moiety, oligonucleotide, oligosaccharide, lipid-related compound or traditional organic drug-like small molecule. The reporter moiety is preferably a dye that interacts with light in the wavelength region from the ultraviolet to the infrared part of the electromagnetic spectrum.

Hepatocyte growth factor (HGF), also known as scatter factor (SF), is a growth factor which is involved in various physiological processes, such as wound healing and angiogenesis. The HGF interaction with its high affinity receptor (c-Met) is implicated in tumour growth, invasion and metastasis.

Knudsen *et al* have reviewed the role of HGF and c-Met in prostate cancer, with possible implications for imaging and therapy [Adv.Cancer Res., 91, 31-67 (2004)]. Labelled anti-met antibodies for diagnosis and therapy are described in WO 03/057155.

WO 2004/078778 discloses polypeptides or multimeric peptide constructs which bind c-Met or a complex comprising c-Met and HGF. Approximately 10 different structural classes of peptide are described. WO 2004/078778 discloses that the peptides can be labelled with a detectable label for *in vitro* and *in vivo* applications, or with a drug for therapeutic applications. The detectable label can be: an enzyme, fluorescent compound, an optical dye, a paramagnetic metal ion, an ultrasound contrast agent or a radionuclide. Preferred labels of WO 2004/078778 are stated to be radioactive or paramagnetic, and most preferably comprise a metal which is chelated by a metal chelator.

WO 2008/139207 discloses optical imaging agents which target c-Met *in vivo*, which comprise a peptide having the amino acid sequence shown labelled with an optical reporter imaging moiety suitable for imaging using light of green to near-infrared wavelength 600-1200 nm:

Cys^a-X¹-Cys^c-X²-Gly-Pro-Pro-X³-Phe-Glu-Cys^d-Trp-Cys^b-Tyr-X⁴-X⁵-X⁶;

wherein X¹ is Asn, His or Tyr;

X² is Gly, Ser, Thr or Asn;

X³ is Thr or Arg;

X⁴ is Ala, Asp, Glu, Gly or Ser;

X⁵ is Ser or Thr;

X⁶ is Asp or Glu;

and Cys^{a-d} are each cysteine residues such that residues a and b as well as c and d are cyclised to form two separate disulfide bonds;

WO 2008/139207 mentions (page 22) that the imaging agent is administered prior to imaging, e.g. by intravenous injection.

WO 2008/043101 relates to the intraoperative imaging of hepatobiliary structures using optical dyes. WO 2008/043101 uses dyes in free, unconjugated form such as indocyanine green. WO 2008/043101 teaches that the dye administration is *via* subcutaneous injection, intramuscular injection or slow, continuous infusion. WO 2008/043101 teaches that systemic administration is suitable for liver imaging, but that subcutaneous administration is preferable for imaging the bile duct or hepatic duct. WO 2008/043101 does not use a targeted molecular imaging agent.

The Present Invention.

The present invention relates to a method of optical imaging of a mammalian subject *in vivo*, wherein the imaging agent has been previously administered *via* intravenous infusion. The imaging agent comprises a peptide which targets c-Met labelled with an optical reporter group suitable for imaging in the red to near-infrared region.

The prior art (WO 2008/139207) contemplates administering a c-Met targeted optical imaging agent as a bolus injection. The agent is delivered to tissue, which comprises the site of interest and tissue background. The present applicants have established that clearance of the agent from background tissue needs to occur before positive contrast is seen at the site of interest (eg. a colorectal cancer lesion). That clearance necessitates a time delay before imaging can commence post-administration of the agent to the mammalian subject. It is expected that clearance is faster from tissues with low or no expression of c-Met when compared to tissues with high expression of the receptor. In the case of colorectal cancer imaging following intravenous bolus injection, a delay of 3 hours post injection is necessary before the medical imaging (i.e. the start of the colonoscopy procedure) can commence. That delay is largely due to the background washout period.

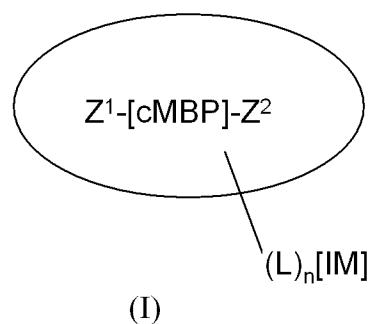
By giving an infusion of the compound instead of a bolus injection, the agent may be delivered to the target site of interest in sufficient quantity, but without ‘overfilling’ the background tissue. Less time is therefore needed to achieve background clearance, while still delivering enough compound to the lesion. Hence the waiting time (from injection to start of endoscopy/imaging procedure) can be reduced. A reduction in waiting time would be of substantial benefit for the clinical workflow – benefiting both patient throughput for the hospital/clinic involved, and the patient (reduced waiting time). It is believed that benefit would outweigh the additional complication (additional instrumentation and patient preparation required for infusion), in terms of both cost (patient throughput) and patient time (reducing the patient time at hospital from say 4 hours down to e.g. 2 hours).

By slow infusion of the imaging agent over say 15 to 25 minutes instead of bolus injection, it is anticipated that the waiting time before starting fluorescence endoscopy could be reduced from 3 hours to 1 to 1.5 hours. An additional advantage may be a

reduction in overall dose of the imaging agent to the mammalian subject, whilst achieving comparable imaging results.

5 Detailed Description of the Invention.

In a first aspect, the present invention provides a method of optical imaging which comprises imaging a mammalian subject to obtain images of sites of c-Met over-expression or localisation *in vivo*, wherein said subject had been previously administered *via* intravenous infusion with an imaging agent which comprises a
10 conjugate of Formula I:



where:

Z^1 is attached to the N-terminus of cMBP, and is H or M^{IG} ;

Z^2 is attached to the C-terminus of cMBP and is OH, OB^c , or M^{IG} ,

15 where B^c is a biocompatible cation;

cMBP is a c-Met binding cyclic peptide of 17 to 30 amino acids which comprises the amino acid sequence (SEQ-1):

$Cys^a-X^1-Cys^c-X^2-Gly-Pro-Pro-X^3-Phe-Glu-Cys^d-Trp-Cys^b-Tyr-X^4-X^5-X^6$;

wherein X^1 is Asn, His or Tyr;

20 X^2 is Gly, Ser, Thr or Asn;

X^3 is Thr or Arg;

X^4 is Ala, Asp, Glu, Gly or Ser;

X^5 is Ser or Thr;

X^6 is Asp or Glu;

25 and Cys^{a-d} are each cysteine residues such that residues a and b as well as c and d are cyclised to form two separate disulfide bonds;

M^{IG} is a metabolism inhibiting group which is a biocompatible group which inhibits or suppresses *in vivo* metabolism of the cMBP peptide;

L is a synthetic linker group of formula $-(A)_m-$ wherein each A is

independently -CR₂-, -CR=CR-, -C≡C-, -CR₂CO₂-, -CO₂CR₂-, -NRCO-,
 -CONR-, -NR(C=O)NR-, -NR(C=S)NR-, -SO₂NR-, -NRSO₂-, -CR₂OCR₂-,
 -CR₂SCR₂-, -CR₂NRCR₂-, a C₄₋₈ cycloheteroalkylene group, a C₄₋₈
 cycloalkylene group, a C₅₋₁₂ arylene group, or a C₃₋₁₂ heteroarylene group, an
 5 amino acid, a sugar or a monodisperse polyethyleneglycol (PEG) building
 block;
 each R is independently chosen from H, C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl,
 C₁₋₄ alkoxyalkyl or C₁₋₄ hydroxyalkyl;
 m is an integer of value 1 to 20;
 10 n is an integer of value 0 or 1;
 IM is an optical reporter imaging moiety suitable for imaging the mammalian
 body *in vivo* using light of wavelength 600-1200 nm.

By the term “sites of c-Met over-expression or localisation *in vivo*” is meant locations
 15 within the mammalian subject where Hepatocyte Growth factor (HGF) high affinity
 receptor (known as c-Met) are localised or over-expressed. HGF is involved in
 various physiological processes, such as wound healing and angiogenesis. Any such
 over-expression of c-Met is implicated in various disease states such as tumour
 growth, tumour invasion and tumour metastasis. Thus, Trusolino *et al* suggest that c-
 20 Met is associated with colorectal cancer progression [Nat. Rev. Cancer, 2: 289-300
 (2002)]. Several studies have reported amplification and overexpression of c-Met in
 cancer cells [see e.g. Umeki *et al*, Oncology, 56: 314-321, (1999)].

By the term “optical imaging” is meant any method that forms an image for detection,
 25 staging or diagnosis of disease, follow up of disease development or for follow up of
 disease treatment (including surgical resection), based on interaction with light in the
 red to near-infrared region (wavelength 600-1200 nm). Optical imaging further
 includes all methods from direct visualization without use of any device and involving
 use of devices such as various scopes, catheters and optical imaging equipment, eg.
 30 computer-assisted hardware for tomographic presentations. The modalities and
 measurement techniques include, but are not limited to: luminescence imaging;
 endoscopy; fluorescence endoscopy; optical coherence tomography; transmittance
 imaging; time resolved transmittance imaging; confocal imaging; nonlinear
 microscopy; photoacoustic imaging; acousto-optical imaging; spectroscopy;

- reflectance spectroscopy; interferometry; coherence interferometry; diffuse optical tomography and fluorescence mediated diffuse optical tomography (continuous wave, time domain and frequency domain systems), and measurement of light scattering, absorption, polarization, luminescence, fluorescence lifetime, quantum yield, and quenching. Further details of these techniques are provided by: (Tuan Vo-Dinh (editor): *Biomedical Photonics Handbook* (2003), CRC Press LCC; Mycek & Pogue (editors): *Handbook of Biomedical Fluorescence* (2003), Marcel Dekker, Inc.; Splinter & Hopper: *An Introduction to Biomedical Optics* (2007), CRC Press LCC.
- 10 By the term “imaging agent” is meant a compound suitable for imaging the mammalian body *in vivo* (or tissue samples removed and imaged *ex-vivo*). Preferably, the mammal is a human subject. The imaging may be invasive (e.g. intra-operative or endoscopic) or non-invasive. The preferred imaging method is endoscopy.
- 15 The terms “comprising” or “comprises” have their conventional meaning throughout this application and imply that the composition must have the components listed, but that other, unspecified compounds or species may be present in addition. These terms include as a preferred subset “consisting essentially of” which means that the composition has the components listed without other compounds or species being
- 20 present.

By the term “previously administered” is meant that the step involving the clinician, wherein the imaging agent is given to the mammalian subject already been carried out prior to imaging. The imaging may start at any time after the infusion has

25 commenced. Hence, the imaging can occur either during the infusion, or preferably after completion of the infusion.

The term “intravenous infusion” has its conventional meaning, i.e. a slow administration of the agent over a period of several minutes into a vein of the

30 mammalian subject, typically *via* a catheter or needle. The infusion time is suitably at least 2 minutes, preferably at least 5 minutes, more preferably at least 10 minutes. That is in contrast to conventional administration of 100% of the agent *via* a single bolus injection, in a fraction of a second to a few seconds. The infusion rate can be steady (i.e. the molar amount of agent delivered per minute is constant throughout

until 100% administration has been achieved), or variable. In clinical practice, infusion rates extend over a wide range depending on the application. Thus, rates vary from 1 mL per hour (e.g. keeping open a central venous catheter, infusion for infants) and >1,000 mL per hour for adult patients. An optimum profile for imaging agent administration may comprise a high infusion rate (bolus period) at the beginning of the administration followed by a longer period with a continuous, low infusion rate.

The infusion of the first aspect is suitably carried out using a gravity drip or programmable infusion pump. Infusion is mainly carried out using such devices with infusion accessories, such as connecting tubing, catheters, cannula and/or needles. A programmable infusion pump is preferred, since such pumps can provide a variable infusion rate profile which can be optimised for imaging. The conventional parenteral administration system normally comprises a container of liquid, an elongated flexible tube and a cannula or catheter which is inserted usually into the cardiovascular system of the patient. Intravenous infusion techniques are known in – see eg. Quinn C (2008) *Intravenous flow control and infusion devices*; Dougherty L and Lamb J in *Intravenous therapy in nursing practice*. (Oxford, Wiley Blackwell) and *Handbook of Infusion Therapy*, Rita M. Doyle (editor), Springhouse Pub Co, (1999).

The concentration of imaging agent for use in the infusion is suitably in the range 0.01 to 10 mg/mL, preferably 0.05 to 9 mg/mL, more preferably 0.4 to 8 mg/mL, most preferably 0.5 to 5 mg/mL. The total patient dose will be in the range 0.05 to 0.2, preferably 0.10 to 0.16, more preferably 0.12 to 0.14 mg/kg body weight. The total dose of contrast agent can delivered either at low volume (eg. 0.1 to 0.3 ml/min) of infusion medium per minute (for concentrations at the higher end of the above ranges), or larger volume (eg. 1.0 to 5 ml/min) for lower concentrations. As noted above, although the concentration of contrast agent within the infusion medium is suitably constant, the infusion rate can be varied during administration to the patient.

The Z^1 group substitutes the amine group of the last amino acid residue. Thus, when Z^1 is H, the amino terminus of the cMBP terminates in a free NH_2 group of the last amino acid residue. The Z^2 group substitutes the carbonyl group of the last amino acid residue. Thus, when Z^2 is OH, the carboxy terminus of the cMBP terminates in

the free CO₂H group of the last amino acid residue, and when Z² is OB^c that terminal carboxy group is ionised as a CO₂B^c group.

By the term “metabolism inhibiting group” (M^{IG}) is meant a biocompatible group which inhibits or suppresses *in vivo* metabolism of the cMBP peptide at either the amino terminus (Z¹) or carboxy terminus (Z²). Such groups are well known to those skilled in the art and are suitably chosen from, for the peptide amine terminus:
N-acylated groups –NH(C=O)R^G where the acyl group –(C=O)R^G has R^G chosen from: C₁₋₆ alkyl, C₃₋₁₀ aryl groups or comprises a polyethyleneglycol (PEG) building block. Suitable PEG groups are described for the linker group (L), below. Preferred such PEG groups are the biomodifiers of Formula IA or IB. Preferred such amino terminus M^{IG} groups are acetyl, benzyloxycarbonyl or trifluoroacetyl, most preferably acetyl.

Suitable metabolism inhibiting groups for the peptide carboxyl terminus include: carboxamide, *tert*-butyl ester, benzyl ester, cyclohexyl ester, amino alcohol or a polyethyleneglycol (PEG) building block. A suitable M^{IG} group for the carboxy terminal amino acid residue of the cMBP peptide is where the terminal amine of the amino acid residue is N-alkylated with a C₁₋₄ alkyl group, preferably a methyl group. Preferred such M^{IG} groups are carboxamide or PEG, most preferred such groups are carboxamide.

Formula I denotes that the -(L)_n[IM] moiety can be attached at any suitable position of Z¹, Z² or cMBP. For Z¹ or Z², the -(L)_n[IM] moiety may either be attached to the M^{IG} group when either of Z¹/Z² is a M^{IG}. When Z¹ is H or Z² is OH, attachment of the -(L)_n[IM] moiety at the Z¹ or Z² position gives compounds of formulae [IM]-(L)_n-[cMBP]-Z² or Z¹-[cMBP]-(L)_n-[IM] respectively. Inhibition of metabolism of the cMBP at either peptide terminus may also be achieved by attachment of the -(L)_n[IM] moiety in this way, but -(L)_n[IM] is outside the definition of M^{IG} of the present invention.

The -(L)_n- moiety of Formula I may be attached at any suitable position of the IM. The -(L)_n- moiety either takes the place of an existing substituent of the IM, or is covalently attached to the existing substituent of the IM. The -(L)_n- moiety is preferably attached *via* a carboxyalkyl substituent of the IM.

By the term “c-Met binding cyclic peptide” (cMBP) is meant a peptide which binds to the hepatocyte growth factor (HGF) high affinity receptor, also known as c-Met (c-Met or hepatocyte growth factor receptor). Suitable cMBP peptides of the present invention have an apparent K_D for c-Met of c-Met/HGF complex of less than about 20 nM. The cMBP peptides comprise proline residues, and it is known that such residues can exhibit *cis/trans* isomerisation of the backbone amide bond. The cMBP peptides of the present invention include any such isomers.

By the term “biocompatible cation” (B^c) is meant a positively charged counterion which forms a salt with an ionised, negatively charged group, where said positively charged counterion is also non-toxic and hence suitable for administration to the mammalian body, especially the human body. Examples of suitable biocompatible cations include: the alkali metals sodium or potassium; the alkaline earth metals calcium and magnesium; and the ammonium ion. Preferred biocompatible cations are sodium and potassium, most preferably sodium.

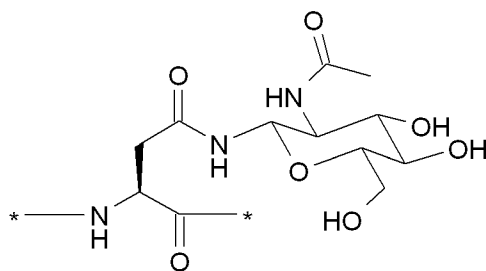
By the term “amino acid” is meant an *L*- or *D*- amino acid, amino acid analogue (eg. naphthylalanine) or amino acid mimetic which may be naturally occurring or of purely synthetic origin, and may be optically pure, i.e. a single enantiomer and hence chiral, or a mixture of enantiomers. Conventional 3-letter or single letter abbreviations for amino acids are used herein. Preferably the amino acids of the present invention are optically pure. By the term “amino acid mimetic” is meant synthetic analogues of naturally occurring amino acids which are isosteres, i.e. have been designed to mimic the steric and electronic structure of the natural compound. Such isosteres are well known to those skilled in the art and include but are not limited to depsipeptides, retro-inverso peptides, thioamides, cycloalkanes or 1,5-disubstituted tetrazoles [see M. Goodman, *Biopolymers*, 24, 137, (1985)].

By the term “optical reporter imaging moiety” (IM) is meant a fluorescent dye or chromophore which is capable of detection either directly or indirectly in an optical imaging procedure using light of green to near-infrared wavelength (500-1200 nm, preferably 600-1000 nm). Preferably, the IM has fluorescent properties.

It is envisaged that one of the roles of the linker group $-(A)_m-$ of Formula I is to

distance the IM from the active site of the cMBP peptide. This is particularly important when the imaging moiety is relatively bulky, so that interaction with the enzyme is not impaired. This can be achieved by a combination of flexibility (eg. simple alkyl chains), so that the bulky group has the freedom to position itself away from the active site and/or rigidity such as a cycloalkyl or aryl spacer which orientate the IM away from the active site. The nature of the linker group can also be used to modify the biodistribution of the imaging agent. Thus, e.g. the introduction of ether groups in the linker will help to minimise plasma protein binding. When $-(A)_m-$ comprises a polyethyleneglycol (PEG) building block or a peptide chain of 1 to 10 amino acid residues, the linker group may function to modify the pharmacokinetics and blood clearance rates of the imaging agent *in vivo*. Such “biomodifier” linker groups may accelerate the clearance of the imaging agent from background tissue, such as muscle or liver, and/or from the blood, thus giving a better diagnostic image due to less background interference. A biomodifier linker group may also be used to favour a particular route of excretion, eg. *via* the kidneys as opposed to *via* the liver.

By the term “sugar” is meant a mono-, di- or tri- saccharide. Suitable sugars include: glucose, galactose, maltose, mannose, and lactose. Optionally, the sugar may be functionalised to permit facile coupling to amino acids. Thus, eg. a glucosamine derivative of an amino acid can be conjugated to other amino acids *via* peptide bonds. The glucosamine derivative of asparagine (commercially available from NovaBiochem) is one example of this:



By the term “peptide” is meant a compound comprising two or more amino acids, as defined above, linked by a peptide bond (ie. an amide bond linking the amine of one amino acid to the carboxyl of another). The term “peptide mimetic” or “mimetic” refers to biologically active compounds that mimic the biological activity of a peptide

or a protein but are no longer peptidic in chemical nature, that is, they no longer contain any peptide bonds (that is, amide bonds between amino acids). Here, the term peptide mimetic is used in a broader sense to include molecules that are no longer completely peptidic in nature, such as pseudo-peptides, semi-peptides and peptoids.

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Preferred features.

The method of the first aspect employs a dosage of the imaging agent suitable for medical diagnostic imaging. The method preferably does not comprise any additional step involving light irradiation of the mammalian subject designed to achieve a therapeutic effect *via* photodynamic therapy (PDT).

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The green to near-infrared region light is preferably of wavelength 600-1000 nm. The optical imaging method of the first aspect is preferably fluorescence endoscopy.

15 The molecular weight of the imaging agent is suitably up to 8000 Daltons. Preferably, the molecular weight is in the range 2800 to 6000 Daltons, most preferably 3000 to 4500 Daltons, with 3200 to 4000 Daltons being especially preferred.

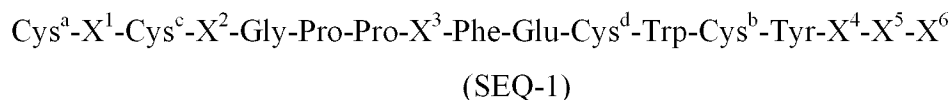
Preferred imaging agents of the present invention have both peptide termini protected by M^{IG} groups, ie. preferably both Z^1 and Z^2 are M^{IG} , which will usually be different. As noted above, either of Z^1/Z^2 may optionally equate to $-(L)_n[IM]$. Having both peptide termini protected in this way is important for *in vivo* imaging applications, since otherwise rapid metabolism would be expected with consequent loss of selective binding affinity for c-Met. When both Z^1 and Z^2 are M^{IG} , preferably Z^1 is acetyl and Z^2 is a primary amide. Most preferably, Z^1 is acetyl and Z^2 is a primary amide and the $-(L)_n[IM]$ moiety is attached to the epsilon amine side chain of a lysine residue of cMBP.

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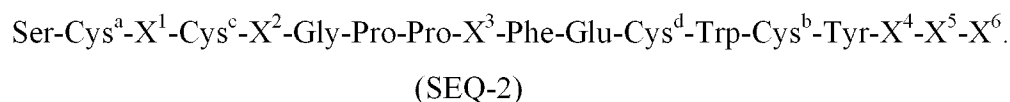
Preferred cMBP peptides of the present invention have a K_D for binding of c-Met to c-Met/HGF complex of less than about 10 nM (based on fluorescence polarisation assay measurements), most preferably in the range 1 to 5 nM, with less than 3nM being the ideal. The peptide sequence (SEQ-1)

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of the cMBP of Formula I is a 17-mer peptide sequence, which is primarily responsible for the selective binding to c-Met. When the cMBP peptide of the present invention comprises more than 17 amino acid residues, the remaining amino acids can be any amino acid apart from cysteine. Additional, unprotected cysteine residues could cause unwanted scrambling of the defined Cys^a-Cys^b and Cys^c-Cys^d disulfide bridges. The additional peptides preferably comprise at least one amino acid residue with a side chain suitable for facile conjugation of the -(L)_n[IM] moiety. Suitable such residues include Asp or Glu residues for conjugation with amine-functionalised -(L)_n[IM] groups, or a Lys residue for conjugation with a carboxy- or active ester-functionalised -(L)_n[IM] group. The amino acid residues for conjugation of -(L)_n[IM] are suitably located away from the 17-mer binding region of the cMBP peptide (SEQ-1), and are preferably located at the C- or N- terminus. Preferably, the amino acid residue for conjugation is a Lys residue.

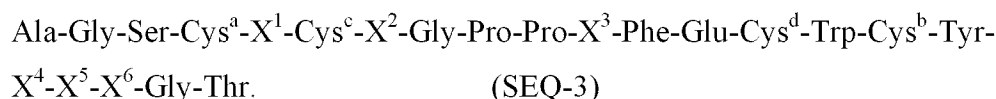
It is preferred that the cMBP peptide further comprises an *N*-terminal serine residue, giving the 18-mer (SEQ-2):



In addition to SEQ-1, or preferably SEQ-2, the cMBP most preferably further comprises either:

- (i) an Asp or Glu residue within 4 amino acid residues of either the C- or N-peptide terminus of the cMBP peptide, and -(L)_nIM is functionalised with an amine group which is conjugated to the carboxyl side chain of said Asp or Glu residue to give an amide bond;
- (ii) a Lys residue within 4 amino acid residues of either the C- or N- peptide terminus of the cMBP peptide, and -(L)_nIM is functionalised with a carboxyl group which is conjugated to the epsilon amine side chain of said Lys residue to give an amide bond.

Preferred cMBP peptides comprise the 22-mer amino acid sequence (SEQ-3):



The cMBP peptides of the present invention preferably have X^3 equal to Arg.

The cMBP peptide preferably further comprises in addition to SEQ-1, SEQ-2 or SEQ-3, at either the N- or C- terminus a linker peptide which is chosen from:

- 5 –Gly-Gly-Gly-Lys- (SEQ-4),
 –Gly-Ser-Gly-Lys- (SEQ-5), or
 –Gly-Ser-Gly-Ser-Lys- (SEQ-6).

The Lys residue of the linker peptide is a most preferred location for conjugation of the $-(L)_n[IM]$ moiety. Especially preferred cMBP peptides comprise SEQ-3 together with the linker peptide of SEQ-4, having the 26-mer amino acid sequence (SEQ-7):

Ala-Gly-Ser-Cys^a-Tyr-Cys^c-Ser-Gly-Pro-Pro-Arg-Phe-Glu-Cys^d-Trp-Cys^b-
 Tyr-Glu-Thr-Glu-Gly-Thr-Gly-Gly-Gly-Lys. (SEQ-7)

cMBP peptides of SEQ-1, SEQ-2, SEQ-3 and SEQ-7 preferably have $Z^1 = Z^2 = M^{IG}$, and most preferably have $Z^1 = \text{acetyl}$ and $Z^2 = \text{primary amide}$.

15 The $-(L)_n[IM]$ moiety is suitably attached to either of the Z^1 or Z^2 groups or an amino acid residue of the cMBP peptide which is different to the c-Met binding sequence of SEQ-1. Preferred amino acid residues and sites of conjugation are as described above. When the $-(L)_n[IM]$ moiety is attached to Z^1 or Z^2 , it may take the place of Z^1 or Z^2 by conjugation to the N- or C- terminus, and block *in vivo* metabolism in that way.

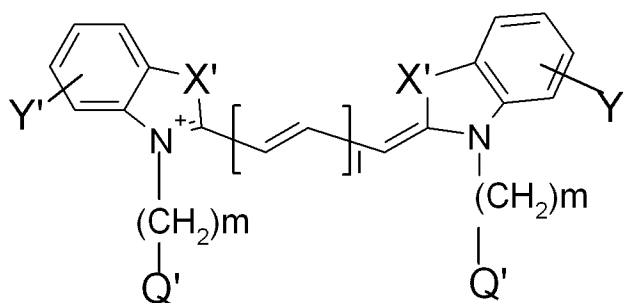
Preferred IM groups have an extensive delocalized electron system, eg. cyanines, merocyanines, indocyanines, phthalocyanines, naphthalocyanines, triphenylmethines, porphyrins, pyrilium dyes, thiapyrilium dyes, squarylium dyes, croconium dyes, azulenium dyes, indoanilines, benzophenoxazinium dyes, benzothiaphenothiazinium dyes, anthraquinones, naphthoquinones, indathrenes, phthaloylacridones, trisphenoquinones, azo dyes, intramolecular and intermolecular charge-transfer dyes and dye complexes, tropones, tetrazines, *bis*(dithiolene) complexes, *bis*(benzene-dithiolate) complexes, iodoaniline dyes, *bis*(S,O-dithiolene) complexes. Fluorescent proteins, such as green fluorescent protein (GFP) and modifications of GFP that have different absorption/emission properties are also useful. Complexes of certain rare earth metals (e.g., europium, samarium, terbium or dysprosium) are used in certain contexts, as are fluorescent nanocrystals (quantum dots). The IM is chosen such that the conjugate of Formula (I) is completely soluble in the infusion medium.

Particulate reporters are therefore less preferred, since they may present solubility problems for the conjugate.

Particular examples of chromophores which may be used include fluorescein, sulforhodamine 101 (Texas Red), rhodamine B, rhodamine 6G, rhodamine 19, indocyanine green, Cy2, Cy3B, Cy3.5, Cy5, Cy5.5, Cy7, Cy7.5, Marina Blue, Pacific Blue, Oregon Green 488, Oregon Green 514, tetramethylrhodamine, and Alexa Fluor 350, Alexa Fluor 430, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, and Alexa Fluor 750. The cyanine dyes are particularly preferred. Licha *et al* have reviewed dyes and dye conjugates for *in vivo* optical imaging [Topics Curr.Chem., 222, 1-29 (2002); Adv.Drug Deliv.Rev., 57, 1087-1108 (2005)].

15

Preferred cyanine dyes which are fluorophores are of Formula II:



(II)

wherein:

20 each X' is independently selected from: -C(CH₃)₂-, -S-, -O- or

-C[(CH₂)_aCH₃][(CH₂)_bM]-, wherein a is an integer of value 0 to 5, b is an integer of value 1 to 5, and M is group G or is selected from SO₃M¹ or H;

each Y' independently represents 1 to 4 groups selected from the group consisting of:

H, -CH₂NH₂, -SO₃M¹, -CH₂COOM¹, -NCS and F, and wherein the Y' groups

are placed in any of the positions of the aromatic ring;

Q' is independently selected from the group consisting of: H, SO₃M¹, NH₂, COOM¹,

ammonium, ester groups, benzyl and a group G;

M¹ is H or B^c;

5 l is an integer from 1 to 3;

and m is an integer from 1 to 5;

wherein at least one of X', Y' and Q' comprises a group G;

G is a reactive or functional group suitable for attaching to the cMBP peptide.

- 10 The G group reacts with a complementary group of the cMBP peptide forming a covalent linkage between the cyanine dye fluorophore and the cMBP peptide. G may be a reactive group that may react with a complementary functional group of the peptide, or alternatively may include a functional group that may react with a reactive group of the cMBP peptide. Examples of reactive and functional groups include:
- 15 active esters; isothiocyanate; maleimide; haloacetamide; acid halide; hydrazide; vinylsulphone; dichlorotriazine; phosphoramidite; hydroxyl; amino; sulphhydryl; carbonyl; carboxylic acid and thiophosphate. Preferably G is an active ester.

- By the term "activated ester" or "active ester" is meant an ester derivative of the associated carboxylic acid which is designed to be a better leaving group, and hence
- 20 permit more facile reaction with nucleophile, such as amines. Examples of suitable active esters are: *N*-hydroxysuccinimide (NHS), sulpho-succinimidyl ester, pentafluorophenol, pentafluorothiophenol, *para*-nitrophenol, hydroxybenzotriazole and PyBOP (ie. benzotriazol-1-yl-oxytripyrrolidinophosphonium
- 25 hexafluorophosphate). Preferred active esters are *N*-hydroxysuccinimide or pentafluorophenol esters, especially *N*-hydroxysuccinimide esters.

In a preferred embodiment of Formula II:

each X' is selected from the group of $-\text{C}(\text{CH}_3)_2-$ and $-\text{C}(\text{CH}_3)[(\text{CH}_2)_4\text{M}]-$,

wherein M is a G group or $-\text{SO}_3\text{M}^1$;

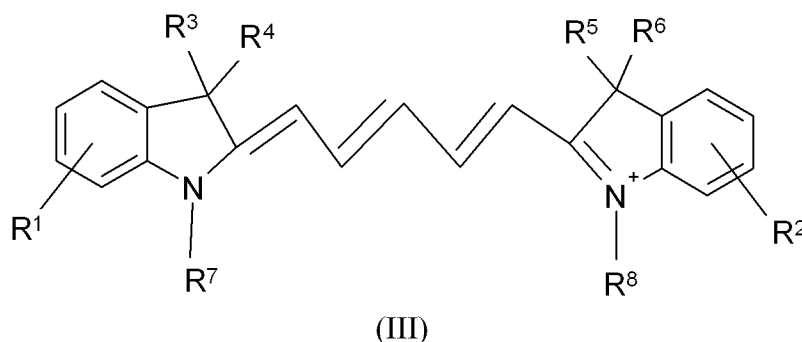
each Y' represents SO_3M^1 , H or 1 to 4 F atoms;

5 each Q' is selected from a G group and SO_3M^1 ;

l is preferably 2 and m is preferably 3, 4 or 5;

wherein when either X' or Q' is a G group, it is most preferably a succinimidyl ester.

Particularly preferred cyanine dyes are of Formula III:



where:

R^1 and R^2 are independently H or SO_3M^1 , and at least one of R^1 and R^2 is SO_3M^1 , where M^1 is H or B^c ;

R^3 and R^4 are independently C_{1-4} alkyl or C_{1-6} carboxyalkyl;

R^5 , R^6 , R^7 and R^8 are independently R^a groups;

wherein R^a is C_{1-4} alkyl, C_{1-6} carboxyalkyl or $-(\text{CH}_2)_k\text{SO}_3\text{M}^1$, where k is an integer of value 3 or 4;

with the proviso that the cyanine dye has a total of 1 to 4 SO_3M^1 substituents in the R^1 , R^2 and R^a groups.

20 Preferred dyes of Formula III are chosen such that at least one C_{1-6} carboxyalkyl group is present, in order to facilitate conjugation to the cMBP.

Preferred individual dyes of Formula III are summarised in Table 1:

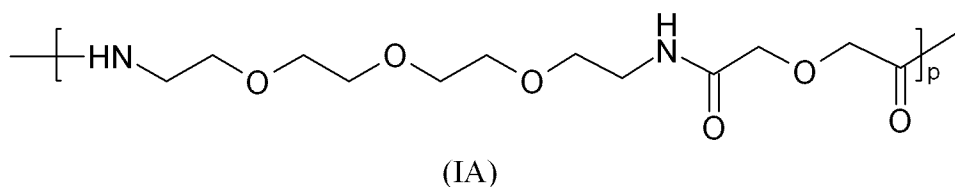
Table 1: chemical structures of individual cyanine dyes.

Table 1	Dye name			
	Cy5(1)	Cy5(2)	Cy5**	Alexa647
R¹	H	SO ₃ H	SO ₃ H	SO ₃ H
R²	SO ₃ H	SO ₃ H	SO ₃ H	SO ₃ H
R³	CH ₃	CH ₃	CH ₃	R ^f
R⁴	CH ₃	CH ₃	CH ₃	CH ₃
R⁵	CH ₃	CH ₃	CH ₃	CH ₃
R⁶	CH ₃	CH ₃	-(CH ₂) ₄ SO ₃ H	CH ₃
R⁷	R ^f	R ^f	R ^f	-(CH ₂) ₃ SO ₃ H
R⁸	CH ₃	Et	-(CH ₂) ₄ SO ₃ H	-(CH ₂) ₃ SO ₃ H

where R^f = -(CH₂)₅COOH.

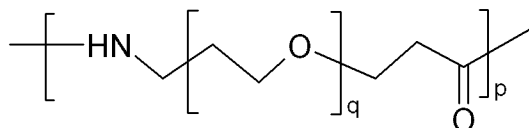
- 5 Especially preferred dyes of Formula II are Cy5** and Alexa647, with Cy5** being the ideal.

When a synthetic linker group (L) is present, it preferably comprises terminal functional groups which facilitate conjugation to [IM] and Z¹-[cMBP]-Z². When L comprises a peptide chain of 1 to 10 amino acid residues, the amino acid residues are preferably chosen from glycine, lysine, arginine, aspartic acid, glutamic acid or serine. When L comprises a PEG moiety, it preferably comprises units derived from oligomerisation of the monodisperse PEG-like structures of Formulae IA or IB:



- 15 17-amino-5-oxo-6-aza-3, 9, 12, 15-tetraoxaheptadecanoic acid of Formula IA

wherein p is an integer from 1 to 10. Alternatively, a PEG-like structure based on a propionic acid derivative of Formula IB can be used:



(IB)

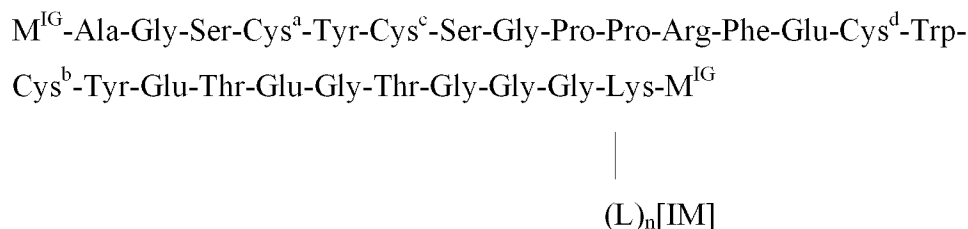
where p is as defined for Formula IA and
q is an integer from 3 to 15.

- 5 In Formula IB, p is preferably 1 or 2, and q is preferably 5 to 12.

When the linker group does not comprise PEG or a peptide chain, preferred L groups have a backbone chain of linked atoms which make up the $-(A)_m-$ moiety of 2 to 10 atoms, most preferably 2 to 5 atoms, with 2 or 3 atoms being especially preferred. A
10 minimum linker group backbone chain of 2 atoms confers the advantage that the imaging moiety is well-separated so that any undesirable interaction is minimised.

In Formula I, n is preferably 0 or 1, most preferably 0, i.e. no linker group is present.

- 15 Preferred imaging agents of the present invention are of Formula IV:



- 20 (IV)

wherein the $(\text{L})_n[\text{IM}]$ group is attached to the epsilon amino group of the Lys residue. Preferred imaging agents of Formula IV have M^{IG} (N-terminal Ala) equal to acetyl and M^{IG} (C-terminal Lys) equal to primary amide. In Formula IV, n is preferably zero
25 and IM is preferably a cyanine dye, most preferably a cyanine dye of Formula II. Especially preferred imaging agents of Formula IV have IM = Cy5** or Alexa647, ideally Cy5**.

The imaging agent of the method of the first aspect is preferably used as a
30 pharmaceutical composition which comprises said imaging agent together with a

biocompatible carrier, in a form suitable for mammalian administration.

The “biocompatible carrier” is a fluid, especially a liquid, in which the imaging agent can be suspended or dissolved, such that the composition is physiologically tolerable, i.e. can be administered to the mammalian body without toxicity or undue discomfort. The biocompatible carrier is suitably an injectable carrier liquid such as sterile, pyrogen-free water for injection; an aqueous solution such as saline (which may advantageously be balanced so that the final product for injection is isotonic); an aqueous solution of one or more tonicity-adjusting substances (e.g. salts of plasma cations with biocompatible counterions), sugars (e.g. glucose or sucrose), sugar alcohols (e.g. sorbitol or mannitol), glycols (e.g. glycerol), or other non-ionic polyol materials (e.g. polyethyleneglycols, propylene glycols and the like). Preferably the biocompatible carrier is pyrogen-free water for injection or isotonic saline.

The imaging agents and biocompatible carrier are each supplied in suitable vials or vessels which comprise a sealed container which permits maintenance of sterile integrity and/or radioactive safety, plus optionally an inert headspace gas (e.g. nitrogen or argon), whilst permitting addition and withdrawal of solutions by syringe or cannula. A preferred such container is a septum-sealed vial, wherein the gas-tight closure is crimped on with an overseal (typically of aluminium). The closure is suitable for single or multiple puncturing with a hypodermic needle (e.g. a crimped-on septum seal closure) whilst maintaining sterile integrity. Such containers have the additional advantage that the closure can withstand vacuum if desired (eg. to change the headspace gas or degas solutions), and withstand pressure changes such as reductions in pressure without permitting ingress of external atmospheric gases, such as oxygen or water vapour. Further practical details of infusion are provided by the textbooks cited above, plus *Nursing Standards of Practice* (2011), Infusion Nurses Society, Untreed Reads Publishing LLC.

The pharmaceutical composition may optionally contain additional excipients such as an antimicrobial preservative, pH-adjusting agent, filler, stabiliser or osmolality adjusting agent. By the term “antimicrobial preservative” is meant an agent which inhibits the growth of potentially harmful micro-organisms such as bacteria, yeasts or moulds. The antimicrobial preservative may also exhibit some bactericidal properties,

- depending on the dosage employed. The main role of the antimicrobial preservative(s) of the present invention is to inhibit the growth of any such micro-organism in the pharmaceutical composition. The antimicrobial preservative may, however, also optionally be used to inhibit the growth of potentially harmful micro-organisms in one or more components of kits used to prepare said composition prior to administration. Suitable antimicrobial preservative(s) include: the parabens, i.e. methyl, ethyl, propyl or butyl paraben or mixtures thereof; benzyl alcohol; phenol; cresol; cetrimide and thiomersal. Preferred antimicrobial preservative(s) are the parabens.
- 10 The term “pH-adjusting agent” means a compound or mixture of compounds useful to ensure that the pH of the composition is within acceptable limits (approximately pH 4.0 to 10.5) for human or mammalian administration. Suitable such pH-adjusting agents include pharmaceutically acceptable buffers, such as tricine, phosphate or TRIS [ie. *tris*(hydroxymethyl)aminomethane], and pharmaceutically acceptable bases such as sodium carbonate, sodium bicarbonate or mixtures thereof.
- 15

By the term “filler” is meant a pharmaceutically acceptable bulking agent which may facilitate material handling during production and lyophilisation. Suitable fillers include inorganic salts such as sodium chloride, and water soluble sugars or sugar alcohols such as sucrose, maltose, mannitol or trehalose.

20

The pharmaceutical compositions of the second aspect may be prepared under aseptic manufacture (ie. clean room) conditions to give the desired sterile, non-pyrogenic product. It is preferred that the key components, especially the associated reagents plus those parts of the apparatus which come into contact with the imaging agent (eg. vials) are sterile. The components and reagents can be sterilised by methods known in the art, including: sterile filtration, terminal sterilisation using e.g. gamma-irradiation, autoclaving, dry heat or chemical treatment (e.g. with ethylene oxide). It is preferred to sterilise some components in advance, so that the minimum number of manipulations needs to be carried out. As a precaution, however, it is preferred to include at least a sterile filtration step as the final step in the preparation of the pharmaceutical composition.

25

30

In the method of the first aspect, the imaging agent is preferably administered at a dosage in the range 0.01 to 2, more preferably 0.02 to 1, most preferably 0.03 to 0.5, mmol per minute. The infusion is preferably into the bloodstream of a peripheral vein of said subject. The total dosage of imaging agent is approximately 40 nmol/kg body weight. Patient weight may range from ca. 50 kg (2 mmol total dose), to 200 kg (8 mmol total dose).

The infusion of the first aspect is preferably carried out using an infusion pump, more preferably a programmable infusion pump. Such infusion pumps are commercially available.

Optical reporter dyes (IM) functionalised suitable for conjugation to peptides are commercially available from GE Healthcare Limited, Atto-Tec, Dyomics, Molecular Probes and others. Most such dyes are available as NHS esters.

The imaging agents used in the first aspect can be prepared by a method which comprises one of steps (i) to (iv):

(i) reaction of a cMBP peptide of formula Z^1 -[cMBP]- Z^2 wherein Z^1 is H and Z^2 is a M^{IG} with a compound of formula Y^1 -(L)_n-[IM], to give the imaging agent of Formula I wherein [IM] is conjugated at the Z^1 position;

(ii) reaction of a cMBP peptide of formula Z^1 -[cMBP]- Z^2 wherein $Z^1 = Z^2 = M^{IG}$ and cMBP comprises an Asp or Glu residue within 4 amino acid residues of either the C- or N- cMBP peptide terminus, and all other Asp/Glu residues of the cMBP peptide are protected, with a compound of formula Y^2 -(L)_n-[IM], to give the imaging agent of Formula I wherein [IM] is conjugated at said Asp or Glu residue of the cMBP peptide;

(iii) reaction of a cMBP peptide of formula Z^1 -[cMBP]- Z^3 wherein Z^1 is M^{IG} and Z^3 is a Z^2 group or an activated ester and all other Asp/Glu residues of the cMBP peptide are protected, with a compound of formula Y^2 -(L)_n-[IM], to give the imaging agent of Formula I wherein [IM] is conjugated at the Z^2 position;

(iv) reaction of a cMBP peptide of formula Z^1 -[cMBP]- Z^2 wherein $Z^1 = Z^2 = M^{IG}$ and cMBP comprises a Lys within 4 amino acid residues of either the C- or N- cMBP peptide terminus, with a compound of formula Y^1 -(L)_n-[IM], to

give the imaging agent of Formula I wherein [IM] is conjugated at a Lys residue of the cMBP peptide;

wherein Z^1 , cMBP, Z^2 , M^{IG} , L, n and IM are as defined above, and

Z^3 is a Z^2 group or an activated ester;

5 Y^1 is a carboxylic acid, activated ester, isothiocyanate or thiocyanate group;

Y^2 is an amine group.

Peptides of formula Z^1 -[cMBP]- Z^2 of the present invention may be obtained by a method of preparation which comprises:

- 10 (i) solid phase peptide synthesis of a linear peptide which has the same peptide sequence as the desired cMBP peptide and in which the Cys^a and Cys^b are unprotected, and the Cys^c and Cys^d residues have thiol-protecting groups;
- (ii) treatment of the peptide from step (i) with aqueous base in solution to give a monocyclic peptide with a first disulfide bond linking Cys^a and Cys^b;
- 15 (iii) removal of the Cys^c and Cys^d thiol-protecting groups and cyclisation to give a second disulfide bond linking Cys^c and Cys^d, which is the desired bicyclic peptide product Z^1 -[cMBP]- Z^2 .

By the term "protecting group" is meant a group which inhibits or suppresses
20 undesirable chemical reactions, but which is designed to be sufficiently reactive that it may be cleaved from the functional group in question under mild enough conditions that do not modify the rest of the molecule. After deprotection the desired product is obtained. Amine protecting groups are well known to those skilled in the art and are suitably chosen from: Boc (where Boc is tert-butyloxycarbonyl), Fmoc (where Fmoc
25 is fluorenylmethoxycarbonyl), trifluoroacetyl, allyloxycarbonyl, Dde [i.e. 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl] or Npys (i.e. 3-nitro-2-pyridine sulphenyl). Suitable thiol protecting groups are Trt (Trityl), Acn (acetamidomethyl), *t*-Bu (tert-butyl), *tert*-Butylthio, methoxybenzyl, methylbenzyl or Npys (3-nitro-2-pyridine sulphenyl). The use of further protecting groups are described in '*Protective Groups in*
30 *Organic Synthesis*', Theodor W. Greene and Peter G. M. Wuts, (John Wiley & Sons, 1991). Preferred amine protecting groups are Boc and Fmoc, most preferably Boc. Preferred amine protecting groups are Trt and Acn.

Examples 1 and 2 provide further specific details. Further details of solid phase

peptide synthesis are described in P. Lloyd-Williams, F. Albericio and E. Girald; *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC Press, 1997. The cMBP peptides are best stored under inert atmosphere and kept in a freezer. When used in solution, it is best to avoid pH above 7 since that may cause scrambling
5 of the disulfide bridges.

Methods of conjugating suitable optical reporters (IM), in particular dyes, to amino acids and peptides are described by Licha (*vide supra*), as well as Flanagan *et al* [Bioconj.Chem., 8, 751-756 (1997)]; Lin *et al*, [*ibid*, 13, 605-610 (2002)] and Zaheer
10 [Mol.Imaging, 1(4), 354-364 (2002)]. Methods of conjugating the linker group (L) to the cMBP peptide use analogous chemistry to that of the dyes alone (see above), and are known in the art.

15 In a second aspect, the present invention provides a method of detection, staging, diagnosis, monitoring of disease progression or monitoring of treatment of cancer of the mammalian body which comprises the optical imaging method of the first aspect.

Preferred aspects of the subject, infusion and imaging agent in the second aspect are
20 as described for the first aspect (above).

The method of the second aspect is preferably used when the cancer is colorectal cancer (CRC), more preferably to help facilitate the management of colorectal cancer. By the term "management of CRC" is meant use in the: detection, staging, diagnosis,
25 monitoring of disease progression or the monitoring of treatment.

In a third aspect, the present invention provides an infusion apparatus, which comprises a supply of the imaging agent or pharmaceutical composition thereof, as
30 described in the first aspect. Preferred aspects of the infusion apparatus, imaging agent and pharmaceutical composition in the third aspect are as described for the first aspect (above). The supply of imaging agent or pharmaceutical composition thereof in the third aspect is suitably provided in a container as described in the first aspect.

In a fourth aspect, the present invention provides an infusion container suitable for use in the method of the first aspect, or the method of detection, staging, diagnosis or monitoring of the second aspect, which comprises a supply of the imaging agent or pharmaceutical composition thereof as described in the first aspect.

5

Preferred aspects of the container, imaging agent and pharmaceutical composition in the fourth aspect are as described for the first aspect (above).

The container of the fourth aspect is preferably suitable for use with the infusion apparatus of the third aspect.

10

In a fifth aspect, the present invention provides the use of the imaging agent or pharmaceutical composition thereof as described in the first aspect in the method of imaging of the first aspect, or the method of detection, staging, diagnosis or monitoring of the second aspect.

15

Preferred aspects of the imaging agent and pharmaceutical composition in the fifth aspect are as described for the first aspect (above).

In a sixth aspect, the present invention provides the use of an infusion apparatus in the method of imaging of the first aspect, or the method of detection, staging, diagnosis or monitoring of the second aspect.

20

Preferred aspects of the imaging agent and pharmaceutical composition in the sixth aspect are as described for the first aspect (above).

25

The invention is illustrated by the non-limiting Examples detailed below. Example 1 provides the synthesis of a cMBP peptide of the invention (Compound 1). Example 2 provides the synthesis of cyanine dye Cy5**, a preferred dye of the invention. Example 3 provides the synthesis of an active ester of Cy5**. Example 4 provides the conjugation of cyanine dyes of the invention to peptides (cMBP peptide and control). Compounds 2 to 6 were prepared in this way. Example 5 provides a method of determination of the affinity of the peptides to c-Met *in vitro*. The results show that the binding is selective, even when an optical reporter imaging moiety (a cyanine dye)

30

is attached. Example 6 provides data on the *in vivo* testing of Compound 3 in an animal model of cancer. It was found that the agent (Compound 3) washes out more rapidly from background tissue compared to the lesion. There was greater uptake in the lesion (due to increased level of c-Met compared to background). Example 7
5 provides a summary of clinical imaging studies with Compound 3 in colorectal cancer patients. Lesion contrast enhancement was consistent with the data obtained in the preclinical animal model. Example 8 provides an analysis of uptake in lesion vs background. See also Figure 1. It is concluded that an infusion administration would allow the preferential uptake in the lesion to be attained without having to wait for
10 background clearance from normal tissue.

Abbreviations.

Conventional single letter or 3-letter amino acid abbreviations are used.

	Acm:	Acetamidomethyl
15	ACN (or MeCN):	Acetonitrile
	Boc:	<i>tert</i> -Butyloxycarbonyl
	DCM:	Dichloromethane
	DMF:	Dimethylformamide
	DMSO:	Dimethylsulfoxide
20	Fmoc:	9-Fluorenylmethoxycarbonyl
	HBTU:	O-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate
	HPLC:	High performance liquid chromatography
	HSPyU	O-(N-succinimidyl)-N,N,N',N'-tetramethyleuronium hexafluorophosphate
	NHS:	<i>N</i> -hydroxy-succinimide
25	NMM:	<i>N</i> -Methylmorpholine
	NMP:	1-Methyl-2-pyrrolidinone
	Pbf:	2,2,4,6,7-Pentamethyldihydrobenzofuran- 5-sulfonyl
	PBS:	Phosphate-buffered saline
	tBu:	<i>t</i> -butyl
30	TFA:	Trifluoroacetic acid
	TIS:	Triisopropylsilane
	Trt:	Trityl.

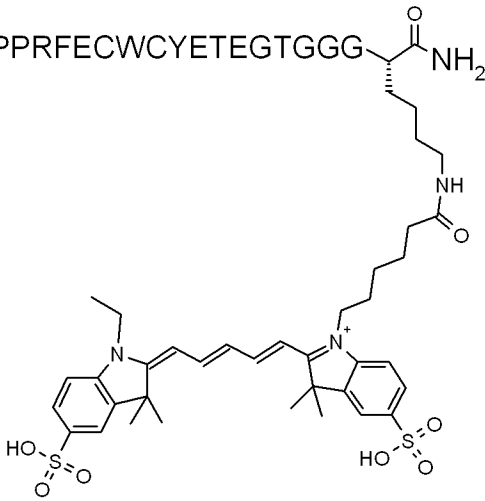
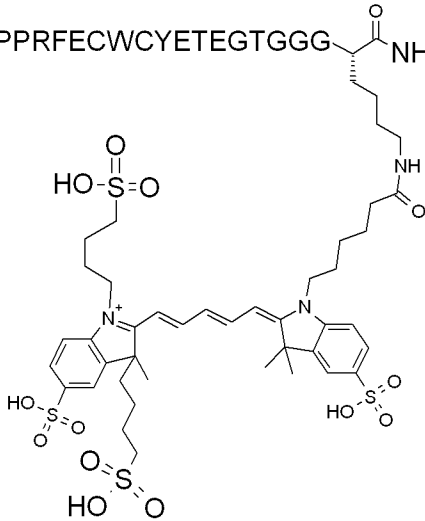
No.	Structure of Compounds.
1	Ac-AGSCYCSGPPRFECWCYETEGTGGGK-NH ₂
2	Ac-AGSCYCSGPPRFECWCYETEGTGGG  Ac-AGSCYCSGPPRFECWCYETEGTGGGK(ε-Cy5)-NH₂
3	Ac-AGSCYCSGPPRFECWCYETEGTGGG  Ac-AGSCYCSGPPRFECWCYETEGTGGGK(ε-Cy5**)-NH₂
4	Ac-AGSCYCSGPPRFECWCYETEGTGGGK(ε-Alexa647)-NH ₂

Table 2: structures of compounds of the invention.

Example 1: Synthesis of Compound 1.Step (a): synthesis of protected precursor linear peptide.

- 5 The precursor linear peptide has the structure:

Ac-Ala-Gly-Ser-Cys-Tyr-Cys(Acm)-Ser-Gly-Pro-Pro-Arg-Phe-Glu-Cys(Acm)-Trp-

Cys-Tyr-Glu-Thr-Glu-Gly-Thr-Gly-Gly-Gly-Lys-NH₂

The peptidyl resin H-Ala-Gly-Ser(tBu)-Cys(Trt)-Tyr(tBu)-Cys(Acm)-Ser(tBu)-Gly-Pro-Pro-Arg(Pbf)-Phe-Glu(OtBu)-Cys(Acm)-Trp(Boc)-Cys(Trt)-Tyr(tBu)-

- 5 Glu(OtBu)-Thr($\psi^{\text{Me,Me}}$ pro)-Glu(OtBu)-Gly-Thr(tBu)-Gly-Gly-Gly-Lys(Boc)-Polymer was assembled on an Applied Biosystems 433A peptide synthesizer using Fmoc chemistry starting with 0.1 mmol Rink Amide Novagel resin. An excess of 1 mmol pre-activated amino acids (using HBTU) was applied in the coupling steps. Glu-Thr pseudoproline (Novabiochem 05-20-1122) was incorporated in the sequence. The resin was transferred to a nitrogen bubbler apparatus and treated with a solution of acetic anhydride (1 mmol) and NMM (1 mmol) dissolved in DCM (5 mL) for 60 min. The anhydride solution was removed by filtration and the resin washed with DCM and dried under a stream of nitrogen.

- 15 The simultaneous removal of the side-chain protecting groups and cleavage of the peptide from the resin was carried out in TFA (10 mL) containing 2.5 % TIS, 2.5 % 4-thiocresol and 2.5 % water for 2 hours and 30 min. The resin was removed by filtration, TFA removed *in vacuo* and diethyl ether added to the residue. The formed precipitate was washed with diethyl ether and air-dried affording 264 mg of crude peptide.

- Purification by preparative HPLC (gradient: 20-30 % B over 40 min where A = H₂O/0.1 % TFA and B = ACN/0.1 % TFA, flow rate: 10 mL/min, column: Phenomenex Luna 5 μ C18 (2) 250 x 21.20 mm, detection: UV 214 nm, product retention time: 30 min) of the crude peptide afforded 100 mg of pure Compound 1 linear precursor. The pure product was analysed by analytical HPLC (gradient: 10-40 % B over 10 min where A = H₂O/0.1 % TFA and B = ACN/0.1 % TFA, flow rate: 0.3 mL/min, column: Phenomenex Luna 3 μ C18 (2) 50 x 2 mm, detection: UV 214 nm, product retention time: 6.54 min). Further product characterisation was carried out using electrospray mass spectrometry (MH₂²⁺ calculated: 1464.6, MH₂²⁺ found: 1465.1).

Step (b): Formation of Monocyclic Cys4-16 disulfide bridge.

Cys4-16; Ac-Ala-Gly-Ser-Cys-Tyr-Cys(Acm)-Ser-Gly-Pro-Pro-Arg-Phe-Glu-

Cys(Acm)-Trp-Cys-Tyr-Glu-Thr-Glu-Gly-Thr-Gly-Gly-Gly-Lys-NH₂.

The linear precursor from step (a) (100 mg) was dissolved in 5 % DMSO/water (200 mL) and the solution adjusted to pH 6 using ammonia. The reaction mixture was stirred for 5 days. The solution was then adjusted to pH 2 using TFA and most of the solvent removed by evaporation *in vacuo*. The residue (40 mL) was injected in portions onto a preparative HPLC column for product purification.

Purification by preparative HPLC (gradient: 0 % B for 10 min, then 0-40 % B over 40 min where A = H₂O/0.1 % TFA and B = ACN/0.1 % TFA, flow rate: 10 mL/min, column: Phenomenex Luna 5 μ C18 (2) 250 x 21.20 mm, detection: UV 214 nm, product retention time: 44 min) of the residue afforded 72 mg of pure Compound 1 monocyclic precursor.

The pure product (as a mixture of isomers P1 to P3) was analysed by analytical HPLC (gradient: 10-40 % B over 10 min where A = H₂O/0.1 % TFA and B = ACN/0.1 % TFA, flow rate: 0.3 mL/min, column: Phenomenex Luna 3 μ C18 (2) 50 x 2 mm, detection: UV 214 nm, product retention time: 5.37 min (P1); 5.61 min (P2); 6.05 min (P3)). Further product characterisation was carried out using electrospray mass spectrometry (MH₂²⁺ calculated: 1463.6, MH₂²⁺ found: 1464.1 (P1); 1464.4 (P2); 1464.3 (P3)).

Step (c): Formation of Second Cys6-14 disulfide bridge (Compound 1).

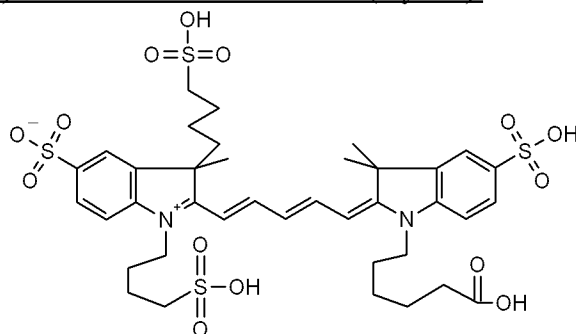
The monocyclic precursor from step (b) (72 mg) was dissolved in 75 % AcOH/water (72 mL) under a blanket of nitrogen. 1 M HCl (7.2 mL) and 0.05 M I₂ in AcOH (4.8 mL) were added in that order and the mixture stirred for 45 min. 1 M ascorbic acid (1 mL) was added giving a colourless mixture. Most of the solvents were evaporated *in vacuo* and the residue (18 mL) diluted with water/0.1 % TFA (4 mL) and the product purified using preparative HPLC.

Purification by preparative HPLC (gradient: 0 % B for 10 min, then 20-30 % B over 40 min where A = H₂O/0.1 % TFA and B = ACN/0.1 % TFA, flow rate: 10 mL/min, column: Phenomenex Luna 5 μ C18 (2) 250 x 21.20 mm, detection: UV 214 nm, product retention time: 43-53 min) of the residue afforded 52 mg of pure Compound 1. The pure product was analysed by analytical HPLC (gradient: 10-40 % B over 10 min

where A = H₂O/0.1 % TFA and B = ACN/0.1 % TFA, flow rate: 0.3 mL/min, column: Phenomenex Luna 3 μ C18 (2) 50 x 2 mm, detection: UV 214 nm, product retention time: 6.54 min). Further product characterisation was carried out using electrospray mass spectrometry (MH₂²⁺ calculated: 1391.5, MH₂²⁺ found: 1392.5).

5

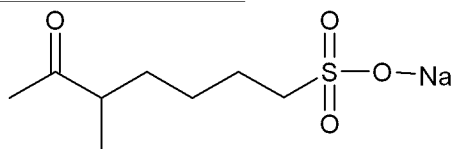
Example 2: Synthesis of the Cyanine Dye 2-[(1E,3E,5E)-5-[1-(5-carboxypentyl)-3,3-dimethyl-5-sulfo-1,3-dihydro-2H-indol-2-ylidene]penta-1,3-dienyl]-3-methyl-1,3-bis(4-sulfobutyl)-3H-indolium-5-sulfonate (Cy5).**



10

Cy5**

(3a) 5-Methyl-6-oxoheptane-1-sulfonic acid.

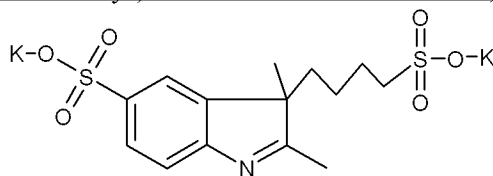


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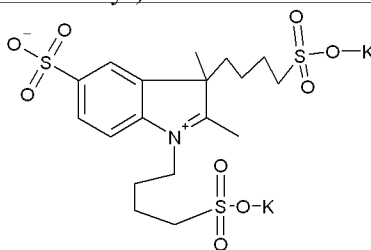
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25

Ethyl 2-methylacetoacetate (50g) in DMF (25ml) was added to a suspension of sodium hydride (12.0g of 60% NaH in mineral oil) in DMF (100ml), dropwise with ice-bath cooling over 1 hour, (internal temperature 0-4°C). This mixture was allowed to warm to ambient temperature for 45mins with stirring before re-cooling. A solution of 1,4-butanediol (45g) in DMF (25ml) was then added dropwise over 15 minutes. The final mixture was heated at 60°C for 18hours. The solvent was removed by rotary evaporation and the residue partitioned between water and diethyl ether. The aqueous layer was collected, washed with fresh diethyl ether and rotary evaporated to yield a sticky foam. This intermediate was dissolved in water (100ml) and sodium hydroxide (17.8g) added over 15 minutes with stirring. The mixture was heated at 90°C for 18 hours. The cooled reaction mixture was adjusted to ~pH2 by the addition of concentrated hydrochloric acid (~40ml). The solution was rotary evaporated and dried under vacuum. The yellow solid was washed with ethanol containing 2% hydrochloric acid (3x150ml). The ethanolic solution was filtered, rotary evaporated and dried under vacuum to yield a yellow solid. Yield 70g.

(3b) 2,3-Dimethyl-3-(4-sulfobutyl)-3H-indole-5-sulfonic acid, dipotassium salt.

4-Hydrazinobenzenesulfonic acid (40g), 5-methyl-6-oxoheptane-1-sulfonic acid (from 3a; 60g) and acetic acid (500ml) were mixed and heated under reflux for 6hrs. The solvent was filtered, rotary evaporated and dried under vacuum. The solid was dissolved in methanol (1L). To this was added 2M methanolic potassium hydroxide (300ml). The mixture was stirred for 3 hours and then the volume of solvent reduced by 50% using rotary evaporation. The resulting precipitate was filtered, washed with methanol and dried under vacuum. Yield 60g. MS (LCMS) : MH^+ 362. Acc. Mass: Found, 362.0729. $MH^+ = C_{14}H_{20}NO_6S_2$ requires m/z 362.0732 (-0.8ppm).

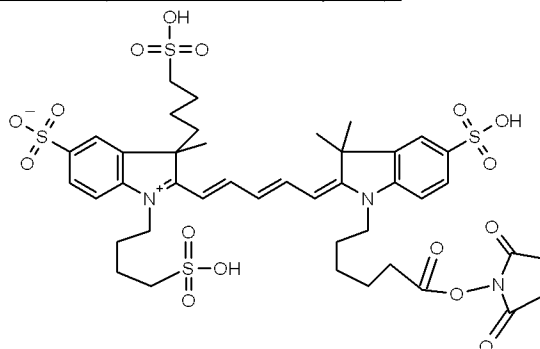
(3c) 2,3-Dimethyl-1,3-bis(4-sulfobutyl)-3H-indolium-5-sulfonate, dipotassium salt.

2,3-Dimethyl-3-(4-sulfobutyl)-3H-indole-5-sulfonic acid (from 3b; 60g) was heated with 1,4 butane sultone (180g) and tetramethylene sulfone (146ml) at 140°C for 16 hours. The resulting red solid was washed with diethyl ether, ground into a powder and dried under vacuum. Yield 60g.

(3d) Cy5**, as TFA salt.

1-(5'-Carboxypentyl)-2,3,3-trimethyl-indolenium bromide-5-sulfonic acid, K^+ salt (2.7g), malonaldehyde bis(phenylimine) monohydrochloride (960mg), acetic anhydride (36ml) and acetic acid (18ml) were heated at 120°C for 1 hour to give a dark brown-red solution. The reaction mixture was cooled to ambient temperature. 2,3-Dimethyl-1,3-bis(4-sulfobutyl)-3H-indolium-5-sulfonate (from 3c; 8.1g) and potassium acetate (4.5g) were added to the mixture, which was stirred for 18 hours at ambient temperature. The resulting blue solution was precipitated using ethyl acetate and dried under vacuum. The crude dye was purified by liquid chromatography (RPC₁₈. Water + 0.1% TFA/ MeCN + 0.1%TFA gradient). Fractions containing the principal dye peak were collected, pooled and evaporated under vacuum to give the title dye, 2g. UV/Vis (Water+0.1%TFA): 650nm. MS (MALDI-TOF): MH^+ 887.1. $MH^+ = C_{38}H_{50}N_2O_{14}S_4$ requires m/z 887.1.

Example 3: Synthesis of 2-[(1*E*,3*E*,5*E*)-5-(1-{6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl})-3,3-dimethyl-5-sulfo-1,3-dihydro-2*H*-indol-2-ylidene)penta-1,3-dienyl]-3-methyl-1,3-bis(4-sulfobutyl)-3*H*-indolium-5-sulfonate, diisopropylethylamine salt (NHS Ester of Cy5).**



5

Cy5** (Example 2; 10mg) was dissolved in anhydrous DMSO (3ml); to this were added HSPyU (20mg) and N,N'-diisopropylethylamine (80μl). The resulting solution was mixed for 3 hours, whereupon TLC (RPC18. Water/MeCN) revealed complete reaction. The dye was isolated by precipitation in ethyl acetate/diethyl ether, filtered, washed with ethyl acetate and dried under vacuum. UV/Vis (Water) 650nm. MS (MALDI-TOF) MH⁺ 983.5. MH⁺ = C₄₂H₅₃N₃O₁₆S₄ requires m/z 984.16.

10

Example 4: Conjugation of Dyes, Synthesis of Compounds 3 to 7.

Cys4-16, 6-14; Ac-Ala-Gly-Ser-Cys-Tyr-Cys-Ser-Gly-Pro-Pro-Arg-Phe-Glu-Cys-Trp-Cys-Tyr-Glu-Thr-Glu-Gly-Thr-Gly-Gly-Gly-Lys(Cy5)-NH₂ (Compound 3).

Compound 1 (10 mg), NMM (4 μL) and Cy5 NHS ester (5.7 mg; GE Healthcare PA15104) were dissolved in NMP (1 mL) and the reaction mixture stirred for 7 hrs. The reaction mixture was then diluted with 5 % ACN/water (8 mL) and the product purified using preparative HPLC. Purification by preparative HPLC (gradient: 5-50 % B over 40 min where A = H₂O/0.1 % HCOOH and B = ACN/0.1 % HCOOH, flow rate: 10 mL/min, column: Phenomenex Luna 5μ C18 (2) 250 x 21.20 mm, detection: UV 214 nm, product retention time: 35.5 min) of the crude peptide afforded 8.1 mg of pure Compound 3. The pure product was analysed by analytical HPLC (gradient: 5-50 % B over 10 min where A = H₂O/0.1 % HCOOH and B = ACN/0.1 % HCOOH, flow rate: 0.3 mL/min, column: Phenomenex Luna 3μ C18 (2) 50 x 2 mm, detection: UV 214 nm, product retention time: 8.15 min). Further product characterisation was carried out using electrospray mass spectrometry (MH₂²⁺ calculated: 1710.6, MH₂²⁺ found: 1711.0).

30

Other dye-peptide conjugates (Compounds 3 and 4) were prepared by analogous methods. Alexa647 was purchased from Molecular Probes (A20106):

Compound 3 (MH_2^{2+} calculated: 1825.7, MH_2^{2+} found: 1825.9),

Compound 4 (MH_2^{2+} calculated: 1811.7, MH_2^{2+} found: 1812.0).

5

Example 5: In Vitro Fluorescence polarisation assay.

The principle of the fluorescence polarisation method can briefly be described as follows:

- 10 Monochromatic light passes through a horizontal polarizing filter and excites fluorescent molecules in the sample. Only those molecules that oriented properly in the vertically polarized plane adsorb light, become excited, and subsequently emit light. The emitted light is measured in both horizontal and vertical planes. The anisotropy value (A), is the ratio between the light intensities following the equation

15

$$A = \frac{\text{Intensity with horizontal polarizer} - \text{Intensity with vertical polarizer}}{\text{Intensity with horizontal polarizer} + 2 * \text{Intensity with vertical polarizer}}$$

20

The fluorescence anisotropy measurements were performed in 384-well microplates in a volume of 10 μL in binding buffer (PBS, 0.01% Tween-20, pH 7.5) using a Tecan Safire fluorescence polarisation plate reader (Tecan, US) at ex646/em678 nm. The concentration of dye-labelled peptide was held constant (20 nM) and the concentrations of the human or mouse c-Met/ Fc chimera (R&D Systems) or Semaphorin 6A (R&D Systems) were varied from 0-150 nM. Binding mixtures were

25 equilibrated in the microplate for 10 min at 30°C. The observed change in anisotropy was fit to the equation

$$r_{\text{obs}} = r_{\text{free}} + (r_{\text{bound}} - r_{\text{free}}) \frac{(K_D + c_{\text{Met}} + P) - \sqrt{(K_D + c_{\text{Met}} + P)^2 - 4 \cdot c_{\text{Met}} \cdot P}}{2 \cdot P}$$

30

where r_{obs} is the observed anisotropy, r_{free} is the anisotropy of the free peptide, r_{bound} is the anisotropy of the bound peptide, K_D is the dissociation constant, c-Met is the total c-Met concentration, and P is the total dye-labelled peptide concentration. The equation assumes that the synthetic peptide and the receptor form a reversible complex in solution with 1:1 stoichiometry. Data fitting was done *via* nonlinear regression using GraphPad Prism software to obtain the K_D value (one-site binding). Compound 2 was tested for binding towards human and mouse c-Met (Fc chimera).

The results showed a K_D of 3 +/- 1 nM for binding to human c-Met, and no binding to mouse c-Met in the tested range. Using the same method, Compound 3 was found to have a K_D for human c-Met of 1.1 nM.

5

Example 6: *In Vivo* testing of Compound 3.

(a) Animal Model.

54 Female BALB c/A nude (Bom) mice were used in the study. The use of the animals was approved by the local ethics committee. BALB c/A nude is an inbred
10 immunocompromised mouse strain with a high take rate for human tumours as compared to other nude mice strains. The mice were 4 weeks old upon arrival and with a body weight of approx. 20 grams at the start of the study. The animals were housed in individually ventilated cages (IVC, Scanbur BK) with HEPA filtered air. The animals had ad libitum access to "Rat and Mouse nr. 3 Breeding" diet (Scanbur
15 BK) and tap water acidified by addition of HCl to a molar concentration of 1 mM (pH 3.0).

The colon cancer cell HCT-15 is derived from human colon carcinomas and is reported to express c-Met according to Zeng *et al* [Clin. Exp. Metastasis, 21, 409-417. (2004)]. The cell line was proven to be tumorigenic when inoculated subcutaneously
20 into nude mice [Flatmark *et al*, Eur. J. Cancer 40,1593-1598 (2004)].

HCT-15 cells were grown and prepared for subcutaneous inoculation in RPMI (Sigma Cat # R0883) with 10% serum and penicillin/streptomycin. Stocks were made at passage number four (P4) and frozen down for storage in liquid nitrogen at 3×10^7
25 cells/vial in the culture media containing 5% DMSO. On the day of the transplantation, the cells were thawed quickly in a 37°C water bath (approx. 2 min), washed and resuspended in PBS/2% serum (centrifugation at 1200 rpm for 10 min). Thorough mixing of cells in the vials was ensured every time cells were aspirated into the dosing syringe. Volumes of 0.1 ml of cell suspension were injected s.c. at the
30 shoulder and at the back using a fine bore needle (25 G) while the animals were under light gas anaesthesia. The animals were then returned to their cages and the tumours were allowed to grow for 13-17 days. The animals were allowed an acclimatisation period of at least 5 days before the inoculation procedure.

(b) procedure.

All test substances were reconstituted with PBS from freeze-dried powder. A small stack of white printer paper was imaged to obtain a flat field image which was used to correct for illumination inhomogeneities. The test substances were injected intravenously in the lateral tail vein during physical fixation. The injection volume was 0.1ml, which corresponds to a dose of 1nmol test substance per animal. After injection the animals were returned to their cages. The animals were sacrificed immediately before imaging by cervical dislocation. The optimal imaging time point for each test substance was estimated based on comparison of wash out rates in the skin and in muscle tissue in a limited number of animals (n=1-6). The imaging time point for Compounds 3 and 4 was 120 minutes post injection. For each animal the subcutaneously grown tumours were excised post mortem. A thin slice, approximately 1.6mm thick and 3-4mm in diameter, was cut off the edge of one of the tumours. The tumour slice was then imaged against an area of normal colon from the same animal.

(c) Imaging.

Imaging was performed through a clinical laparoscope adapted to use a light source to excite the reporter and a filtering system to extract the fluorescence component. A 635nm laser was used for excitation of the reporter molecule. A Hamamatsu ORCA ERG CCD camera was used as the detector. The camera was operated in 2x2 binning mode with 0 gain. Standard exposure time for colon imaging was 10s. System calibration measurements indicate that the 10s exposure time with the animal imaging system corresponds to 40ms exposure with a clinically relevant light source, field of view, and distance to the tissue surface. The intensity distribution in the image was corrected for illumination inhomogeneities through system calibration data. A target to background ratio was computed from regions of interest placed over the tumour, and normal colon background. The images were visually scored using the standard scoring system employed for receiver operating characteristic analysis.

(d) Results.

Compound 3 had a tumour to background ratio of 1.46:1. Receiver operating characteristic analysis gave a sensitivity of 87% a specificity of 100% and an area under the curve of 0.99.

Example 7: *In Vivo* Imaging with Compound 3 in Human Patients.

Imaging studies with Compound 3 were carried out in colorectal cancer patients. The 0.13mg/kg body weight dose of Compound 3 was given as a bolus intravenous injection. Colonoscopies were started 3 hours post administration. A total of 101 lesions were observed during colonoscopy imaging of 15 patients with suspected colorectal cancer. The proportion of lesions seen in white light and with mild or clear positive fluorescence contrast was 72/101. The proportion of lesions that were isofluorescent (same fluorescence as background) was 12/101. The proportion of lesions with positive fluorescence contrast which were not detected in white light was 17/101. No lesions were scored as possessing negative fluorescence contrast (reduced fluorescence compared to background).

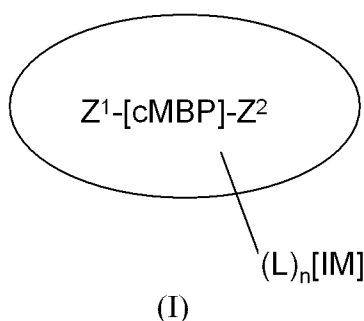
Example 8: Time/Uptake Profile.

Based on Example 6, it was found that the concentration of Compound 3 in the lesion compared to the background was closer to 2:1 (and gave rise to an observed lesion:background image contrast of 1.5:1). The following assumptions were made: (i) that the time to peak signal (wash-in phase) is approximately 30 minutes; (ii) the wash-out phase begins at ~30 minutes; (iii) there is slightly higher uptake in the c-Met rich lesion compared to the background; (iv) washout from the lesion is slower than the background. A rough estimate for the imaging time (time post injection) that a concentration difference of 2:1 is obtained is based on waiting one half-time after the start of the washout stage. This is shown schematically in Figure 1. This also corresponds to the optimal imaging time point in pre-clinical murine model (2 hours = 30 minutes to peak + 90 minute washout half-time) from Example 6.

The estimation of the imaging time to start patient imaging was determined using the above assumptions. Two different measurement methods were used in the dose escalation part of Example 7 to estimate this time. 1: A 'contact' measurement, where the endoscope was placed gently on the colon wall to gain a signal related to the concentration of Compound 3 in the tissue directly under the endoscope tip. 2: Use of a calibrated fluorescence reference standard that is passed through the working channel of the endoscope and placed on the colon wall to provide a calibrated fluorescence reference standard to compare the background fluorescence levels.

CLAIMS.

1. A method of optical imaging which comprises imaging a mammalian subject
 5 to obtain images of sites of c-Met over-expression or localisation *in vivo*,
 wherein said subject had been previously administered *via* intravenous infusion with
 an imaging agent which comprises a conjugate of Formula I:



10 where:

Z^1 is attached to the N-terminus of cMBP, and is H or M^{IG} ;

Z^2 is attached to the C-terminus of cMBP and is OH, OB^c , or M^{IG} ,

where B^c is a biocompatible cation;

cMBP is a c-Met binding cyclic peptide of 17 to 30 amino acids which
 15 comprises the amino acid sequence (SEQ-1):

$Cys^a-X^1-Cys^c-X^2-Gly-Pro-Pro-X^3-Phe-Glu-Cys^d-Trp-Cys^b-Tyr-X^4-X^5-X^6$;

wherein X^1 is Asn, His or Tyr;

X^2 is Gly, Ser, Thr or Asn;

X^3 is Thr or Arg;

20 X^4 is Ala, Asp, Glu, Gly or Ser;

X^5 is Ser or Thr;

X^6 is Asp or Glu;

and Cys^{a-d} are each cysteine residues such that residues a and b as well
 as c and d are cyclised to form two separate disulfide bonds;

25 M^{IG} is a metabolism inhibiting group which is a biocompatible group which
 inhibits or suppresses *in vivo* metabolism of the peptide;

L is a synthetic linker group of formula $-(A)_m-$ wherein each A is
 independently $-CR_2-$, $-CR=CR-$, $-C\equiv C-$, $-CR_2CO_2-$, $-CO_2CR_2-$, $-NRCO-$,

-CONR- , -NR(C=O)NR-, -NR(C=S)NR-, -SO₂NR- , -NRSO₂- , -CR₂OCR₂- ,
 -CR₂SCR₂- , -CR₂NRCR₂- , a C₄₋₈ cycloheteroalkylene group, a C₄₋₈
 cycloalkylene group, a C₅₋₁₂ arylene group, or a C₃₋₁₂ heteroarylene group, an
 amino acid, a sugar or a monodisperse polyethyleneglycol (PEG) building
 5 block;
 each R is independently chosen from H, C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl,
 C₁₋₄ alkoxyalkyl or C₁₋₄ hydroxyalkyl;
 m is an integer of value 1 to 20;
 n is an integer of value 0 or 1;
 10 IM is an optical reporter imaging moiety suitable for imaging the mammalian
 body *in vivo* using light of wavelength 600-1200 nm.

2. The method of Claim 1, where in addition to SEQ-1, the cMBP further
 comprises an Asp or Glu residue within 4 amino acid residues of either the C- or N-
 15 cMBP peptide terminus, and -(L)_nIM is functionalised with an amine group which is
 conjugated to the carboxyl side chain of said Asp or Glu residue to give an amide
 bond.

3. The method of either of Claim 1 or Claim 2, where in addition to SEQ-1, the
 20 cMBP comprises a Lys residue within 4 amino acid residues of either the C- or N-
 cMBP peptide terminus, and -(L)_nIM is functionalised with a carboxyl group which is
 conjugated to the epsilon amine side chain of said Lys residue to give an amide bond.

4. The method of any one of Claims 1 to 3, wherein cMBP comprises the
 25 amino acid sequence of either SEQ-2 or SEQ-3:

Ser-Cys^a-X¹-Cys^c-X²-Gly-Pro-Pro-X³-Phe-Glu-Cys^d-Trp-Cys^b-Tyr-X⁴-X⁵-X⁶.
 (SEQ-2);

Ala-Gly-Ser-Cys^a-X¹-Cys^c-X²-Gly-Pro-Pro-X³-Phe-Glu-Cys^d-Trp-Cys^b-Tyr-
 X⁴-X⁵-X⁶-Gly-Thr (SEQ-3).

30

5. The method of any one of Claims 1 to 4, wherein X³ is Arg.

6. The method of any one of Claims 1 to 5, wherein in addition to SEQ-1, SEQ-2
 or SEQ-3, cMBP further comprises at either the N- or C- terminus a linker peptide

which is chosen from -Gly-Gly-Gly-Lys- (SEQ-4), -Gly-Ser-Gly-Lys- (SEQ-5) or -Gly-Ser-Gly-Ser-Lys- (SEQ-6).

7. The method of Claim 6, where cMBP has the amino acid sequence (SEQ-7):

5 Ala-Gly-Ser-Cys^a-Tyr-Cys^c-Ser-Gly-Pro-Pro-Arg-Phe-Glu-Cys^d-Trp-Cys^b-Tyr-Glu-Thr-Glu-Gly-Thr-Gly-Gly-Gly-Lys.

8. The method of any one of Claims 1 to 7, where both Z¹ and Z² are independently M^{IG}.

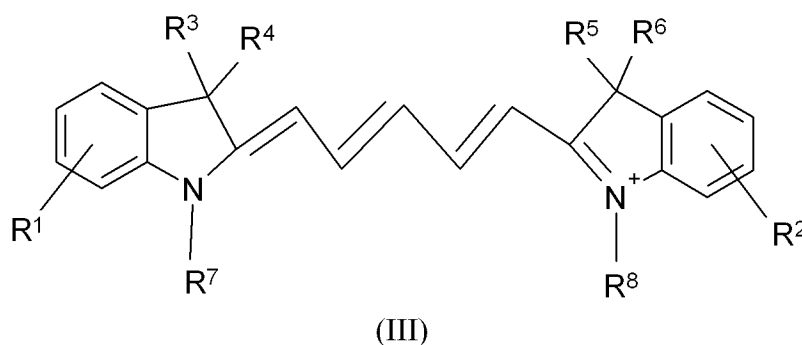
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9. The method of any one of Claims 1 to 8, where IM is a dye having an absorbance maximum in the range 600-1000 nm.

10. The method of Claim 9, where IM is a cyanine dye.

15

11. The method of Claim 10, where the cyanine dye is of Formula III:



where:

R¹ and R² are independently H or SO₃M¹, and at least one of R¹ and R² is SO₃M¹, where M¹ is H or B^c;

20

R³ and R⁴ are independently C₁₋₄ alkyl or C₁₋₆ carboxyalkyl;

R⁵, R⁶, R⁷ and R⁸ are independently R^a groups;

wherein R^a is C₁₋₄ alkyl, C₁₋₆ carboxyalkyl or -(CH₂)_kSO₃M¹, where k is an integer of value 3 or 4;

25

with the proviso that the cyanine dye has a total of 1 to 4 SO₃M¹ substituents in the R¹, R² and R^a groups.

12. The method of any one of Claims 1 to 11, where the imaging agent is administered as a pharmaceutical composition, which comprises said imaging agent together with a biocompatible carrier, in a form suitable for mammalian administration.

5

13. The method of any one of claims 1 to 12, where the imaging agent is administered at a dosage in the range 0.01 to 2 mmol per minute.

14. The method of any one of claims 1 to 13, where the infusion is into the
10 bloodstream of a peripheral vein of said subject.

15. The method of any one of claims 1 to 14, where the infusion is carried out using an infusion pump.

15 16. The method of any one of claims 1 to 15, where the optical imaging method comprises fluorescence endoscopy.

17. A method of detection, staging, diagnosis, monitoring of disease progression or monitoring of treatment of cancer of the mammalian body which comprises the
20 optical imaging method of any one of claims 1 to 16.

18. An infusion apparatus, which comprises a supply of the imaging agent as defined in any one of claims 1 to 11, or the pharmaceutical composition as defined in claim 12.

25

19. An infusion container suitable for use in the method of any one of claims 1 to 16, or the method of detection, staging, diagnosis or monitoring of claim 17, which comprises a supply of the imaging agent as defined in any one of claims 1 to 11, or the pharmaceutical composition as defined in claim 12.

30

20. The container of claim 19, which is suitable for use with the infusion apparatus of claim 18.

21. Use of the imaging agent as defined in any one of claims 1 to 11, or the pharmaceutical composition as defined in claim 12 in the method of imaging of any one of claims 1 to 16, or the method of detection, staging, diagnosis or monitoring of
5 claim 17.

22. Use of an infusion apparatus in the method of imaging of any one of claims 1 to 16, or the method of detection, staging, diagnosis or monitoring of claim 17.

Figure 1: Schematic of the Logarithm of the concentration $[c]$ of Compound 3 in tissue versus time in the lesion and background. $t_{1/2}$ is the half-life of Compound 3 in background tissue. $t_{start\ w/o}$ is the time of the start of the exponential washout phase. $t_{start\ w/o} + t_{1/2}$ is the time of the start of the exponential washout plus the half-life of Compound 3 in background tissue.

