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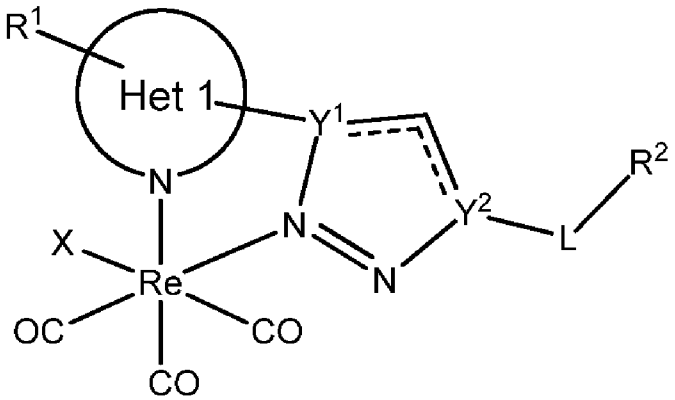

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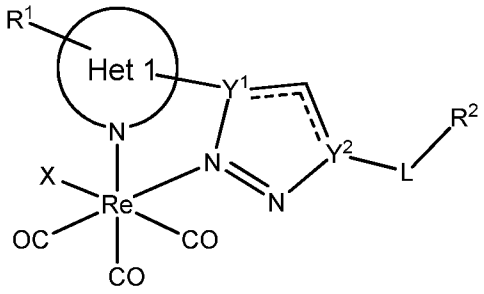
(57) Abstract: The present invention relates to a X-ray fluorescence molecular probe of formula I or a salt thereof, wherein X, Het 1, R^1, Y^1, Y^2, L and R^2 are as defined in the claims. The invention also relates to the use of the probes of the invention for X-ray fluorescence imaging, including in the context of multimodal imaging. The probes of the invention are useful in biological applications of X-ray fluorescence imaging, especially for imaging intracellular organelles and to label biomolecules. The probes of the invention may be functionalized or functionalizable.

FUNCTIONALIZABLE MOLECULAR PROBE FOR X-RAY FLUORESCENCE IMAGING AND MULTIMODAL IMAGING

FIELD OF INVENTION

The present invention relates to a X-ray fluorescence molecular probe of formula I



(I)

or a salt thereof, wherein **X**, **Het 1**, $\mathbf{R^1}$, $\mathbf{Y^1}$, $\mathbf{Y^2}$, **L** and $\mathbf{R^2}$ are as defined below. The invention relates to the use of the probes of the invention for X-ray fluorescence spectroscopy and/or X-ray fluorescence imaging, including in the context of multimodal imaging. The probes of the invention are useful in biological applications of X-ray fluorescence imaging, especially for imaging intracellular organelles and to label biomolecules. The probes of the invention may be functionalized or functionalizable.

BACKGROUND OF INVENTION

Characterization and observation of complex media, at macroscopic or microscopic scales, requires the use of adapted imaging techniques. It is often necessary to label the studied object with a probe to be able identifying it within complex media. This is especially the case for the study of biological media. Besides, molecular probes tuned to recognize a specific target may be introduced in the biological media to image the target.

Various imaging methods may be used, among which nuclear imaging methods, fluorescence spectroscopy or infrared spectroscopy are commonly used. All these methods are more or less attractive in terms of sensitivity, selectivity, resolution, cost, safety or facilities.

Another interesting method of imaging is X-ray fluorescence (XRF). XRF is commonly used in the field of material analysis and it is of growing interest in biology.

Excitation of an atom with photons in the hard X-ray region (>1 keV) leads to the ejection of a core-shell electron. The resulting vacancy is filled through a higher-shell electron, a process that results in emission of a photon whose energy is equal to the difference in binding energies of the two shells involved in the transition. Because the binding energy is proportional to the squared nuclear charge, the emitted photon energy is characteristic for each element. The XRF technique enables to map heavy elements (Z>14). The XRF signal is specific of a given element and its intensity is proportional to its amount. All the elements that can be excited at the working energy may be detected in a single experiment.

XRF spectroscopy thus presents the advantage to enable quantification and to provide a high detection sensitivity, a high specificity, a good spatial resolution and is thus adapted for imaging biological samples.

Especially, with the development of synchrotrons, quantitative mapping at sub-cellular scale is now rendered possible, with submicron spot sizes. A few number of X-ray microscopes with submicron resolution are today available in the world, wherein the detection limit for trace elements has been estimated to range between 5.0×10^{-20} and 3.9×10^{-19} mol.mm^{-2}, corresponding to just a few thousand atoms within the irradiated section of the sample.

Scanning of the sample and acquisition of the entire X-ray spectrum yields quantitative topographical maps for a wide range of elements, including most biologically relevant metals. It is especially useful to map the intracellular distribution of endogenous metallic cations in biological environments and cells.

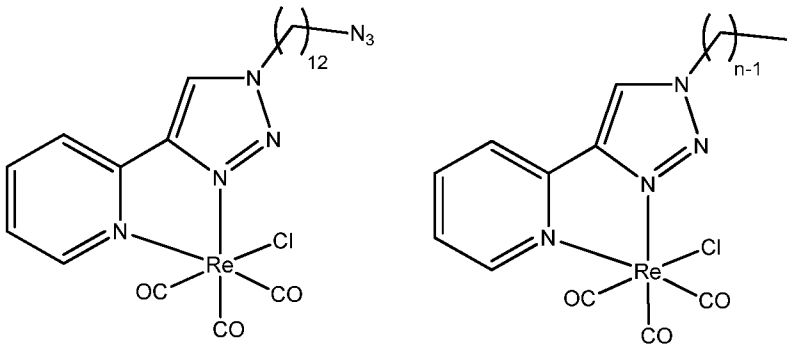
Among currently available imaging modalities, XRF is the only technique that is compatible with fully hydrated biological samples such as whole cells or tissue sections, while simultaneously offering trace element sensitivity and submicron spatial resolution.

The development of XRF in the biological field renders desirable to be able to detect specific objects or cell organelles by XRF spectroscopy. There is thus a need for probes able to label biomolecules and for probes targeting biological targets, which are detectable by XRF spectroscopy.

However, to the knowledge of the Applicant, there is nowadays no probe for such purposes, i.e. to tag specific biomolecules or to dye specific organelles, which are detectable by XRF to provide XRF images.

The Applicant previously developed metal-carbonyl complexes for infrared and fluorescence bimodal imaging, some of which targeting specific organelles such as the Golgi apparatus (Clède S. and Policar C., Chem. Eur. J., 2015, 21, 942-958; Clède et al., Chem. Commun., 2015, 51, 2687-2689; Fernandez et al., Colloids and Surfaces B : Biointerfaces, 2015, 131, 102-107 ; Clède et al., Chem. Eur. J., 2014, 20, 8714-8722; Bertrand H. et al., Inorg. Chem., 2014, 53, 6204-6223; Mattson et al., Analyst, 2013, 138, 5610-5618; Clède et al., Analyst, 2013, 138, 5627-5638; Clède S. et al., Chem. Commun., 2012, 48, 7729-7731).

Examples of metal-carbonyl complexes developed by the Applicant for infrared and fluorescence bimodal imaging are represented below (Clède et al., Chem. Eur. J., 2014, 20, 8714-8722; Clède S. et al., Chem. Commun., 2012, 48, 7729-7731):



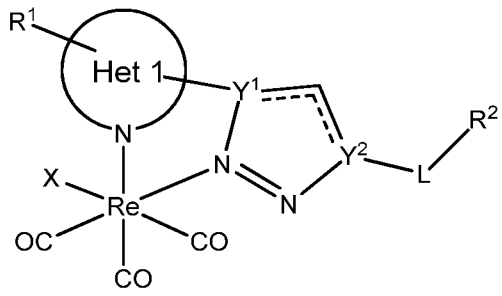
n = 4, 8, 12

The presence of the CO moieties provides above metal-carbonyl complexes with vibrational properties that enable infrared spectroscopy; while the coordination with an ancillary bi-heteroaryl ligand bearing low π^* orbitals enables fluorescence detection.

Infrared and fluorescence spectroscopies rely on physical properties different from those implied in XRF.

The Applicant evidenced that this kind of metal-carbonyl complexes display an unexpected high response in XRF of rhenium.

Therefore, the present invention relates to X-ray fluorescence probes of formula I



(I)

or a salt thereof, wherein **X**, **Het 1**, $\mathbf{R^1}$, $\mathbf{Y^1}$, $\mathbf{Y^2}$, **L** and $\mathbf{R^2}$ are as defined below.

The invention also relates to the use of the XRF probes of formula I for XRF spectroscopy and/or XRF imaging.

The XRF probes of the invention are centered on rhenium. Rhenium is present *in vivo* as an ultra-trace element (Rodushkin et al., Anal. Bioanal. Chem., 2004, 247). Therefore, the XRF probes of the invention can be detected with very few background noise and thus with a very high contrast.

The XRF probes of the invention can be easily functionalized at R^2 position and thus enable to label small molecules of biological interest, biomolecules, polymers or materials. The XRF probes of the invention thus present the advantage of being functionalizable or functionalized.

The XRF probes of the invention were evidenced to maintain their integrity in cells.

The invention further relates to the use of the XRF probes of formula I for multimodal imaging, including XRF and infrared and/or fluorescence spectroscopies. These 3 modalities are implemented using the same molecular probe. Multimodal probes allow taking advantage of the benefits of each modality and offsetting their drawbacks.

XRF spectroscopy exhibits a high detection sensitivity, a high specificity and a high spatial resolution; it does not induce photo-bleaching; but XRF requires the use of high energetic radiation and is applicable to dead cells only. Infrared (IR) spectroscopy is selective for a chemical function; it enables deep tissue penetration; it is less energetic than fluorescence or XRF; it does not induce photo-beaching; but IR has a low resolution and a moderate sensitivity. Interestingly, biological media are almost transparent in the range 2200-1800 cm^{-1} while metal-carbonyl complexes show intense CO absorption bands in this transparent region. Classical fluorescence spectroscopy is of high resolution, in the range of 100 nm, and may be done in real time; but it has a low tissue penetration; artifacts due to spectra overlapping may arise; photo-bleaching may occur and quantitative measures are very complicated to set up contrary to XRF and infrared spectroscopies.

SUMMARY

This invention thus relates to the use of a X-ray fluorescence probe of formula I for performing X-ray fluorescence imaging, wherein formula I is as defined below.

According to one embodiment, the invention relates to the use a X-ray fluorescence probe of formula Ia or Ib for performing X-ray fluorescence imaging, wherein formulae Ia and Ib are as defined below. According to one embodiment, the invention relates to the use a X-ray fluorescence probe of formula Ia1 or Ib1 for performing X-ray fluorescence imaging, wherein formulae Ia1 and Ib1 are as defined below. According to one embodiment, the invention relates to the use a X-ray fluorescence probe of formula Ia2 or Ib2 for performing X-ray fluorescence imaging, wherein formulae Ia2 and Ib2 are as defined below. According to one embodiment, the invention relates to the use a X-ray fluorescence probe of formula Ia3 or Ib3 for performing X-ray fluorescence imaging, wherein formulae Ia3 and Ib3 are as defined below. According to one embodiment, the invention relates to the use a X-ray fluorescence probe of formula Ia4 or Ib4 for performing X-ray fluorescence imaging, wherein formulae Ia4 and Ib4 are as defined below. According to one embodiment, the invention relates to the use a X-ray fluorescence probe of formula Ia5 or Ib5 for performing X-ray fluorescence imaging,

wherein formulae Ia5 and Ib5 are as defined below. According to one embodiment, the invention relates to the use a X-ray fluorescence probe selected in Table 1 below, for performing X-ray fluorescence imaging.

According to one embodiment, the use according to the invention further comprises performing infrared spectroscopy and/or fluorescence spectroscopy.

According to one embodiment, the use according to the invention is for imaging cellular environment, and comprises:

- adding a X-ray fluorescence probe of formula I to a sample containing at least one cell;
- incubating the sample for a time sufficient for the X-ray fluorescence probe to be loaded onto and/or into the cell;
- exciting the sample at an energy that generates a X-ray fluorescence response from the X-ray fluorescence probe;
- detecting the X-ray fluorescence response.

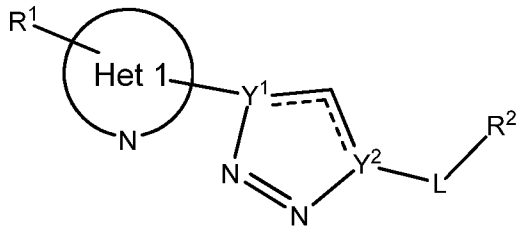
According to one embodiment, the use according to the invention is for imaging cell organelles.

The invention also relates to a X-ray fluorescence probe for imaging of formula I, wherein formula I is as defined below. According to one embodiment, the X-ray fluorescence probe for imaging is selected from compounds n°1-37 of Table 1 below.

The invention also relates to a compound of formula I, characterized in that it is a X-ray fluorescence probe for imaging. According to one embodiment, the compound of formula I characterized in that it is a X-ray fluorescence probe for imaging is selected from compounds n°1-37 and 63-65 of Table 1 below.

The invention further relates to a process for manufacturing a X-ray fluorescence probe of formula I according to the invention, said process comprising:

1) reacting a ligand of formula II



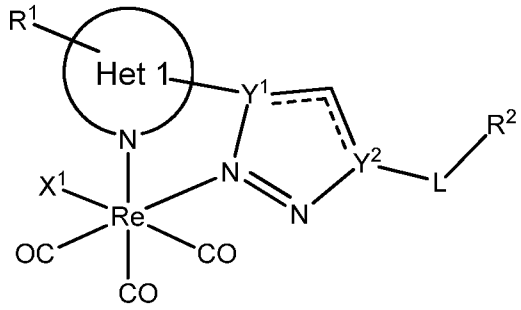
(II)

wherein **Het 1**, $\mathbf{Y^1}$, $\mathbf{Y^2}$, **L** and $\mathbf{R^2}$ are as defined in formula I;

with a rhenium-containing reactant of formula III

(III) $ReX^1(CO)_5$, wherein X^1 represents a halogen atom;

to afford compound of formula I'



(I')

wherein **Het 1**, $\mathbf{Y^1}$, $\mathbf{Y^2}$, **L** and $\mathbf{R^2}$ are as defined in formula I; and $\mathbf{X^1}$ represents halo;

2) and optionally:

- replacing $\mathbf{X^1}$ moiety by **X** as defined in formula I; and/or
- modifying and/or functionalizing –L-R^2;

to form a compound of formula I.

The invention also relates to a kit for performing X-ray fluorescence imaging, comprising a X-ray fluorescence probe for imaging of formula I according to the invention.

DEFINITIONS

In the present invention, the following terms have the following meanings:

- "**about**", preceding a figure, means plus or less 10% of the value of said figure.

- "**activated carbonate**" refers to a carbonate moiety in which one oxygen is replaced by an electron-withdrawing group, for example N-hydroxysuccinimide, N-hydroxyglutarimide, N-hydroxybenzotriazole or maleimide.
- "**activated carboxylic acid**" refers for example to acid anhydride or acid halide.
- "**activated ester**" refers to an ester in which the alkoxy group is replaced by an electron-withdrawing group, for example N-hydroxysuccinimide ester, N-hydroxyglutarimide ester, N-hydroxybenzotriazole ester, maleimide ester or pentafluorophenyl ester.
- "**alkenyl**" by itself or as part of another substituent refers to an unsaturated hydrocarbyl group, which may be linear or branched, wherein the unsaturation arises from the presence of one or more carbon-carbon double bonds. Suitable alkenyl groups comprise between 2 and 6 carbon atoms. Non-limiting examples of alkenyl groups are ethenyl, propenyl, butenyl, pentenyl and hexenyl.
- "**alkoxy**", by itself or as part of another substituent, refers to any group –O-alkyl, wherein alkyl is as herein defined. Suitable alkoxy groups include for example methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, f-butoxy, sec-butoxy, and n-pentoxy.
- "**alkyl**", by itself or as part of another substituent, refers to a hydrocarbyl radical of formula C_nH_{2n+1} wherein n is a number greater than or equal to 1. Generally, alkyl groups of this invention comprise from 1 to 20 carbon atoms, preferably from 1 to 12 carbon atoms, more preferably from 1 to 6 carbon atoms. Alkyl groups may be linear or branched and may be substituted as indicated herein. Suitable alkyl groups include methyl, ethyl, propyl (n-propyl, i-propyl, n- butyl), butyl (i-butyl, s-butyl and t-butyl), pentyl and its isomers (e.g. n-pentyl, iso-pentyl), and hexyl and its isomers (e.g. n- hexyl, iso-hexyl).
- "**alkylamino**" refers to the groups -NHR or –NRR' wherein R and R' are alkyl groups.
- "**alkylaryl**" refers to an aryl group substituted by an alkyl group, which may be represented as alkyl-aryl-.

- "**alkynyl**", by itself or as part of another substituent, refers to a class of monovalent unsaturated hydrocarbyl groups, wherein the unsaturation arises from the presence of one or more carbon-carbon triple bonds. Alkynyl groups typically, and preferably, have the same number of carbon atoms as described above in relation to alkyl groups. Non limiting examples of alkynyl groups are ethynyl, propynyl, butynyl, pentynyl, hexynyl.

- "**amido**" refers to the moieties –CO-NRR' or –NR-CO-R', wherein R and R' represent preferably H, alkyl or aryl. According to a specific embodiment, "amido" refers to the $-CO-NH_2$ moiety.

- "**amino**" refers to the groups $-NH_2$, $-NH_3^+$ and corresponding amino-protected groups. Amino-protected groups refers to a $-NH_2$ moiety protected by an "amino-protected group". The term "**amino-protected group**" is known in general terms and relates to groups which are suitable for protecting (blocking) an amino group against chemical reactions, but which are easy to remove after the desired chemical reaction has been carried out elsewhere in the molecule. Typical of such groups are, in particular, unsubstituted or substituted acyl, aryl, aralkoxymethyl or aralkyl groups. Since the amino-protecting groups are removed after the desired reaction (or reaction sequence), their type and size are furthermore not crucial; however, preference is given to those having 1-20, in particular 1-8, carbon atoms. The term "acyl group" is to be understood in the broadest sense in connection with the present process. It includes acyl groups derived from aliphatic, araliphatic, aromatic or heterocyclic carboxylic acids or sulfonic acids, and, in particular, alkoxycarbonyl, aryloxycarbonyl and especially aralkoxycarbonyl groups. Examples of such acyl groups are alkanoyl, such as acetyl, propionyl and butyryl; aralkanoyl, such as phenylacetyl; aroyl, such as benzoyl and tolyl; aryloxyalkanoyl, such as POA; alkoxycarbonyl, such as methoxycarbonyl, ethoxycarbonyl, 2,2,2-trichloroethoxycarbonyl, BOC (tert-butoxycarbonyl) and 2-iodoethoxycarbonyl; aralkoxycarbonyl, such as CBZ ("carbobenzoxy"), 4-methoxybenzyloxycarbonyl and FMOC; and arylsulfonyl, such as Mtr. Preferred amino-protecting groups are BOC and Mtr, furthermore CBZ, Fmoc, benzyl and acetyl.

- "**antibody**" refers to gamma globulin proteins that are found in blood or other bodily fluids of vertebrates, and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. Antibodies consist of two pairs of polypeptide chains, called heavy chains and light chains that are arranged in a Y-shape. The two tips of the Y are the regions that bind to antigens and deactivate them. The term "antibody" as used herein includes monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity.

- "**aryl**", by itself or as part of another substituent, refers to a polyunsaturated, aromatic hydrocarbyl group having a single ring (i.e. phenyl) or multiple aromatic rings fused together (e.g. naphtyl) or linked covalently, typically containing 5 to 20 atoms; preferably 6 to 12, wherein at least one ring is aromatic. The aromatic ring may optionally include one to two additional rings (either cycloalkyl, heterocyclyl or heteroaryl) fused thereto. Aryl is also intended to include the partially hydrogenated derivatives of the carbocyclic systems enumerated herein. Non- limiting examples of aryl comprise phenyl group, the biphenyl group, the 1-naphthyl group, the 2-naphthyl group, the tetrahydronaphthyl group, the indanyl group and the binaphthyl group.

- "**arylalkyl**" refers to an alkyl group substituted by an aryl group, which may be represented as aryl-alkyl-.

- "**carbene**" refers a molecule containing a neutral carbon atom with a valence of two and two unshared valence electrons. The general formula is R-(C:)-R' or R=C:, wherein R and R' preferably represent independently H or alkyl. According to a specific embodiment, "carbine" refers to the specific compound H_2C:, also called methylene.

- "**cell organelle**" refers to a cell compartment such as nucleus, mitochondria, Golgi apparatus, lysosome, endosome, endoplasmic reticulum.

- "**cell organelle targeting group**" refers to a moiety (organic group, complex, peptide, protein…) leading to an accumulation into a defined cell organelle of the compound to which it is attached.

- "**chelating moiety**" refers to a polydentate chemical moiety able to form coordinate bonds with a metallic ion to form a complex.

- "**complex of metallic ion**" refers to a molecule binding a metallic ion. Complexation involves the formation or presence of two or more separate coordinate bonds between a polydentate (multiple bonded) molecule (i.e. "chelating moiety") and a single central atom.

- "**cycloalkyl**", by itself or as part of another substituent, refers to a cyclic alkyl group, that is to say, a monovalent, saturated, or unsaturated hydrocarbyl group having 1 or 2 cyclic structures. Cycloalkyl includes monocyclic or bicyclic hydrocarbyl groups. Cycloalkyl groups may comprise 3 or more carbon atoms in the ring and generally, according to this invention comprise from 3 to 10, more preferably from 3 to 8 carbon atoms still more preferably from 3 to 6 carbon atoms. Examples of cycloalkyl groups include but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl.

- "**fluorophore**" refers to a chemical substance able to emit fluorescent light after excitation. Preferably, fluorophores are molecules comprising several conjugated aromatic rings or planar cyclic molecules having one or more π bond. Examples of fluorophores are: coumarines (hydroxycoumarine, aminocoumarine, methoxycoumarine); fluoresceine, rhodamines (X-rhodamine, lissamine rhodamine B), cyanine derivatives (Cy3, Cy5). Fluorophores also comprise fluorescent proteines, such as for example GFP and derivatives thereof.

- "**halo**" refers to fluoro, chloro, bromo, or iodo.

- "**heteroaryl**", by itself or as part of another substituent, refers to 5 to 12 carbon-atom aromatic rings or ring systems containing 1 to 2 rings which are fused together or linked covalently, typically containing 5 to 6 atoms; at least one of which is aromatic, in which one or more carbon atoms in one or more of these rings is replaced by oxygen, nitrogen and/or sulfur atoms; where the nitrogen and sulfur heteroatoms may optionally be oxidized; and the nitrogen heteroatoms may optionally be quaternized. Such rings may be fused to an aryl, cycloalkyl, heteroaryl or heterocyclyl ring. Non-limiting examples of such heteroaryl, include: furanyl, thiophenyl, pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl,

thiadiazolyl, tetrazolyl, oxatriazolyl, thiatriazolyl, pyridinyl, pyrimidyl, pyrazinyl, pyridazinyl, oxazinyl, dioxinyl, thiazinyl, triazinyl, imidazo[2,1-b][1,3]thiazolyl, thieno[3,2-b]furanyl, thieno[3,2-b]thiophenyl, thieno[2,3-d][1,3]thiazolyl, thieno[2,3-d]imidazolyl, tetrazolo[1,5-a]pyridinyl, indolyl, indolizinyl, isoindolyl, benzofuranyl, isobenzofuranyl, benzothiophenyl, isobenzothiophenyl, indazolyl, benzimidazolyl, 1,3-benzoxazolyl, 1,2- benzisoxazolyl, 2,1-benzisoxazolyl, 1,3-benzothiazolyl, 1,2-benzoisothiazolyl, 2,1-benzoisothiazolyl, benzotriazolyl, 1,2,3-benzoxadiazolyl, 2,1,3-benzoxadiazolyl, 1,2,3-benzothiadiazolyl, 2,1,3-benzothiadiazolyl, thienopyridinyl, purinyl, imidazo[1,2-a]pyridinyl, 6-oxo-pyridazin-l(6H)-yl, 2-oxopyridin-1(2H)-yl, 6-oxo-pyridazin-1(6H)-yl, 2-oxopyridin-1(2H)-yl, 1,3-benzodioxolyl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, quinoxalinyl.

According to a preferred embodiment of the invention, in the probe of formula I, **Het** represents a heteroaryl group comprising at least one nitrogen atom. Non limitative examples of heteroaryl groups comprising at least one nitrogen atom are: pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, thiadiazolyl, tetrazolyl, oxatriazolyl, thiatriazolyl, pyridinyl, pyrimidyl, pyrazinyl, pyridazinyl, oxazinyl, thiazinyl, triazinyl, imidazo[2,1-b][1,3]thiazolyl, thieno[2,3-d][1,3]thiazolyl, thieno[2,3-d]imidazolyl, tetrazolo[1,5-a]pyridinyl, indolyl, indolizinyl, isoindolyl, indazolyl, benzimidazolyl, 1,3-benzoxazolyl, 1,2-benzisoxazolyl, 2,1-benzisoxazolyl, 1,3-benzothiazolyl, 1,2-benzoisothiazolyl, 2,1-benzoisothiazolyl, benzotriazolyl, 1,2,3-benzoxadiazolyl, 2,1,3-benzoxadiazolyl, 1,2,3-benzothiadiazolyl, 2,1,3-benzothiadiazolyl, thienopyridinyl, purinyl, imidazo[1,2-a]pyridinyl, 6-oxo-pyridazin-l(6H)-yl, 2-oxopyridin-1(2H)-yl, 6-oxo-pyridazin-1(6H)-yl, 2-oxopyridin-1(2H)-yl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, quinoxalinyl.

- "**heterocyclyl**", by itself or as part of another substituent, refers to non-aromatic, fully saturated or partially unsaturated cyclic groups (for example, 3 to 7 member monocyclic, 7 to 11 member bicyclic, or containing a total of 3 to 10 ring atoms) which have at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2, 3 or

4 heteroatoms selected from nitrogen, oxygen and/or sulfur atoms, where the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatoms may optionally be quaternized. Any of the carbon atoms of the heterocyclic group may be substituted by oxo (for example piperidone, pyrrolidinone). The heterocyclic group may be attached at any heteroatom or carbon atom of the ring or ring system, where valence allows. The rings of multi-ring heterocycles may be fused, bridged and/or joined through one or more spiro atoms. Non limiting exemplary heterocyclic groups include oxetanyl, piperidinyl, azetidinyl, 2-imidazolinyl, pyrazolidinyl imidazolidinyl, isoxazolinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl, isothiazolidinyl, piperidinyl, 3H- indolyl, indolinyl, isoindolinyl, 2-oxopiperazinyl, piperazinyl, homopiperazinyl, 2-pyrazolinyl, 3-pyrazolinyl, tetrahydro-2H-pyranyl, 2H-pyranyl, 4H-pyranyl, 3,4-dihydro-2H-pyranyl, 3-dioxolanyl, 1,4-dioxanyl, 2,5-dioximidazolidinyl, 2- oxopiperidinyl, 2-oxopyrrolodinyl, indolinyl, tetrahydropyranyl, tetrahydrofuranyl, tetrahydroquinolinyl, tetrahydroisoquinolin- 1-yl, tetrahydroisoquinolin-2-yl, tetrahydroisoquinolin-3-yl, tetrahydroisoquinolin-4-yl, thiomorpholin-4-yl, thiomorpholin-4-ylsulf oxide, thiomorpholin-4-ylsulfone, 1,3- dioxolanyl, 1,4-oxathianyl, lH-pyrrolizinyl, tetrahydro-l,l-dioxothiophenyl, N-formylpiperazinyl, and morpholin-4-yl.

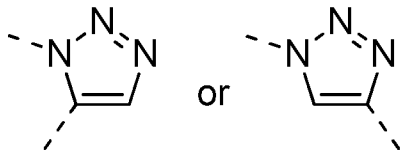
- "**hormone**" refers to any member of a class of signaling molecules produced by glands in multicellular organisms that are transported by the circulatory system to target distant organs to regulate physiology and behavior. Hormones may have diverse chemical structures that include eicosanoids, steroids, amino acid derivatives, peptides, and proteins. Examples of hormones include, but are not limited to, melatonin, thyroxine, TRH, vasopressin, insulin, growth hormone, luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, estradiol, testosterone, and cortisol.

- "**linker**" refers to a single covalent bond or a moiety comprising series of stable covalent bonds, the moiety often incorporating 1-40 plural valent atoms selected from the group consisting of C, N, O, S and P, that covalently attach a reactive group or bioactive group to the probe of the invention. The number of plural valent atoms in a linker may be, for example, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 25, 30 or a larger number

up to 40 or more. A linker may be linear or non-linear; some linkers have pendant side chains or pendant functional groups (or both). Examples of such pendant moieties are hydrophilicity modifiers, for example solubilizing groups like, e.g. sulfo (-SO_3H or -SO_3^-), carboxy (-COOH or –COO^-), hydroxy.

In one embodiment, L is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds and carbon-sulfur bonds. Linkers may by way of example consist of a combination of moieties selected from alkyl, -C(O)NH-, -C(O)O-, -NH-, -S-, -O-, -C(O) -, -$S(O)_n$- where n is 0, 1 or 2; -O-, 5- or 6- membered monocyclic rings and optional pendant functional groups, for example sulfo, hydroxy and carboxy.

The reactive group may be reacted with a substance reactive therewith, whereby the linker becomes bonded to a bioactive group. In this case, the linker typically contains a residue of a reactive group (such as for example the carbonyl group of an ester after reaction with a nucleophile; a triazolo group resulting from a click reaction between an azide and an alkyne; an amide link resulting from a reaction between an amine and an acid; a thiourea resulting from the coupling between an amine and an isothiocyanate; a –O-C(O)- moiety remaining for example after reaction of an activated carbonate with a nucleophile, etc.). By “triazolo group” it is referred to the following moiety:



The linker may also contain a component of an Ugi reaction which occurs between a ketone or aldehyde, an amine, an isocyanide and a carboxylic acid to form a bis-amide. An example of such reaction and related residue is provided in example 11 below, wherein the aldehyde is isobutyraldehyde; the amine is the reactive function of the rhenium complex; the isocyanide is cyclohexylisocyanide and the carboxylic acid is the reactive function of the hyaluronic acid used as bioactive group.

- "**lipid**" refers to hydrophobic or amphiphilic small molecules, which are naturally occurring and include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides and phospholipids. Lipids may be divided into eight categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides (derived from condensation of ketoacyl subunits); sterol lipids and prenol lipids (derived from condensation of isoprene subunits).

- "**molecular probe**" or "**probe**" herein refers to a molecule that emits radiations to produce an observable detectable signal. "**XRF probe**" refers to a probe that emits X-ray fluorescent radiations.

- "**microparticle**" refers to a particle having a size ranging from 0.1 to 100 micrometers.

- "**nanoparticle**" refers to a particle having a size ranging from 1 to 100 nanometers.

- "**oxyanion**" refers to an ion with the generic formula $A_xO_y^{z-}$, wherein A represents a chemical element and O represents an oxygen atom. Oxoanions may be formed by a large majority of the chemical elements. The formulae of simple oxoanions are determined by the octet rule. Non-limitative examples of oxyanions are borate, carbonate, nitrate, nitrite, phosphate, phosphite, hypophosphite, sulfate, sulfite, hyposulfite, chromate, arsenate, arsenite, hypochlorite, chlorite, chlorate, perchlorate, hypobromite, bromite, bromate, perbromate, iodate, periodate, permanganate, molybdate.

- "**peptide**" refers to a linear polymer of amino acids.

- "**polyethylene glycol**" refers to the moiety $-(O\text{-}CH_2\text{-}CH_2)_n\text{-}OH$ wherein n ranges from 2 to 2000.

- "**polypropylene glycol**" refers to the moiety $-(O\text{-}CH_2\text{-}CH(CH_3))_n\text{-}OH$ wherein n ranges from 2 to 2000.

- "**polysaccharide**" refers to a polymeric carbohydrate molecule composed of long chains of monosaccharide units bound together by glycosidic linkages; which may be linear or branched. Examples include starch, glycogen, cellulose, chitin, hyaluronic

acid and chondroitin sulfate. a particularly preferred polysaccharide is hyaluronic acid, more preferably low weight hyaluronic acid ($400 \leq MW \leq 1000$ kDa).

- "**protein**" specifically refers to a functional entity formed of one or more peptides, and optionally of non-polypeptides cofactors.
- "**reactive group**" refers to a group capable of reacting with another chemical group to form a covalent bond, i.e. is covalently reactive under suitable reaction conditions, and generally represents a point of attachment for another substance. The reactive group is a moiety on the compounds of the present invention that is capable of chemically reacting with a functional group on a different compound to form a covalent linkage. Reactive groups generally include nucleophiles, electrophiles and photoactivatable groups.
- "**saccharide**" or "**monosaccharide**" refers to polyhydroxy aldehydes or polyhydroxy ketones, comprising at least carbon atoms, and which are not hydrolizable. Non limitative examples of monosaccharides are trioses (glyceraldehyde, dihydroxyacetone); tetroses (erythrose, threose, erythrulose); pentoses (desoxyribose, ribose, arabinose, xylose, lyxose, ribulose, xylulose); hexoses (allose, altrose, galactose, glucose, gulose, idose, mannose, talose, fructose, psicose, sorbose, tagatose); desoxy-hexoses (fucose, rhamnose); heptoses (sedoheptulose, mannoheptulose); nonoses (neuraminic acid or sialic acid).
- "**salt**" of the compounds of the invention includes the acid addition and base salts thereof. Suitable acid addition salts are formed from acids which form non-toxic salts. Non-limiting examples include the acetate, trifluoroacetate, adipate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, borate, tetrafluoroborate, camsylate, citrate, cyclamate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulphate, naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, pyroglutamate, saccharate, stearate, succinate, tannate, tartrate, tosylate, trifluoroacetate and xinofoate salts.

Suitable base salts are formed from bases which form non-toxic salts. Non-limiting examples include the aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine, 2-(diethylamino)ethanol, ethanolamine, morpholine, 4- (2- hydroxyethyl)morpholine and zinc salts. Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts. Preferred, pharmaceutically acceptable salts include hydrochloride/chloride, hydrobromide/bromide, bisulphate/sulphate, nitrate, citrate, and acetate.

- "**therapeutic ingredient**" describes a molecule or a substance whose administration to a subject slows down or stops the progression, aggravation, or deterioration of one or more symptoms of a disease, or condition; alleviates the symptoms of a disease or condition; cures a disease or condition. According to one embodiment, the therapeutic ingredient is a small molecule, either natural or synthetic. According to another the therapeutic ingredient is a biological molecule such as for example an oligonucleotide, a siRNA, a miRNA, a DNA fragment, an aptamer, an antibody and the like.

- "**thiol**" refers to a group –SH.

- "**thiolato**" refers to a group comprising a deprotonated thiol: $R\text{-}S^-$, wherein R represents an aryl or an alkyl group.

- "**thioether**" refers to a group –S-R wherein R represents an aryl or an alkyl group.

- "**XRF imaging**" refers to the generation of X-ray fluorescence emission of selected elements, herein rhenium, and its recording to provide a 2D or 3D map. In other words, XRF imaging refers to the generation of 2D or 3D images from X-ray fluorescence spectral data. In this context, the X-ray fluorescence spectral data may refer to the whole XRF spectrum or to a part of this spectrum. In the case wherein a part of the X-ray fluorescence spectrum is used, the XANES energy range is preferably used.

- "**XRF spectroscopy**" refers to the detection of emitted X-ray photons from a sample after irradiation at a given energy (X-rays or gamma-rays). The energy of the emitted photons is characteristic for each element.

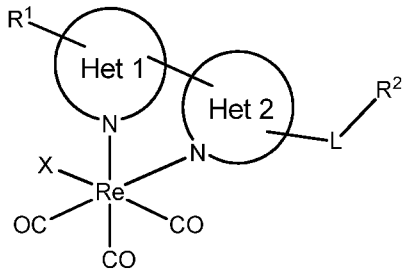
Unless indicated otherwise, the nomenclature of substituents that are not explicitly defined herein are arrived at by naming the terminal portion of the functionality followed by the adjacent functionality toward the point of attachment. For example, the substituent "arylalkyl" refers to the group (aryl)-(alkyl)-.

Where groups may be substituted, such groups may be substituted with one or more substituents. Substituents may be selected from but not limited to, for example, the group comprising halogen, hydroxyl, oxo, nitro, amido, carboxy, amino, dialkylamino, cyano, alkyl, aryl, haloalkyl and hydroxyalkyl.

DETAILED DESCRIPTION

X-ray fluorescence (XRF) probes

The present invention relates to a XRF probe of formula A:



(A)

or a salt thereof, wherein

X represents halo, optionally substituted pyridin-1-yl, carbene, thiolato, alkynyl, carboxylate, phosphine, phosphonate, sulfonate, OH_2, NC-alkyl, oxyanion such as phosphate;

Het 1 represents a heteroaryl group comprising at least one nitrogen atom; preferably **Het 1** is selected from pyridinyl, quinolynyl, quinoxalinyl, benzothiadiazolyl, benzimidazole, benzoxazole, bipyridine;

R^1 is either absent or represents one or more substituent selected from halo, nitro, alkyl, aryl, phosphate, sulfate, cyano;

Het 2 represents a heteroaryl group comprising at least one nitrogen atom; preferably **Het 2** is selected from triazolyl and pyridinyl;

L represents a single bound or a linker selected from alkyl, aryl, heteroaryl, heterocyclyl, alkylaryl, arylalkyl, alkoxy, alkenyl, alkynyl, polyethylene glycol, polypropylene glycol, polyamines or a combination thereof; said groups being optionally interrupted or terminated by one or more -O-, -S-, -S(O)-, $-S(O)_2-$, -C(O)-NH-, -NH-C(O)-, -C(O)O-, -C(O)-, -NH- or a combination thereof; said groups optionally further comprising pendant groups selected from carboxylic acid, carboxylate, sulfonic acid, sulfonate, hydroxy; the linker optionally additionally comprising a residue of a reactive group through which **L** is bound to R^2;

R^2 represents a group selected from:

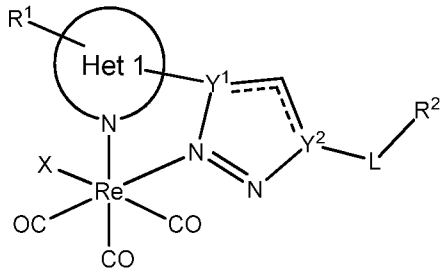
a hydrogen atom;

a reactive group selected from azide, alkynyl, amino, alkylamino, amido, maleimide, thiol, hydroxy, ester, activated ester, carboxylic acid, activated carboxylic acid, halo, nitro, nitrile, isonitriles, acrylamide, aldehyde, ketone, acetals, ketals, anhydride, glutaric anhydride, succinic anhydride, maleic anhydride, thiocyanate, isothiocyanate, isocyanate, hydrazide, hydrazines, hydrazones, ethers, oxides, cyanates, diazo, diazonium, sulfides, disulfides, sulfoxides, sulfones, sulfonic acids, sulfinic acids, sulfates, sulfenic acids, amidines, imides, imidates, nitrones, hydroxylamines, oximes, hydroxamic acids, thiohydroxamic acids, alkenes, ortho esters, sulfites, enamines, ynamines, ureas, pseudoureas, semicarbazides, carbodiimides, carbamates, carbonate, activated carbonate, imines, phosphonium, chelating moiety;

a bioactive group selected from steroid, peptide, protein, amino acid, nucleic acid, nucleoside, nucleotide, oligonucleotide, antibody, saccharide, polysaccharide, lipid, hormone, biotin, avidin, therapeutic ingredient,

complex of metallic ion, microparticle, nanoparticle, fluorophore, a cell organelle targeting group and combinations thereof.

According to one embodiment, the invention relates to a XRF probe of formula I:



(I)

or a salt thereof, wherein

X represents halo, optionally substituted pyridin-1-yl, carbene, thiolato, alkynyl, carboxylate, phosphine, phosphonate, sulfonate, OH_2, NC-alkyl, oxyanion such as phosphate;

Het 1 represents a heteroaryl group comprising at least one nitrogen atom; preferably **Het 1** is selected from pyridinyl, quinolynyl, quinoxalinyl, benzothiadiazolyl, benzimidazole, benzoxazole, bipyridine;

$\mathbf{R^1}$ is either absent or represents one or more substituent selected from halo, nitro, alkyl, aryl, phosphate, sulfate, cyano;

$\mathbf{Y^1}$ represents C and $\mathbf{Y^2}$ represents N; or $\mathbf{Y^1}$ represents N and $\mathbf{Y^2}$ represents C;

====== represents a single bound or a double bound depending on $\mathbf{Y^1}$ and $\mathbf{Y^2}$ definitions;

L represents a single bound or a linker selected from alkyl, aryl, heteroaryl, heterocyclyl, alkylaryl, arylalkyl, alkoxy, alkenyl, alkynyl, polyethylene glycol, polypropylene glycol, polyamines or a combination thereof; said groups being optionally interrupted or terminated by one or more -O-, -S-, -S(O)-, $-S(O)_2-$, -C(O)-NH-, -NH-C(O)-, -C(O)O-, -C(O)-, -NH- or a combination thereof; said groups optionally further comprising pendant groups selected from carboxylic acid, carboxylate, sulfonic acid, sulfonate, hydroxy; the linker optionally additionally comprising a residue of a reactive group through which **L** is bound to $\mathbf{R^2}$;

R^2 represents a group selected from:

a hydrogen atom;

a reactive group selected from azide, alkynyl, amino, alkylamino, amido, maleimide, thiol, hydroxy, ester, activated ester, carboxylic acid, activated carboxylic acid, halo, nitro, nitrile, isonitriles, acrylamide, aldehyde, ketone, acetals, ketals, anhydride, glutaric anhydride, succinic anhydride, maleic anhydride, thiocyanate, isothiocyanate, isocyanate, hydrazide, hydrazines, hydrazones, ethers, oxides, cyanates, diazo, diazonium, sulfides, disulfides, sulfoxides, sulfones, sulfonic acids, sulfinic acids, sulfates, sulfenic acids, amidines, imides, imidates, nitrones, hydroxylamines, oximes, hydroxamic acids, thiohydroxamic acids, alkenes, ortho esters, sulfites, enamines, ynamines, ureas, pseudoureas, semicarbazides, carbodiimides, carbamates, carbonate, activated carbonate, imines, phosphonium, chelating moiety;

a bioactive group selected from steroid, peptide, protein, amino acid, nucleic acid, nucleoside, nucleotide, oligonucleotide, antibody, saccharide, polysaccharide, lipid, hormone, biotin, avidin, therapeutic ingredient, complex of metallic ion, microparticle, nanoparticle, fluorophore, a cell organelle targeting group and combinations thereof.

According to a specific embodiment, **X** represents halo, preferably chloro or bromo.

According to a specific embodiment, **X** represents optionally substituted pyridinyl, preferably pyridin-1-yl.

According to a specific embodiment, **Het 1** represents pyridinyl, quinolynyl, quinoxalinyl, benzothiadiazolyl, benzimidazole, benzoxazole, bipyridine, preferably **Het 1** represents pyridinyl, quinolynyl, quinoxalinyl, benzothiadiazolyl, benzimidazole, more preferably **Het 1** represents pyridinyl.

According to a specific embodiment, **R^1** is absent. In this case **Het 1** is not further substituted other than by the triazolo group also chelating the metal ion.

According to a specific embodiment, **R1** represents one or more substituent selected from halo, nitro, alkyl, aryl, phosphate, sulfonate, cyano; preferably **R1** represents one substituent selected from halo, nitro, alkyl; more preferably **R1** represents one substituent selected from chloro, nitro, methyl.

According to a specific embodiment, **R1** represents one substituent as defined above.

According to a specific embodiment, **R1** represents at least two substituents as defined above.

According to a specific embodiment, **L** represents a single bound.

According to another specific embodiment, **L** represents a linker selected from alkyl, aryl, heteroaryl, heterocyclyl, alkylaryl, arylalkyl, alkoxy, alkenyl, alkynyl, polyethylene glycol, polypropylene glycol, polyamines or a combination thereof; said groups being optionally interrupted or terminated by one or more -O-, -S-, -S(O)-, -$S(O)_2$-, -C(O)-NH-, -NH-C(O)-, -C(O)O-, -C(O)-, -NH- or a combination thereof.

According to one embodiment, **L** represents a linker comprising at least one pendant group, preferably selected from carboxylic acid, carboxylate, sulfonic acid, sulfonate, hydroxyl.

According to one embodiment, the linker optionally additionally comprising a residue of a reactive group through which **L** is bound to **R2**. Preferably such residue is present at the extremity of the linker through which **L** is bound to **R2**.

According to a preferred embodiment, **L** represents a linker which is an alkyl group.

According to a specific embodiment, **L** represents a linker which is an alkyl group comprising a residue of a reactive group through which **L** is bound to **R2**, such as for example the carbonyl group of an ester after reaction with a nucleophile; a triazolo group resulting from a click reaction between an azide and an alkyne; an amide link resulting from a reaction between an amine and an acid; a thiourea resulting from the coupling

between an amine and an isothiocyanate; a –O-C(O)- moiety remaining after reaction of an activated carbonate with a nucleophile.

According to a specific embodiment, **L** represents a linker which is an alkyl group interrupted by at least one -C(O)-NH- , -NH-C(O)- or –O- moiety.

According to a specific embodiment, **L** represents a linker which is an alkyl group terminated at least one extremity by –O- moiety.

According to a specific embodiment, **L** represents a linker which is a polyethylene group.

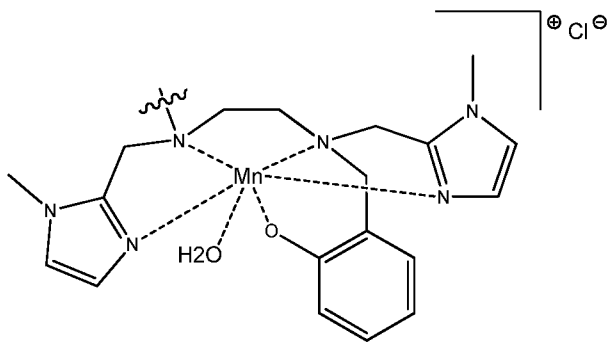
According to a preferred embodiment, **L** represents a linker which is an aryl group.

According to a specific embodiment, $\mathbf{R^2}$ represents a hydrogen atom.

According to a specific embodiment, $\mathbf{R^2}$ represents a reactive group selected from azide, amino, maleimide, hydroxy, ester, activated ester, carboxylic acid, halo, nitro, aldehyde, activated carbonate, chelating moiety.

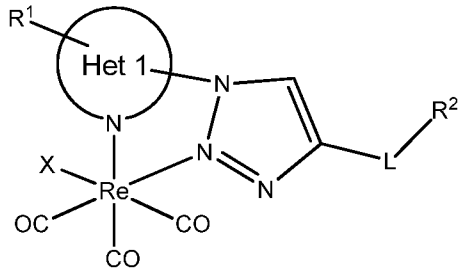
According to a specific embodiment, $\mathbf{R^2}$ represents a bioactive group selected from steroid, peptide, complex of metallic ion, fluorophore, a cell organelle targeting group and combinations thereof.

According to a specific embodiment, $\mathbf{R^2}$ represents a bioactive group which is a complex of metal, preferably a complex of manganese, more preferably a complex of manganese which is a superoxide dismutase mimic, even more preferably a complex of manganese which is a superoxide dismutase mimic of following formula:

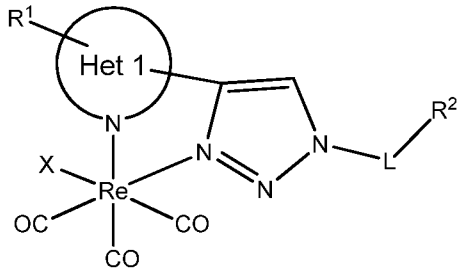


According to a specific embodiment, **R²** represents a bioactive group which is a superoxide dismutase mimic. According to another specific embodiment, **R²** represents a bioactive group which is a cell penetrating peptide.

According to one embodiment, the XRF probes of the invention are of formula Ia or Ib:



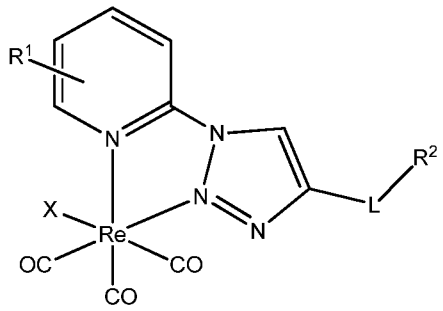
(Ia)



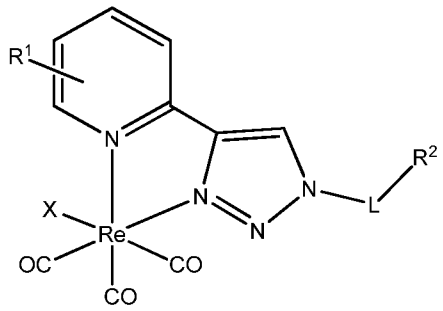
(Ib)

or a salt thereof, wherein **X**, **Het 1**, **R¹**, **L** and **R²** are as defined above.

According to one embodiment, the XRF probes of the invention are of formula Ia1 or Ib1:



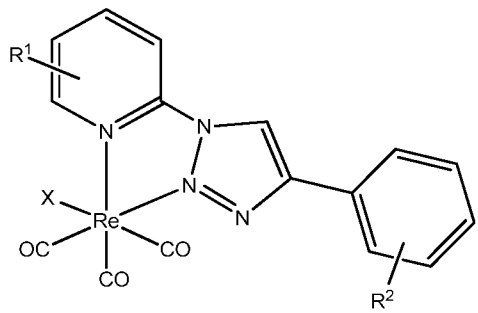
(Ia1)



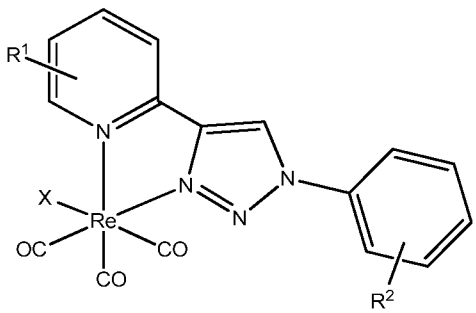
(Ib1)

or a salt thereof, wherein **X**, **R¹**, **L** and **R²** are as defined above.

According to one embodiment, the XRF probes of the invention are of formula Ia1-1 or Ib1-1:



(Ia1-1)



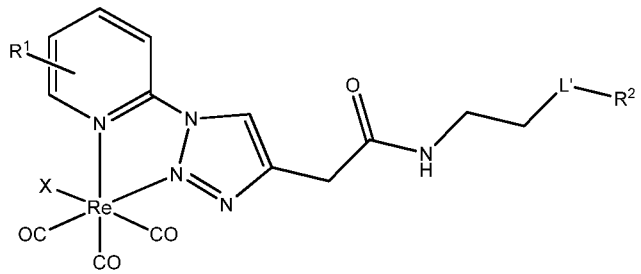
(Ib1-1)

or a salt thereof, wherein **X**, **R¹**, **L** and **R²** are as defined above.

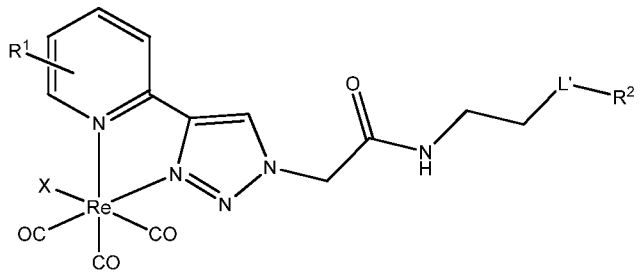
According to a specific embodiment, in formulae Ia1-1 and Ib1-1, R^2 is not H.

According to a specific embodiment, in formulae Ia1-1 and Ib1-1, R^2 is in meta position. According to a preferred embodiment, in formulae Ia1-1 and Ib1-1, R^2 represents halo or nitro and is preferably in position in meta.

According to one embodiment, the XRF probes of the invention are of formula Ia1-2 or Ib1-2:



(Ia1-2)

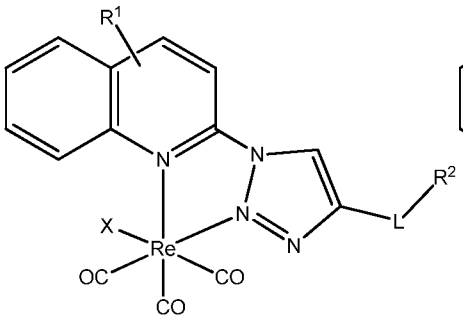


(Ib1-2)

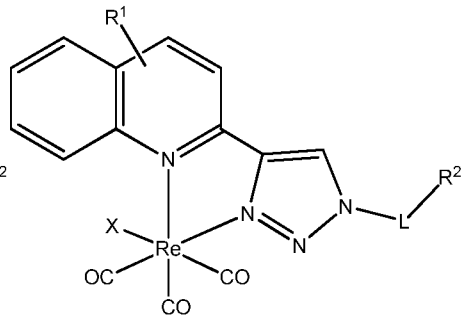
or a salt thereof, wherein **X**, **R^1** and **R^2** are as defined above and wherein **L'** is either absent or represents a residue of a reactive group through which **R^2** is attached to the rest of the molecule.

According to one embodiment, in formulae Ia1-2 and Ib1-2, L' is absent. According to another embodiment, in formulae Ia1-2 and Ib1-2, L' represents a carbonyl group.

According to one embodiment, the XRF probes of the invention are of formula Ia2 or Ib2:



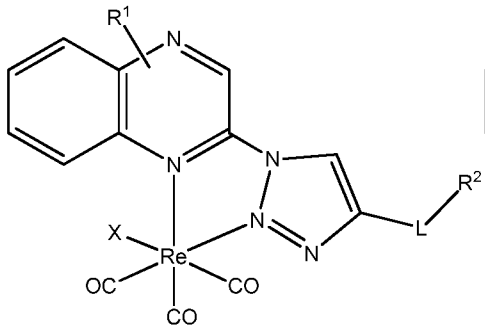
(Ia2)



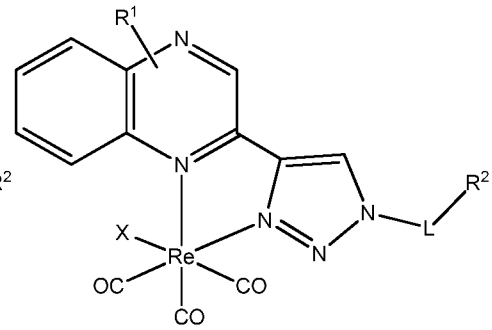
(Ib2)

or a salt thereof, wherein **X**, **R**^{1}, **L** and **R**^{2} are as defined above.

According to one embodiment, the XRF probes of the invention are of formula Ia3 or Ib3:



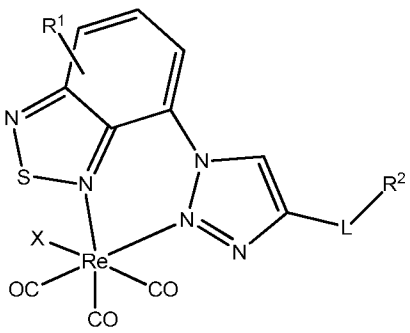
(Ia3)



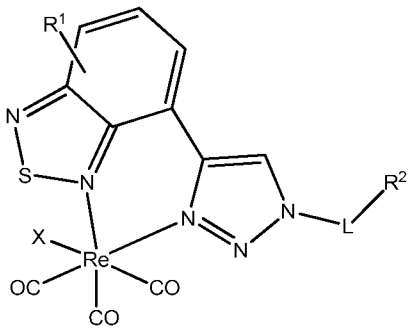
(Ib3)

or a salt thereof, wherein **X**, **R**^{1}, **L** and **R**^{2} are as defined above.

According to one embodiment, the XRF probes of the invention are of formula Ia4 or Ib4:



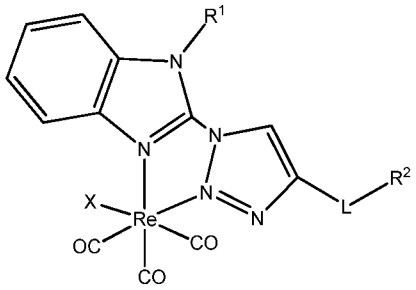
(Ia4)



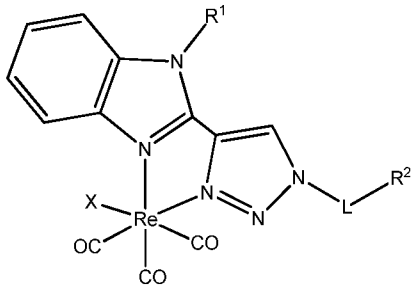
(Ib4)

or a salt thereof, wherein **X**, **R**^{1}, **L** and **R**^{2} are as defined above.

According to one embodiment, the XRF probes of the invention are of formula Ia5 or Ib5:



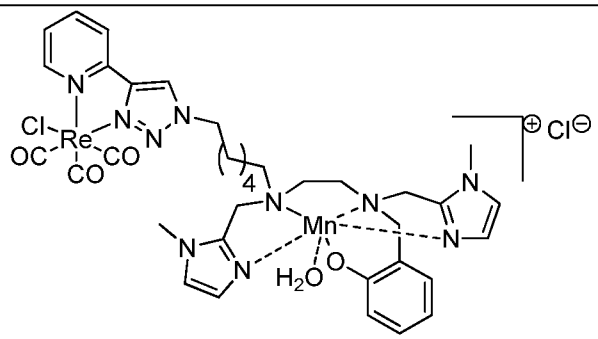
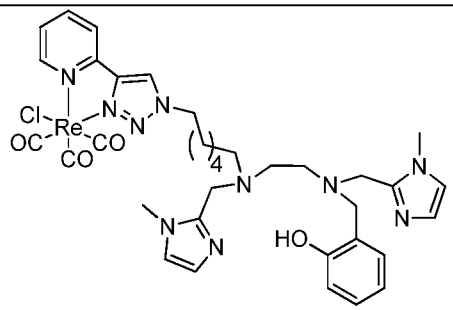
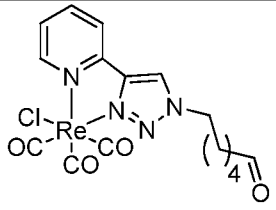
(Ia5)

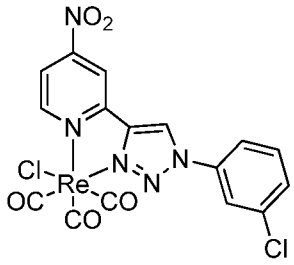
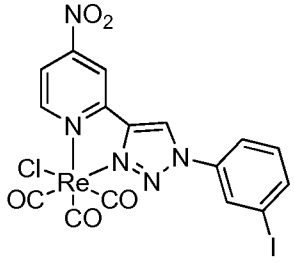
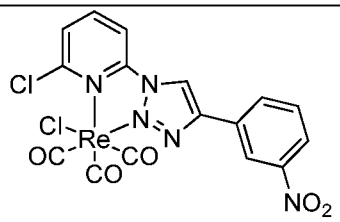
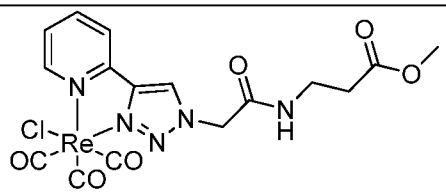
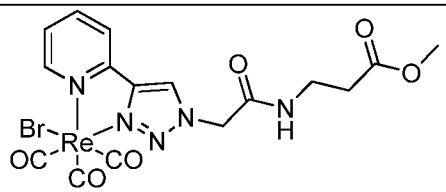
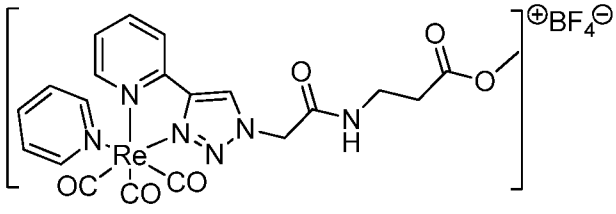
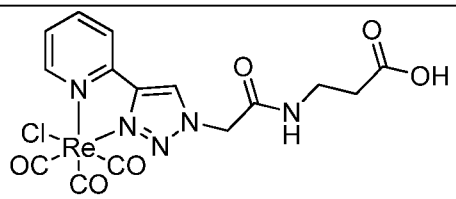


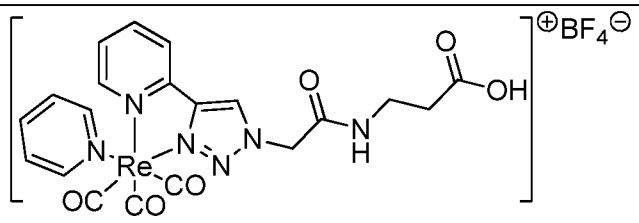
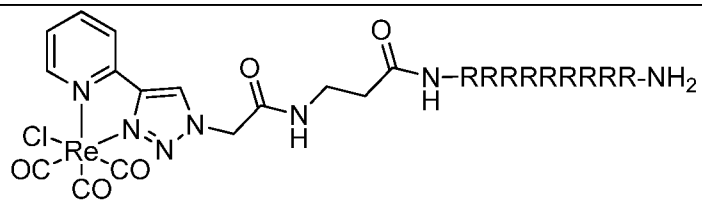
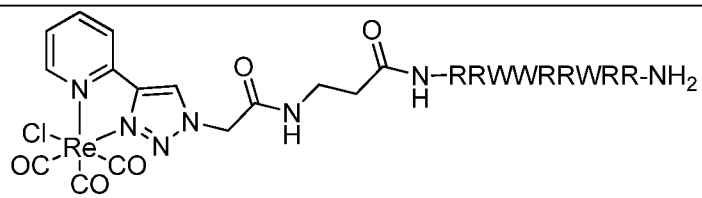
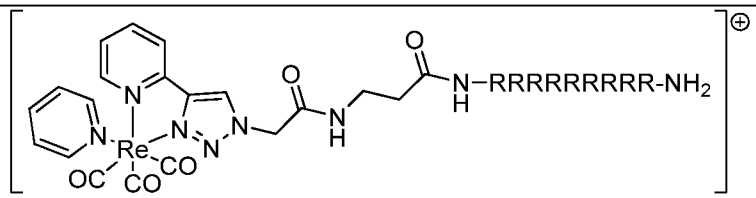
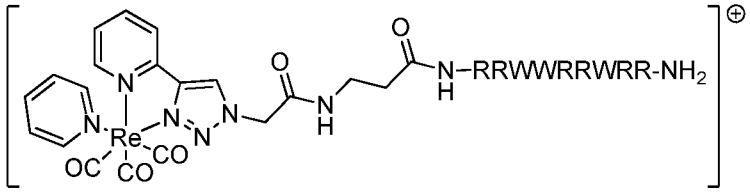
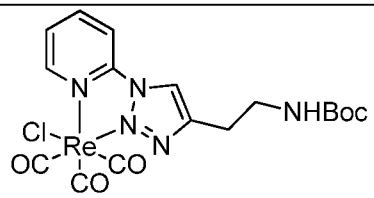
(Ib5)

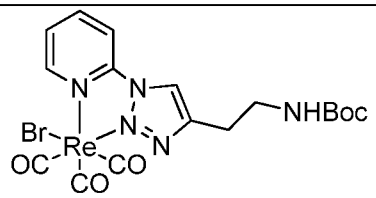
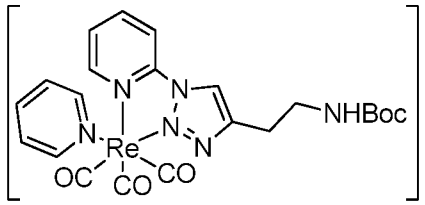
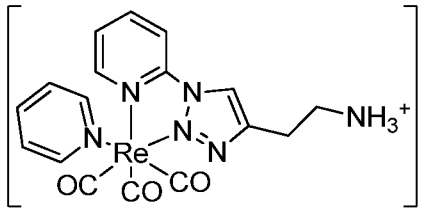
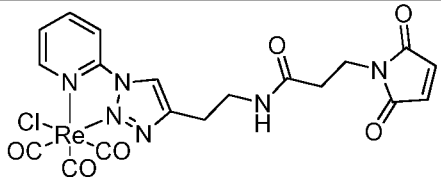
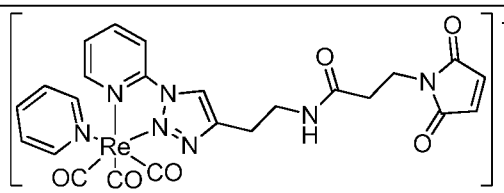
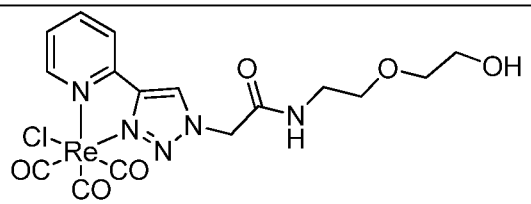
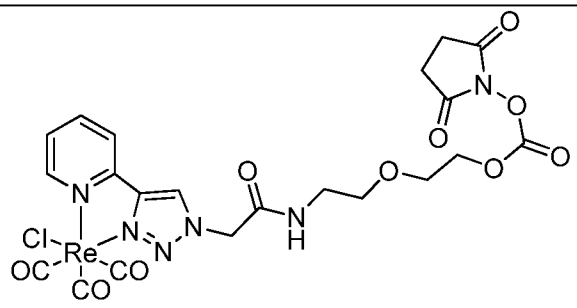
or a salt thereof, wherein **X**, **R^1**, **L** and **R^2** are as defined above.

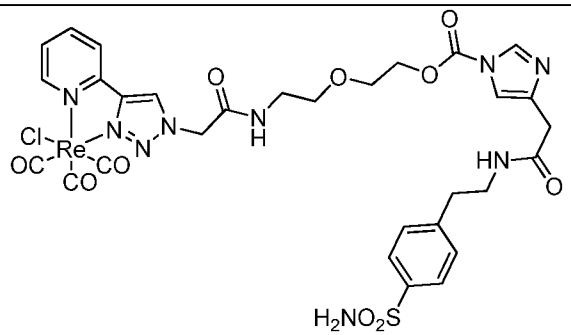
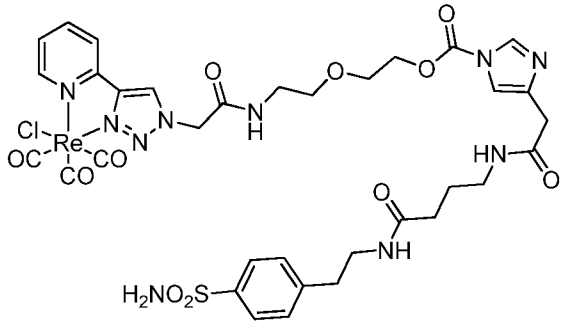
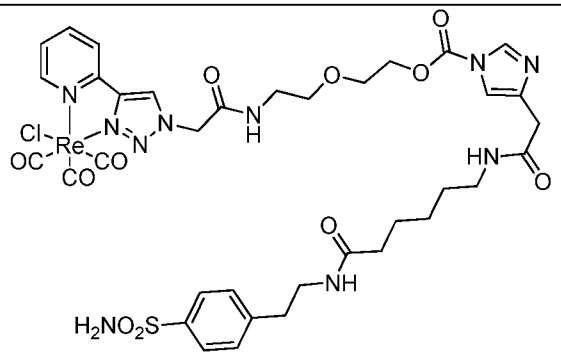
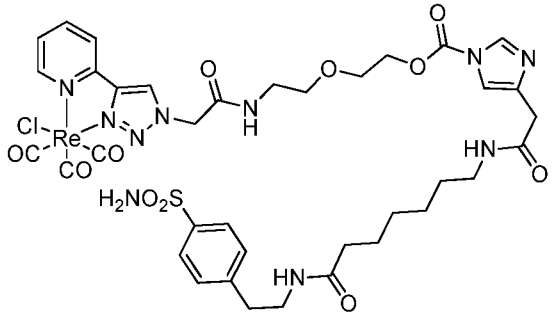
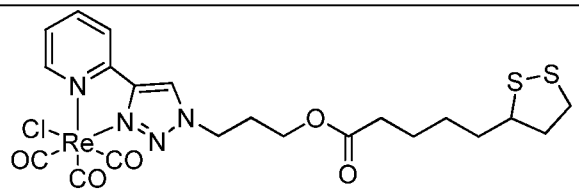
According to one embodiment, the XRF probe of the invention is selected from the group comprising compounds of the Table 1 below:

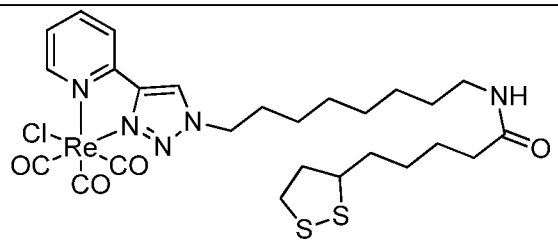
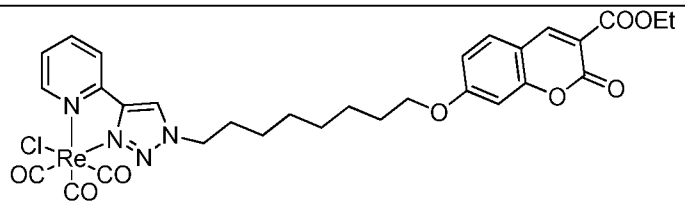
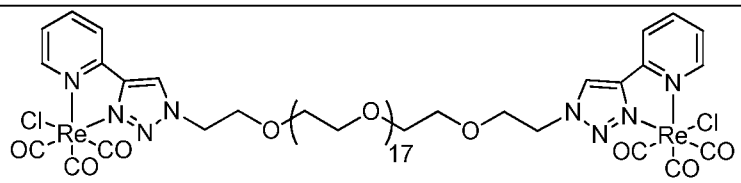
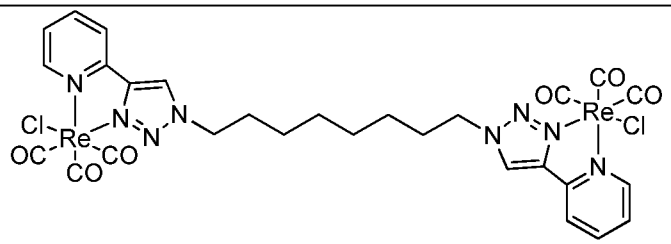
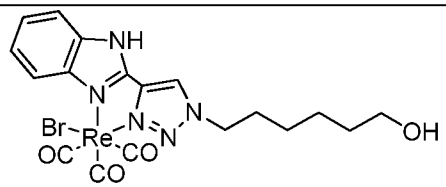
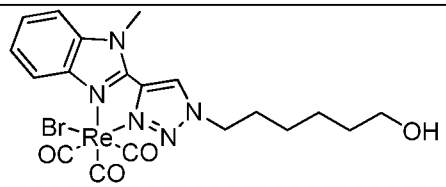
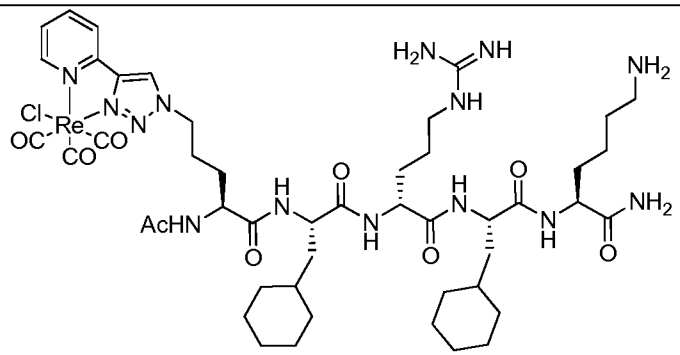
Cpd n°	Formula
1	
2	
3	

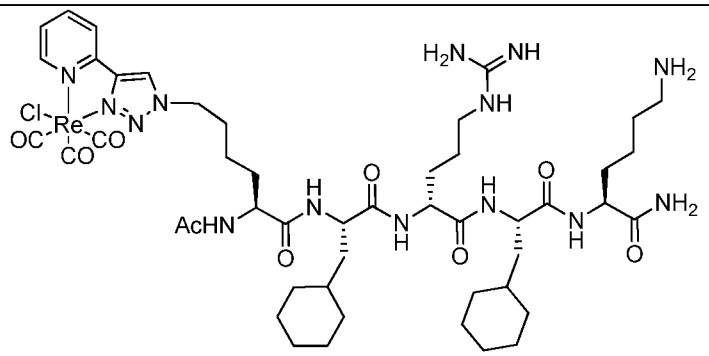
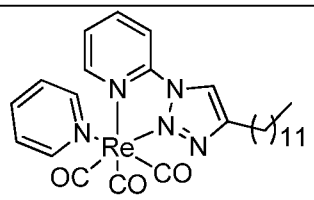
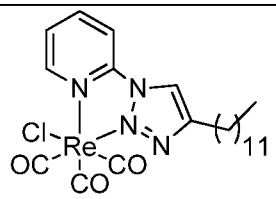
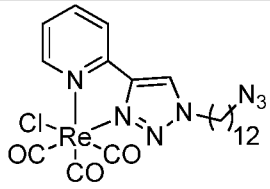
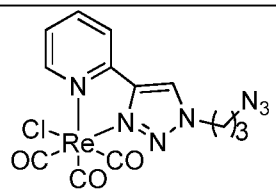
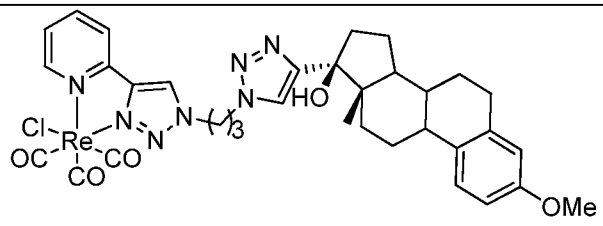
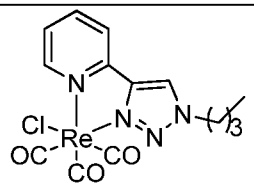
4	
5	
6	
7	
8	
9	
10	

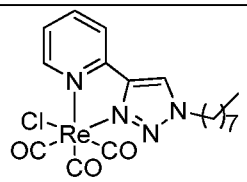
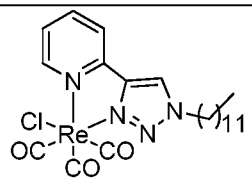
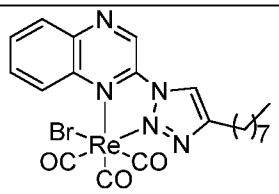
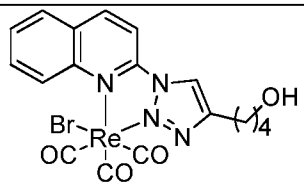
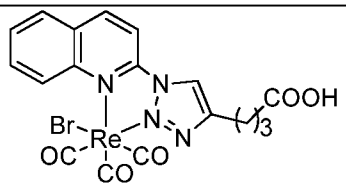
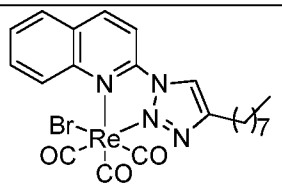
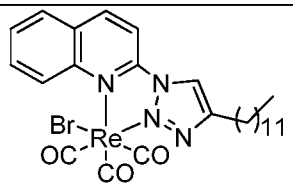
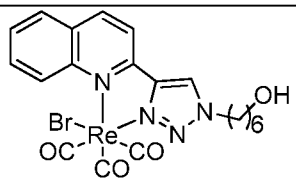
11	
12	 wherein "R" represents the arginine amino acid
13	 wherein "R" represents the arginine amino acid and "W" represents the tryptophan amino acid
14	 wherein "R" represents the arginine amino acid
15	 wherein "R" represents the arginine amino acid and "W" represents the tryptophan amino acid
16	

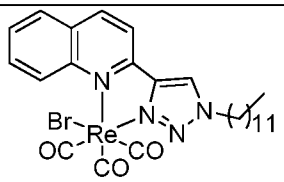
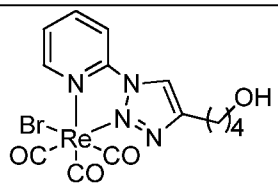
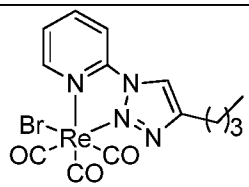
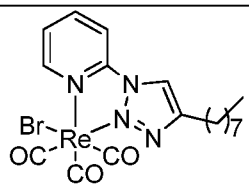
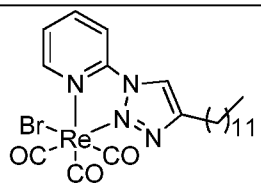
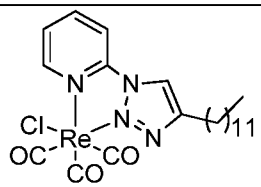
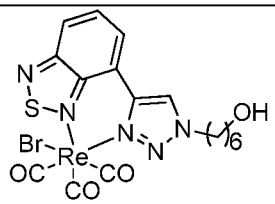
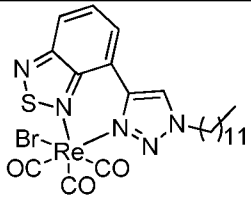
17	
18	
19	
20	
21	
22	
23	

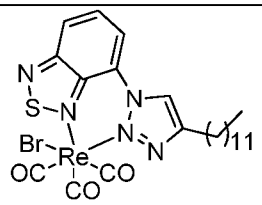
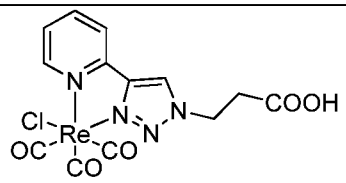
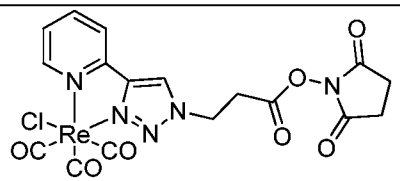
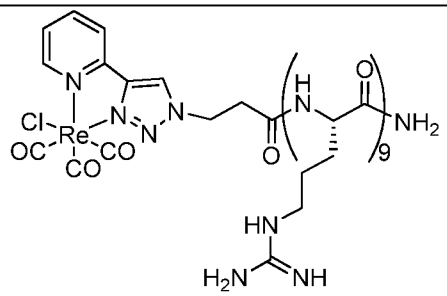
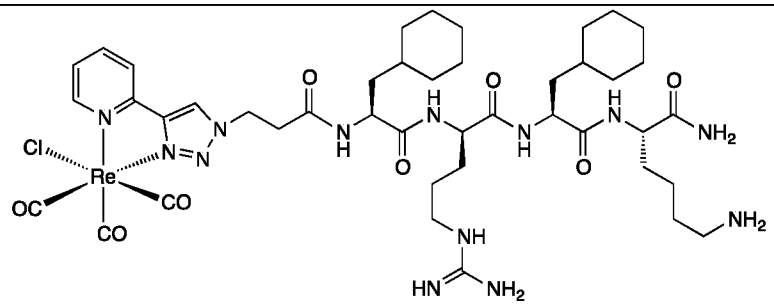
24	
25	
26	
27	
28	

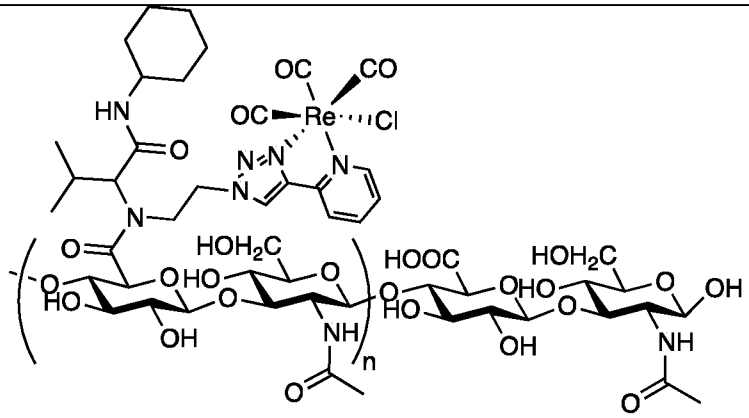
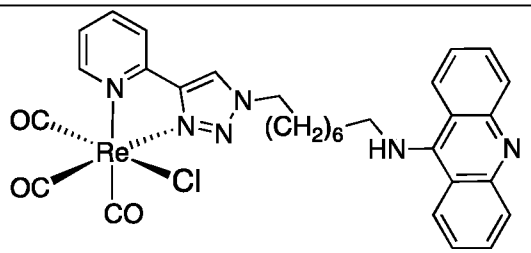
29	
30	
31	
32	
33	
34	
35	

36	
37	
38	
39	
40	
41	
42	

43	
44	
45	
46	
47	
48	
49	
50	

51	
52	
53	
54	
55	
56	
57	
58	

59	
60	
61	
62	
63	

64	 wherein n represents an integer corresponding to a hyaluronic acid having a molecular weight ranging from 400 kDa to 1000 kDa;
65	

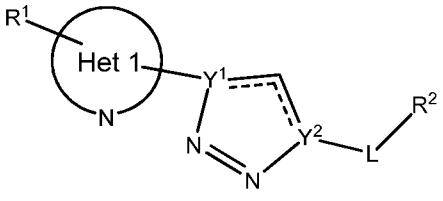
According to one embodiment, the XRF probe of the invention is selected from compounds n°1-62 of Table 1 above. According to one embodiment, the XRF probe of the invention is selected from compounds n°1-65 of Table 1 above. According to a specific embodiment, the XRF probe of the invention is selected from compounds n°1-37 of Table 1 above. According to a specific embodiment, the XRF probe of the invention is selected from compounds n°1-37 and 63-35 of Table 1 above. According to a specific embodiment, the XRF probe of the invention is selected from compounds n°38-62 of Table 1 above.

Process for manufacturing the XRF probes

The compounds of the invention may be prepared using any suitable reactions known by those skilled in the art.

The invention further relates to a process for manufacturing the compounds of the invention. According to one embodiment, the invention relates to a process for manufacturing a compound of formula I as defined above, comprising:

1) reacting a ligand of formula II:



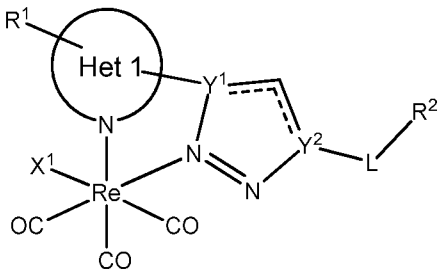
(II)

wherein **Het 1**, $\mathbf{Y^1}$, $\mathbf{Y^2}$, **L** and $\mathbf{R^2}$ are as defined in formula I;

with a rhenium-containing reactant of formula III

(III) $ReX^1(CO)_5$, wherein X^1 represents a halogen atom;

to afford compound of formula I':



(I')

wherein **Het 1**, $\mathbf{Y^1}$, $\mathbf{Y^2}$, **L** and $\mathbf{R^2}$ are as defined in formula I; and $\mathbf{X^1}$ represents halo;

2) and optionally:

- replacing $\mathbf{X^1}$ moiety by **X** as defined in formula I; and/or
- modifying and/or functionalizing –L-R^2;

to form a compound of formula I as defined above.

According to one embodiment, the optional step of replacement of $\mathbf{X^1}$ moiety by **X** as defined in formula I comprises reacting compound of formula I' with silver tetrafluoroborate and then adding a compound corresponding to **X** moiety.

According to one embodiment, the optional step of modification and/or functionalization of –L-R^2 includes protection/deprotection steps of reactive functions, coupling reactions of reactive R^2 groups with bioactive molecules, etc.

Use of the XRF probes

The present invention further relates to the use of a compound comprising at least one rhenium atom for performing XRF spectroscopy and/or XRF imaging. According to one embodiment, the invention relates to the use of a compound comprising at least one rhenium atom for performing XRF spectroscopy. According to one embodiment, the invention relates to the use of a compound comprising at least one rhenium atom for performing XRF imaging.

The present invention further relates to the use of the XRF probes of the invention for performing XRF spectroscopy and/or XRF imaging. According to one embodiment, the invention relates to the use of the XRF probes of the invention for performing XRF spectroscopy. According to one embodiment, the invention relates to the use of the XRF probes of the invention for performing XRF imaging.

The XRF imaging of a sample containing the XRF probe of the invention enables to map the presence of said XRF probe at different scales, either at a microscopic scale or at a macroscopic scale. The XRF probe of the invention may be quantified by XRF spectroscopy, by integration of its emission bands.

According to one embodiment, an appropriated excitation energy is applied to the sample, preferably of about 12 keV. According to one embodiment, the excitation of the sample may be performed using a synchrotron source of hard X-rays (5-32 keV). According to one embodiment, the detection of the XRF response may be performed using an XRF multi-element detector.

According to one embodiment, the XRF probes of the invention are used to perform XRF spectroscopy and/or XRF imaging on biological tissues, especially on cells. According to one embodiment, the invention relates to a method for imaging cellular environment comprising incubating cells in presence of a XRF probe of the invention. By “cellular environment” it is referred to any part of the cell, including cytoplasm, nucleus and membranes. The cytoplasm corresponds to the biological material present between the cellular membrane and the nucleus envelop. The cytoplasm comprises organelles and

structures in suspension in the cytosol, such as for example the Golgi apparatus, vesicles, endosomes, lysosomes, mitochondria or endoplasmic reticulum. According to one embodiment, "membranes" both refers to the internal and external parts of cell membranes. Membranes include extracellular and membranous receptors.

According to one embodiment, the invention relates to a method of imaging cellular environment by XRF spectroscopy, comprising:

- adding a XRF probe according to the invention to a sample containing at least one cell;
- incubating the sample for a time sufficient for the XRF probe to be loaded onto and/or into the cell;
- exciting the sample at an energy that generates a XRF response from the XRF probe;
- detecting the XRF response.

According to one embodiment, cells of potential interest for performing XRF imaging include, but are not limited to, primary culture of mammalian cells, cells dissociated from mammalian tissues, and bacteria. Cell types may include white blood cell, hepatocytes, pancreatic beta cells, neurons, smooth muscle cells, intestinal epithelial cells, cardiac myocytes, glial cells, and the like. Non-limitative examples of bacteria include Escherichia Coli, Lactobacillus, etc.

According to one embodiment, the XRF probe of the invention is functionalized. This refers to cases wherein R^2 moiety of the XRF probe of the invention represents a bioactive group. In this case, XRF imaging enables to locate the functionalized XRF probe of the invention in a biological sample or to study biological processes.

The bioactive group may be a moiety enabling to direct the XRF probe to a target of biological interest, especially direct the XRF probe to specific cell organelles. According to one embodiment, the bioactive group may be a biological molecule, such as for example a protein, an oligonucleotide, an antibody, a polysaccharide such as hyaluronic acid, a hormone. The bioactive group may also be a moiety that recognizes a target of biological interest, such as for example an agonist or an antagonist of a receptor, a peptide, a therapeutic agent.

According to one embodiment, the bioactive group is a superoxide dismutase (SOD) mimic, a cell penetrating peptide, a peptide targeting mitochondria, an inhibitor targeting carbonic anhydrase, a fluorophore.

According to a specific embodiment, the XRF probes of the invention enable the imaging of cell organelles, such as for example the Golgi apparatus of a cell or mitochondria; or targeting the nucleus of a cell.

In a specific embodiment, the –L-R^2 moiety of the XRF probe of the invention comprises a "ligand-directed acyl imidazole" (LDAI). In this case, the linker L comprises an acyl imidazole moiety that enables to covalently *in situ* label a biological target having a nucleophilic residue.

In a specific embodiment, the XRF probe of the invention, when having R^2 group being a N_3 reactive function, may be co-administered with modified biomacromolecules to operate intracellular click reaction, as suggested by Takei et al. (Takei et al., ChemComm, 2013, 49, 7313-7315). Especially, the probe of the invention may be administered with a bibenzylcyclooctyl-modified biomacromolecule. The click reaction between the azide function and the alkyne moiety of the modified biomacromolecule aims at retaining the probe in the cytosol of tested cells.

The invention further relates to the use of the XRF probes of the invention for multimodal imaging. According to one embodiment, the XRF probes of the invention are used for performing XRF spectroscopy and infrared spectroscopy; thereby providing XRF and IR bimodal images. According to one embodiment, the XRF probes of the invention are used for performing XRF spectroscopy and fluorescence spectroscopy; thereby providing XRF and fluorescence bimodal images. According to one embodiment, the XRF probes of the invention are used for performing XRF spectroscopy, infrared spectroscopy and fluorescence spectroscopy; thereby providing XRF, IR and fluorescence trimodal images.

In one embodiment, the invention does not relate to the use of a probe of formula I according to the invention to perform X-ray crystallography.

The present invention further relates to a kit for performing XRF spectroscopy and/or XRF imaging, comprising a XRF probe according to the invention. According to one embodiment, the present invention provides a kit for performing XRF imaging, comprising a XRF probe for imaging according to the invention. The kit of the invention may comprise a XRF probe of the invention either present as a pure compound, or in a suitable carrier composition, or dissolved in an appropriate stock solution. The kit may further comprise instructions for the use of the XRF probe of the invention. The kit may further comprise one or more additional components, such as an additional detection reagent. According to an embodiment, the kit further comprises calibration standards.

According to one embodiment, the XRF probe of the invention may be present in the kit associated with a surface, such as for example a chip, microplate well, or other solid or semi-solid matrix.

According to one embodiment, the kit further comprises buffers and/or stabilizers. According to another embodiment, the kit further comprises indicator solutions, blotters, culture media, cuvettes, and the like.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the X-ray fluorescence spectrum of compound n°1.

Figure 2 is a series of X-ray fluorescence images showing the localization of potassium (A), manganese (B) and rhenium (C) in a HT29 MD2 cell incubated with compound n°1 (100µM, 2h), cryofixed and freeze-dried. Excitation at 12 kev; integration time: 4s/pixel; pixel size: 200 × 200 nm^2.

Figure 3 is a series of X-ray fluorescence images showing the localization of calcium, zinc, phosphor and rhenium in CHO cells incubated with labelled proteins **Cys-NLS-HD-ReCl** or **Cys-HD-ReCl** of example 3. Excitation at 12 kev; integration time: 3s/pixel; pixel size: 500 × 500 nm^2.

EXAMPLES

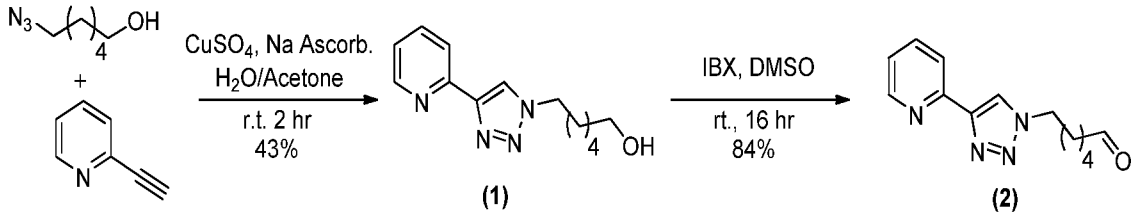
The present invention is further illustrated by the following examples.

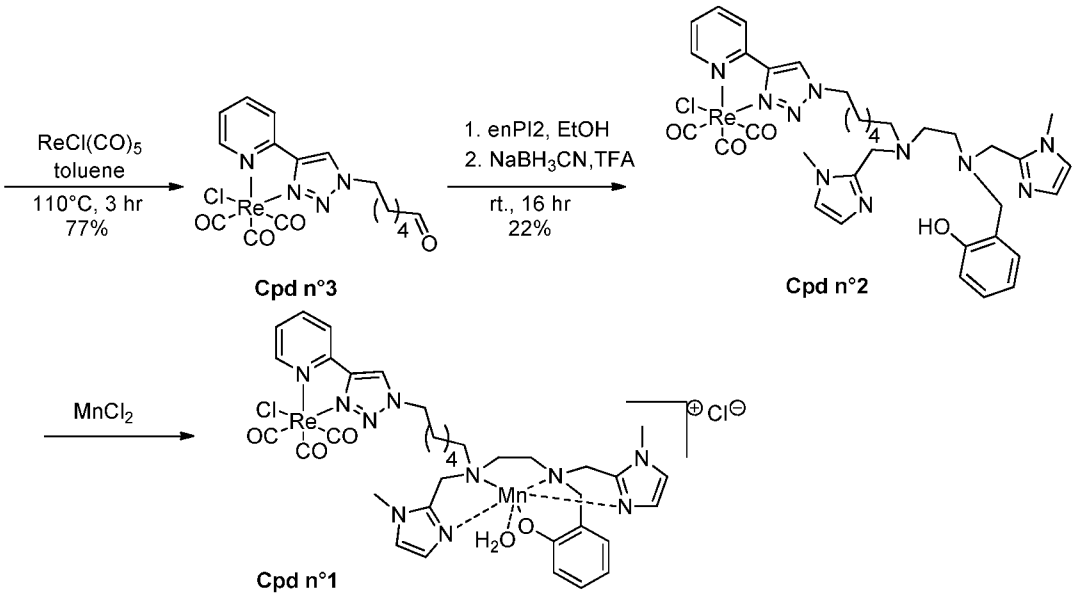
CHEMISTRY

Material and methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 or 600 spectrometer using solvent residuals as internal references. The following abbreviations are used: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), doublet of triplets (td), quintuplet (quint), sextuplet (sext), and multiplet (m). Mass spectrometry services were performed at the ICMMO (Université Paris Sud, Orsay, France) and at UMR7201 (Université Pierre et Marie Curie). The following abbreviations are used: MS (mass spectrometry), HRMS (high resolution mass spectrometry), electrospray (ESI), time-of-flight (TOF). TLC analysis was carried out on silica gel (Merck 60F-254) with visualization at 254 and 366 nm. Preparative flash chromatography was carried out with Merck silica gel (Si 60, 40–63 μm). Reagents and chemicals were purchased from Sigma-Aldrich, Alfa Aesar, or Strem Chemicals. Dry solvents (dichloromethane (CH_2Cl_2 or DCM), toluene, tetrahydrofuran(THF), and dimethylformamide (DMF)) were purchased from Sigma and used without further purification. UV–vis absorption spectra were recorded on a Varian Cary 300 Bio spectrophotometer, luminescence emission spectra on a Jasco FP-8300 spectrofluorometer, and IR spectra on a Perkin-Elmer Spectrum 100 FT-IR spectrometer. Analytical HPLC measurements were run on a Dionex Ultimate 3000 instrument using C8A or C18A ACE columns.

1. Re complexes functionalized with an aldehyde: application to the conjugation with a SOD (superoxide dismutase) mimic





Intermediate (1): 2-(1-(1-hydroxyhexyl)-1H-1,2,3-triazol-4-yl)pyridine

To a solution of 6-azidohexan-1-ol (0.593 g, 4.147 mmol, 1 eq) in acetone/water (90 mL, 2:1) were added 2-ethynylpyridine (0.42 mL, 4.147 mmol, 1 eq), copper sulfate (0.259 g, 1.037 mmol, 0.25 eq) and sodium ascorbate (0.409 g, 2.065 mmol, 0.5 eq) at room temperature. The reaction was stirred for 2h at room temperature under nitrogen atmosphere and then ammonia 28% was added (50 mL). The aqueous phase was extracted thrice with ethyl acetate (100 mL), the organic phase was dried over Na_2SO_4, filtered and concentrated. Column chromatography (AcOEt/MeOH 98:2, 850 mL) was applied to the organic fraction and afforded the product as a white-yellow powder (502 mg, 2.024 mmol, 49%). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ ppm: 8.58 (ddd, J = 4.9, 1.8, 0.9 Hz, 1H), 8.27 – 8.07 (m, 2H), 7.79 (td, J = 7.8, 1.8 Hz, 1H), 7.25 – 7.20 (m, 1H), 4.43 (t, J = 7.1 Hz, 2H), 3.63 (t, J = 6.3 Hz, 2H), 2.00 (dt, J = 19.1, 6.5 Hz, 2H), 1.67 – 1.30 (m, 4H).

Intermediate (2): 2-(1-(1-hexanal)-1H-1,2,3-triazol-4-yl)pyridine

To a solution of **(1)** (253 mg, 1.020 mmol, 1 eq) in DMSO (6 mL) was added 2-iodoxybenzoic acid (0.343 g, 1.225 mmol, 1.2 eq). The reaction was stirred under nitrogen atmosphere overnight at room temperature. Water (50 mL) was added and the resulting solution was filtered over a sintered filter and thoroughly washed with ethyl acetate. The organic phase was extracted thrice with ethyl acetate, washed with $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The desired product appeared as a yellow

solid and used in the next step without further purification (213 mg, 0.873 mmol, 86%). **^{1}H NMR** (300 MHz, $CDCl_3$): δ ppm: 9.76 (t, J = 1.5 Hz, 1H), 8.58 (ddd, J = 4.9, 1.8, 1.0 Hz, 1H), 8.22 – 8.08 (m, 2H), 7.78 (ddd, J = 7.9, 7.5, 1.8 Hz, 1H), 7.23 (ddd, J = 7.6, 4.9, 1.2 Hz, 1H), 4.43 (t, J = 7.0 Hz, 2H), 2.45 (td, J = 7.2, 1.5 Hz, 2H), 1.99 (dt, J = 14.8, 7.2 Hz, 2H), 1.68 (dt, J = 15.1, 7.3 Hz, 2H), 1.50 – 1.28 (m, 2H).

***Cpd n°3*: 2-(1-(1-hexanal)-1H-1,2,3-triazol-4-yl)pyridine chlorotricarbonylrhenium**

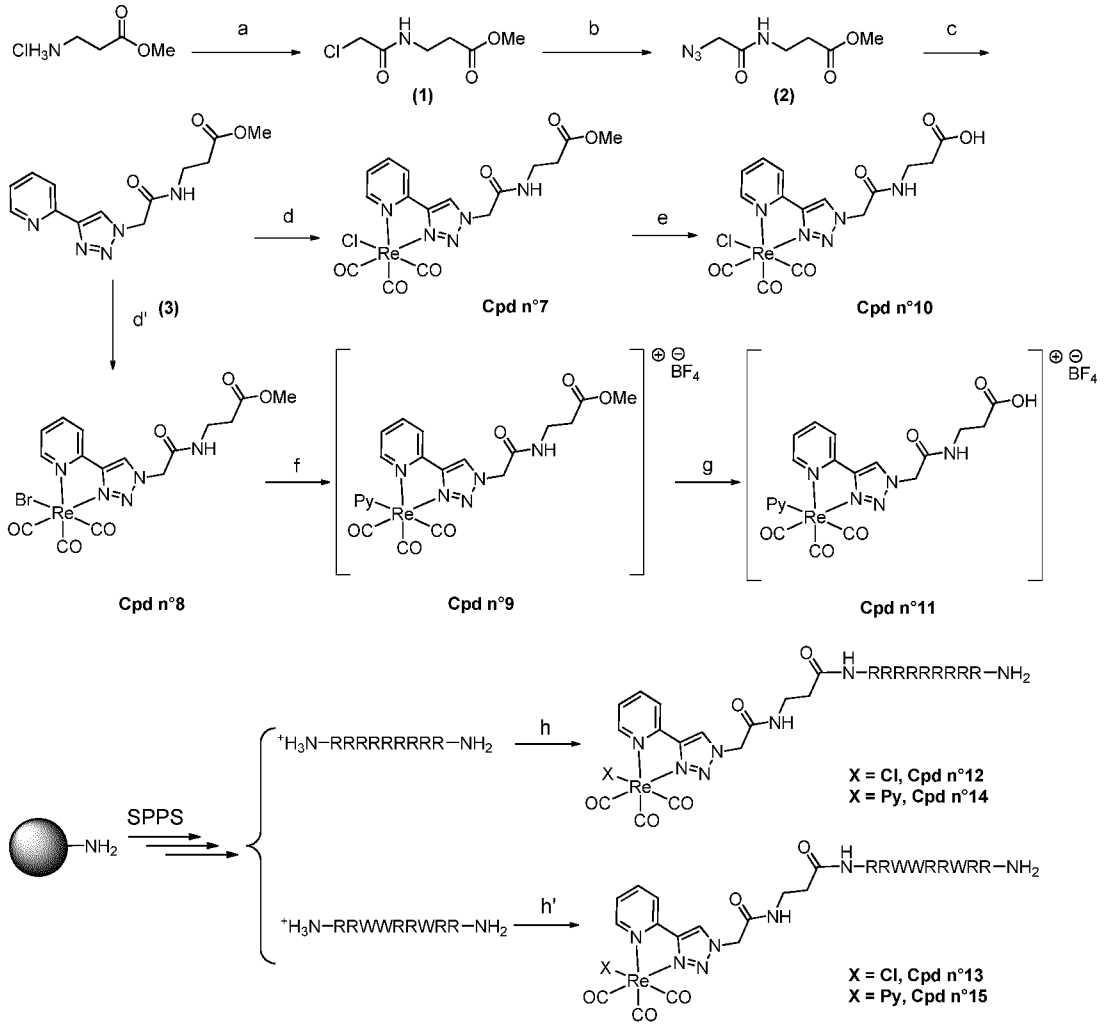
To a solution of **(2)** (0.210 g, 0.860 mmol, 1 eq) in toluene (10.5 mL) was added rhenium-pentacarbonyl chloride (0.311 g, 0.860 mmol, 1 eq) at room temperature. The reaction was refluxed at 110°C for 3 hours. After cooling the reaction, the solvent was evaporated. Column chromatography (EtOAc/MeOH 95/5, 750 mL) was applied to the brown solid and afforded the product the desired product as a yellow oil (364 mg, 77%). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 9.79 (t, J = 1.2 Hz, 1H), 9.03 (d, J = 5.6 Hz, 1H), 8.29 (s, 1H), 8.05 – 7.70 (m, 2H), 7.51 – 7.35 (m, 1H), 4.69 – 4.27 (m, 2H), 2.62 – 2.40 (m, 2H), 2.04 (t, J = 7.4 Hz, 2H), 1.82 – 1.59 (m, 2H), 1.59 – 1.31 (m, 6H), 1.26 (s, 1H).

***Cpd n°2*: 2-(1-(1-hexEnPI2)-1H-1,2,3-triazol-4-yl)pyridine chlorotricarbonylrhenium**

To a solution of **Cpd n°3** (298 mg, 0.512 mmol, 1 eq) in absolute ethanol (26 mL) was added triethylamine (70 µL, 0.512 mmol, 1 eq) and **enPI2 (**Cisnetti et al., Eur. J. Inorg. Chem., 2007, 4472-4480) (0.512 mmol, 1 eq). The reaction was stirred under nitrogen atmosphere overnight. To this solution sodium cyanoborohydride (77.3 mg, 1.229 mmol, 2.4 eq) and trifluoroacetic acid (80 µL, 1.025 mmol, 2 eq) were added, the reaction was stirred overnight. The pH was set at 8±0.4 with a saturated solution of sodium hydrogenocarbonate, and ethanol was evaporated under reduced pressure. Then dichloromethane and water (4:1 v:v, 30 mL) were added and the pH was set at 9.6±0.2. The aqueous phase was extracted thrice with dichloromethane and the combined organic fractions were dried upon Na_2SO_4, filtered and concentrated. The resulting yellow oil was purified by preparative HPLC (acetonitrile/water + TFA 0.1%, gradient from 10 to 100 over 30 minutes, t_R = 5.9 min) to obtained 120 mg (26%) of the expected compound.

***Cpd n°1*:** The Mn(II) complex is then obtained by addition of 1 equivalent of $MnCl_2$ to **Cpd n°2**, as described by Cisnetti et al. (Eur. J. Inorg. Chem., 2007, 4472-4480).

2. Re complexes functionalized with a carboxylic acid group: application to the conjugation with Cell Penetrating Peptides (CPP) or with a Mitochondria Penetrating Peptide (MPP)



Intermediate (1): N-(2-chloroacetyl) β-alanine methyl ester

β-alanine methyl ester hydrochloride salt (1.33 g, 9.5 mmol, 1.2 equiv) was suspended in dry DCM (15 mL) under argon. Dry DIEA (3.5 mL, 20 mmol, 2.5 equiv) was added, and the suspension was cooled down in an ice bath. Chloroacetyl chloride (0.64 mL, 8 mmol, 1 equiv) was added dropwise at 0°C, and the reaction mixture was stirred for one hour at room temperature. The organic layer was then diluted with DCM (15 mL), washed once with 0.1N HCl aqueous solution (30 mL), once with 10% $NaHCO_3$ aqueous solution (30 mL) and once with brine (30 mL). It was then dried over $MgSO_4$, filtered, and concentrated to yield the expected compound as a colorless oil (0.947 g, 5.3 mmol, 66%).

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.17 (s, 1H), 4.03 (s, 2H), 3.72 (s, 3H), 3.58 (q, J = 6.0 Hz, 2H), 2.58 (t, J = 6.0 Hz, 2H). **^{13}C-NMR** (101 MHz, $CDCl_3$): δ 172.40, 166.14, 51.68, 42.40, 35.14, 33.32. **HRMS** (ESI+): m/z calculated for $[C_6H_{10}ClNO_3+Na]^+$ 202.02414, found 202.02438 error: 1.2 ppm.

Intermediate (2): N-(2-azidoacetyl) β-alanine methyl ester

Intermediate **(1)** (0.947 g, 5.3 mmol, 1 equiv) was dissolved in a 3:1 v:v mixture of acetone (15.6 mL) and water (5.2 mL). Sodium azide (0.69 g, 10.6 mmol, 2 equiv) and sodium iodide (0.079 g, 0.53 mmol, 0.1 equiv) were then added, and the mixture was stirred at 35°C (bath temperature) for 16 h. Acetone was removed by rotary evaporation and the solution was diluted with DCM (15 mL) and water (5 mL). The mixture was then decanted, the organic layer dried over $MgSO_4$, filtered and concentrated to yield compound 2 as a colorless oil (0.78 g, 4.2 mmol, 79%). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ 6.87 (s, 1H), 3.97 (s, 2H), 3.72 (s, 3H), 3.57 (q, J = 6.0 Hz, 2H), 2.57 (t, J = 6.0 Hz, 2H). **^{13}C-NMR** (101 MHz, $CDCl_3$): δ 172.18, 166.94, 52.00, 51.43, 34.67, 33.26.

Intermediate (3): 2-(1-acetamide-(N-3-methoxy-3-oxopropyl))-1H-1,2,3-triazol-4-yl)pyridine

Intermediate **(2)** (0.51 g, 2.74 mmol, 1 equiv) was dissolved in a 2:1 v:v mixture of acetone (36 mL) and water (18 mL). 2-ethynylpyridine (0.28 mL, 2.74 mmol, 1 equiv), copper sulfate (0.17 g, 0.69 mmol, 0.25 equiv) and sodium ascorbate (0.14 g, 0.69 mmol, 0.25 equiv) were then added, and the suspension was sonicated for a few minutes, during which a light brownish precipitate formed. The reaction mixture was then stirred for 2h at room temperature (until the solution turned greenish). The solution was then poured into a 28% ammonia solution and extracted three times with DCM. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated. The resulting brown solid was purified by silica gel column chromatography (DCM:EtOAc:MeOH 50:50:0 to 0:98:2 v:v:v) to yield compound 3 (0.56 g, 70%) as a white solid. **^{1}H-NMR** (400 MHz, CDCl3): δ 8.56 (ddd, J = 4.9, 1.8, 0.9 Hz, 1H), 8.28 (s, 1H), 8.10 (dt, J = 7.9, 1.0 Hz, 1H), 7.78-7.73 (m, 1H), 7.22 (ddd, J = 7.5, 4.9, 1.2 Hz, 1H), 6.88 (s, 1H), 5.11 (s, 2H), 3.62 (s, 3H), 3.53 (q, J = 6.2 Hz, 2H), 2.53 (t, J = 6.2 Hz, 2H). **^{13}C-NMR** (101 MHz, $CDCl_3$): δ 172.49, 165.19, 149.58, 136.98, 123.77, 123.16, 120.37, 53.16, 51.97, 35.42, 33.57.

HRMS (ESI+): m/z calculated for $[C_{13}H_{15}N_5O_3+H]^+$ 290.12477, found 290.1125527, error: 1.7 ppm.

Cpd n°7: 2-(1-acetamide-(N-3-methoxy-3-oxopropyl)-1H-1,2,3-triazol-4-yl)pyridine chlorotricarbonylrhenium

Intermediate **(3)** (36.3 mg, 0.10 mmol, 1 equiv) was dissolved in hot toluene (1.3 mL). $Re(CO)_5Cl$ (29 mg, 0.1mmol, 1 equiv) was added, and the suspension was refluxed for 6h. The reaction mixture was cooled down to room temperature, the resulting yellow precipitate was filtered and washed with cold toluene, to give pure compound **4** (59.5 mg, 0.10 mmol, 100 %) which was used without further purification. **^{1}H-NMR** (300 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 8.64 (d, J = 5.4 Hz, 1H), 8.50 (s, 1H), 7.80-7.72 (m, 2H), 7.21-7.16 (m, 1H), 4.93 (q, J = 14.5 Hz, 2H), 3.37 (s, 3H), 3.21 (t, J = 6.5 Hz, 2H), 2.27 (t, J = 6.6 Hz, 2H). **^{13}C-NMR** (101 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 197.55, 196.12, 189.27, 173.12, 165.43, 153.49, 149.87, 149.46, 140.71, 127.06, 126.64, 123.20, 53.88, 52.27, 36.11, 34.04. **HRMS (ESI+)**: m/z calculated for $[C_{16}H_{15}ClN_5O_6Re+Na]^+$ 616.01326, found 616.01333, error: 0.1 ppm.

Cpd n°10: 2-(1-acetamide-(N-2-carboxyethyl)-1H-1,2,3-triazol-4-yl)pyridine chloro tricarbonylrhenium

Methyl ester **Cpd n°7** (59.4 mg, 0.100 mmol, 1 equiv) was dissolved in a 2:1 mixture of THF/H_2O (1 mL). $LiOH \cdot H_2O$ (5.0 mg, 0.119 mmol, 1 equiv) was added, and the reaction mixture was stirred at room temperature for 45 min. THF was removed by rotary evaporation (bath temperature 45°C). A 1M aqueous solution of HCl was added dropwise to the resulting solution, until pH reached 1 (about 0.5 mL). 1 mL of distilled water was added, and the aqueous solution was extracted with EtOAc (15 mL). The organic layer was washed once with brine (2 mL). The aqueous layers were extracted twice more with EtOAc (15 mL). Organic layers were pooled, dried over Na_2SO_4, filtered and concentrated to give the desired compound **5** as a yellow powder (46.5 mg, 0.080 mmol, 80%). **^{1}H-NMR** (400 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 8.65 (d, J = 5.4 Hz, 1H), 8.51 (s, 1H), 7.80-7.74 (m, 2H), 7.19 (td, J = 6.3, 1.4 Hz, 1H), 4.95 (q, J = 19.9 Hz, 2H), 3.22 (t, J = 6.6 Hz, 2H), 2.25 (t, J = 6.6 Hz, 2H). **^{13}C-NMR** (101 MHz, $CDCl_3$/MeOD 1:1 v:v): δ

196.67, 195.10, 188.44, 173.64, 164.45, 152.79, 149.18, 148.71, 139.74, 125.94, 125.79, 122.21, 52.95, 35.40, 33.17. **HRMS (ESI+)**: m/z calculated for $[C_{15}H_{13}ClN_5O_6Re+Na]^+$ 601.99761, found 601.99786, error: 0.4 ppm.

Cpd n°8: 2-(1-acetamide-(N-3-methoxy-3-oxopropyl)-1H-1,2,3-triazol-4-yl)pyridine bromotricarbonylrhenium

Intermediate **(3)** (146 mg, 0.50 mmol, 1 equiv) was dissolved in hot toluene (4 mL). $Re(CO)_5Br$ (205 mg, 0.5 mmol, 1 equiv) was added, and the suspension was refluxed for 6 h. The reaction mixture was cooled down to room temperature, the resulting yellow precipitate was filtered and washed with cold toluene, to give pure compound **6** (323 mg, 0.5 mmol, 100 %). **^{1}H-NMR** (300 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 8.99 (d, J = 5.5 Hz, 1H), 8.84 (s, 1H), 8.10-8.08 (m, 2H), 7.53-7.48 (m, 1H), 5.27 (q, J = 17.4 Hz, 2H), 3.70 (s, 3H), 3.54 (t, J = 6.4 Hz, 2H), 2.60 (t, J = 6.5 Hz, 2H). **^{13}C-NMR** (75 MHz, $CDCl_3$/MeOD 1:1 v:v): δ 172.10, 164.40, 152.74, 149.02, 148.53, 139.35, 125.73, 125.49, 121.95, 52.69, 51.17, 35.10, 32.99. **HRMS** (ESI+): m/z calculated for $[C_{16}H_{15}BrN_5O_6Re+Na]^+$ 659.96275, found 659.96321, error: 0.7 ppm.

Cpd n°9: 2-(1-acetamide-(N-3-methoxy-3-oxopropyl)-1H-1,2,3-triazol-4-yl)pyridine pyridiumtricarbonylrhenium tetrafluoroborate salt

Rhenium tricarbonyl bromide **Cpd n°8** (252.3 mg, 0.394 mmol, 1 equiv) was dissolved in acetonitrile (49 mL) under argon. Silver tetrafluoroborate (78.9 mg, 0.405 mmol, 1 equiv) was added and the mixture was refluxed for 5 h, resulting in the formation of a fine dark suspension. Solvent was evaporated to dryness, and the residue was dissolved in THF (58 mL). Pyridine (96 µL, 1.18 mmol, 3 equiv) was added, and the reaction mixture was refluxed for 20 h. The suspension was filtered over celite and the solvent was removed by rotary evaporation. The crude was co-evaporated three times with toluene, dissolved in the minimal amount of methanol and precipitated in diethyl ether, to give the desired compound as a yellow powder (224.7 mg, 0.310 mmol, 79 %). **^{1}H-NMR** (300 MHz, MeOD): δ 8.39 (dd, J = 6.5, 1.5 Hz, 2H), 8.26 (td, J = 7.8, 1.5 Hz, 1H), 8.17-8.13 (m, 1H), 7.92 (tt, J = 7.7, 1.5 Hz, 1H), 7.75 (ddd, J = 7.6, 5.6, 1.5 Hz, 1H), 7.43-7.38 (m, 2H), 5.47 (s, 2H), 3.71 (s, 3H), 3.56 (t, J = 6.4 Hz, 2H), 2.63 (t, J = 6.6 Hz, 2H).

^{13}C-NMR (75 MHz, MeOD): δ 173.67, 166.35, 155.04, 153.40, 150.60, 150.51, 142.75, 141.06, 129.06, 128.68, 127.99, 124.33, 54.72, 52.29, 36.75, 34.45. **HRMS (ESI+)**: m/z calculated for $[C_{21}H_{20}ClN_6O_6Re]^+$ 637.09684, found 637.09735, error: 0.8 ppm.

***Cpd n°11*: 2-(1-acetamide-(N-2-carboxyethyl)-1H-1,2,3-triazol-4-yl)pyridine pyridiniumtricarbonylrhenium tetrafluoroborate salt**

Compound **Cpd n°9** was prepared according to the same procedure as compound 5: compound 7 (148.6 mg, 0.205 mmol, 1 equiv) was dissolved in a 2:1 v:v mixture of THF (1.3 mL) and water (0.65 mL). Lithium hydroxide monohydrate (8.8 mg, 0.210 mmol, 1 equiv) was then added, and the reaction mixture was stirred for 1 h at room temperature. THF was removed by rotary evaporation, and the solution was acidified to pH<1 with 1N HCl aqueous solution. The aqueous layer was extracted three times with EtOAc (5 mL). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated to give compound **8** (127.2 mg, 0.179 mmol, 87%) as a yellow powder. **^{1}H-NMR** (300 MHz, MeOD): δ 9.31 (d, J = 5.4 Hz, 1H), 8.98 (s, 1H), 8.41-8.39 (m, 2H), 8.28 (t, J = 7.8 Hz, 1H), 8.18-8.15 (m, 1H), 7.94 (d, J = 7.6 Hz, 1H), 7.79-7.74 (m, 1H), 7.42 (t, J = 6.7 Hz, 2H), 5.48 (s, 2H), 3.72 (s, 3H), 3.58 (t, J = 6.5 Hz, 2H), 2.64 (t, J = 6.5 Hz, 2H). **^{13}C-NMR** (101 MHz, MeOD): δ 196.64, 195.17, 192.13, 174.95, 166.27, 154.94, 153.32, 150.49, 150.43, 142.69, 140.98, 128.98, 128.65, 127.93, 124.28, 54.71, 36.81, 34.42. **HRMS** (ESI+): m/z calculated for $[C_{20}H_{18}ClN_6O_6Re]^+$ 623.08119, found 623.08152, error: 0.5 ppm.

Cpd n°15*: *R6W3PytaPy

A solution of EDC in DMF (33.3 mg/mL, 187 µL, 32.5 µmol, 1.5 equiv) were added to compound **Cpd n°11** (15.4 mg, 21.7 µmol, 1 equiv). The mixture was stirred for 20 min before addition of a solution of R6W3 in DMF (462 mg/mL, 108 µL, 21.6 µmol, 1 equiv) and of DIEA (7.5 µL, 43.3 µmol, 2 equiv). The mixture was stirred overnight at room temperature and the crude was purified by HPLC.

Cpd n°13: R6W3PytaCl

The procedure is similar to the one used for **Cpd n°15**. A solution of EDC in DMF (33.3 mg/mL, 187 µL, 32.5 µmol, 1.5 equiv) were added to compound **Cpd n°10** (12.6 mg, 21.7 µmol, 1 equiv). The mixture was stirred for 20 min before addition of a solution of R6W3 in DMF (462 mg/mL, 108 µL, 21.6 µmol, 1 equiv) and of DIEA (7.5 µL, 43.3 µmol, 2 equiv). The mixture was stirred overnight at room temperature and the crude was purified by HPLC. The collected pure fraction was frozen and freeze-dried immediately after purification in order to prevent the exchange of the chloride.

Cpd n°14: R9PytaPy

Compound **Cpd n°11** (17.7 mg, 24.5 µmol, 1.5 equiv) and N-hydroxysuccinimide (4.8 mg, 42.6 µmol, 2.5 equiv) were dissolved in a solution of DCC in DMF (4.13 mg/mL, 850 µL, 17.7 µmol, 1.1 equiv). The mixture was stirred for 20 min, followed by addition of an aqueous solution of R9 (50 mg/mL, 680 µL, 16.8 µmol, 1 equiv) and DIEA (8.5 µL, 49.1 µmol, 2.9 equiv). The reaction mixture was stirred overnight at room temperature and then purified by HPLC.

Cpd n°12: R9PytaCl

Compound **Cpd n°10** (16.1 mg, 27.7 µmol, 1.6 equiv) and N-hydroxysuccinimide (3.9 mg, 33.8 µmol, 2 equiv) were dissolved in a solution of DCC in DMF (4.13 mg/mL, 850 µL, 17.7 µmol, 1.1 equiv). The mixture was stirred for 20 min, followed by addition of an aqueous solution of R9 (50 mg/mL, 680 µL, 16.8 µmol, 1 equiv) and DIEA (8.5 µL, 49.1 µmol, 2.9 equiv). The reaction mixture was stirred overnight at room temperature and then purified by HPLC. Right after purification, the collected pure fraction was frozen and freeze-dried to prevent the exchange of the chloride.

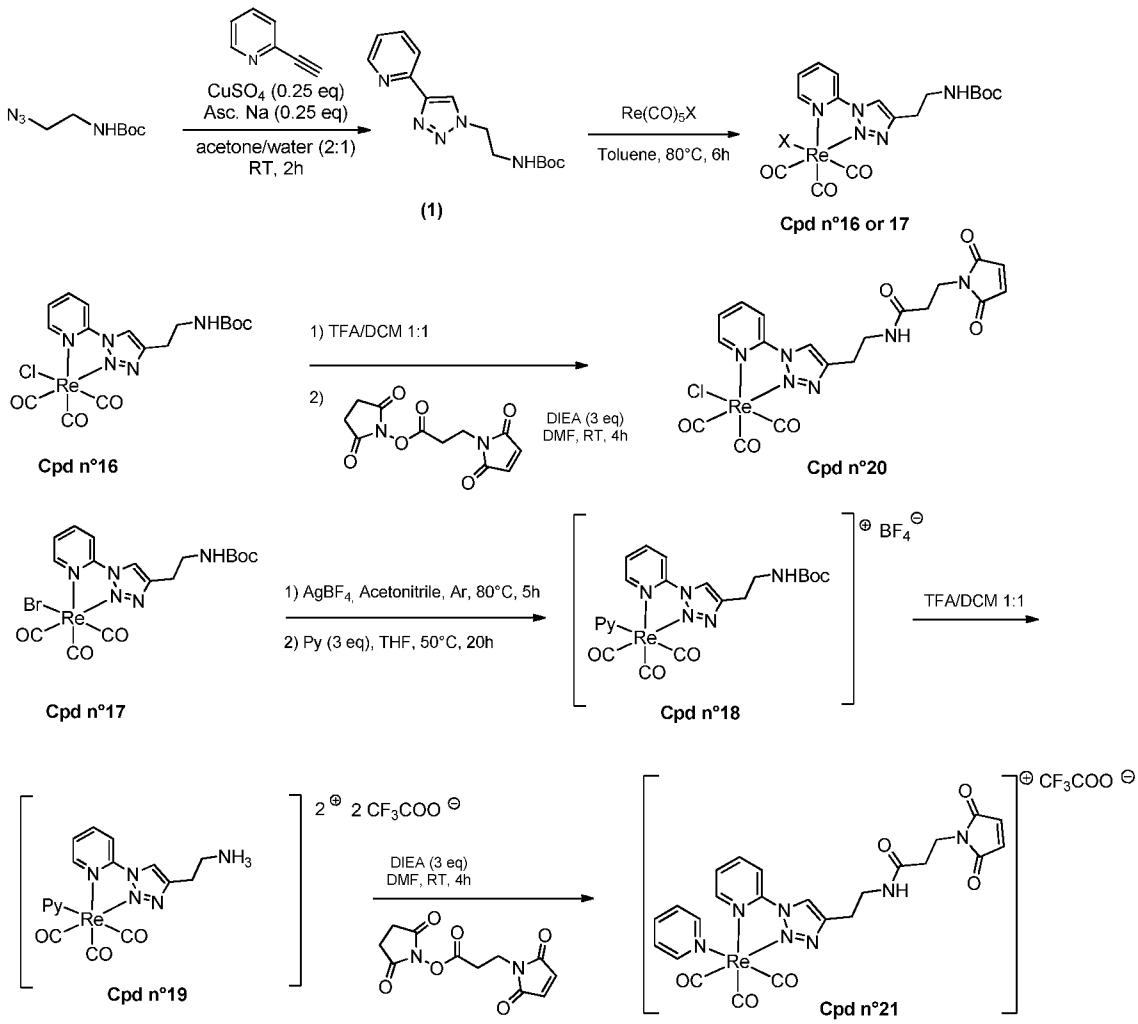
Cpd n°63: MPP-PytaCl

MPP synthesis: The peptide was assembled by stepwise solid-phase synthesis using standard protocols for Fmoc chemistry: amino acid activation with HBTU/HOBt, Fmoc deprotection with 20% piperidine in N-methyl-2-pyrrolidone. The peptide was cleaved

from the resin by treatment with a 95/2.5/2.5 mixture of TFA/water/triisopropylsilane or 95/5 mixture of TFA/ triisopropylsilane for 3 hours at room temperature, and precipitation in diethylether. It was purified by preparative HPLC (preparative C18 column, acetonitrile/ H_2O 15/85 to 50/50 over 30 min containing 0.1% of TFA, flow: 16 mL/min). The pure fractions of the expected peptide were combined and freeze-dried to afford the desired compound. **MS** (Maldi+, HCCA matrix): *m/z* 1078 $(M\text{-}Cl+H)^+$.

Compound **Cpd n°63** was then obtained by a procedure similar to the one used for compound **Cpd n°12 or Cpd n°13**.

3. Re complexes functionalized with a maleimide: application to the conjugation with the cysteine of a protein



Intermediate (1): 2-(1-(2-ethyl-N-(tert-Butoxycarbonyl)amine)-1H-1,2,3-triazol-4-yl)pyridine

N-(2-azidoethyl)-N-(tert-Butoxycarbonyl)amine (568 mg, 3.05 mmol, 1 equiv.) was dissolved in a mixture of acetone and water (30 mL acetone:water 2:1 v:v). Anhydrous copper sulfate (131 mg, 0.82 mmol, 0.25 equiv.) and sodium ascorbate (153.8 mg, 0.77 mmol, 0.25 equiv.), followed by 2-ethynylpyridine (310 µL, 3.07 mmol, 1 equiv.), were added. The mixture was sonicated for a few minutes, and reaction mixture was stirred at room temperature for 2 hours. A light brown precipitate formed. Acetone was removed by rotary evaporation, and the aqueous layer was diluted with 28% aqueous ammonia solution. The aqueous layer was extracted once with DCM. The organic layer was then washed once more with 28% ammonia solution, and once with brine. It was then dried over Na_2SO_4, filtered and concentrated. The resulting brown solid was purified by automated silica gel flash column chromatography (0-100% EtOAc in cyclohexane) to yield the desired compound as a white solid (692 mg, 2.39 mmol, 78%). **^{1}H-NMR** (300 MHz, $CDCl_3$) δ (ppm): 8.33 (d, J = 4.6 Hz, 1H), 8.01 (s, 1H), 7.90 (d, J = 7.9 Hz, 1H), 7.57 (td, J = 7.7, 1.7 Hz, 1H), 7.03 (ddd, J = 7.5, 4.9, 0.9 Hz, 1H), 5.99 (br, 1H), 4.41 (t, J = 5.4 Hz, 2H), 3.56 (q, J = 5.4 Hz, 2H), 1.30 (s, 9H). **^{13}C-NMR** (75 MHz, $CDCl_3$) δ (ppm): 155.91, 149.74, 149.01, 147.79, 136.60, 122.74, 119.82, 79.41, 50.11, 40.57, 28.19.

<u>Cpd n°16</u>: 2-(1-(2-ethyl-N-(tert-Butoxycarbonyl)amine)-1H-1,2,3-triazol-4-yl)pyridine chlorotricarbonylrhenium [Re(CO)$_3$(Cl)(PytaNHBoc)]

The ligand **(1)** (49.5 mg, 0.171 mmol, 1 equiv.) was dissolved in warm toluene (5 mL). Rhenium pentacarbonyl chloride (62.9 mg, 0.174 mmol, 1 equiv.) was added, and the mixture was heated at 80°C (bath temperature) for 6 hours, during which a yellow precipitate formed. The yellow precipitate was filtered and washed with cold toluene to obtain the desired compound (99.7 mg, 0.168 mmol, 98%). **^{1}H-NMR** (300 MHz, $CDCl_3$:MeOD 1:1 v:v) δ (ppm): 8.95 (d, J = 5.5 Hz, 1H), 8.77 (s, 1H), 8.10-8.02 (m, 2H), 7.48 (ddd, J = 6.8, 5.6, 2.1 Hz, 1H), 4.64-4.60 (m, 2H), 3.62 (t, J = 5.8 Hz, 2H), 1.38 (s, 9H). **^{13}C-NMR** (75 MHz, $CDCl_3$:MeOD 1:1 v:v) δ (ppm): 197.3, 195.8, 188.8, 157.4,

153.7, 150.0, 149.4 , 140.4, 126.5, 125.6, 122.9, 80.5, 52.4, 40.5, 28.5. **HRMS** (ESI+): *m/z* calculated for $[C_{17}H_{19}ClN_5O_5Re+Na]^+$ 616.04965, found 616.05007, error: 0.7 ppm.

***Cpd n°20:* 2-(1-(2-ethyl-N-(2,5-dihydro-2,5-dioxo-H-Pyrrole-1-propanamide))-1H-1,2,3-triazol-4-yl)pyridine chlorotricarbonylrhenium [$Re(CO)_3$(Cl)(PytaNH-maleimide)]**

[$Re(CO)_3$(Cl)(PytaNHBoc)] derivative **Cpd n°16** (30.5 mg, 51.3 µmol, 1.3 equiv.) was dissolved in DCM (1 mL) and TFA (1 mL) was added drop wise. The reaction mixture was stirred at room temperature for 1 hour, then precipitated in cold Et_2O. The resulting solid was dried under vacuum, put under argon, and dissolved in dry DMF (1.2 mL). 3-maleimidopropionic acid N-hydroxysuccinimide ester (10.6 mg, 39.8 µmol, 1 equiv.), then DIEA (27 µL, 156 µmol, 3.9 equiv.), were added. Reaction mixture was stirred overnight at room temperature. DMF was removed by rotary evaporation and the resulting crude was directly purified by HPLC. The collected fraction was frozen in liquid nitrogen immediately after purification to prevent exchange of the chloride ligand. **^{1}H-NMR** (400 MHz, $CDCl_3$:MeOD, 1:1 v:v) δ (ppm): 8.96 (ddd, J = 5.6, 1.4, 0.9, 1H), 8.83 (s, 1H), 8.10-8.08 (m, 1H), 8.07 (dd, J = 1.7, 0.9 Hz, 1H), 7.51 (ddd, J = 7.3, 5.6, 1.7 Hz, 1H), 6.72 (s, 2H), 4.64 (t, J = 5.7 Hz, 2H), 3.76-3.72 (m, 4H), 2.42 (td, J = 6.8 Hz, 1.0, 2H). **^{13}C-NMR** (101 MHz, $CDCl_3$:MeOD 1:1 v:v) δ (ppm): 197.4, 195.9, 189.3, 172.9, 171.4, 153.7, 150.0, 149.5 , 140.5, 134.9, 126.6, 126.0, 122.9, 51.7, 39.5, 35.17, 34.99. **HRMS** (ESI+): *m/z* calculated for $[C_{19}H_{16}ClN_6O_6Re+Na]^+$ 667.02416, found 667.02445, error: 0.4 ppm.

***Cpd n°17:* 2-(1-(2-ethyl-N-(tert-Butoxycarbonyl)amine)-1H-1,2,3-triazol-4-yl)pyridine bromotricarbonylrhenium [$Re(CO)_3$(Br)(PytaNHBoc)]**

The ligand **(1)** (206 mg, 0.712 mmol, 1 equiv.) was dissolved in warm toluene (30 mL, 80°C). Rhenium pentacarbonyl bromide (289 mg, 0.712 mmol, 1 equiv.) was added, and the mixture was heated at 80°C (bath temperature) for 6 hours, during which a yellow precipitate formed. The yellow precipitate was cooled to room temperature, filtered and washed with cold toluene to obtain the desired compound (428 mg, 0.669 mmol, 94%). **^{1}H-NMR** (400 MHz, $CDCl_3$:MeOD 1:1 v:v) δ (ppm): 9.30 (d, J = 5.4 Hz, 1H), 9.09 (s,

1H), 8.43-8.36 (m, 2H), 7.82 (ddd, J = 7.3, 5.6, 1.7 Hz, 1H), 4.96 (t, J = 5.8 Hz, 2H), 3.96 (t, J = 5.6 Hz, 2H), 1.73 (s, 9H). **^{13}C-NMR** (101 MHz, $CDCl_3$:MeOD 1:1 v:v) δ (ppm): 197.25, 195.68, 188.78, 157.44, 153.71, 149.98, 149.40, 140.33, 126.47, 125.57, 122.84, 80.48, 52.37, 40.53, 28.53. **HRMS** (ESI+): *m/z* calculated for $[C_{17}H_{19}BrN_5O_5Re+Na]^+$ 659.99913, found 659.99998, error: 1.0 ppm.

***Cpd n°18*: 2-(1-(2-ethyl-N-(tert-Butoxycarbonyl)amine)-1H-1,2,3-triazol-4-yl)pyridine pyridiniumtricarbonylrhenium tetrafluoroborate salt $[Re(CO)_3(Py)(PytaNHBoc)]^+BF_4^-$**

Cpd n°17 (88.3 mg, 0.138 mmol, 1 equiv.) was dissolved in acetonitrile (25 mL) under argon. Silver tetrafluoroborate (29.5 mg, 0.138 mmol, 1 equiv.) was added, and the reaction mixture was stirred for 3 days at 65°C (bath temperature), during which a dark precipitate formed. Solvent was then removed by rotary evaporation, and the solid was dried under vacuum. It was then dissolved in non-distilled tetrahydrofuran (20 mL), and pyridine (35 µL, 0.434 mmol, 3 equiv.) was added. The reaction mixture was stirred 36h at 60°C. It was then cooled to room temperature and filtered over celite. The solvent was removed by rotary evaporation, and the resulting yellow oil was co-evaporated three times with toluene. The crude was then dissolved in the minimal amount of methanol and precipitated in cold diethyl ether to give compound (68.3 mg, 0.094 mmol, 68%) as a pale brown solid. **^{1}H-NMR** (400MHz, MeOD) δ (ppm): 9.29 (d, J = 5.5 Hz, 1H), 8.99 (s, 1H, 8.45, d, J = 5.1 Hz, 2H), 8.26 (td, J = 7.8 Hz, 1.2, 1H), 8.15 (d, J = 7.9 Hz, 1H), 7.93 (tt, J = 7.7, 1.5 Hz, 1H), 7.75 (ddd, J = 7.4, 5.9, 1.3 Hz, 1H), 7.42 (t, J = 7.1 Hz, 2H), 4.74-4.70 (m, 2H), 3.77-3.66 (m, 2H), 1.23 (s, 9H). **^{13}C-NMR** (101 MHz, MeOD) δ (ppm): 158.0, 155.0, 153.5, 150.76, 150.58, 142.7, 140.9, 128.6, 128.03, 127.86, 124.1, 80.4, 54.1, 41.3, 28.5. **HRMS** (ESI+): *m/z* calculated for $[C_{22}H_{24}N_6O_5Re]^+$ 637.13323, found 637.13369, error: 0.7 ppm.

Cpd n°19: **2-(1-(2-ethyl-N-(tert-Butoxycarbonyl)amine)-1H-1,2,3-triazol-4-yl)pyridine pyridiniumtricarbonylrhenium ditrifluoroacetate salt $[Re(CO)_3(Py)(PytaNH_3)]^{2+}$, 2 CF_3COO^-**

Boc-protected complex **Cpd n°18** (70 mg, 0.096 mmol, 1 equiv.) was dissolved in dichloromethane, and trifluoroacetic acid (0.5 mL) was added carefully to the stirred solution. The reaction mixture was stirred 30 min at room temperature, then solvent was removed under reduced pressure. The resulting crude was dissolved in the minimal amount of methanol and precipitated in diethyl ether to give the desired compound as a solid (64 mg, 83.7 µmol, 87%). **^{1}H-NMR** (400MHz, MeOD) δ (ppm): 9.35 (ddd, J = 5.6, 1.5, 0.8 Hz, 1H), 9.11 (s, 1H), 8.47-8.45 (m, 2H), 8.32 (dd, J = 7.8, 1.5 Hz, 1H), 8.22-8.20 (m, 1H), 7.97 (dd, J = 8.5, 7.0 Hz, 1H), 7.81 (ddd, J = 7.7, 5.6, 1.4 Hz, 1H), 7.46-7.43 (m, 2H), 5.08-4.97 (m, 2H), 3.77-3.67 (m, 2H).

Cpd n°21: ***2-(1-(2-ethyl-N-(2,5-dihydro-2,5-dioxo-H-Pyrrole-1-propanamide))-1H-1,2,3-triazol-4-yl)pyridine pyridiniumtricarbonylrhenium trifluoroacetate salt*** **$[Re(CO)_3(Py)(PytaNH\text{-}maleimide)]^+$, CF_3COO^-**

Cpd n°19 (61 mg, 79.8 µmol, 1.2 equiv.) was dissolved in dry DMF under argon. 3-maleimidopropionic acid N-hydroxysuccinimide ester (17.9 mg, 83.7 µmol, 1 equiv.) and DIEA (33 µL, 191 µmol, 2.8 equiv.) were added. The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, and the resulting oil was dilute in DCM and washed twice with aqueous citric acid (5%). The crude was then purified by HPLC (C8, 20 to 80% ACN (0.1% TFA) in 40 min). The product was obtained as a pale yellow powder (18.5 mg, 23.0 µmol, 34%). **^{1}H-NMR** (400 MHz, MeOD) δ (ppm): 9.29 (d, *J* = 5.6 Hz, 1H), 9.01 (s, 1H), 8.44-8.42 (m, 2H), 8.27 (td, *J* = 7.8, 1.5 Hz, 1H), 8.19-8.15 (m, 1H), 7.94 (tt, *J* = 7.7, 1.5 Hz, 1H), 7.75 (ddd, *J* = 7.7, 5.6, 1.4 Hz, 1H), 7.45-7.41 (m, 2H), 6.77 (s, 2H), 4.78-4.75 (m, 2H), 3.91-3.71 (m, 3H), 3.67 (td, *J* = 7.0, 2.4 Hz, 2H), 2.43 (td, *J* = 7.0, 0.6 Hz, 2H). **^{13}C-NMR** (101 MHz, MeOD) δ (ppm): 173.53, 172.11, 155.00, 153.51, 150.62, 142.70, 141.08, 135.47, 128.61, 128.01, 124.33, 53.45, 40.02, 35.56, 35.30. **HRMS** (ESI+): *m/z* calculated for $[C_{24}H_{21}ClN_7O_6Re]^+$ 688.10774, found 688.10903, error: 1.9 ppm.

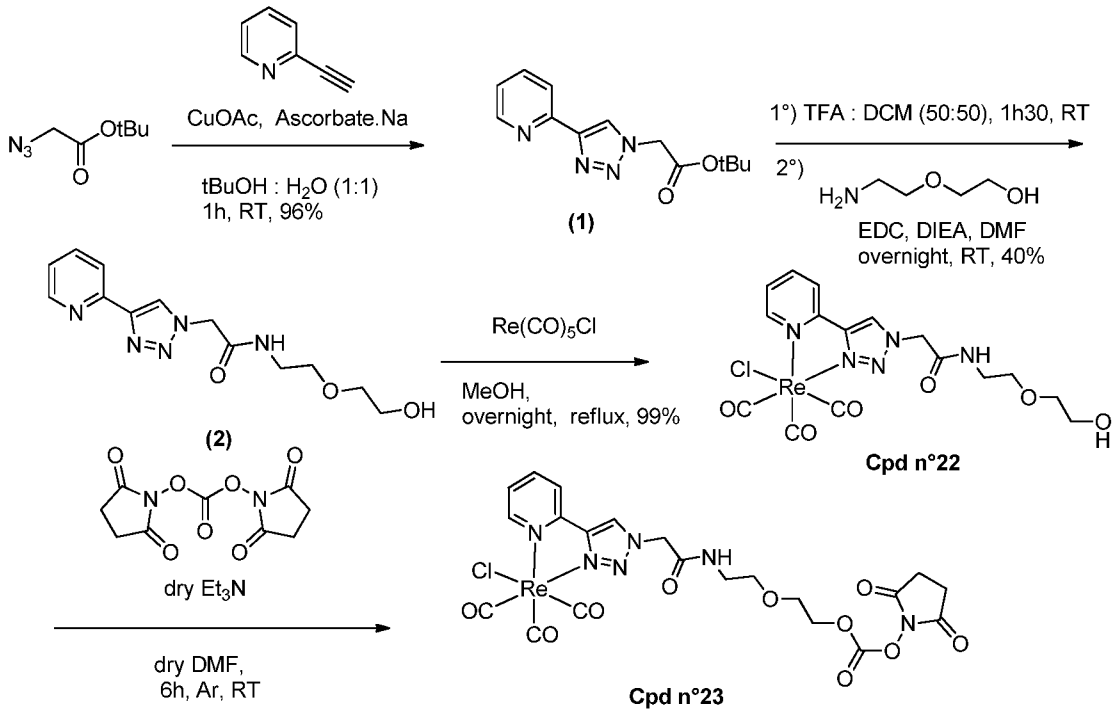
Labelling of proteins Cys-HD and Cys-NLS-HD

Engrailed homeodomain has been used as a model to label and image a protein inside cell using X-ray fluorescence. Two *Engrailed* homeodomain constructions were used: the first one consisted of the homeodomain, the second presented an extended sequence comprising a suspected nuclear localization signal (NLS). *Engrailed* homeodomains do not contain cystein, consequently a single cystein was introduced at the *N*-terminus of both proteins, in order to allow diverse protein labelling through thiol-maleimide coupling. The proteins (Cys-HD and Cys-NLS-HD) were expressed and purified.

A solution of protein (100-200µM) in reaction buffer (50 mM phosphate buffer, 150 mM NaCl, 10 mM EDTA, pH 6.7) was degased and put under argon. 1 equivalent of TCEP was added, and the solution wad incubated for 30 min-1h at room temperature. 5 equivalents of **Cpd n°20** ***([Re(CO)$_3$(Cl)(PytaNH-maleimide)])*** **or 21** (**[Re(CO)$_3$(Py)(PytaNH-maleimide)]$^+$, CF_3COO^-**) were then added (stock solution in DMSO, final concentration of DMSO <1% v:v), and the solution was incubated overnight at room temperature under inert atmosphere. Excess of reagent and TCEP were removed by ultrafiltration (cut 3kDa) with EDTA-free phosphate buffer. Labelled proteins Cys-NLS-HD-ReCl, Cys-HD-ReCl, Cys-NLS-HD-RePy and Cys-HD-RePy were obtained. XRF imaging of **Cys-NLS-HD-ReCl** and **Cys-HD-ReCl** in CHO cells is reported further below.

4. Re complexes functionalized with an activated carbonate: application to the conjugation with inhibitors of carbonic anhydrase IX (CAIX) for CAIX imaging by CAIX labeling.

4.1. Synthesis of Cpd n°22 and 23



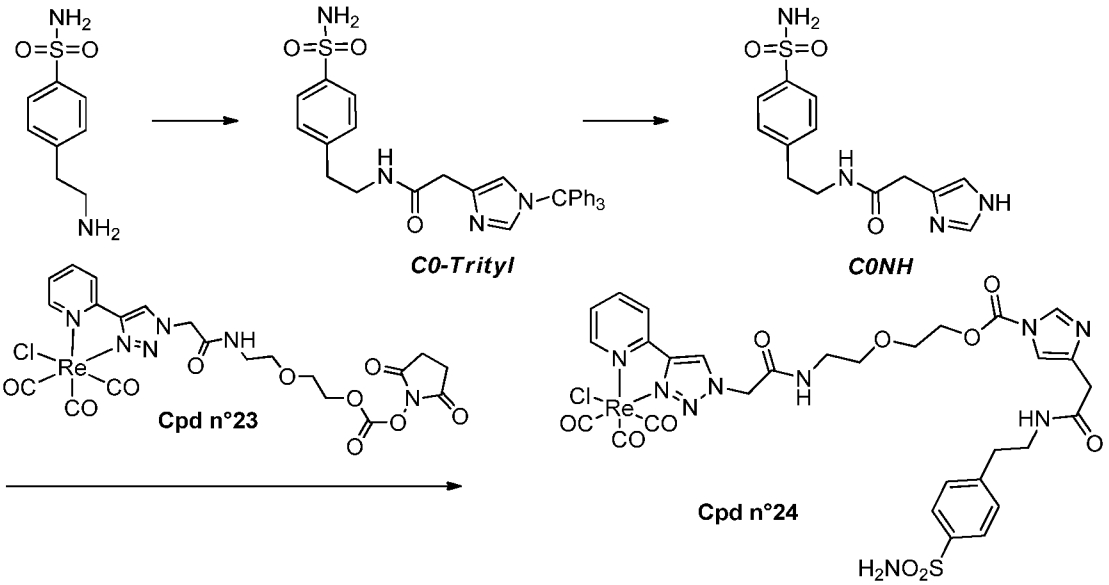
Intermediate (1): 2-(1-[acetic acid tert-butyl ester]-1H-1,2,3-triazol-4-yl)pyridine (PytaCOOtBu) – 2-azidoacetic acid tert-butyl ester (902.1 mg, 5.74 mmol, 1 equiv.) was dissolved in a 1:1 mixture of tBuOH (28 mL) and water (28 mL). Copper acetate (278 mg, 1.40 mmol, 0.25 equiv.), sodium ascorbate (276.8 mg, 1.40 mmol, 0.25 equiv.) and 2-ethynylpyridine (560 µL, 5.54 mmol, 0.96 equiv.) were successively added. The mixture was sonicated for a few tens of seconds, during which a pale brown precipitate formed. The mixture was then stirred at room temperature until the solution turned green (1h30). The solution was diluted with DCM (60 mL) and extracted once. The organic layer was washed twice with aqueous saturated (28%) ammonia solution (2 × 60 mL) and once with brine (60 mL). The aqueous layers were extracted once more with DCM (60 mL), then both organic layers were pooled, dried over Na_2SO_4, filtered and concentrated to the desired compound as a white solid (1.3821 g, 5.31 mmol, 96%). **^{1}H-NMR** (300 MHz, $CDCl_3$) δ (ppm): 8.42 (d, J = 4.7 Hz, 1H), 8.18 (s, 1H), 8.00 (d, J = 7.9 Hz, 1H), 7.60 (td, J = 7.7, 1.4 Hz, 1H), 7.06 (dd, J = 6.8, 5.3 Hz, 1H), 5.02 (s, 2H), 1.31 (s, 9H). **^{13}C-NMR** (75MHz, $CDCl_3$) δ (ppm): 164.99, 149.90, 149.19, 148.37, 136.58, 123.47, 122.63, 119.93, 83.43, 51.43, 27.67.

Intermediate (2) 2-(1-acetamide-(N-2-(2-hydroxyethoxy)ethyl)-1H-1,2,3-triazol-4-yl)pyridine (Pyta-OH) - Intermediate **(1)** (1.597 g, 6.1 mmol, 1 equiv.) was dissolved in DCM (20 mL), and TFA (20 mL) was added slowly to the solution. The mixture was stirred at room temperature for 2h. Solvents were co-evaporated three times with toluene (3 × 30 mL). EDC.HCl (1.61 g, 8.4 mmol, 1.4 equiv.) was added to the resulting solid, and both solids were dissolved in dry DMF (30 mL) under argon. After 5-10 min stirring, 2-(2-aminoethoxy)ethanol (0.86 mL, 8.6 mmol, 1.4 equiv.) then DIEA (3.2 mL, 18.5 mmol, 3 equiv.) were added. Reaction mixture was stirred overnight at room temperature, and solvent was removed. The resulting crude was purified by flash silica gel column chromatography (0 to 10% MeOH in DCM) to yield the desired compound as a white solid (721 mg, 2.5 mmol, 40%). **^{1}H-NMR** (300 MHz, $CDCl_3$:MeOD 1:1 v:v) δ (ppm): 8.29 (ddd, J = 5.0, 1.8, 0.9 Hz, 1H), 8.16 (s, 1H), 7.86 (dt, J = 8.0, 1.1 Hz, 1H), 7.61 (td, J = 7.8, 1.8 Hz, 1H), 7.07 (ddd, J = 7.6, 5.0, 1.2 Hz, 1H), 4.93 (s, 2H), 3.48-3.45 (m, 2H), 3.34-3.29 (m, 4H), 3.23-3.20 (m, 2H). **^{13}C-NMR** (75 MHz, $CDCl_3$:MeOD 1:1 v:v) δ (ppm): 165.46, 149.23, 148.74, 147.28, 137.39, 123.92, 123.02, 120.31, 71.88, 68.81, 60.77, 52.00, 39.27. **HRMS** (ESI+): *m/z* calculated for $[C_{13}H_{17}ClN_5O_3+Na]^+$ 314.12236, found 314.12230, error: -0.2 ppm.

Cpd n°22: ***2-(1-acetamide-(N-2-(2-hydroxyethoxy)ethyl)-1H-1,2,3-triazol-4-yl) pyridine chlorotricarbonyrhenium [Re(CO)$_3$(Cl)(Pyta-OH)]*** - Ligand Pyta-OH **(2)** (105.3 mg, 0.361 mmol, 1 equiv.) was dissolved in warm MeOH (1 mL). $Re(CO)_5Cl$ (133.1 mg, 0.368 mmol, 1 equiv.) was added, and the mixture was refluxed overnight. The yellow precipitate that formed during this time was filtered and dried to give the desired complex (214.2 mg, 0.359 mmol, 99%). **^{1}H-NMR** (400 MHz, $CDCl_3$:MeOD 1:1 v:v) δ (ppm): 8.96 (ddd, J = 5.6, 1.4, 1.0 Hz, 1H), 8.83 (s, 1H), 8.11-8.05 (m, 2H), 7.50 (ddd, J = 7.0, 5.6, 2.0 Hz, 1H), 5.28 (q, J = 20.7 Hz, 2H), 3.70 (t, J = 4.6 Hz, 2H), 3.60-3.56 (m, 4H), 3.48-3.45 (m, 2H). **^{13}C-NMR** (101 MHz, $CDCl_3$:MeOD 1:1 v:v) δ (ppm): 197.5, 195.9, 189.3, 165.3, 153.6, 150.1, 149.6, 140.5, 126.76, 126.58, 123.0, 72.8, 69.7, 61.7, 53.8, 40.3. **HRMS** (ESI+): *m/z* calculated for $[C_{16}H_{17}ClN_5O_6Re+Na]^+$ 618.02918, found 618.02891, error: 0.4 ppm.

Cpd n°23: ***2-(1-acetamide-(N-2-(((((2,5-dioxo-1-pyrrolidinyl)oxy)carbonyl)oxy) ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)pyridine chlorotricarbonylrhenium [Re(CO)$_3$(Cl) (Pyta-OC(O)ONHS)]*** - Rhenium complex **Cpd n°22** (149 mg, 0.25 mmol, 1 equiv.) and *N,N'*-disuccinimidyl carbonate (DSC) (187.4 mg, 0.73 mmol, 2.9 equiv.) were dissolved in dry DMF (2.4 mL) under Argon. Dry Et_3N (99.3 µL, 0.71 µmol, 2.9 equiv.) was added and the mixture was stirred at room temperature. After 2h30, more DSC was added (121.8 mg, 0.475 mmol, 1.9 equiv.) and the mixture was stirred at 50°C (bath temperature) for another 3h30. DMF was removed by rotary evaporation. The resulting crude was dissolved in EtOAc and washed once with a saturated aqueous solution of $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and concentrated. It was then purified by silica gel column chromatography (0 to 3 to 5 to 10% MeOH in DCM) to yield the desired compound as a yellow solid containing traces of impurities (106 mg). **^{1}H-NMR** (300 MHz, $CDCl_3$) δ (ppm): 8.96 (d, J = 5.3 Hz, 1H), 8.60 (s, 1H), 8.04-7.95 (m, 2H), 7.41 (ddd, J = 7.1, 5.6, 1.7 Hz, 1H), 5.14 (q, J = 16.9 Hz, 2H), 4.48 (tdt, J = 13.0, 8.9, 4.4 Hz, 2H), 3.73 (t, J = 4.3 Hz, 2H), 3.58 (t, J = 4.6 Hz, 2H), 3.50-3.45 (m, 2H), 2.88 (s, 4H). **^{13}C-NMR** (75MHz, $CDCl_3$) δ (ppm): 197.1, 196.3, 188.9, 169.7 (2C), 164.2, 153.1, 151.7, 149.4, 148.8, 139.8, 126.05, 125.93, 122.6, 69.9, 69.5, 68.4, 53.4, 39.9, 25.76. **HRMS** (ESI+): *m/z* calculated for $[C_{21}H_{20}ClN_6O_{10}Re+Na]^+$ 759.03512, found 759.03574, error: 0.8 ppm.

4.2. Synthesis of Cpd n°24



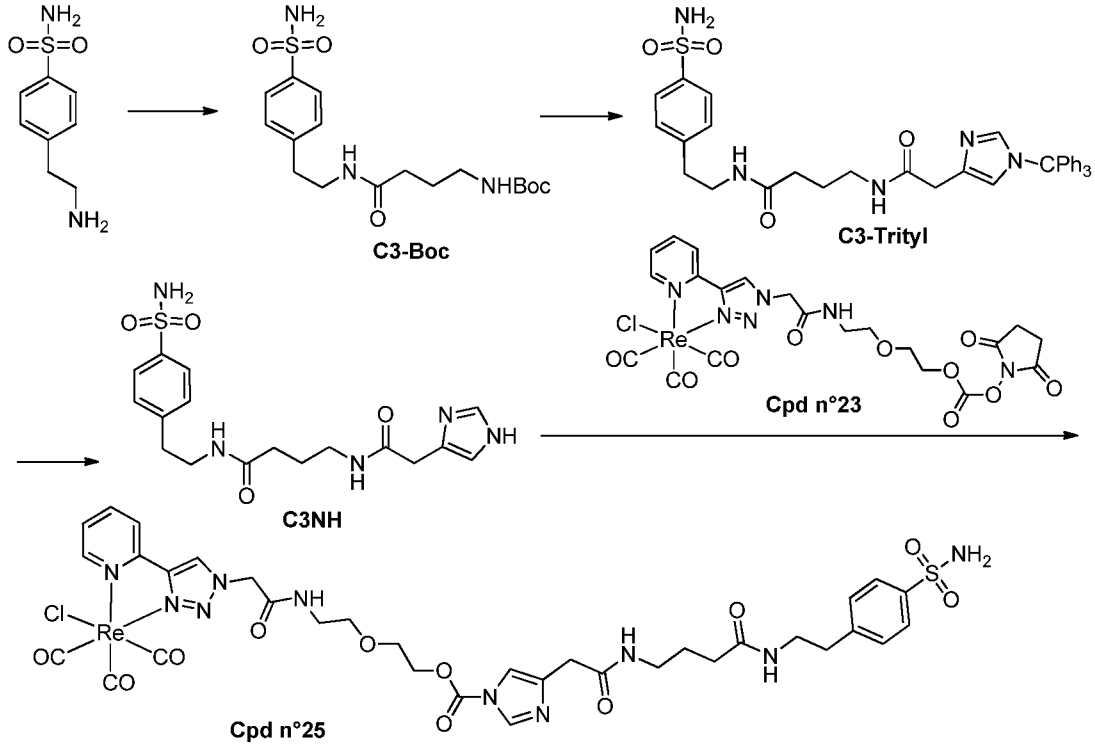
C0-Trityl **-** 1-(triphenylmethyl)-1*H*-imidazole-4-acetic acid (221.7 mg, 0.60 mmol, 1.2 equiv.), EDC.HCl (146.2 mg, 0.76 mmol, 1.5 equiv.) and HOBT.H_2O (115.8 mg, 0.76 mmol, 1.5 equiv.) were dissolved in dry DMF (2.5 mL) under argon. After 5-10 minutes stirring, 4-(2-aminoethyl)benzene sulfonamide (100.1 mg, 0.50 mmol, 1 equiv.) and DIEA (260 µL, 1.5 mmol, 3 equiv.) were added and reaction mixture was stirred overnight at room temperature. Solvent was removed by rotary evaporation and the resulting sticky solid was taken up in a 1:1 mixture of EtOAc and saturated aqueous solution of $NaHCO_3$. After decantation, the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by automated silica gel flash column chromatography (5% MeOH in DCM). The resulting oil was co-evaporated with Et_2O until obtention of the desired compound as a white solid, with traces of impurities (109.1 mg, 0.20 mmol, 40%). **^{1}H-NMR** (400MHz, MeOD) δ (ppm): 7.79 (d, J = 8.5 Hz, 2H), 7.41-7.33 (m, 9H+2H+1H), 7.16 (ddt, J = 5.1, 2.3, 1.2 Hz, 6H), 6.85 (s, 1H), 3.43 (t, J = 7.2 Hz, 2H), 3.41 (d, J = 1.7 Hz, 2H,), 2.84 (t, J = 7.3 Hz, 2H). **^{13}C-NMR** (75 MHz, MeOD) δ (ppm): 172.95, 145.28, 143.60, 143.04, 142.90, 130.86, 130.38, 129.34, 129.26, 127.33, 76.97, 41.70, 36.44, 36.23.

C0NH **-** Trityl imidazole derivative **(C0-Trityl)** (50.2 mg, 0.091 mmol, 1 equiv.) was dissolved in DCM (0.8 mL). TFA (0.2 mL) was added drop wise, followed by TIS (18.7 µL, 0.091 mmol, 1 equiv.). The mixture was stirred for 1 hour at room temperature, then precipitated in Et_2O to give the desired compound as a white solid/oil containing traces of water (34.6 mg of hydrated compound, 93%). **^{1}H-NMR** (300 MHz, MeOD) δ (ppm): 8.80 (s, 1H), 7.80 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.35 (s, 1H), 3.67 (s, 2H), 3.49 (t, J = 7.2 Hz, 2H), 2.90 (t, J = 7.2 Hz, 2H). **^{13}C-NMR (75 MHz, MeOD) δ (ppm):** 169.9, 145.2, 143.1, 135.0, 130.4 (2C), 129.2, 127.3 (2C), 118.5, 41.6, 36.1, 32.0.

Cpd n°24 **-** C0-imidazole **(C0NH)** (8.6 mg, 27.9 µmol, 1.1 equiv.) and the rhenium complex **Cpd n°23** (18.6 mg, 25.2 µmol, 1 equiv.) were dissolved in dry DMF (2 mL) under argon. Dry pyridine (2.2 µL, 27.3 µmol, 1.1 equiv.). Solvent was removed by rotary evaporation and the resulting crude was purified by HPLC (ACN:10 mM NH_4OAc pH = 7). **^{1}H-NMR** (400 MHz, $CDCl_3$:MeOD, 1:1 v:v) δ (ppm): 8.96 (d, J = 5.5 Hz, 1H), 8.82 (s, 1H), 8.14 (s, 1H), 8.11-8.05 (m, H + H), 7.74 (d, J = 8.3 Hz, 2H), 7.50 (ddd, J = 7.1, 5.4, 1.8 Hz, 1H), 7.36 (s, 1H), 7.27 (d, J = 8.3 Hz, 2H), 5.25 (q, J = 22.2 Hz, 2H), 4.55

(m, 2H, under water signal), 3.84-3.82 (m, 2H), 3.64 (t, J = 5.3 Hz, 2H), 3.48-3.41 (m + d + s, 2H + 2H + 2H), 2.84 (t, J = 7.0 Hz, 2H). **HRMS** (ESI+): *m/z* calculated for $[C_{30}H_{31}ClN_9O_{10}ReS+Na]^+$ 952.10249, found 952.10229, error: -0.2 ppm.

4.3. Synthesis of Cpd n°25



C3-Boc **-** Boc-4-aminobutanoic acid (104.6 mg, 0.51 mmol, 1 equiv.), EDC.HCl (143.8 mg, 0.75 mmol, 1.5 equiv.) and HOBT.H_2O (114.6 mg, 0.76 mmol, 1.5 equiv.) were dissolved in dry DMF (2.6 mL) under argon. After 5-10 minutes stirring, 4-(2-aminoethyl)benzene sulfonamide (100.1 mg, 0.50 mmol, 1 equiv.) and DIEA (260 µL, 1.5 mmol, 3 equiv.) were added and reaction mixture was stirred overnight at room temperature. Solvent was removed by rotary evaporation and the resulting sticky solid was taken up in a 1:1 mixture of EtOAc and saturated aqueous solution of $NaHCO_3$. After decantation, the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by automated silica gel flash column chromatography (5% MeOH in DCM) to give the desired compound as a white solid (133.2 mg, 0.35 mmol, 68%). **^{1}H-NMR** (300 MHz, MeOD) δ (ppm): 7.83 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 3.43 (t, J = 7.1 Hz, 2H), 3.00 (t, J = 6.8 Hz, 2H), 2.88 (t, J

= 7.1 Hz, 2H), 2.16 (t, J = 7.5 Hz, 2H), 1.70 (quintet, J = 7.2 Hz, 2H), 1.43 (s, 9H). **^{13}C-NMR** (75 MHz, MeOD) δ (ppm): 175.5, 158.4, 145.3, 142.9, 130.4, 127.2, 79.9, 41.3, 40.7, 36.2, 34.3, 28.8, 27.2.

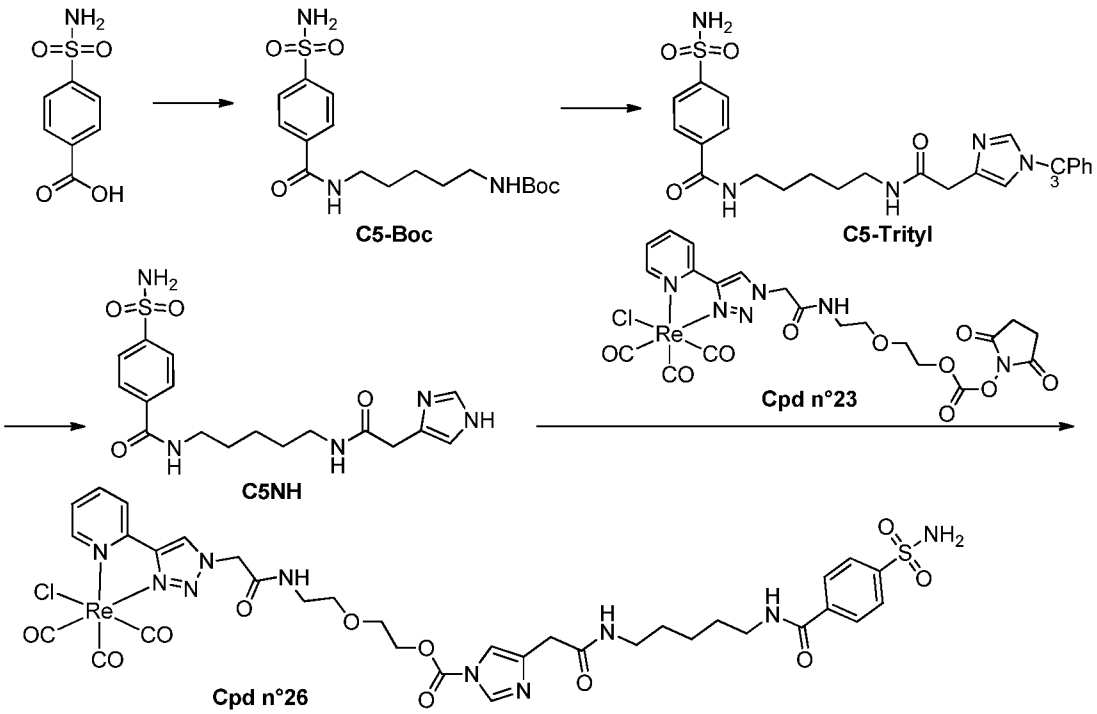
***C3-Trityl* - C3-Boc** (100 mg, 0.26 mmol, 1 equiv.) was dissolved in DCM (1 mL), and TFA (1 mL) was added drop wise. The solution was stirred for 1h at room temperature, then precipitated in cold Et_2O (40 mL) to give a white solid. 1-(triphenylmethyl)-1H-imidazole-4-acetic acid (117.3 mg, 0.32 mmol, 1.2 equiv.), EDC.HCl (75.2 mg, 0.39 mmol, 1.5 equiv.) and HOBT.H_2O (60.5 mg, 0.39 mmol, 1.5 equiv.) were dissolved in dry DMF (1.3 mL) under argon. After 5-10 minutes stirring, the solution was added to the deprotected amine. DIEA (135 µL, 0.78 mmol, 3 equiv.) was added and reaction mixture was stirred overnight at room temperature. Solvent was removed by rotary evaporation and the resulting sticky solid was taken up in a 1:1 mixture of EtOAc and saturated aqueous solution of $NaHCO_3$. After decantation, the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by automated silica gel flash column chromatography (5% MeOH in DCM). The resulting oil was co-evaporated with Et_2O until obtention of the desired compound as a white solid (88.7 mg, 0.14 mmol, 54%). **^{1}H-NMR** (400MHz, MeOD) δ (ppm): 7.81 (d, J = 8.4 Hz, 2H), 7.43 (s, 1H), 7.36 (ddt, J = 5.9, 2.3, 2.8 Hz, 10H), 7.17-7.14 (m, 5H), 6.87 (s, 1H), 3.43 (t, J = 7.1 Hz, 4H), 3.11 (dd, J = 8.8, 5.0 Hz, 2H), 2.86 (t, J = 7.2 Hz, 2H), 2.13 (t, J = 7.4 Hz, 2H), 1.70 (t, J = 7.2 Hz, 2H). **^{13}C-NMR** (75 MHz, MeOD) δ (ppm): 175.47, 158.40, 145.31, 142.89, 130.40, 127.24, 79.92, 41.35, 40.72, 36.18, 34.28, 28.76, 27.25.

***C3NH* -** Trityl imidazole derivative **C3-Trityl** (60.6 mg, 0.095 mmol, 1 equiv.) was dissolved in DCM (0.8 mL). TFA (0.2 mL) was added drop wise, followed by TIS (19.5 µL, 0.095 mmol, 1 equiv.). The mixture was stirred for 1 hour at room temperature, then precipitated in Et_2O to give the desired compound as a white solid containing traces of water (39.7 mg of hydrated compound, 85%). **^{1}H-NMR** (300 MHz, MeOD) δ (ppm): 8.82 (d, J = 1.4 Hz, 1H), 7.81 (d, J = 8.5 Hz, 2H), 7.39 (d + s, J = 8.5 Hz, 2H + 1H), 3.72 (s, 2H), 3.45 (t, J = 7.1 Hz, 2H), 3.13 (t, J = 7.1 Hz, 2H), 2.88 (t, J = 7.1 Hz, 2H), 2.17 (t, J = 7.4 Hz, 2H), 1.73 (quintet, J = 7.2 Hz, 2H). **^{13}C-NMR** (75 MHz, MeOD) δ (ppm):

175.3, 169.9, 145.4, 142.9, 135.0, 130.49 (2C), 129.2, 127.2 (2C), 118.6, 41.3, 40.1, 36.2, 34.3, 32.0, 26.6.

Cpd n°25 - C3-imidazole **(C3NH)** (11.9 mg, 30.2 µmol, 1.1 equiv.) and the rhenium complex **Cpd n°23** (19.1 mg, 25.9 µmol, 1 equiv.) were dissolved in dry DMF (2 mL) under argon. Dry pyridine (2.2 µL, 27.3 µmol, 1.1 equiv.). Solvent was removed by rotary evaporation and the resulting crude was purified by HPLC (ACN:10 mM NH_4OAc pH = 7). **^{1}H-NMR (400 MHz, $CDCl_3$:MeOD, 1:1 v:v) δ (ppm):** 8.96 (dt, J = 5.5 Hz, 1.1, 1H), 8.83 (s, 1H) 8.17 (s, 1H), 8.13-8.06 (m, 2H), 7.78 (d, J = 8.4 Hz, 2H), 7.51 (ddd, J = 6.9, 5.6, 2.1 Hz, 1H), 7.45 (s, 1H), 7.33 (d, J = 8.5 Hz, 2H), 5.26 (q, J = 22.4 Hz, 2H), 4.58-4.56 (m, 2H, partly hidden by water signal), 3.83-3.80 (m, 2H), 3.63 (t, J = 5.2 Hz, 2H), 3.47-3.40 (m + d + s, 2H + 2H + 2H), 3.07 (t, J = 7.0 Hz, 2H,), 2.85 (t, J = 7.0 Hz, 2H), 2.10 (t, J = 7.3 Hz, 2H), 1.73-1.64 (m, 2H). **HRMS** (ESI+): *m/z* calculated for $[C_{34}H_{38}ClN_{10}O_{11}ReS+Na]^+$ 1037.15525, found 1037.15545, error: 0.2 ppm.

4.4. Synthesis of Cpd n°26



C5-Boc - 4-carboxybenzenesulfonamide (120.8 mg, 0.6 mmol, 1.25 equiv.), EDC.HCl (143.8 mg, 0.75 mmol, 1.6 equiv.) and HOBT.H2O (114.5 mg, 0.75 mmol, 1.6 equiv.)

were dissolved in dry DMF (2.5 mL) under argon. After 5-10 minutes stirring, *N*-Boc-1,5-diaminopentane (0.1 mL, 0.48 mmol, 1 equiv.) and DIEA (260 µL, 1.5 mmol, 3.1 equiv.) were added. Reaction mixture was stirred overnight at room temperature. Solvent was removed by rotary evaporation and the resulting sticky solid was taken up in a 1:1 mixture of EtOAc and saturated aqueous solution of $NaHCO_3$. After decantation, the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by automated silica gel flash column chromatography (5% MeOH in DCM) to give the desired compound as a white solid (173.8 mg, 0.45 mmol, 94%). **^{1}H-NMR** (400MHz, MeOD) δ (ppm): 7.98-7.93 (m, 4H), 3.39 (t, J = 7.1 Hz, 2H), 3.05 (t, J = 6.9 Hz, 2H), 1.65 (quintet, J = 7.3 Hz, 2H), 1.56-1.49 (m, 2H), 1.42-1.38 (m, 9H + 2H). **^{13}C-NMR** (101 MHz, MeOD) δ (ppm): 168.71, 158.53, 147.53, 139.16, 128.90, 127.26, 79.80, 41.02, 30.03, 28.77, 25.18.

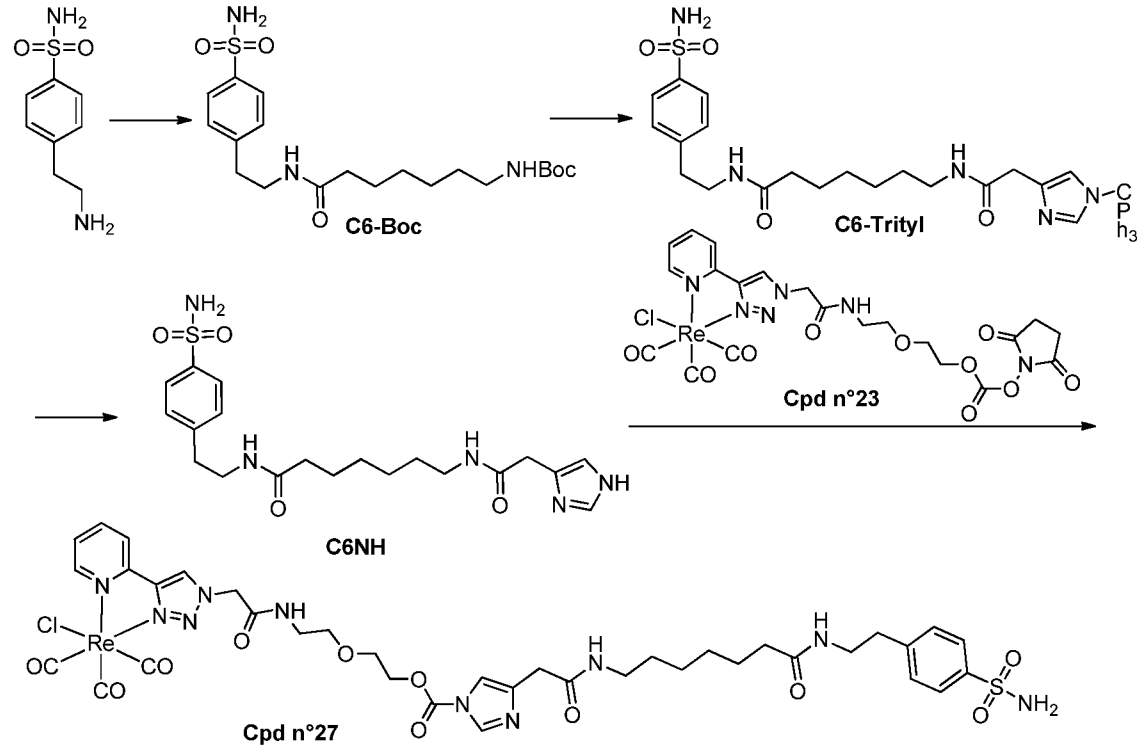
C5-Trityl - Boc-protected compound **C5-Boc** (112.7 mg, 0.29 mmol, 1 equiv.) was dissolved in DCM (1.2 mL), and TFA (1.2 mL) was added drop wise. The solution was stirred for 1h at room temperature, then precipitated in cold Et2O (40 mL) to give the deprotected ammonium salt as a white solid. 1-(triphenylmethyl)-1H-imidazole-4-acetic acid (132.6 mg, 0.36 mmol, 1.2 equiv.), EDC.HCl (88.3 mg, 0.46 mmol, 1.5 equiv.) and HOBT.H2O (69.3 mg, 0.45 mmol, 1.3 equiv.) were dissolved in dry DMF (1.6 mL) under argon. After 5-10 minutes stirring, the solution was added to the deprotected amine. DIEA (156 µL, 0.9 mmol, 3 equiv.) was added and reaction mixture was stirred overnight at room temperature. Solvent was removed by rotary evaporation and the resulting sticky solid was taken up in a 1:1 mixture of EtOAc and saturated aqueous solution of $NaHCO_3$. After decantation, the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by automated silica gel flash column chromatography (5% MeOH in DCM). The resulting oil was co-evaporated with Et2O until obtention of the desired compound as a white solid (63.4 mg, 0.10 mmol, 34%). **^{1}H-NMR** (400 MHz, MeOD): δ (ppm) 7.95 (q, *J* = 7.1 Hz, 4H), 7.41 (s, 1H), 7.37 (dt, *J* = 4.3, 2.8 Hz, 9H), 7.15 (dd, *J* = 6.8, 3.0 Hz, 6H), 6.85 (s, 1H), 3.43 (s, 2H), 3.36 (t, *J* = 7.1 Hz, 2H), 3.19 (t, *J* = 7.0 Hz, 2H), 1.67-1.59 (m, 2H), 1.53 (dt, *J* = 14.4, 7.2 Hz, 2H), 1.38 (dt, *J* = 15.4, 7.7 Hz, 2H). **^{13}C-NMR** (101 MHz, MeOD): δ (ppm) 172.89, 168.72,

147.61, 143.61, 139.18, 130.87, 129.35, 129.25, 128.93, 127.30, 121.28, 77.00, 41.00, 40.38, 29.99, 29.94, 25.24.

C5NH **-** Trityl imidazole derivative **C5-Trityl** (67.4 mg, 0.106 mmol, 1 equiv.) was dissolved in DCM (0.8 mL). TFA (0.2 mL) was added drop wise, followed by TIS (23 µL, 0.112 mmol, 1 equiv.). The mixture was stirred for 1 hour at room temperature, then precipitated in Et_2O to give the desired compound as a white solid containing traces of water (43.2 mg of hydrated compound, 0.095 mmol, 90%). **^{1}H-NMR** (300 MHz, MeOD) δ (ppm): 8.81 (s, 1H), 7.95 (s, 4H), 7.39 (s, 1H), 3.71 (s, 2H), 3.39 (t, J = 6.7 Hz, 2H), 3.22 (t, J = 6.3 Hz, 2H), 1.61 (td, J = 13.6, 6.7 Hz, 2H + 2H), 1.41 (t, J = 6.6 Hz, 2H). **^{13}C-NMR** (75 MHz, MeOD) δ (ppm): 169.80, 168.74, 147.54, 139.12**,** 134.96, 129.36, 128.92 (2C), 127.27 (2C), 118.49, 40.94, 40.58, 31.96, 29.99-29.91 (C + C), 25.27.

Cpd n°26 **-** C5-imidazole (***C5NH)*** (12.4 mg, 31.5 µmol, 1.3 equiv.) and the rhenium complex **Cpd n°23** (18.3 mg, 24.8 µmol, 1 equiv.) were dissolved in dry DMF (2 mL) under argon. Dry pyridine (2.2 µL, 27.3 µmol, 1.1 equiv.). Solvent was removed by rotary evaporation and the resulting crude was purified by HPLC (ACN:10 mM NH4OAc pH = 7). **^{1}H NMR** (400MHz, MeOD:$CDCl_3$) d (ppm) : 8.96-8.94 (m, 1H), 8.81 (s, 1H), 8.13 (s, 1H), 8.11-8.04 (m, 2H, H + H), 7.94-7.89 (m, 4H), 7.50 (ddd, J = 7.3, 5.5, 1.8 Hz, 1H), 7.42 (s, 1H), 5.25 (q, J = 23.0 Hz, 2H), 4.57-4.55 (m, 2H, partly hidden by water signal), 3.81 (td, J = 3.1, 2.3 Hz, 2H), 3.62 (t, J = 5.4 Hz, 2H), 3.45 (m + s, 2H + 2H), 3.38-3.33 (m, 2H), 3.18 (t, J = 7.0 Hz, 2H), 1.61 (dt, J = 14.6, 7.3 Hz, 2H), 1.52 (dd, J = 14.5, 6.9 Hz, 2H), 1.38 (dd, J = 16.1, 7.6 Hz, 2H). **HRMS** (ESI+): *m/z* calculated for $[C_{34}H_{38}ClN_{10}O_{11}ReS+Na]^+$ 1037.15525, found 1037.15464, error: -0.6 ppm.

4.5. Synthesis of C6-SCoMPI



C6-Boc - Boc-7-aminoheptanoic acid (101.8 mg, 0.51 mmol, 1 equiv.), EDC.HCl (145.7 mg, 0.76 mmol, 1.5 equiv.) and HOBT.H2O (115.6 mg, 0.76 mmol, 1.5 equiv.) were dissolved in dry DMF (2.6 mL) under argon. After 5-10 minutes stirring, 4-(2-aminoethyl)benzene sulfonamide (249.7 mg, 1.25 mmol, 1 equiv.) and DIEA (260{\uL, 1.5 mmol, 3 equiv.) were added and reaction mixture was stirred overnight at room temperature. Solvent was removed by rotary evaporation and the resulting sticky solid was taken up in a 1:1 mixture of EtOAc and saturated aqueous solution of $NaHCO_3$. After decantation, the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by automated silica gel flash column chromatography (5% MeOH in DCM) to give the desired compound as a white solid (146.1 mg, 0.34 mmol, 67%). **^{1}H-NMR** (400MHz, MeOD) δ (ppm): 7.84 (d, J = 8.5 Hz, 2H), 7.41 (d, J = 8.6 Hz, 2H), 3.46 (t, J = 7.2 Hz, 2H), 3.02 (t, J = 7.0 Hz, 2H), 2.90 (t, J = 7.1 Hz, 2H), 2.15 (t, J = 7.5 Hz, 2H), 1.57 (t, J = 7.1 Hz, 2H), 1.47-1.44 (m, 9H+ 2H), 1.30 (dt, J = 6.7, 3.1 Hz, 4H). **^{13}C-NMR** (101 MHz, MeOD) δ (ppm): 176.2, 158.5, 145.4,

143.1, 130.4 (2C), 127.3 (2C), 79.8, 41.3 (C + C), 37.0, 36.3, 30.8, 29.9, 28.8 (3C), 27.5, 26.9.

C6-Trityl **-** Boc-protected compound **C6-Boc** (120 mg, 0.28 mmol, 1 equiv.) was dissolved in DCM (1 mL), and TFA (1 mL) was added drop wise. The solution was stirred for 1h at room temperature, then precipitated in cold Et_2O (40 mL) to give the deprotected ammonium salt as a white solid. 1-(triphenylmethyl)-1H-imidazole-4-acetic acid (121.2 mg, 0.33 mmol, 1.2 equiv.), EDC.HCl (90.2 mg, 0.47 mmol, 1.5 equiv.) and HOBT.H2O (65.0 mg, 0.42 mmol, 1.3 equiv.) were dissolved in dry DMF (1.5 mL) under argon. After 5-10 minutes stirring, the solution was added to the deprotected amine. DIEA (146 µL, 0.78 mmol, 3 equiv.) was added and reaction mixture was stirred overnight at room temperature. Solvent was removed by rotary evaporation and the resulting sticky solid was taken up in a 1:1 mixture of EtOAc and saturated aqueous solution of $NaHCO_3$. After decantation, the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by automated silica gel flash column chromatography (5% MeOH in DCM). The resulting oil was co-evaporated with Et_2O until obtention of the desired compound as a white solid (79.8 mg, 0.12 mmol, 42%). **^{1}H-NMR** (400 MHz, MeOD) δ (ppm): 7.84-7.81 (m, 2H), 7.40-7.34 (m, 12H), 7.16-7.14 (m, 6H), 6.85 (s, 1H), 3.43 (d, *J* = 7.1 Hz, 4H), 3.15 (t, *J* = 6.9 Hz, 2H), 2.87 (t, *J* = 7.1 Hz, 3H), 2.11 (t, *J* = 7.4 Hz, 3H), 1.51 (ddd, *J* = 30.5, 15.9, 7.1 Hz, 5H), 1.30-1.21 (m, 6H). **^{13}C-NMR (101 MHz, MeOD) δ (ppm):** 176.14, 145.35, 143.61, 143.07, 130.86, 130.41, 129.33, 129.24, 127.28, 41.28, 40.64, 40.45, 36.95, 36.23, 30.15, 29.75, 27.51, 26.84.

C6NH **-** Trityl imidazole derivative **C6-Trityl** (53.6 mg, 0.079 mmol, 1 equiv.) was dissolved in DCM (0.8 mL). TFA (0.2 mL) was added drop wise, followed by TIS (16.2 µL, 0.079 mmol, 1 equiv.). The mixture was stirred for 1 hour at room temperature, then precipitated in Et_2O to give the desired compound as a white solid containing traces of water (35.6 mg of hydrated compound, 0.070 mmol, 88%). **^{1}H-NMR** (300 MHz, MeOD) δ (ppm): 8.81 (s, 1H), 7.82 (d, J = 8.4 Hz, 2H), 7.41-7.38 (d + s, J = 8.3, 2H + 1H), 3.72 (s, 2H), 3.46 (t, J = 7.0 Hz, 2H), 3.18 (t, J = 7.1 Hz, 2H), 2.89 (t, J = 7.0 Hz, 2H), 2.13 (t, J = 7.4 Hz, 2H), 1.51 (tt, J = 13.7, 6.9 Hz, 2H + 2H), 1.33-1.24 (m, 4H, 2H

+ 2H). **^{13}C-NMR** (75 MHz, MeOD) δ (ppm): 176.2, 169.8, 145.4, 143.0, 135.0, 130.46 (2C), 129.4, 127.2 (2C), 118.5, 41.2, 40.7, 36.9, 36.1, 32.0, 30.1, 29.7, 27.6, 26.8.

Cpd n°27 - C6-imidazole **(C6NH)** (12.8 mg, 29.4 µmol, 1.1 equiv.) and the rhenium complex **Cpd n°23** (19.4 mg, 26.3 µmol, 1 equiv.) were dissolved in dry DMF (2 mL) under argon. Dry pyridine (2.2 µL, 27.3 µmol, 1.1 equiv.). Solvent was removed by rotary evaporation and the resulting crude was purified by HPLC (ACN:10 mM NH_4OAc pH = 7). **^{1}H-NMR** (400MHz, $CDCl_3$:MeOD 1:1 v:v) δ (ppm): 8.96 (dd, J = 5.6, 0.5 Hz, 1H), 8.82 (s, 1H), 8.16 (s, 1H), 8.10-8.06 (m, 2H), 7.79 (dd, J = 8.5, 1.4 Hz), 7.50 (ddd, J = 7.0, 5.5, 2.0 Hz, 1H), 7.45 (s, 1H), 7.33 (d, J = 8.5 Hz, 2H), 5.25 (q, J = 22.5 Hz, 2H), 4.56-4.55 (m, 2H, partly hidden by water signal), 3.82-3.80 (m, 2H), 3.62 (t, J = 5.3 Hz, 2H), 3.44 (dt, J = 12.3, 6.2 Hz, m + d + s, 2H + 2H + 2H), 3.13 (t, J = 7.1 Hz, 2H), 2.85 (t, J = 7.1 Hz, 2H), 2.09 (t, J = 7.4 Hz, 2H), 1.51 (dt, J = 14.4 Hz, 7.6, 2H), 1.43 (dt, J = 14.6 Hz, 7.4, 2H), 1.28-1.19 (m, 2H + 2H). **HRMS** (ESI+): *m/z* calculated for $[C_{37}H_{44}ClN_{10}O_{11}ReS+Na]^+$ 1079.20220, found 1079.20160, error: -0.6 ppm.

4.6. Labelling of Carbonic Anhydrases

***In vitro* labelling of human Carbonic Anhydrase 1 (hCA1)**

A solution of hCA1 (10µM in 50mM HEPES pH 7.2 was incubated at 37°C for 6h with 2 or 20 equivalents of compounds of the invention **Cpd n°24-27**. At given time points, aliquots of the incubation solution were taken, treated and analyzed either by MALDI-TOF MS or fluorescence gel imaging. A control experiment consisting in incubation solution + 10 equivalents EZA was performed for each experiment, and analyzed at 6h.
Treatment for MALDI-TOF MS analysis: 10µL of sample solution were quenched and purified using a ZipTip (C18) and 0.1% aqueous TFA, then spotted on a MALDI plate. Peak areas of labelled and unlabelled protein were determined and percentage of labelling calculated.

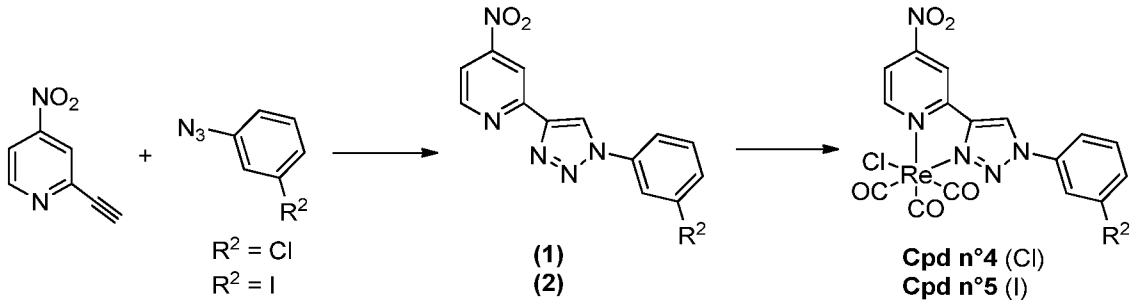
Treatment for gel imaging analysis: 30 µL of sample solution were diluted with 30 µL 2X denaturing Laemmli buffer and stored at -30°C prior to gel analysis. Sample were

heated at 70°C for 20 min and loaded on a 12.5% SDS polyacrylamide gel. Fluorescence gel Imaging was performed prior to BBB staining.

***In cellulo* labelling of CA IX and CA XII for imaging**

A549 cells were deposed in 6-wells plates in presence of glass of CaF_2 slides (10^5 cells/well). After 24h, cells were put under hypoxic conditions for 24h using Oxoid Anaerogen kit. Cells were then incubated at 37°C with compounds of the invention **Cpd n°24-27** under normoxic conditions. Typical incubation conditions are 10 µM reagent for 3-6h.

5. Re complexes with substituted pyridine and triazole



Intermediate (1): 2-(4-(3-Chlorophenyl)-1H-1,2,3-triazol-1-yl)-4-nitropyridine

2-Ethynyl-4-nitropyridine (30.0 mg, 0.20 mmol, 1.0 equiv.) was dissolved in tert-butanol (2.8 mL). An aqueous solution of $CuSO_4$ $5H_2O$ (0.1 equiv. from a 4.12 $mg.mL^{-1}$ solution in water), an aqueous solution of sodium ascorbate (0.3 equiv. from a 8.7 $mg.mL^{-1}$ solution in water) and 1-azido-3-chlorobenzene (31.1 mg, 0.20 mmol, 1.0 equiv.) were then added. The resulting mixture was stirred in a sealed tube at 110 °C for 24 hrs. The obtained suspension was cooled down and filtered. The residue was washed with water and tert-butanol and dried under vacuum to afford 2-(4-(3-chlorophenyl)-1H-1,2,3-triazol-1-yl)-4-nitropyridine as a clear brown solid (58.0 mg, 95% yield). **^{1}H-NMR** (300 MHz, DMSO-d_6) δ 8.45 (t app, J = 2.1 Hz, 2H), 8.34 (ddd, J = 1.2, 2.4, 8.1 Hz, 2H), 8.10 (dt, J = 1.2, 8.1 Hz, 2H), 7.76 (t, J = 8.1 Hz, 1H).

Cpd n°4: ***2-(4-(3-Chlorophenyl)-1H-1,2,3-triazol-1-yl)-4-nitropyridine chloro tricarbonylrhenium***

It was obtained according to general procedure B described below starting from 2-(4-(3-chlorophenyl)-1H-1,2,3-triazol-1-yl)-4-nitropyridine (20.0 mg, 66.2 mmol, 1.0 equiv.) and chloropentacarbonylrhenium(I) (24.2 mg, 66.8 mmol, 1.01 equiv.) after filtration and washing with toluene as a deep red solid (23.0 mg, 57% chemical yield). **MS** (ESI+) m/z (%): 629.9322 (46) $[M+Na]^+$, 589.9846 (100) $[M-Cl+H_2O]^+$, 571.9747 (69) $[M-Cl]^+$, 324.0251 (23) $[M-Re(CO)_3Cl+Na]^+$; **HRMS** (ESI+): calcd for $C_{16}H_8Cl_2N_5NaO_5Re$: 629.9337, found: 629.9322; **IR** n_{max}/cm^{-1} 2027, 1899 (CO).

Intermediate (2): 2-(4-(3-Iodophenyl)-1H-1,2,3-triazol-1-yl)-4-nitropyridine

2-Ethynyl-4-nitropyridine (30.0 mg, 0.20 mmol, 1.0 equiv.) was dissolved in tert-butanol (2.8 mL). An aqueous solution of $CuSO_4$ $5H_2O$ (0.1 equiv. from a 4.12 $mg.mL^{-1}$ solution in water), an aqueous solution of sodium ascorbate (0.3 equiv. from a 8.7 $mg.mL^{-1}$ solution in water) and 1-azido-3-iodobenzene (49.6 mg, 0.20 mmol, 1.0 equiv.) were then added. The resulting mixture was stirred in a sealed tube at 110 °C for 24 hrs. The obtained suspension was cooled down and filtered. The residue was washed with water and tert-butanol and dried under vacuum to afford 2-(4-(3-iodophenyl)-1H-1,2,3-triazol-1-yl)-4-nitropyridine as a brown solid (63.0 mg, 79% yield). **^{1}H-NMR** (300 MHz, DMSO-d_6) δ 9.64 (s, 1H), 9.06 (s, 1H), 8.65 (s, 1H), 8.21-8.06 (m, 3H), 7.67-7.63 (m, 2H).

Cpd n°5: 2-(4-(3-Iodophenyl)-1H-1,2,3-triazol-1-yl)-4-nitropyridine chloro tricarbonylrhenium

It was obtained according to general procedure B described below starting from 2-(4-(3-iodophenyl)-1H-1,2,3-triazol-1-yl)-4-nitropyridine (20.0 mg, 50.8 mmol, 1.0 equiv.) and chloropentacarbonylrhenium(I) (18.6 mg, 51.3 mmol, 1.01 equiv.) after filtration and washing with toluene as a deep red solid (30.0 mg, 84% chemical yield). **MS** (ESI+) m/z (%): 721.8689 (86) $[M+Na]^+$, 681.9218 (100) $[M-Cl+H_2O]^+$, 663.9119 (71) $[M-Cl]^+$;

HRMS (ESI+): calcd for $C_{16}H_8ClIN_5NaO_5Re$: 721.8700, found: 721.8689; **IR** n_{max}/cm^{-1} 2027, 1899 (CO).

6. Re complexes functionalized with a thioctic acid

PytaC3OH **-** 3-Azidopropan-1-ol was directly added (1 g, 9.7 mmol) with 2-ethynylpyridine (1 g, 9.7 mmol) to a mixture of CH_2Cl_2 (5 mL) and water (5 mL) at r.t. Copper (II) Sulfate pentahydrate (250 mg, 1.0 mmol) and sodium ascorbate (500 mg, 2.0 mmol) were then added. After overnight stirring, water (10 mL) and CH_2Cl_2 (10 mL) were poured into the mixture. The product was extracted with CH_2Cl_2 (2 x 30 mL), the organic fractions were combined, dried over anhydrous sulfate magnesium, filtered and evaporated to dryness. The crude was purified by silica gel column chromatography with (CH_2Cl_2/MeOH) (from 1/0 to 95/5) to give 4-(2-pyridyl)-1,2,3-triazole-$(CH_2)_3$-OH (1.6 g, 7.8 mmol, 80% yield) as a white solid. **^{1}H-NMR** ($CDCl_3$, 300 MHz) d (ppm): 8.37 (1H, ddd, J =2.9, 2.1, 1 Hz), 8.05 (1H, s), 8.01-7.86 (1H, m), 7.67-7.41 (1H, m), 7.06-6.90 (1H, m), 4.40-4.09 (2H, m), 3.30 (2H, m), 2.08-1.83 (2H, m). **^{13}C-NMR** ($CDCl_3$, 75 MHz) d (ppm): 150.0, 149.27, 148.20, 136.75, 122.74, 122.37, 119.9, 47.8, 47.15, 29.25.

PytaC3Othioctic **-** Thioctic acid (0.5g, 2.5 mmol), and PytaC3OH (0.5 g, 2.5 mmol) were dissolved in 50 ml of $CHCl_3$. DMAP (305 mg, 2.5 mmol) and DCC (0.5 g, 2.5 mmol) were successively added under stirring at rt. After one night of stirring, 100 ml of water was added. The product was extracted with CH_2Cl_2 (2 x 50 mL). The organic fractions were combined, dried over anhydrous sulfate magnesium, filtered and evaporated to dryness. 30 ml of acetone was added and the suspension was filtrated. The white solid was discarded and the filtrate was purified by silica gel column chromatography with (CH_2Cl_2/ethanol) (90/10) to give 360 mg of a yellow solid (36%). **^{1}H-NMR** (CD_3COCD_3, 300 MHz) δ ppm: 8.97 (d, *J* = 5.4 Hz, 1 H), 8.51 (s, 1H), 7.97 (m, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.44 (m, 1H), 4.54 (m, 2H), 4.16 (t, *J* = 5.8 Hz, 2H), 3.58 (m, 1H), 3.15 (m, 2H), 2.48 (m, 4H), 2.35 (t, 4H), 1.90 (m, 1H), 1.68 (m, 4H), 1.47 (m, 2H). **^{13}C-NMR** (CD_3COCD_3, 75 MHz) δ ppm: 172.65, 150.80, 149.55, 148.00, 139.79, 122.82, 122.67, 119.44, 60.96, 59.28, 47.17, 40.03, 38.20, 34.40, 33.44, 28.72, 28.21, 24.45. **HRMS** (ESI+): calcd for C18H24N4Na1O2S2 ($[M+Na]^+$): m/z 415.1233, found: m/z 415.1208.

Cpd n°28: RePytaC3Othioctique - PytaC3Othioctique (200 mg, 0.5 mmol) and $Re(CO)_5Cl$ (200 mg, 0.55 mmol) were added in a mixture of (toluene / acetone) (50 ml / 10 ml). The reaction mixture was stirred for 3h at 70°C. After cooling down to r.t., the reaction mixture was evaporated on vacuum and the crude purified by silica gel column chromatography with (CH_2Cl_2/acetone) (70/30) to give 250 mg of a yellow solid (71%). **^{1}H-NMR** (CD_3COCD_3, 300 MHz) δ ppm: 9.07 (s, 1H), 9.03 (d, *J* = 5.5 Hz, 1 H), 8.20 (m, 2 H), 7.64 (m, 1 H), 4.78 (t, *J* = 7Hz, 2H), 4.21 (t, *J* = 6 Hz, 2H), 3.56 (ddd, *J* = 12.4, 8.3, 6.3 Hz, 1H), 3.13 (m, 2H), 2.42 (m, 3H), 2.29 (t, 2H), 1.89 (m, 1H), 1.59 (m, 4H), 1.40 (m, 2H). **^{13}C-NMR** ($CDCl_3$, 75 MHz) δ ppm: 197.13, 195.69, 188.92, 173.37, 153.17, 149.13, 148.68, 139.46, 125.92, 124.08, 122.21, 60.41, 59.47, 49.52, 40.28, 38.52, 34.50, 33.85, 29.10, 28.69, 24.54. **HRMS** (ESI+): calcd for C21H24Cl1N4Na1O5Re1S2 ($[M+Na]^+$): m/z 721.0315, found: m/z 721.0313 and ($[M+K]^+$ m/z 737.0069 and ($[M–Cl]^+$ m/z 663.0731).

PytaC8NHthioctic - N_3-$(CH_2)_8$-NHthioctic (160 mg, 0.5 mmol) and 2-ethynylpyridine (120 mg, 1.2 mmol) were dissolved in CH_2Cl_2 (10 mL) and water (10 mL). Sulfate copper (II) pentahydrate (15 mg, 0.05 mmol) and sodium ascorbate (50 mg, 0.25 mmol) were then added. The reaction mixture was stirred and became trouble and light yellow. After 3 days at r.t., 20 ml of CH_2Cl_2 was added and the organic phase was washed with 20 mL of ammoniac (1 mol L^{-1}) then with 50 ml of water. The organic fraction was dried over anhydrous magnesium sulfate, filtered and concentrated. The crude product was purified by a fast and short column chromatography on silica gel with (CH_2Cl_2 /methanol) (10/0 to 9/1). The product was obtained as a cream colored solid (0.170 g, 73%). **^{1}H-NMR** ($CDCl_3$, 300 MHz) δ ppm: 8.46 (dd, *J* = 28, 5 Hz, 1H), 8.08 (s, *J* = 5.5 Hz, 1 H), 8.10 (m, 1 H), 7.71 (m, 1 H), 7.16 (m, 1 H), 6.04 (m, 1 H), 4.34 (t, *J* = 7.1 Hz, 2H), 3.47(dt, *J* = 13, 6.5, 1 Hz, 1H), 3.06 (m, 2H), 2.36 (dt, *J* = 12.5, 6.4, 1 Hz, 1H), 2.09 (t, *J* = 7.1 Hz, 2H), 1.82 (m, 3H), 1.58(m, 4H), 1.38 (m, 4H) 1.20(m, 8H). **^{13}C-NMR** ($CDCl_3$, 75 MHz) δ ppm: 172.7, 150.28, 149.39, 148.25, 136.89, 122.80, 121.84, 120.07, 56.42, 50.37, 40.17, 39.34, 38.41, 36.37, 34.56, 30.08, 29.50, 28.91, 28.84, 28.73, 26.22, 26.67, 25.45. **HRMS** (ESI+): C23H36N5O1S2 ($[M+H]^+$): calc m/z 462.2361, found: m/z 462.2347d for C23H35N5Na1O1S2 ($[M+Na]^+$) calc m/z 484.2175, found: m/z 484.2180.

Cpd n°29: RePYTA-C8-NHthioctic - PytaC8NHCOthioctic (0.5 mmol, 200 mg) and $Re(CO)_5Cl$ (0.4 mmol, 140 mg) were added in a mixture of (toluene/chloroforme) (20 ml / 5 ml). The reaction mixture was stirred for 3h at 70°C. After cooling down to r.t., the reaction mixture was evaporated and the crude purified by silica gel column chromatography with (CH_2Cl_2/acetone) (80/20) to give 240 mg of a yellow solid (87%). **^{1}H-NMR** (CD_3Cl_3, 300 MHz) δ ppm: 8.91 (d, J = 5.4 Hz, 1 H), 8.62(s, 1H), 7.96 (m, 2H), 7.41 (dt, J = 6, 2.5 Hz, 1H), 4.41 (m, 2H), 3.52 (dt, J = 12.8, 6.3, 1 Hz, 1H), 3.10 (m, 2H), 2.42 (dt, J = 12.4, 6.1 Hz, 1H), 2.12 (m, 2H), 1.87 (m, 3H), 1.60 (m, 4H), 1.40 (m, 4H), 1.28 (m, 8H). **^{13}C-NMR** ($CDCl_3$, 75 MHz) δ ppm: 197.40, 195.95, 189.01, 172.94, 152.97, 149.31, 148.70, 139.62, 125.86, 124.05, 122.39, 56.52, 52.24, 40.24, 39.27, 38.49, 36.40, 34.60, 29.68, 29.40, 28.85, 28.72, 28.45, 26.49, 25.93, 25.52. **HRMS** (ESI+): C26H35N5Na1O4Re1S2$^+$ ([M+Na]$^+$): calc m/z 790.1274, found: m/z 790.1260.

7. Re complexes functionalized with a fluorophore

7-(8-bromoocta)oxycoumarine-3-carboxylic acid ethyl ester

7-hydroxycoumarine-3-carboxylic acid ethyl ester (2.34 g, 10 mmol) was mixed with K_2CO_3 (20 mmol, 2.80 g) and 1,8-dibromooctane (50 mmol, 12 mg). 10 ml of aliquat was added and the mixture was heated at 80°C for 3h and at room temperature for 2 days. 100 ml of CH_2Cl_2 was added, the suspension was filtrated and the filtrate was dried on vacuum. 100 ml of heptane was added and the formed precipitate was isolated by filtration and washed with 100 ml of heptane. The solid was dissolved in a minimum of mixture (CH_2Cl_2 / heptane) (50/50) and purified by silica gel column chromatography with (CH_2Cl_2 / heptane) (50/50) and (CH_2Cl_2) and (CH_2Cl_2/ acétone) (99/1) to obtain (2.6 g, 61%) of a white non-fluorescent solid. **^{1}H-NMR** ($CDCl_3$, 300 MHz) δ ppm: 8.50 (m, 1H), 7.51 (dd, J = 8.7, 1.7 Hz, 1H), 6.87 (dd, J = 8.7, 2.2 Hz, 1H), 6.78 (m, 1H), 4.39 (m, 2H), 4.04 (m, 2H), 3.42 (2H), 1.84 (m, 4H), 1.39 (m, 11H). **^{13}C-NMR** ($CDCl_3$, 75 MHz) δ ppm: 164.7, 163.4, 157.57, 148.95, 130.67, 113.96, 111.47, 100.78, 68.87, 61.62, 33.91, 32.71, 29.07, 28.81, 28.61, 28.02, 25.80, 14.27. **HRMS** (ESI+): calcd for C20H25BrNaO5 ([M+Na]$^+$): m/z 447.0778, found: m/z 447.0767 and calcd for([2M+Na]$^+$ m/z 873.1648, found: m/z 873.1621.

7-(8-azidomoocta)oxycoumarine-3-carboxylic acid ethyl ester

7-(8-bromoocta)oxycoumarine-3-carboxylic acid ethyl ester (2.12 g, 5 mmol) was diluted in 25 ml of DMF. NaN_3 (0.65 g, 10 mmol) was added and the mixture was stirred overnight at 70°C. After cooling at r.t. the reaction mixture was poured in 200 ml of NaCl saturated ice water. The product was extracted with 100 ml of CH_2Cl_2. The organic phase was dried over anhydrous sulfate sodium, filtered and evaporated to dryness. The oily product was precipitated by addition of water (100 ml). The solid was dissolved in ethyl acetate, dried over anhydrous sulfate sodium and evaporated on vacuum to obtain a white solid (2g, quantitative). **^{1}H-NMR** (DMSO-d6, 300 MHz) δ ppm: 8.52 (m, 1H), 7.50 (d, *J* = 8.7 Hz, 1H), 6.89 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.81 (d, *J* = 2.3 Hz), 4.41 (q, *J* = 7.1 Hz, 2H), 4.05 (t, *J* = 6.5 Hz, 2H), 3.28 (t, *J* = 6.9 Hz, 2H), 1.83 (dd, *J* = 14.6, 6.6 Hz, 2H), 1.62 (m, 2H), 1.44 (m, 9H). **^{13}C-NMR** (DMSO-d6, 75 MHz) δ ppm: 164.6, 163.36, 157.52, 148.93, 130.67, 113.91, 111.44, 100.76, 68.85, 61.56, 51.38, 29.09, 28.98, 28.77, 26.58, 25.78, 14.23. **HRMS** (ESI+): calcd for C20H25N3NaO5 ($[M+Na]^+$): m/z 410.1686, found: m/z 410.1687.

Pyta C8-O-coumarineCO_2Et

7-(8-bromoocta)oxycoumarine-3-carboxylic acid ethyl ester (5 mmol, 2 g) was added with 2-ethynylpyridine (0.5 g, 5 mmol) in 10 mL of water and 20 ml of CH_2Cl_2. The mixture was vigorously stirred and $CuSO_4$ (240 mg, 1 mmol) and sodium ascorbate (500 mg, 2 mmol) were added. The reaction mixture was stirred for three days at r.t. 10 ml of concentrated ammoniac solution was added and the product was extracted by (3 x 100 mL) of CH_2Cl_2. The organic fractions were combined, dried over anhydrous sulfate sodium, filtered and evaporated to dryness. The crude was purified by silica gel column chromatography with (CH_2Cl_2 / acetone) (90/10 to 70/30) to give 0.9 g of a white solid (50% yield). **^{1}H-NMR** (DMSO-d6, 300 MHz) δ ppm: 8.59 (d, *J* = 4.8 Hz, 1H), 8.50 (s, 1H), 8.19 (d, *J* = 7.9 Hz, 1H), 8.14 (s, 1Ht), 7.79 (t, *J* = 7.7 Hz, 1H), 7.49 (d, *J* = 8.7 Hz, 1H), 7.24 (m, 1H), 6.87 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.79 (d, *J* = 2.3 Hz, 1H), 4.41 (m, 4H), 4.02 (t, *J* = 6.3 Hz, 2H), 1.83 (m, 6.6 Hz, 2H), 1.80 (m, 2H), 1.4 (m, 9H). **^{13}C-NMR** (DMSO-d6, 75 MHz) δ ppm: 164.6, 163.36, 157.52, 150.38, 149.39, 148.93, 148.38, 136.92, 130.67, 122.82, 121.77, 120.10, 113.97, 111.46, 100.77, 68.81, 61.62, 50.42,

30.15, 28.99, 28.82, 28.77, 26.30, 25.76, 14.27. **HRMS** (ESI+): calcd for C27H30N4NaO5 ([M+Na]$^+$): m/z 513.2108, found: m/z 513.2090.

Cpd n°30: Re Pyta C8-O-coumarineCO$_2$Et

PytaC8-O-coumarineCO$_2$Et (0.2 mmol, 98 mg) and Re(CO)$_5$Cl (72 mg, 0.2 mmol) were added in 20 ml of toluene. The mixture was stirred 3h at 70°C. After cooling at r.t., the product has precipitated and was separated by filtration. Silica gel column chromatography with (CH_2Cl_2/acetone) (20/80) gave 70 mg of a pale yellow solid (43%). **^{1}H-NMR** (DMSO-d6, 300 MHz) δ ppm: 8.92 (d, *J* = 4.8 Hz, 1H), 8.70 (s, 1H), 8.49 (s, 1H), 7.95 (m, 2H), 7.49 (d, *J* = 8.7 Hz, 1H), 7.39(m, 1H), 6.87 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.79 (d, *J* = 2.3 Hz, 1H), 4.42 (m, 2H), 4.34 (q, 2H), 4.02 (t, *J* = 6.3 Hz, 2H), 1.96 (m, 6.6 Hz, 2H), 1.75 (m, 2H), 1.4 (m, 9H). **^{13}C-NMR** (DMSO-d6, 75 MHz) δ ppm: 197.40, 195.92, 189.08, 164.85, 163.15, 157.44, 157.36, 152.92, 149.37, 148.67, 148.67, 136.55, 130.90, 125.77, 124.27, 122.42, 114.01, 111.40, 100.83, 68.89, 61.54, 52.26, 29.78, 28.87, 28.70, 28.65, 26.10, 25.64, 14.26. **HRMS** (ESI+): calcd for C30H30ClNaO8Re ([M+Na]$^+$): m/z 819.1194, found: m/z 819.1197.

8. Multivalent Re complexes

N$_3$-peg900-N$_3$ **-** In 100 mL of DMF were added 1.3 g of NaN_3 and 6 g of Cl-peg900-Cl. After overnight stirring at 90°C, the reaction mixture was partially evaporated on vacuum, until the solution volume was around 20 ml. 100 ml of water was added and the product was extracted by (3 x 100 mL) of CH_2Cl_2. The organic fractions were combined, dried over anhydrous sulfate sodium, filtered and evaporated to dryness. 6 g of colorless oil were obtained (quantitative yield). The product was used without further purification.**^{1}H-NMR** ($CDCl_3$, 300 MHz) δ ppm: 4.60 (m, 4H), 3.92 (m, *J* = 5.1 Hz, 4H), 3.59 (m, 76H). **^{13}C-NMR** ($CDCl_3$, 75 MHz) δ ppm: 70.49, 69.96, 50.59.

Pyta-peg900-Pyta **-** 6 g of N_3-peg900-N_3 and 1.5 g of 2-ethynylpyridine were added in 10 mL of water and 10 ml of CH_2Cl_2. The mixture was vigorously stirred and $CuSO_4$ (500 mg, 2 mmol) and sodium ascorbate (1 g, 4 mmol) were added. After overnight stirring at r.t., two colored phases were obtained. 10ml of concentrated ammoniac solution

was added and the product was extracted with (3 x 100 mL) of CH_2Cl_2. The organic fractions were combined, dried over anhydrous sulfate sodium, filtered and evaporated to dryness. The crude was purified by silica gel column chromatography with (CH_2Cl_2 / methanol) (100/0 to 85/15) to give 3.6 g of a brown oil (50%). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ ppm: 8.55 (d, J = 4.8 Hz, 2H), 8.29 (s, 2H), 8.13 (d, J = 8 Hz, 2H), 7.74 (t, J = 7.7 Hz, 2H), 7.19 (ddd, J = 7.5, 4.9, 1Hz, 2H), 4.60 (m, 4H), 3.92 (m, J = 5.1 Hz, 4H), 3.59 (m, 76H). **^{13}C-NMR** ($CDCl_3$, 75 MHz) δ ppm: 150.43, 149.35, 148.21, 136.79, 123.17, 122.69, 120.14, 70.51, 69.39, 50.41.

Cpd n°31*: *RePytaPEG900PytaRe - Pyta-PEG900-Pyta (290 mg, 0.25 mmol), $Re(CO)_5Cl$ (198 mg, 0.55 mmol) and 20 ml of toluene were mixed. The mixture was stirred 3h at 70°C. After cooling at r.t., toluene was removed and the crude purified by silica gel column chromatography with (CH_2Cl_2/ethanol) (90/10) to give 240 mg of a clear yellow oil (54%). **^{1}H-NMR** ($CDCl_3$, 300 MHz) δ ppm: 8.86 (d, J = 5Hz, 2 H), 8.77 (s, 2H), 7.96 (m, 4H), 7.77 (t, J = 7.7 Hz, 2H), 7.37 (m, 2H), 4.61 (m, J = 5 Hz, 4H), 3.88 (t, J = 5.1 Hz, 4H), 3.56 (m, 76H). **^{13}C-NMR** ($CDCl_3$, 75 MHz) δ ppm: 197.35, 195.86, 189.12, 1452.85, 148.58, 139.64, 125.69, 122.41, 70.36, 68.28, 51.99.

pyta-$(CH_2)_8$-pyta from N_3-$(CH_2)_8$-N_3 - 1,8-diazidooctane (0.98 g, 5 mmol) and 2-ethynylpyridine (1.03 g, 10 mmol) were dissolved in a mixture of CH_2Cl_2 (20 mL) and water (20 mL) at r.t. Copper (II) sulfate pentahydrate (125 mg, 0.5 mmol) and sodium ascorbate (200 mg, 1 mmol) were then added. After 12 h stirring at r.t., water (50 mL), 10 ml of 10% ammoniac solution were added. Aqueous solution was extract three times with CH_2Cl_2 (3 x 100 mL). The organic phases were combined, dried over anhydrous sulfate magnesium filtered and concentrated. The crude was dissolved in a minimum of (CH_2Cl_2/MeOH) (99/1) and purified by column chromatography on silica gel with (CH_2Cl_2/MeOH) (98/2 to 95/5) to give pyta-$(CH_2)_8$-pyta (1.7 g, 85%). **^{1}H-NMR** (DMSO-d6, 300 MHz) δ (ppm): 8.66 (bs, 2H), 8.55 (2H, s), 8.14 (2H, m), 7.92 (2H, m), 7.37 (1H, bs), 4.51 (4H, t, J = 7.12 Hz), 2.02 (4H, m), 1.44 (8H, m). **^{13}C-NMR** (acetone-d6/$CDCl_3$, 75 MHz) δ (ppm): 149.2, 148.5, 147.0, 137.3, 122.8, 122.1, 120.0, 48.7, 29.6, 28.2, 25.7. **HRMS** (ESI+): calcd for $C_{22}H_{26}N_8Na_1$ ($[M + Na]^+$) m/z 425.2173, found: m/z 425.2176.

***Cpd n°32*: Repyta-C8-pytaRe from pyta-C8-pyta -** PytaC8Pyta (115 mg, 0.25 mmol) and $Re(CO)_5Cl$ (118 mg, 0.5 mmol) were added in 20 ml of toluene and 10 ml of acetone. The mixture was stirred for 3h at 70°C. After cooling to r.t., the solvent was evaporated and the crude was purified by silica gel column chromatography with (CH_2Cl_2/acetone) (90/10) to give 210 mg of a pale yellow solid (78%). **^{1}H-NMR** (D_3COCD_3, 300 MHz) δ (ppm): 9.06 (2H, s), 9.05 (2H, s), 8.22 (4H, d), 7.65 (2H, m), 4.65 (4H, t), 1.37 (4H, m), 1.28 (8H, m). **^{13}C-NMR** (D_3COCD_3, 75 MHz) δ (ppm): 197.94, 196.84, 189.54, 153.04, 149.48, 148.67, 140.15, 126.10, 124.91, 122.45, 52.01, 29.55, 29.05, 25.97.

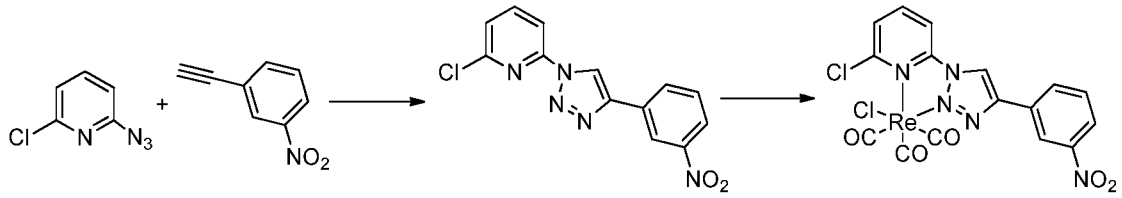
9. Tapy complexes

General procedure A for $(CuOTf)_2 \cdot C_6H_6$ catalyzed CuAAC Click reaction

A vial was charged with tetrazolo- or azido- derivative (1.0 equiv.) and copper(I) trifluoromethanesulfonate benzene complex (10 mol %). After three purges vacuum/N_2, dry toluene (1.0 mL / 0.15 mmol) was added under inert atmosphere, followed by alkyne derivative (1.1 equiv.). The vial was closed with a screwed cap and secured by teflon tape. The reaction mixture was stirred at 100°C. The reaction mixture was diluted with EtOAc or DCM and H_2O. The organic phase was decanted out, washed with water and saturated brine, dried over Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography on silica gel to afford the desired compound.

General procedure B for rhenium coordination

The ligand (1.0 equiv.) was suspended in anhydrous toluene (1.0 mL for 70.0 mmol) and heated at 110 °C under N_2 until complete dissolution. Halopentacarbonylrhenium(I) (1.0 equiv.) was added, the color of the solution changes and gas was released. The solution was stirred at 110°C under N_2 for 6 hrs. The mixture was cooled down to RT and evaporated. The residue was purified as described to afford the desired tricarbonyl rhenium complex.



(1) Cpd n°6

Intermediate (1): 2-(4-Dodecyl-1H-1,2,3-triazol-1-yl)pyridine was obtained following the general procedure A at 120°C for 24 hrs starting from tetrazolo[1,5-a]pyridine (150.0 mg, 1.25 mmol, 1.0 equiv.) and 1-tetradecyne (341 µL, 1.38 mmol, 1.1 equiv.) after column chromatography on silica gel (cyclohexane/EtOAc 80:20) as a pale yellow solid (278.0 mg, 70% chemical yield). *Rf* (cyclohexane/EtOAc 80:20): 0.12; **^{1}- NMR** (300 MHz, $CDCl_3$) δ ppm: 8.37 (d, *J* = 4.1 Hz, 1H), 8.21 (s, 1H), 8.07 (d, *J* = 8.2 Hz, 1H), 7.78 (t, *J* = 8.6 Hz, 1H), 7.2 (dd, 1H), 2.70 (t, *J* = 7.6 Hz, 2H), 1.64 (p, *J* = 7.5 Hz, 2 H), 1.24 (d, *J* = 48.3 Hz, 18 H), 0.77 (t, *J* = 6.5 Hz, 3H); **^{13}C-NMR** (75 MHz, $CDCl_3$) δ ppm: 149.3, 148.8, 148.4, 138.9, 123.1, 118.0, 113.6, 31.9, 29.7, 29.6, 29.5, 29.4, 29.33, 29.25, 29.19, 26.9, 25.6, 22.7, 14.1; **MS** (ESI+) m/z (%): 315.2529 (100) $[M+H]^+$, 337.2349 (69) $[M+Na]^+$.

<u>Cpd n°6</u>: 2-(4-Dodecyl-1H-1,2,3-triazol-1-yl)pyridine chlorotricarbonyl rhenium(I)

Cpd n°6 was obtained according to general procedure B starting from 2-(4-dodecyl-1H-1,2,3-triazol-1-yl)pyridine (28.0 mg, 92.1 mmol, 1.0 equiv.) and chloropentacarbonylrhenium(I) (35.0 mg, 96.7 mmol, 1.05 equiv.) after purification by column chromatography on silica gel (cyclohexane/EtOAc 30:70) as a yellow solid (44.0 mg, 77% chemical yield). **^{1}H-NMR** (300 MHz, $CDCl_3$): δ (ppm). 8.89 (d, *J* = 5.4 Hz, 1H), 8.12 (m, 2H), 7.74 (d, *J* = 8.1 Hz, 1H), 7.47 (dd, *J* = 5.7, 7.2 Hz, 1H), 2.75 (m, 2H), 1.68 (quint, *J* = 7.2 Hz, 2H), 1.23 (m, 18H), 0.81 (t, *J* = 6.9 Hz, 3H); **^{13}C-NMR** (75 MHz, $CDCl_3$): δ (ppm). 153.2, 152.8, 147.7, 141.9, 125.8, 120.2, 113.4, 32.0, 29.79, 29.77, 29.76, 29.6, 29.5, 29.4, 29.36, 28.8, 25.8, 22.8, 14.3; **MS** (ESI+) m/z (%): 899.4322 (52) $[L_2Re(CO)_3]^+$, 643.1432 (100) $[M+Na]^+$, 585.1859 (48) $[M\text{-}Cl]^+$, 337.2349 (48) $[M\text{-}Re(CO)_3Cl+Na]^+$; **HRMS** (ESI+): calcd for $C_{22}H_{30}ClN_4NaO_3Re$: 643.1448, found: 643.1432; HPLC(40 to 100% ACN in 30 min - column C18A): rt 21.754 (100%); **IR** n_{max}/cm^{-1} 2028, 1914 (CO).

2-(1-(3-Nitrophenyl)-1H-1,2,3-triazol-4-yl)-6-chloropyridine was obtained following the general procedure A at 100°C for 24 hrs starting from 1-ethynyl-3-nitrobenzene (50.0 mg, 0.34 mmol, 1.0 equiv.) and 2-azido-6-chloropyridine (52.6 mg, 0.34 mmol, 1.0 equiv.) after column chromatography on silica gel (cyclohexane/EtOAc 60:40) as a colourless solid (69.0 mg, 67% chemical yield). *Rf* (cyclohexane/EtOAc 70:30): 0.35; **^{1}H-NMR** (300 MHz, $CDCl_3$) δ ppm: 8.92 (s, 1H), 8.75 (m, 1H), 8.29 (d, *J* = 7.8 Hz, 1H), 8.24-8.17 (m, 2H), 7.93 (t-app, *J* = 7.8 Hz, 1H), 7.67 (t-app, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H); **^{13}C-NMR** (75 MHz, $CDCl_3$) δ ppm: 150.5, 148.9, 148.5, 146.2, 141.8, 131.8, 131.7, 130.2, 124.4, 123.3, 120.9, 118.0, 112.2.

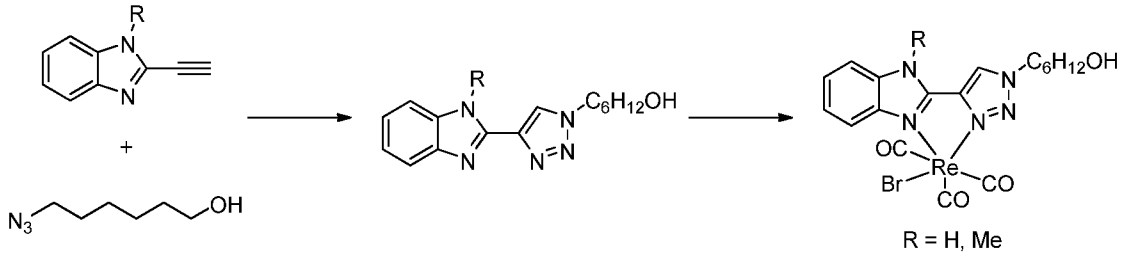
<u>Cpd n°38</u>: 2-(1-(3-Nitrophenyl)-1H-1,2,3-triazol-4-yl)-6-chloropyridine chloro tricarbonylrhenium(I)

It was obtained according to general procedure B starting from 2-(1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)-6-chloropyridine (20.0 mg, 66.2 mmol, 1.0 equiv.) and chloropentacarbonylrhenium(I) (24.2 mg, 66.8 mmol, 1.01 equiv.) after filtration and washing with toluene as a bright yellow solid (33.0 mg, 82% chemical yield). **MS** (ESI+) m/z (%): 629.9330 (59) $[M+Na]^+$, 589.9854 (70) $[M-Cl+H_2O]^+$, 571.9747 (57) $[M-Cl]^+$, 324.0254 (100) $[M-Re(CO)_3Cl+Na]^+$; **HRMS** (ESI+): calcd for $C_{16}H_8Cl_2N_5NaO_5Re$: 629.9337, found: 629.9330; **IR** n_{max}/cm^{-1} 2032, 1939, 1906 (CO).

<u>Cpd n°37</u>: 2-(4-Dodecyl-1H-1,2,3-triazol-1-yl)pyridine pyridiniumtricarbonylrhenium tetrafluoroborate2-(4-dodecyl-1H-1,2,3-triazol-1-yl)pyridine bromotricarbonylrhenium (30.0 mg, 45.0 mmol, 1.0 equiv.) is dissolved in ACN (9.4 mL) and $AgBF_4$ (9.7 mg, 50.0 mmol, 1.1 equiv.) is added in one portion. The solution is degassed, purged with Ar and refluxed for 5 hrs under Ar protected from light. The mixture is evaporated. The residue is taken up in THF (stabilized, 6.5 mL), pyridine (11 mL, 136 mmol, 3.0 equiv.) is added and the mixture is stirred at 50°C under Ar protected from light for 20 hrs. The mixture is filtered on celite and evaporated. The residue is taken up in Et_2O and the resulting solid is filtered and dried under vacuum to afford 2-(4-dodecyl-1H-1,2,3-triazol-1-yl)pyridine pyridiniumtricarbonylrhenium tetrafluoroborate as a pale yellow solid (21.4 mg, 94% chemical yield). **^{1}H-NMR** (300 MHz, CD_3OD): δ (ppm). 9.27 (ddd, *J* = 0.6, 1.5, 5.4 Hz,

1H), 9.11 (s, 1H), 8.46 (m, 3H), 8.26 (dt_{app}, J = 0.9, 8.4 Hz, 1H), 7.95 (m, 2H), 7.86 (ddd, J = 1.2, 5.7, 7.8 Hz, 1H), 7.30 (m, 2H), 2.94 (t, J = 7.5 Hz, 2H), 1.84 (quint, J = 7.5 Hz, 2H), 1.35 (m, 18H), 0.90 (t, J = 6.9 Hz, 3H).

10. Benzimidazoles-triazole complexes



Cpd n°33: 6-(4-(1H-Benzo[d]imidazol-2-yl)-1H-1,2,3-triazol-1-yl)hexan-1-ol-bromotricarbonylrhenium(I)

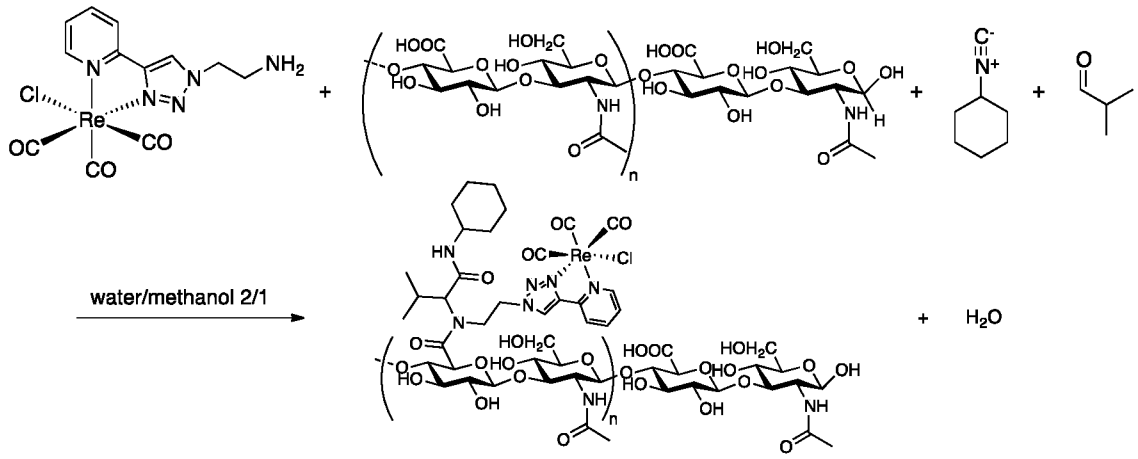
2-Ethynyl-1H-benzo[d]imidazole (50.0 mg, 0.35 mmol, 1.0 equiv.) is suspended in *tert*-butanol (4.6 mL). 6-Azidohexanol (55.4 mg, 0.39 mmol, 1.1 equiv.), an aqueous solution of $CuSO_4.5H_2O$ (2.15 mL, 0.03 mmol, 0.1 equiv. from a 4.12 $mg.mL^{-1}$ solution in water) and an aqueous solution of sodium ascorbate (2.4 mL, 0.11 mmol, 0.3 equiv., from a 8.7 $mg.mL^{-1}$ solution in water) are then added. The resulting mixture is stirred at 100°C under Ar overnight. The mixture is diluted with H_2O and extracted twice with DCM. The organic phase is dried over $MgSO_4$, filtered and evaporated. The residue is purified by column chromatography on silica gel (eluent DCM/MeOH 94:6) to afford 6-(4-(1H-benzo[d]imidazol-2-yl)-1H-1,2,3-triazol-1-yl)hexan-1-ol as a white solid (74.0 mg, 74% chemical yield). R*f* (DCM/MeOH 94:6) = 0.26; **^{1}H-NMR** (300MHz, $CDCl_3$): δ (ppm). 8.51 (s, 1H), 7.20 (dd, J = 3.3 Hz, 6.0 Hz, 2H), 7.99 (q, J = 3.0 Hz, 2H), 4.47 (t, J = 7.2 Hz, 2H), 3.65 (t, J = 6.3 Hz, 2H), 1.98 (quint, J = 6.9 Hz, 2H), 1.55 (m, 2H), 1.40 (m, 4H); **^{13}C-NMR** (75MHz, $CDCl_3$): δ (ppm). 144.1, 139.6, 124.0, 123.6, 62.5, 51.0, 32.5, 30.2, 26.3, 25.3.6-(4-(1H-Benzo[d]imidazol-2-yl)-1H-1,2,3-triazol-1-yl)hexan-1-ol (50.0 mg, 175.0 mmol, 1.0 equiv.) is suspended in toluene (2.2 mL) and heated at 110°C under Ar until complete dissolution. Bromopentacarbonylrhenium(I) (71.2 mg, 175.0 mmol, 1.0 equiv.) is added, gas release is observed. The solution is stirred at 110°C under Ar for 6 hrs. The mixture is cooled down to RT and the resulting precipitate is filtered. The solid is washed with toluene and dried under vacuum to afford 6-(4-(1H-benzo[d]imidazol-2-

yl)-1H-1,2,3-triazol-1-yl)hexan-1-ol-bromotricarbonylrhenium(I) as a white solid (104.0 mg, 91% chemical yield).**^{1}H-NMR** (300MHz, Acetone-d_6): δ (ppm). 8.95 (s, 1H), 7.96 (dt_{app}, J = 0.9 Hz, 8.4 Hz, 1H), 7.79 (dt_{app}, J = 0.9 Hz, 8.1 Hz, 2H), 7.55 (m, 2H), 4.76 (t, J = 7.2 Hz, 2H), 3.55 (t, J = 6.3 Hz, 2H), 2.11 (m, 2H), 1.57-1.45 (m, 6H)**^{13}C-NMR** (75MHz, Acetone-d_6): δ (ppm). 147.9, 142.2, 140.5, 134.9, 126.5, 125.9, 125.5, 119.1, 114.3, 62.4, 53.2, 33.6, 31.0, 27.0, 26.2 (CO non-visible)HPLC(0 to 100% ACN in 30 min – column C8A): rt 20.54 (84.62%).

<u>Cpd n°34</u>: 6-(4-(1-Methyl-1H-benzo[d]imidazol-2-yl)-1H-1,2,3-triazol-1-yl)hexan-1-ol-bromotricarbonylrhenium(I)

2-Ethynyl-1-methyl-1H-benzo[d]imidazole (100.0 mg, 0.64 mmol, 1.0 equiv.) is suspended in *tert*-butanol (8.3 mL). 6-Azidohexanol (101.0 mg, 0.70 mmol, 1.1 equiv.), an aqueous solution of $CuSO_4.5H_2O$ (3.9 mL, 0.06 mmol, 0.1 equiv. from a 4.12 $mg.mL^{-1}$ solution in water) and an aqueous solution of sodium ascorbate (4.4 mL, 0.19 mmol, 0.3 equiv., from a 8.7 $mg.mL^{-1}$ solution in water) are then added. The resulting mixture is stirred at 100°C under Ar overnight. The mixture is diluted with H_2O and extracted twice with DCM. The organic phase is dried over $MgSO_4$, filtered and evaporated. The residue is purified by column chromatography on silica gel (eluent DCM/MeOH 94:6) to afford 6-(4-(1-methyl-1H-benzo[d]imidazol-2-yl)-1H-1,2,3-triazol-1-yl)hexan-1-ol as a beige solid (124.0 mg, 65% chemical yield). R*f* (DCM/MeOH 94:6) = 0.29; **^{1}H-NMR** (300MHz, MeOD): δ (ppm). 8.60 (br s, 1H), 7.72 (br s, 2H), 7.32 (m, 2H), 4.53 (t, J = 6.6 Hz, 2H), 4.17 (s, 3H), 3.54 (t, J = 6.3 Hz, 2H), 2.00 (t, J = 6.3 Hz, 2H), 1.54 (m, 2H), 1.41 (m, 4H); **^{13}C-NMR** (75MHz, MeOD): δ (ppm). 126.9, 123., 119.9, 111.7, 62.7, 51.7, 33.4, 32.7, 31.2, 27.3, 26.3.6-(4-(1-Methyl-1H-benzo[d]imidazol-2-yl)-1H-1,2,3-triazol-1-yl)hexan-1-ol (100.0 mg, 334.0 mmol, 1.0 equiv.) is suspended in toluene (4.1 mL) and heated at 110°C under Ar until complete dissolution. Bromopentacarbonylrhenium(I) (135.7 mg, 334.0 mmol, 1.0 equiv.) is added, gas release is observed. The solution is stirred at 110°C under Ar for 6 hrs. The mixture is cooled down to RT and the resulting precipitate is filtered. The solid is washed with toluene and dried under vacuum to afford 6-(4-(1-methyl-1H-benzo[d]imidazol-2-yl)-1H-1,2,3-triazol-1-yl)hexan-1-ol-bromotricarbonylrhenium(I) as a white solid (189.0 mg, 87% chemical yield). **^{1}H-NMR**

(300 MHz, Acetone-d_6): δ (ppm). 9.36 (s, 1H), 7.94 (m, 1H), 7.85 (m, 1H), 7.60 (m, 2H), 4.80 (t, J = 7.2 Hz, 2H), 4.36 (s, 3H), 3.55 (t, J = 6.0 Hz, 2H), 2.15 (m, 2H), 1.57-1.48 (m, 6H). **^{13}C-NMR** (75 MHz, Acetone-d_6): δ (ppm). 148.6, 141.6, 139.6, 136.4, 125.9, 125.7, 125.6, 119.0, 112.5, 62.1, 53.1, 32.4, 32.3, 30.6, 26.7, 26.0 (CO non-visible).**11. Re complexes functionalized with an amine: application to the conjugation with hyaluronic acid (Cpd n°64)**



*Deprotection of **Cpd n°16***

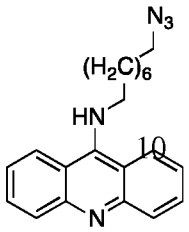
Cpd n°16 (300.0 mg, 504.2 µmol, 1 eq.) was dissolved in dichloromethane (2.5 mL), and trifluoroacetic acid (2.5 mL, 32.7 mmol, 62 eq.) was added carefully to the stirred solution. The mixture became transparent. The reaction mixture was stirred 1 h at room temperature, and monitored by TLC (silica, CH_2Cl_2/EtOH 80/20), then solvents were removed under reduced pressure. The solid was dissolved in a minimum amount of methanol and concentrated HCl was added. It was stirred 10 min and then concentrated under reduced pressure to obtain [$Re(CO)_3(Cl)$Pyta-C_2H_4-$NH_3^+Cl^-$].

Ugi reaction for coupling with hyaluronic acid

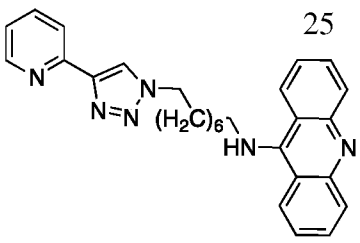
Hyaluronic acid (400 ≤ MW ≤ 1000 kDa) was dissolved in water to 1.25 mg/mL (49.6 mg in 40 mL), then 20 mL of methanol followed by [$Re(CO)_3(Cl)$Pyta-C_2H_4-$NH_3^+Cl^-$] (3.6 mg, 0.072 mmol) dissolved in a minimum of methanol were added to the mixture. Once it was totally dissolved, isobutyraldehyde (40 µL, 0.44 mmol) and cyclohexyl isocyanide (40 µL, 0.080 mmol) dissolved in methanol were added to the reaction mixture. The reaction was monitored by HPLC (50 µL of the mixture were diluted in 450 µL of water), 50 µL injected. Acetonitrile/Water+0,1%TFA 5/95 to 100/0 in 10 min.

After two days of stirring at r.t., 10 mL of the mixture was diluted in 130 mL of a Guanidine-HCl/EtOH mixture (6,8 mL of 3M Guanidine + 123,2 mL absolute EtOH) and precipitated at -20°C overnight. The obtained precipitate was centrifugated and washed three times with ice cold absolute ethanol to provide **Cpd n°64**. **IR** (cm^{-1}): 3335.44, 2931.68, 2031.56, 1926.74, 1655.92, 1606.61, 1403.88, 1376.02.

12. Re complex functionalized with a nucleus targeting molecule (Cpd n°65)

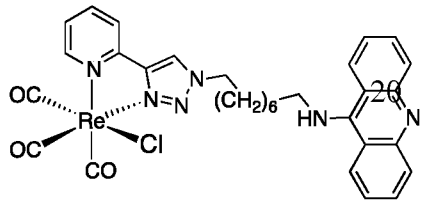


N-(8-azidooctyl)acridin-9-amine. Under argon, 9-chloroacridine (214.8 mg, 1.01 mmol, 1 eq.) was dissolved in 20 mL of DMF and 8-azidooctan-1-amine (409.1 mg, 2,40 mmol, 2.4 eq.) was added. The mixture was heated to 120°C, stirred for 2h30 and monitored by TLC (alumina oxide, CH_2Cl_2/EtOH: 95/5). Solvent was removed under reduced pressure to afford a brown oil, that was purified by column chromatography (silica, CH_2Cl_2/EtOH 100/0 to 90/10, then CH_2Cl_2/EtOH 80/20 + 1% NEt_3). After removing solvents a yellow solid was obtained (282.7 mg, 81%). **^{1}H NMR** (300 MHz, $CDCl_3$): d 9.90 (s, 1H, NH), 8.40 (d, J = 8.7 Hz, 2H, H^4 & H^5), 8.06 (d, J = 1.2 Hz, 2H, H^1 & H^8), 7.49 (ddd, J = 8.3, 6.9, 1.2 Hz, 2H, H^3 & H^6), 7.21 (ddd, J = 8.3, 6.9, 1.2 Hz, 2H, H^2 & H^7), 4.02 (t, J = 7.5 Hz, 2H, NH-CH_2), 3.17 (t, J = 6.9 Hz, 2H, CH_2-N_3), 2.09 – 1.80 (m, 2H, NH-CH_2-CH_2), 1.46 (m, 2H, CH_2-CH_2-N_3), 1.32 – 1.18 (m, 8H, CH_2). **^{13}C NMR** (75 MHz, $CDCl_3$): d (ppm) 156.13 (C9), 141.46, 133.28 (C4,C5), 125.13 (C3,C6), 123.04 (C1,C8), 120.91 (C2,C7), 113.09, 51.38 (CH_2-N_3), 49.07 (NH-CH_2), 30.33 (NH-CH_2-CH_2), 29.09 (CH_2-CH_2-N3), 28.99, 28.75, 26.81, 26.57 (4 x CH_2). **IR:** 3189.2, 2928.8, 2856.6, 2736.0, 2088.5, 1634.5, 1587.2, 1563.3, 1532.8, 1468.5, 1428.7; **HR-MS (ESI+):** m/z calculated for [$C_{21}H_{26}N_5$]: 348.2183, found: 328.2180, error: 0.8 ppm.



N-(8-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl)octyl)acridin-9-amine N-(8-azidooctyl)acridin-9-amine (198.7 mg, 0.57 mmol, 1 eq.) and 2-ethynylpyridine (87.6 mg, 0.85 mmol, 1.5 eq) were dissolved in 4 mL of dichloromethane and 4 mL of water, then copper sulfate (II) (14.0 mg, 88 µmol, 0.15 eq.) and sodium ascorbate (32.5 mg,

0.16 mmol, 0.29 eq) were added to the mixture. It was sonicated, stirred at room temperature overnight and monitored by TLC (Aluminum oxide, CH_2Cl_2/EtOH 98/2). A solution of ammonia (28%) was added to wash the organic layer; it was then washed with more ammonia and brine, and the aqueous layer was extracted with dichloromethane until the organic layer became. Organic layers were dried over sodium sulfate and solvents were removed to give a dark yellow oil (251.7 mg, 98%). **^{1}H NMR** (300 MHz, $CDCl_3$): d (ppm) 8.55 (d, J = 4.0 Hz, 1H, H^a pyta), 8.15 (d, J = 7.9 Hz, 1H, H^d pyta), 8.09 (s, 1H, H^g pyta), 8.05 – 8.08 (m, 4H, $H^{1,8,4,5}$ acridine), 7.72 (td, J = 7.9, 1.5 Hz, 1H, H^c pyta), 7.67 – 7.57 (m, 2H, H^3 H^6 acridine), 7.31 (dd, J = 8.8, 6.5 Hz, 2H, H^2 & H^7 acridine), 7.21 – 7.14 (m, 1H, H^b pyta, J = 1.5, 4.0 Hz), 5.08 (s, 1H, N*H*), 4.33 (t, J = 7.0 Hz, 2H, NH-C*H*$_2$), 3.73 (t, J = 7.2 Hz, 2H, C*H*$_2$-N_3), 1.85 (p, J = 7.0 Hz, 2H, NH-CH_2-C*H*$_2$), 1.68 (p, J = 7.2 Hz, 2H, C*H*$_2$-CH_2-N_3), 1.34 - 1.24 (m, 8H, C*H*$_z$). **^{13}C NMR** (75 MHz, $CDCl_3$): d (ppm) 151.35 (C^f pyta), 150.39 (acridine), 149.40 (Ce pyta), 148.42 (acridine), 136.96 (C^c pyta), 129.86 (C^4 C^5 acridine), 129.53 (C^3 C^6 acridine), 123.01 (C^g pyta), 122.89 (C^1 C^8 acridine), 122.80 (C^b pyta), 121.85 (C^2 C^7), 120.25 (C^d pyta), 116.70 (acridine), 50.86 (*C*H_2-N_3), 50.42 (NH-*C*H_2), 31.71 (NH-CH_2-*C*H_2), 30.13 (*C*H_2-CH_2-N_3), 29.00, 28.77, 26.69, 26.24 (4 x CH_2). **IR:** 3059.4, 2926.7, 2854.1, 1596.3, 1557.7, 1519.1, 1504.4, 1471.3, 1420.2.



Cpd n°65 - [Re(CO)$_3$(Cl)(Pyta-(CH$_2$)$_8$-NH-Acridine)] *N*-(8-(4-(pyridin-2-yl)-1*H*-1,2,3-triazol-1-yl)octyl)acridin-9-amine (72.9 mg, 162 µmol, 1 eq.) was dissolved in 15 mL of warm toluene and dichloromethane was added to solubilize it better. Then, $Re(CO)_5Cl$ (64.9 mg, 179 µmol, 1.1 eq) was added and the reaction mixture was stirred at 80°C for 6 h and monitored by TLC (CH_2Cl_2/EtOH: 98/2). Solvents were removed and the crude was purified by column chromatography (aluminium oxide neutral, CH_2Cl_2/EtOH 98/2) to afford **Cpd n°65** as a yellow solid (47.2 mg, 39%). **^{1}H NMR** (300 MHz, $CDCl_3$): d (ppm) 9.04 (s, 1H), 8.98 (ddd, J = 5.6, 1.6, 0.9 Hz, 1H), 8.35 – 8.30 (m, 4H), 8.20 – 8.08 (m, 2H), 7.98 – 7.94 (m, 2H), 7.63 (ddd, J = 8.8, 6.7, 1.3 Hz, 2H), 7.55 (ddd, J = 7.3, 5.6, 1.6 Hz, 1H), 7.32 (ddd, J = 8.8, 6.7, 1.3 Hz, 2H), 4.54 (t, J = 7.2 Hz, 2H), 3.90 (t, J = 7.2 Hz, 2H), 3.59 (q, J = 7.0 Hz, 1H), 2.06 (p, J = 2.2 Hz, 3H), 1.98 – 1.91 (m, 0H), 1.87 – 1.76 (m, 2H), 1.30 (q,

J = 2.5 Hz, 7H), 1.14 (t, J = 7.0 Hz, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$): d (ppm) 152.16, 146.25, 139.06, 129.82, 125.64, 125.13, 123.99, 123.34, 121.70, 115.04, 51.17, 49.35, 29.93, 29.35, 29.10, 28.84, 28.67, 28.58, 28.33, 28.07, 27.82, 27.58, 25.60, 24.97. **HR-MS (ESI+):** m/z calculated for $[C_{31}H_{31}ClN_6O_3Re]$: 757.1690, found: 757.1742, error: 6.8 ppm.

X-RAY FLUORESCENCE PROPERTIES

XRF Spectroscopy

The X-ray fluorescence spectrum of **Cpd n°1** has been recorded for an excitation at 12 keV at the synchrotron APS at Argonne, Chicago (Fig. 1). The spectrum clearly shows that, even if there is the same amount of manganese and rhenium are present in Cpd n°1, the bands arising from the rhenium are much more intense. Especially, $L\beta_1$ band of rhenium is about 100 times more intense than Mn $K\alpha$ band. This result means that rhenium is more emissive and thus sensitive than manganese in Cpd n°1.

XRF Imaging: Golgi apparatus imaging

Cell culture and cell treatments

HT29 MD2 human cancerous intestinal epithelial cells were used for all experiments. HT29 cells were obtained from the European Collection of Cell Cultures (ECCC) and were stably transfected to overexpress MD2 (Lenoir et al., Life Sciences, 2008, 82, 519).

Cells were cultured in DMEM supplemented with 10% of heat inactivated fetal calf serum, 1% of penicillin-streptomycin (100X) and 0.1% of blasticidin (10 µg/mL) at 37°C in a 5% CO2/air atmosphere.

HT29-MD2 cells were seeded on silicon nitride windows (size: 1 mm x 1 mm, thickness: 500 nm) in 24-wells plate (75000 cells/well). After 36 h, they were incubated with **Cpd n°1** (100 µM) for 2 h. Cells were washed with NaCl 0.9% and a chaotropic shock was performed by adding a solution of NaCl 1 M. They were then washed and treated with

EDTA (50 mM). After two more washings, cells were cryofixed in liquid ethane and freeze-dried.

XRF imaging

Mapping of intracellular manganese, potassium and rhenium was performed on 2-ID-D beamline of Advanced Photon Source synchrotron (Argonne National Laboratory, Chicago, USA). Cells were located using a phase-contrast optical microscope. All measurements were conducted at room temperature, under a He atmosphere using a 6.8 keV or 12 keV monochomatic X-ray incident beam focused to 200 nm diameter. The X-ray fluorescence signal was detected with an integration time of 2 or 4 s per pixel, with a 200 nm pixel size, at 90° to the incident beam using a Vortex EM single element silicon drift detector. Images analyses were performed by using MAPS software from APS.

Due to the last washing of cells with NaCl 0.9% before cryofixation and freeze-drying, some NaCl crystals were present on silicon nitride membranes. The strong signal of Cl was then subtracted from other element signals.

Results

Maps showing the localization of potassium, manganese and rhenium are reported in Figure 2 (A), (B) and (C) respectively.

The potassium map (Fig. 2(A)) enables to localize the cell nucleus due to its intrinsic elevated concentration in potassium.

The Mn map (Fig. 2(B)) shows a homogenous distribution of Mn over the whole cell, very similar to the one of basal Mn in control cells (cells incubated only with the vehicle). The Mn imaging consequently failed to give the accurate distribution of **Cpd n°1** since incubated cells and controls cells were the same due to a too weak concentration of **Cpd n°1**.

Very interestingly, rhenium map (Fig 2(C)) shows a perinuclear distribution of Re. As rhenium is intrinsically present in cells as an ultra-trace element, the rhenium map

corresponds to the distribution of **Cpd n°1** only. Cells were also analyzed by UV-vis fluorescence microscopy, confirming the perinuclear distribution of **Cpd n°1**.

These results clearly highlight the strong sensitivity of X-ray fluorescence of rhenium XRF probes of the invention. Labeling of the SOD mimic Mn complex by the rhenium XRF probe of the invention enabled its observation despite that it was present in weak quantities in incubated cells.

XRF Imaging: nucleus imaging

Cell culture and cell treatments

Chinese Hamster Ovarian (CHO) cells were seeded in 12-well plates containing Silicon nitride slides, and grown for 24 h. Cells were washed once with HBSS buffer and once with fresh DMEM.

Cells were then incubated with 10 μM solutions of **Cys-NLS-HD-ReCl** or **Cys-HD-ReCl** in DMEM for one hour at 37 °C. Cells were washed twice with PBS, and incubated for 8 min with 500 μL of a 4% PFA solution in PBS at room temperature. Finally, cells were washed once with PBS and twice with milliQ water. Slides were left to dry at air prior to examination by microscopy.

XRF imaging

Mapping of intracellular calcium, zinc, phosphor and rhenium was performed on Nanoscopium beamline of synchrotron Soleil (Saclay, France).

Cells were located using a phase-contrast optical microscope. All measurements were conducted at room temperature, under a He atmosphere using a 12 keV monochomatic X-ray incident beam focused to 500 nm diameter. The X-ray fluorescence signal was detected with an integration time of 3 s per pixel, with a 500 nm pixel size, at 90° to the incident beam using a Vortex EM single element silicon drift detector. Images were processed and analysed using PyMCA ImageJ softwares.

Results

Maps showing the localization of calcium, zinc, phosphor and rhenium in incubated CHO cells are reported in Figure 3.

These experiments led to the successful detection of the Re signal and to map the Re distribution in CHO cells incubated with **Cys-NLS-HD-ReCl** or **Cys-HD-ReCl**.

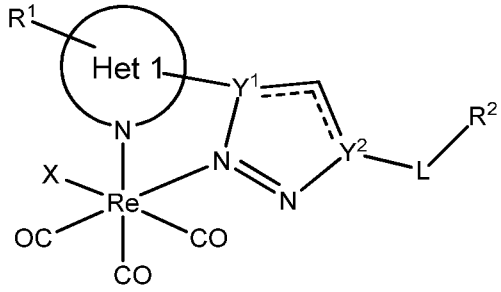
Some cells incubated with **Cys-NLS-HD-ReCl** displayed a very localized area where various elements concentrated, including Zn and P. Ca signal, although more diffuse and present in the whole cell, was also stronger in this area. Interestingly, Re signal mostly concentrated in this area. According to the literature, P and Zn are accumulated in the nucleus and are often used to identify it. This thus suggests that the labelled homeodomain is mainly localized in the nucleus. This is consistent with the presence of the NLS sequence in the sequence of the protein.

On the other hand, cells incubated with **Cys-HD-ReCl**, missing the NLS sequence, displayed weaker signals, which seemed distributed all over the cells.

It should be noted that the resulting maps give a 2D-projection of the signal of the cell. If an element is homogenously distributed in the volume of the cell, its signal will thus be stronger in the thicker areas. This seems to be the case for **Cys-HD-ReCl**.

CLAIMS

1. Use of a X-ray fluorescence probe of formula I for performing X-ray fluorescence imaging, wherein formula I is



(I)

or a salt thereof, wherein

X represents halo, optionally substituted pyridin-1-yl, carbene, thiolato, alkynyl, carboxylate, phosphine, phosphonate, sulfonate, OH_2, NC-alkyl, oxyanion such as phosphate;

Het 1 represents a heteroaryl group comprising at least one nitrogen atom; preferably **Het 1** is selected from pyridinyl, quinolynyl, quinoxalinyl, benzothiadiazolyl, benzimidazole, benzoxazole, bipyridine;

$\mathbf{R^1}$ is either absent or represents one or more substituent selected from halo, nitro, alkyl, aryl, phosphate, sulfate, cyano;

$\mathbf{Y^1}$ represents C and $\mathbf{Y^2}$ represents N; or $\mathbf{Y^1}$ represents N and $\mathbf{Y^2}$ represents C;

====== represents a single bound or a double bound depending on $\mathbf{Y^1}$ and $\mathbf{Y^2}$ definitions;

L represents a single bound or a linker selected from alkyl, aryl, heteroaryl, heterocyclyl, alkylaryl, arylalkyl, alkoxy, alkenyl, alkynyl, polyethylene glycol, polypropylene glycol, polyamines or a combination thereof; said groups being optionally interrupted or terminated by one or more -O-, -S-, -S(O)-, $-S(O)_2-$, -C(O)-NH-, -NH-C(O)-, -C(O)O-, -C(O)-, -NH- or a combination thereof; said groups optionally further comprising pendant groups selected from carboxylic acid, carboxylate, sulfonic acid, sulfonate, hydroxy; the

linker optionally additionally comprising a residue of a reactive group through which **L** is bound to $\mathbf{R^2}$;

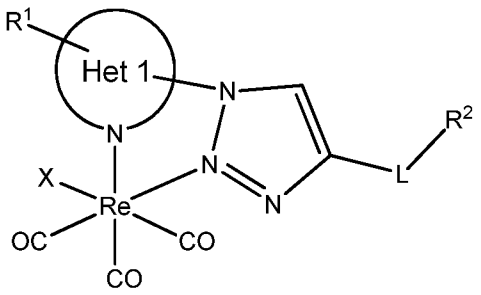
$\mathbf{R^2}$ represents a group selected from:

a hydrogen atom;

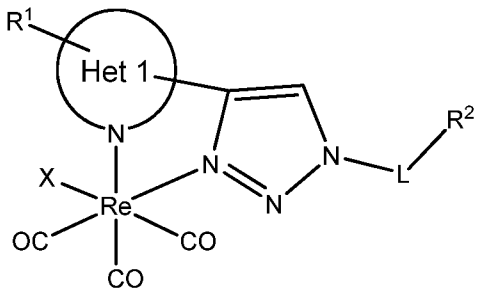
a reactive group selected from azide, alkynyl, amino, alkylamino, amido, maleimide, thiol, hydroxy, ester, activated ester, carboxylic acid, activated carboxylic acid, halo, nitro, nitrile, isonitriles, acrylamide, aldehyde, ketone, acetals, ketals, anhydride, glutaric anhydride, succinic anhydride, maleic anhydride, thiocyanate, isothiocyanate, isocyanate, hydrazide, hydrazines, hydrazones, ethers, oxides, cyanates, diazo, diazonium, sulfides, disulfides, sulfoxides, sulfones, sulfonic acids, sulfinic acids, sulfates, sulfenic acids, amidines, imides, imidates, nitrones, hydroxylamines, oximes, hydroxamic acids, thiohydroxamic acids, alkenes, ortho esters, sulfites, enamines, ynamines, ureas, pseudoureas, semicarbazides, carbodiimides, carbamates, carbonate, activated carbonate, imines, phosphonium, chelating moiety;

a bioactive group selected from steroid, peptide, protein, amino acid, nucleic acid, nucleoside, nucleotide, oligonucleotide, antibody, saccharide, polysaccharide, lipid, hormone, biotin, avidin, therapeutic ingredient, complex of metallic ion, microparticle, nanoparticle, fluorophore, a cell organelle targeting group and combinations thereof.

2. The use according to claim **1**, wherein the X-ray fluorescence probe is of formula Ia or Ib



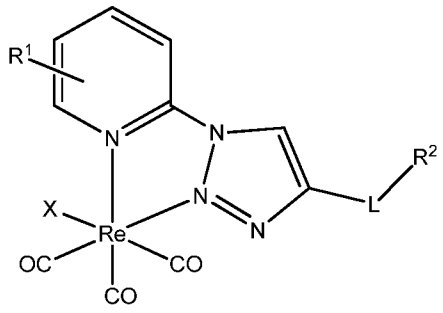
(Ia)



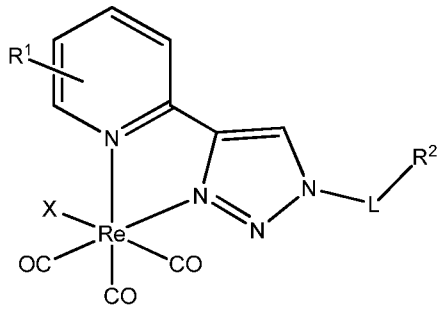
(Ib)

or a salt thereof, wherein **X**, **Het 1**, $\mathbf{R^1}$, **L** and $\mathbf{R^2}$ are as defined in claim **1**.

3. The use according to claim **1** or claim **2**, wherein the X-ray fluorescence probe is of formula Ia1 or Ib1



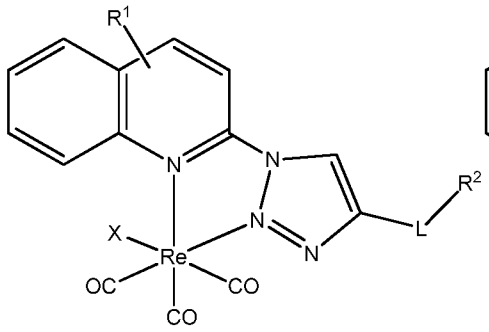
(Ia1)



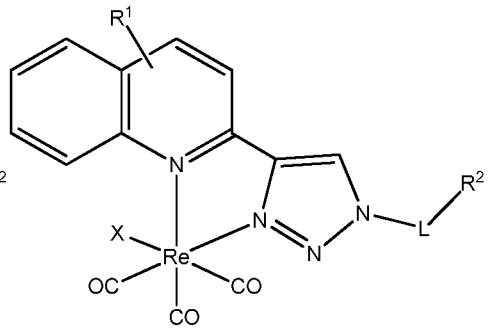
(Ib1)

or a salt thereof, wherein **X**, $\mathbf{R^1}$, **L** and $\mathbf{R^2}$ are as defined in claim **1**.

4. The use according to claim **1** or claim **2**, wherein the X-ray fluorescence probe is of formula Ia2 or Ib2:



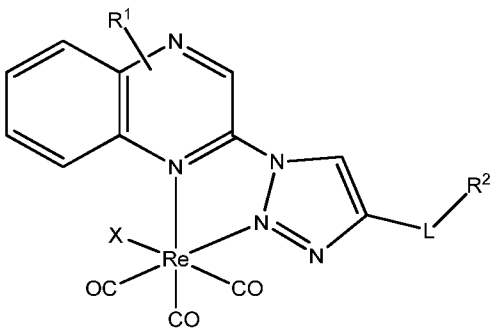
(Ia2)



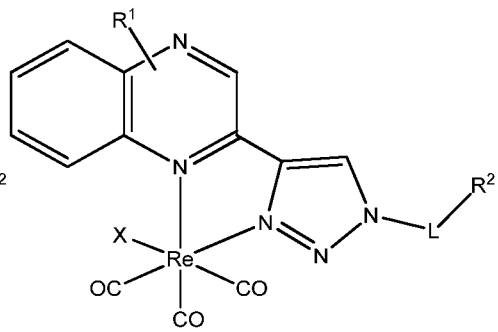
(Ib2)

or a salt thereof, wherein **X**, $\mathbf{R^1}$, **L** and $\mathbf{R^2}$ are as defined in claim **1**.

5. The use according to claim **1** or claim **2**, wherein the X-ray fluorescence probe is of formula Ia3 or Ib3:



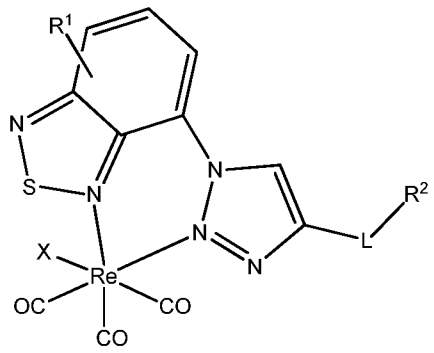
(Ia3)



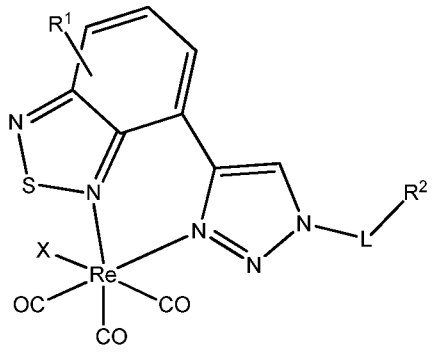
(Ib3)

or a salt thereof, wherein **X**, $\mathbf{R^1}$, **L** and $\mathbf{R^2}$ are as defined in claim **1**.

6. The use according to claim **1** or claim **2**, wherein the X-ray fluorescence probe is of formula Ia4 or Ib4:



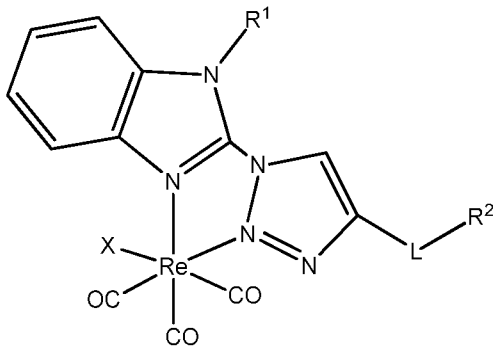
(Ia4)



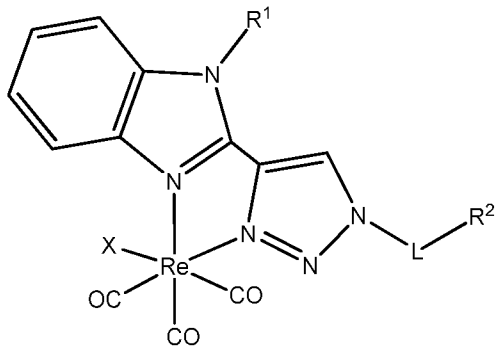
(Ib4)

or a salt thereof, wherein **X**, $\mathbf{R^1}$, **L** and $\mathbf{R^2}$ are as defined in claim **1**.

7. The use according to claim **1** or claim **2**, wherein the X-ray fluorescence probe is of formula Ia5 or Ib5:



(Ia5)

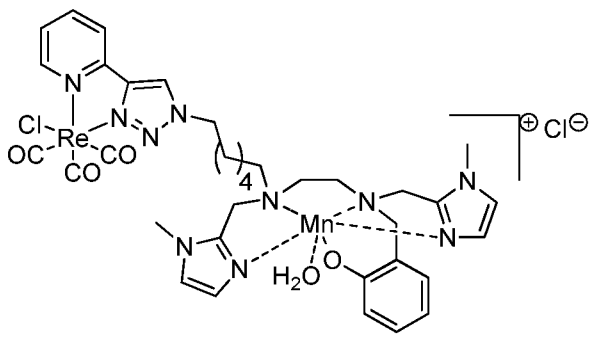


(Ib5)

or a salt thereof, wherein **X**, $\mathbf{R^1}$, **L** and $\mathbf{R^2}$ are as defined in claim **1**.

8. The use according to any one of claims **1** to **7**, wherein the X-ray fluorescence probe is selected from:

1



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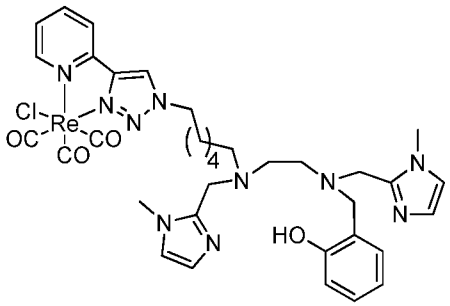

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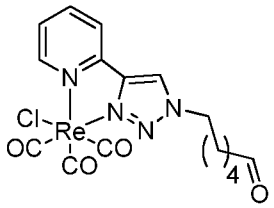

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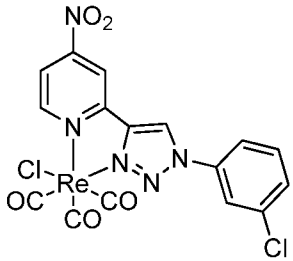

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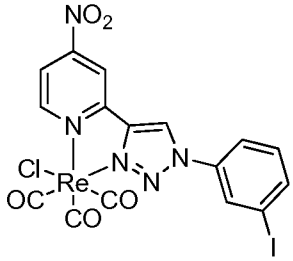

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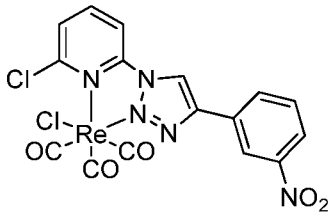

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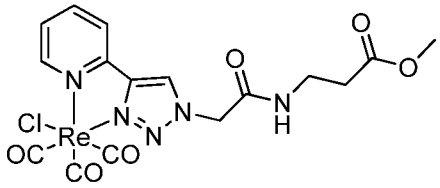

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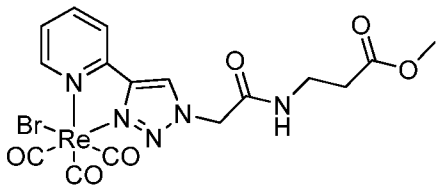

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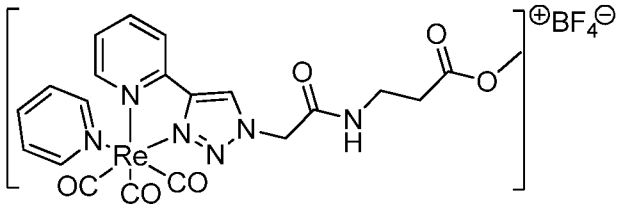

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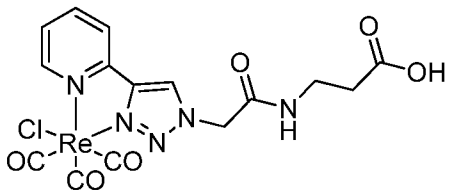

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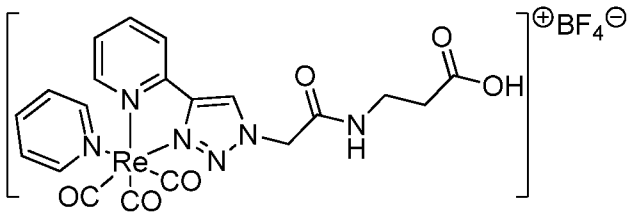

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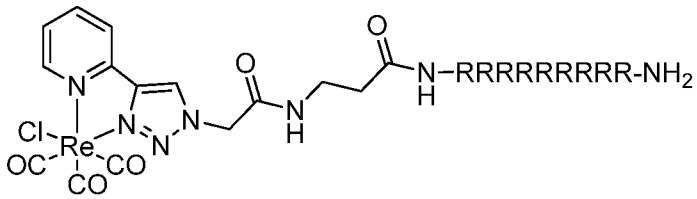

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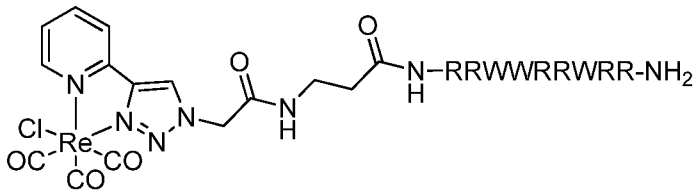

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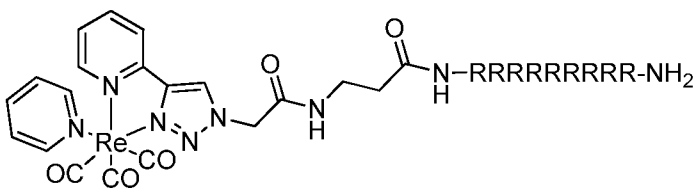

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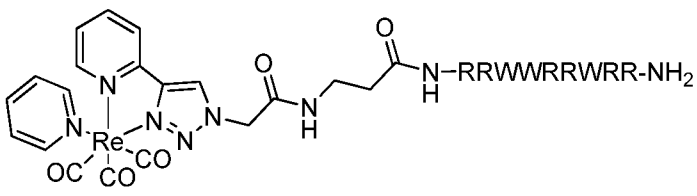

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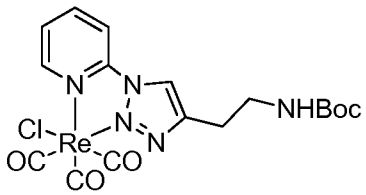

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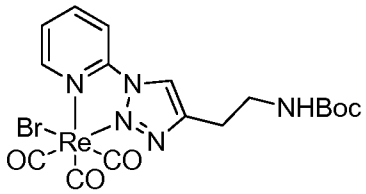

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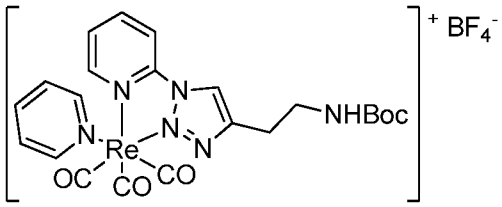

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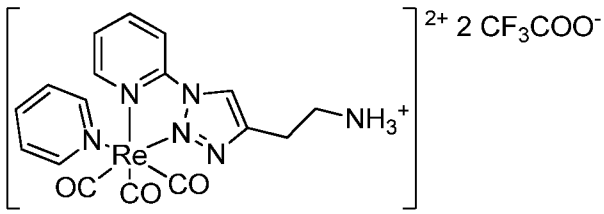

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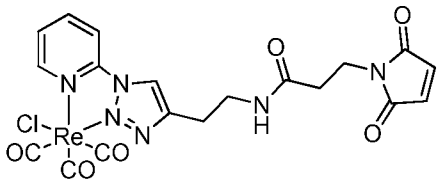

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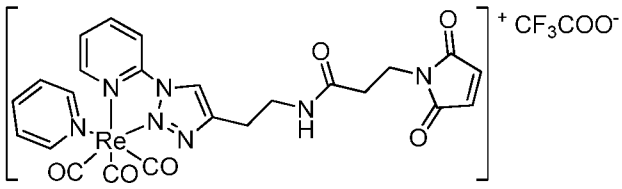

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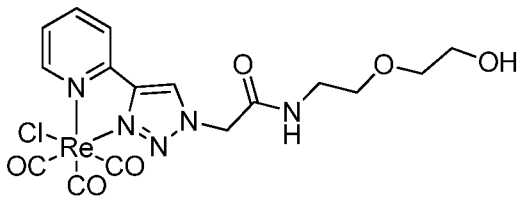

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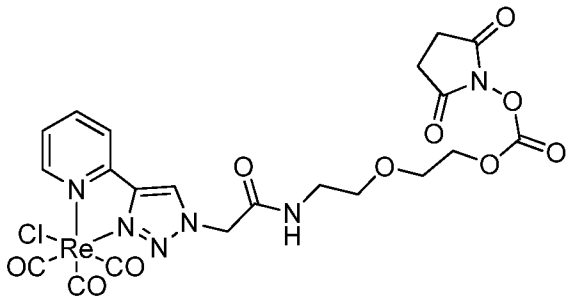

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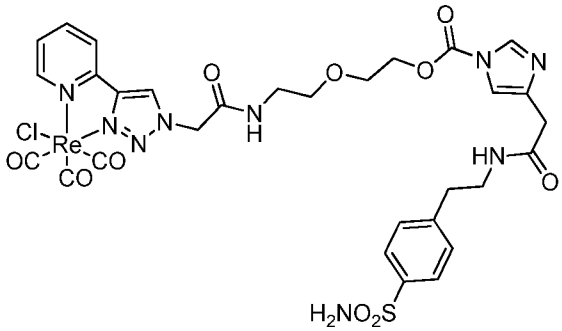

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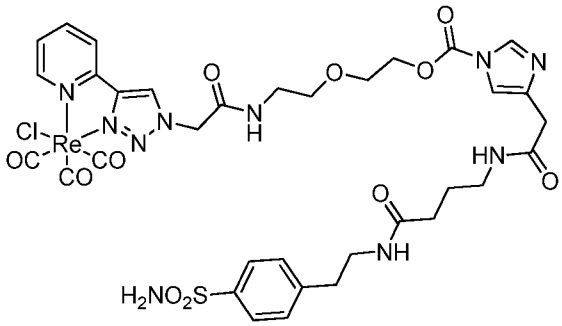

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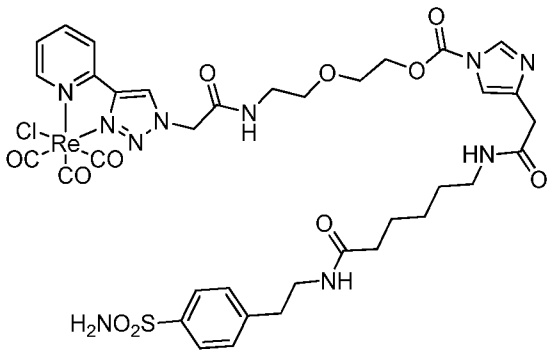

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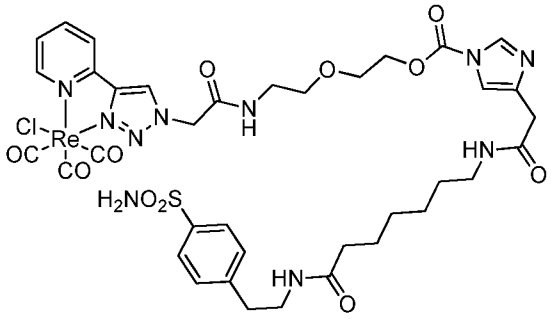

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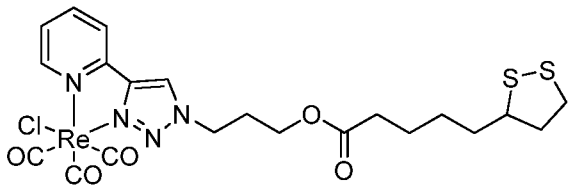

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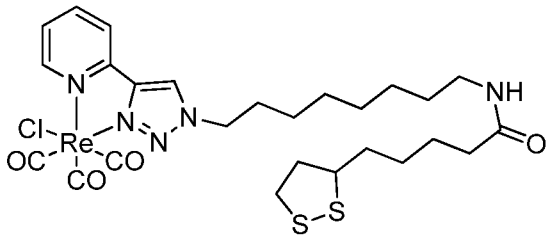

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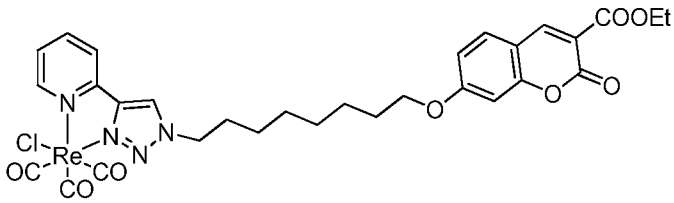

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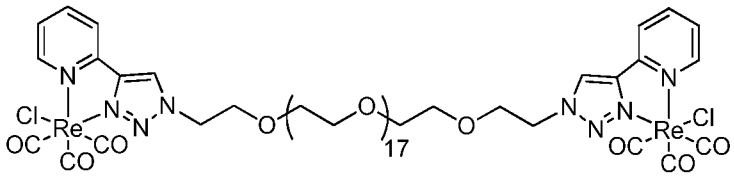

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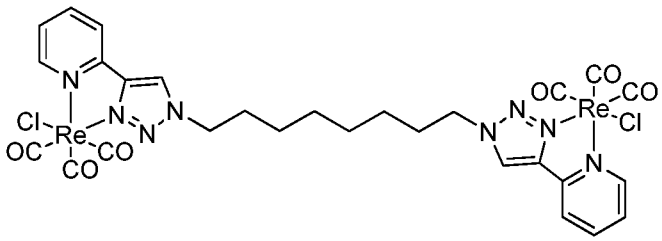

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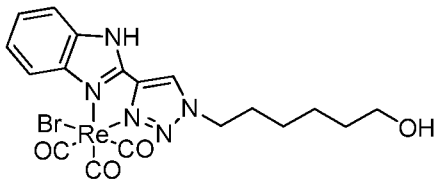

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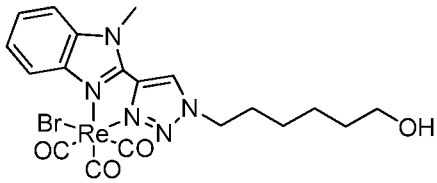

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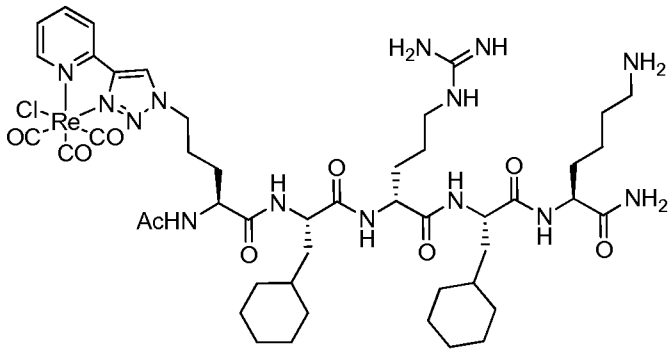

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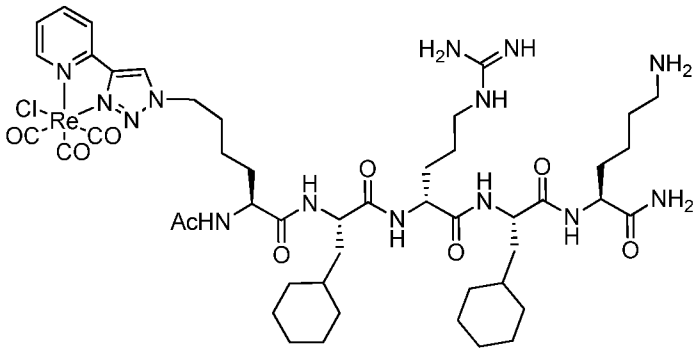

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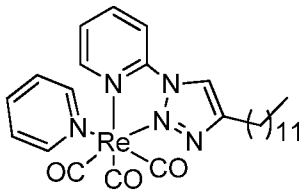

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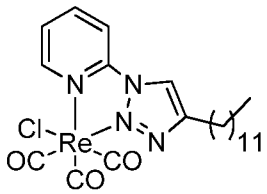

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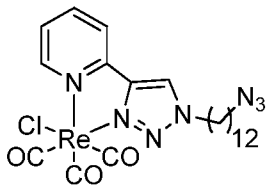

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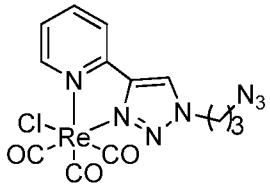

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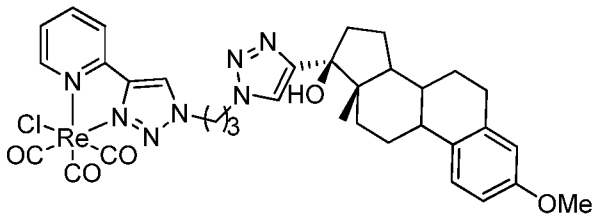

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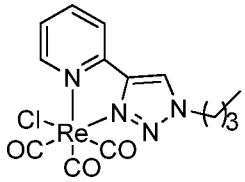

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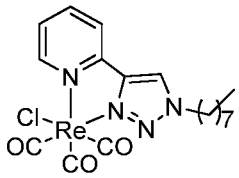

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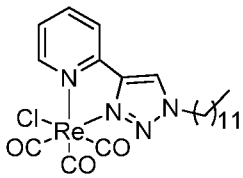

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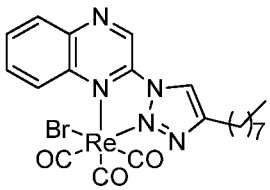

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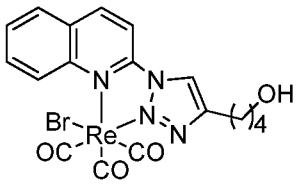

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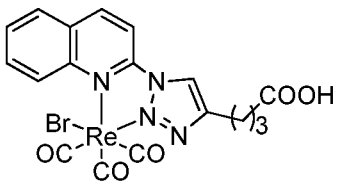

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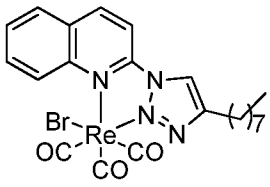

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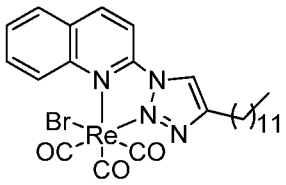

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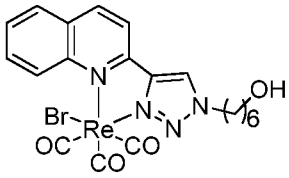

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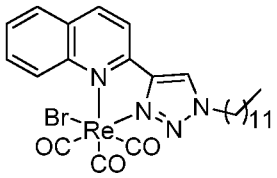

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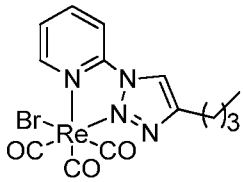

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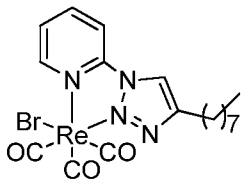

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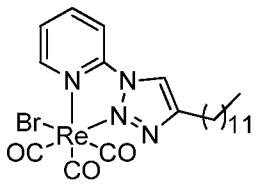

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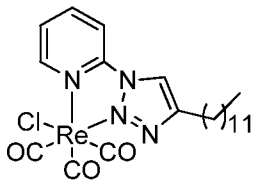

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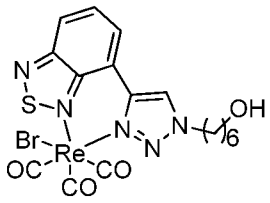

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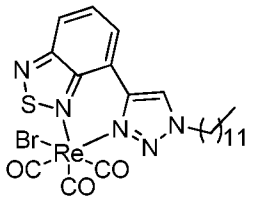

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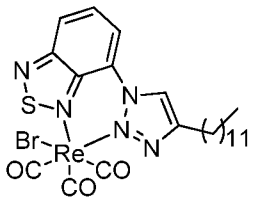

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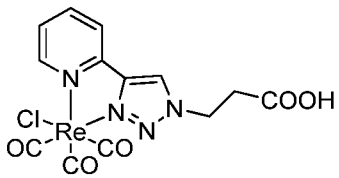

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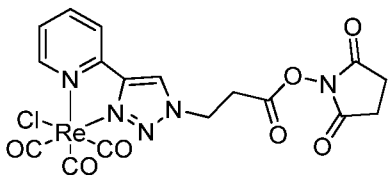

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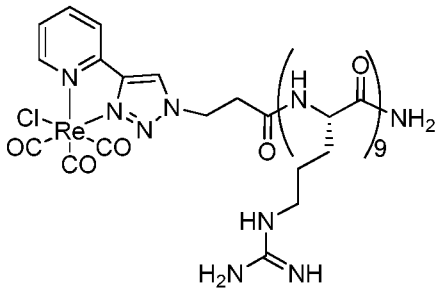

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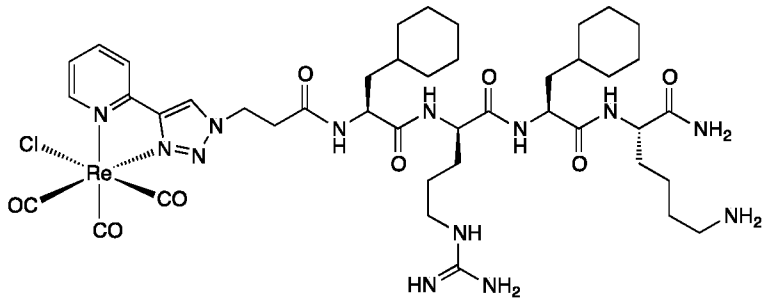

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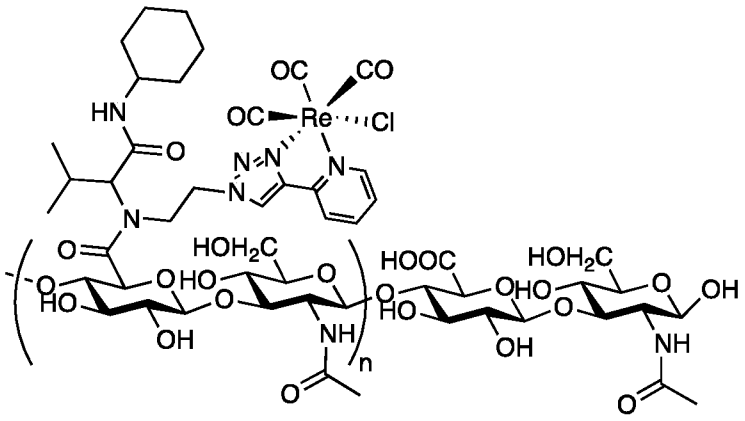
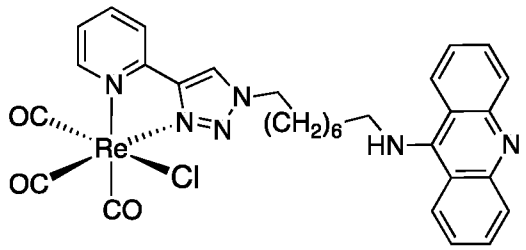


wherein n represents an integer corresponding to a hyaluronic acid having a molecular weight ranging from 400 kDa to 1000 kDa;

65



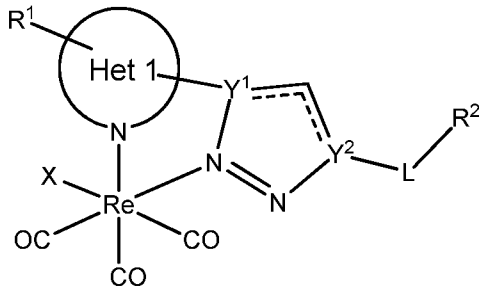
9. The use according to any one of claims **1** to **8**, further comprising performing infrared spectroscopy and/or fluorescence spectroscopy.

10. The use according to any one of claims **1** to **9**, for imaging cellular environment, comprising:

- adding a X-ray fluorescence probe of formula I to a sample containing at least one cell;
- incubating the sample for a time sufficient for the X-ray fluorescence probe to be loaded onto and/or into the cell;
- exciting the sample at an energy that generates a X-ray fluorescence response from the X-ray fluorescence probe;
- detecting the X-ray fluorescence response.

11. The use according to any one of claims **1** to **10**, for imaging cell organelles.

12. X-ray fluorescence probe for imaging of formula I:



(I)

or a salt thereof, wherein

X represents halo, optionally substituted pyridin-1-yl, carbene, thiolato, alkynyl, carboxylate, phosphine, phosphonate, sulfonate, OH_2, NC-alkyl, oxyanion such as phosphate;

Het 1 represents a heteroaryl group comprising at least one nitrogen atom; preferably **Het 1** is selected from pyridinyl, quinolynyl, quinoxalinyl, benzothiadiazolyl, benzimidazole, benzoxazole, bipyridine;

$\mathbf{R^1}$ is either absent or represents one or more substituent selected from halo, nitro, alkyl, aryl, phosphate, sulfate, cyano;

$\mathbf{Y^1}$ represents C and $\mathbf{Y^2}$ represents N; or $\mathbf{Y^1}$ represents N and $\mathbf{Y^2}$ represents C;

------ represents a single bound or a double bound depending on $\mathbf{Y^1}$ and $\mathbf{Y^2}$ definitions;

L represents a single bound or a linker selected from alkyl, aryl, heteroaryl, heterocyclyl, alkylaryl, arylalkyl, alkoxy, alkenyl, alkynyl, polyethylene glycol, polypropylene glycol, polyamines or a combination thereof; said groups being optionally interrupted or terminated by one or more -O-, -S-, -S(O)-, $-S(O)_2-$, -C(O)-NH-, -NH-C(O)-, -C(O)O-, -C(O)-, -NH- or a combination thereof; said groups optionally further comprising pendant groups selected from carboxylic acid, carboxylate, sulfonic acid, sulfonate, hydroxy; the linker optionally additionally comprising a residue of a reactive group through which **L** is bound to $\mathbf{R^2}$;

$\mathbf{R^2}$ represents a group selected from:

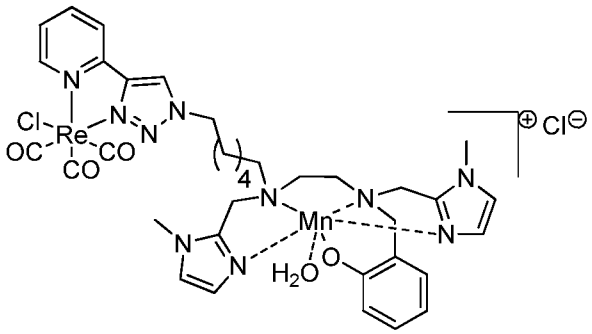
a hydrogen atom;

a reactive group selected from azide, alkynyl, amino, alkylamino, amido, maleimide, thiol, hydroxy, ester, activated ester, carboxylic acid, activated carboxylic acid, halo, nitro, nitrile, isonitriles, acrylamide, aldehyde, ketone, acetals, ketals, anhydride, glutaric anhydride, succinic anhydride, maleic anhydride, thiocyanate, isothiocyanate, isocyanate, hydrazide, hydrazines, hydrazones, ethers, oxides, cyanates, diazo, diazonium, sulfides, disulfides, sulfoxides, sulfones, sulfonic acids, sulfinic acids, sulfates, sulfenic acids, amidines, imides, imidates, nitrones, hydroxylamines, oximes, hydroxamic acids, thiohydroxamic acids, alkenes, ortho esters, sulfites, enamines, ynamines, ureas, pseudoureas, semicarbazides, carbodiimides, carbamates, carbonate, activated carbonate, imines, phosphonium, chelating moiety;

a bioactive group selected from steroid, peptide, protein, amino acid, nucleic acid, nucleoside, nucleotide, oligonucleotide, antibody, saccharide, polysaccharide, lipid, hormone, biotin, avidin, therapeutic ingredient, complex of metallic ion, microparticle, nanoparticle, fluorophore, a cell organelle targeting group and combinations thereof.

13. The X-ray fluorescence probe for imaging according to claim **12**, selected from

1



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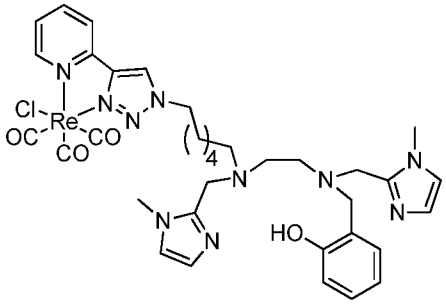

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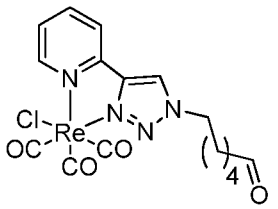

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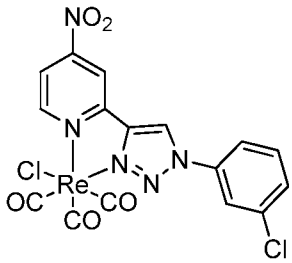

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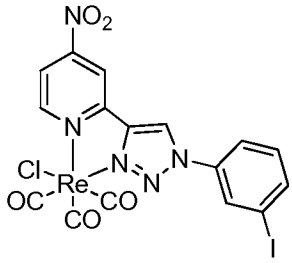

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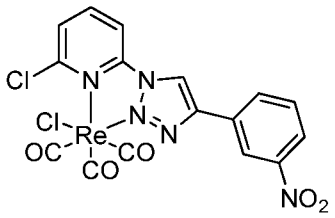

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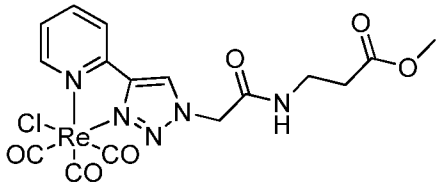

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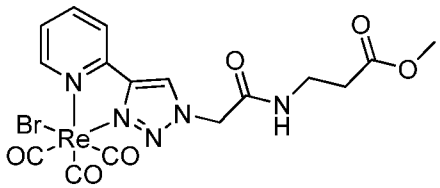

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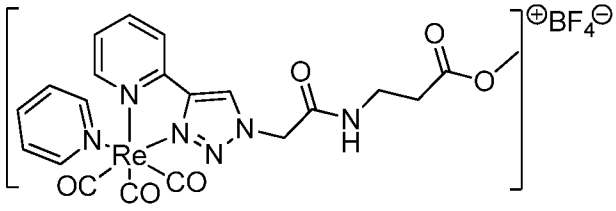

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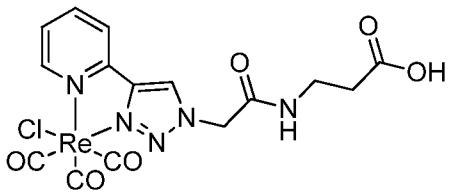

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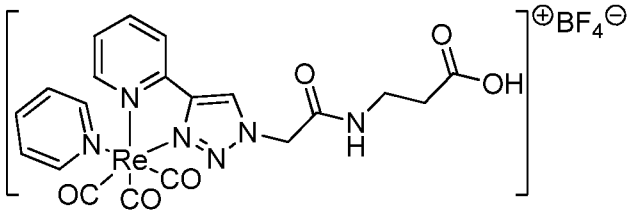

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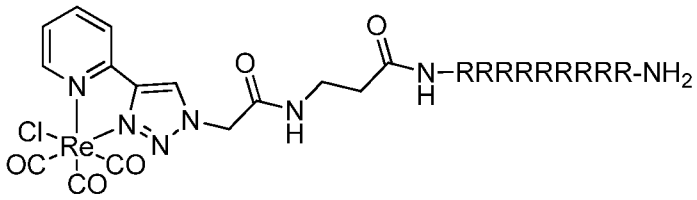

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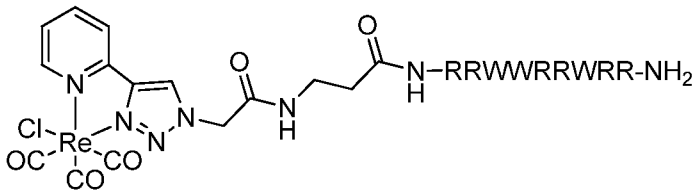

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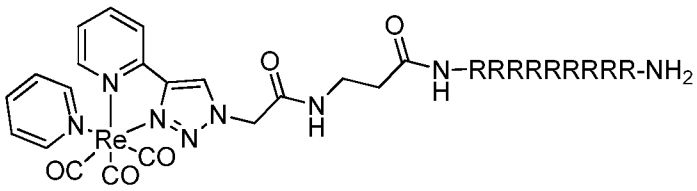

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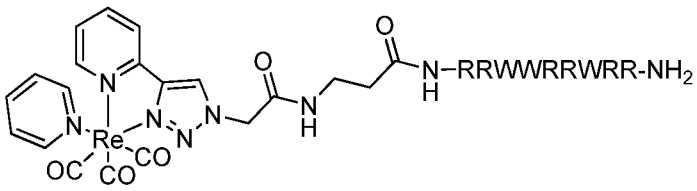

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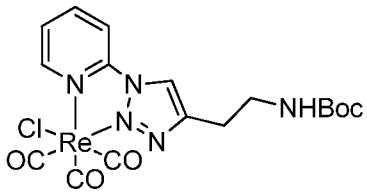

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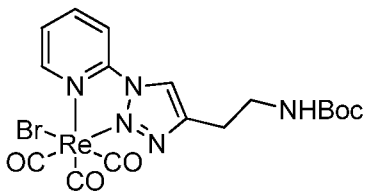

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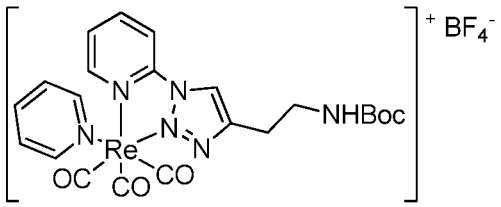

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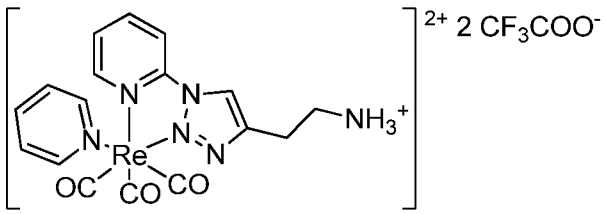

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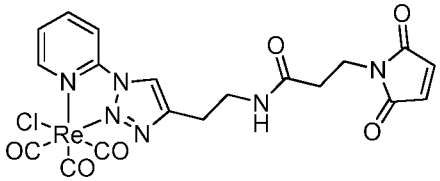

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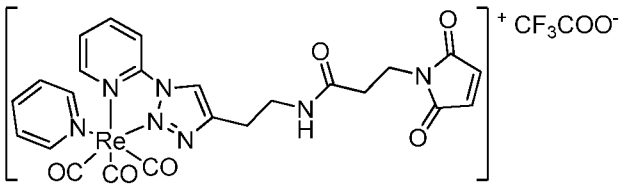

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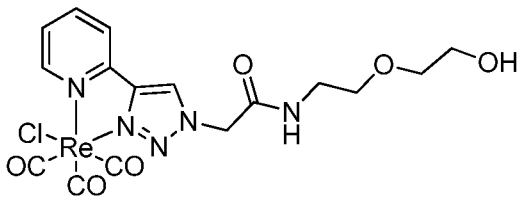

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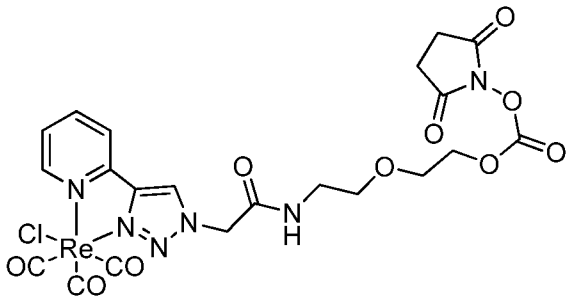

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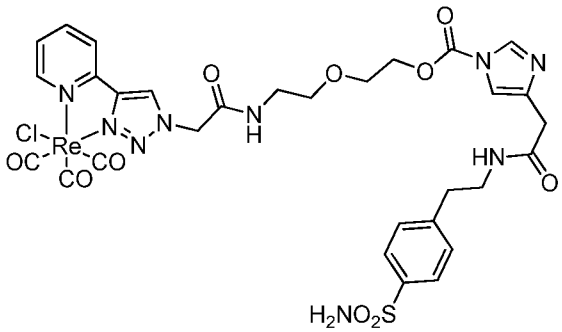

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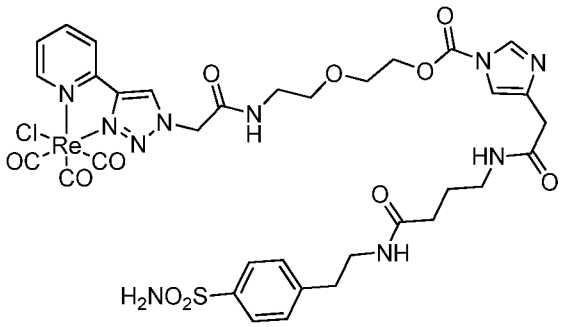

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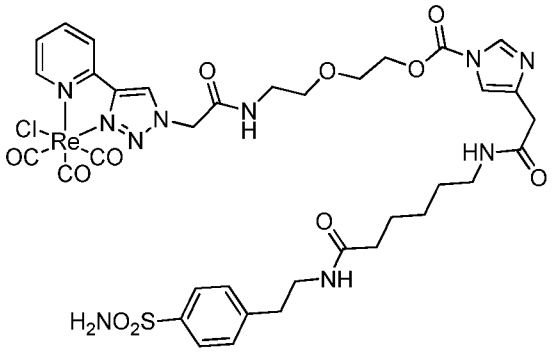

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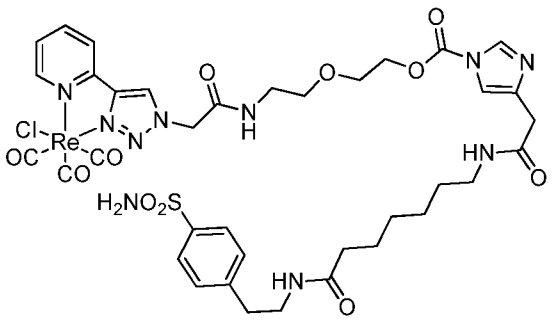

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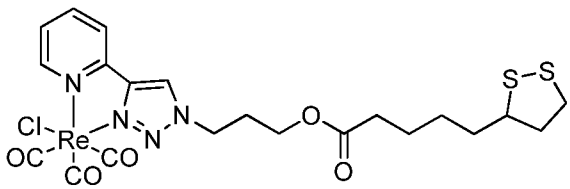

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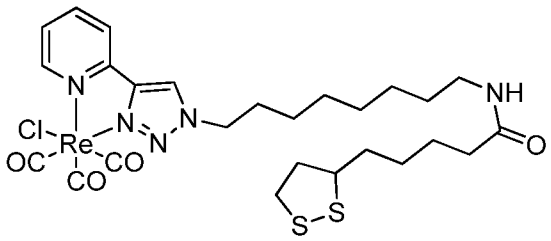

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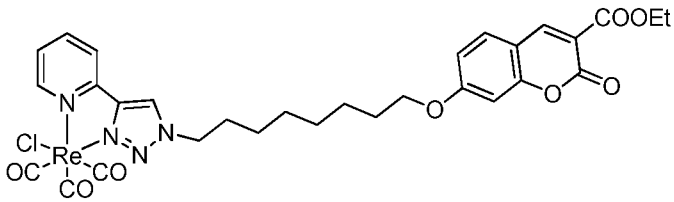

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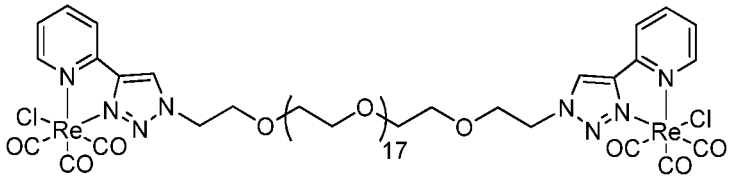

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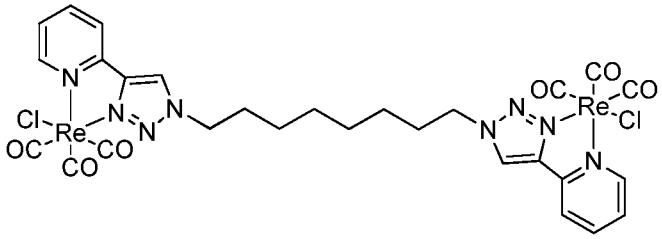

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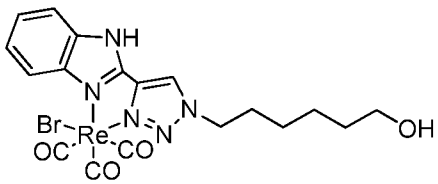

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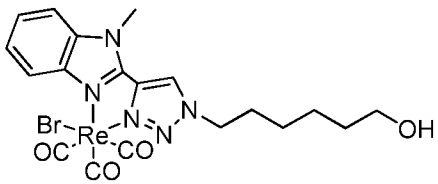

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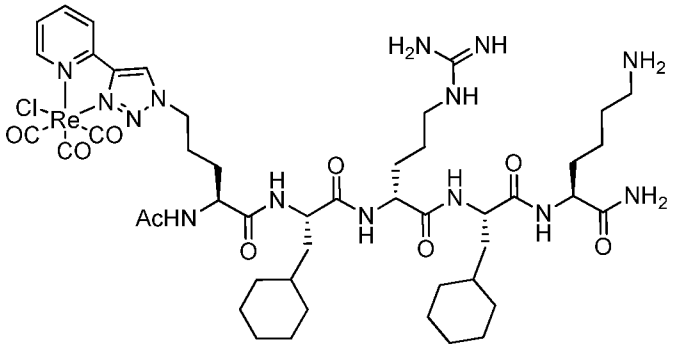

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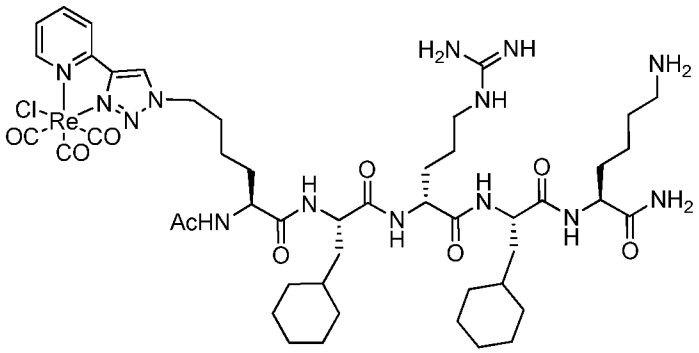

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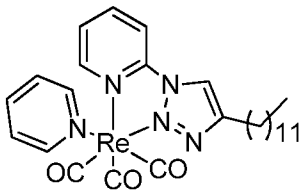

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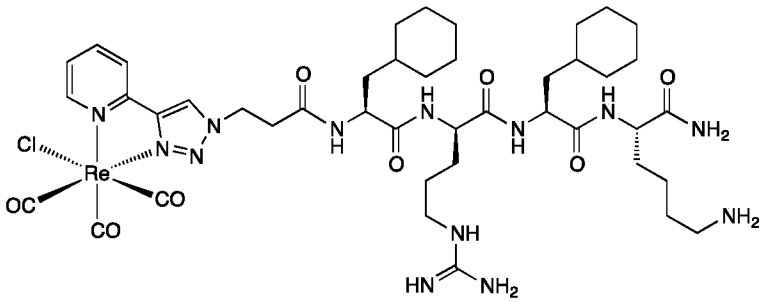

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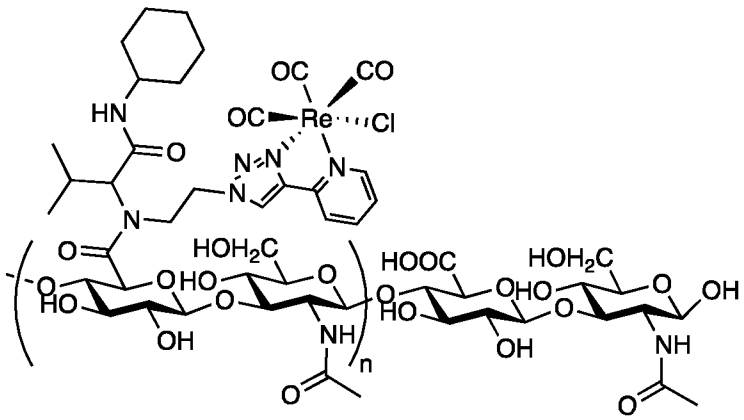
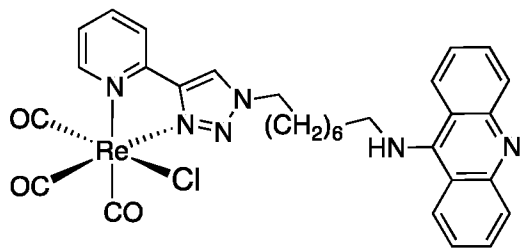


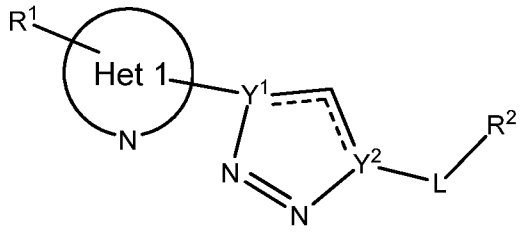
wherein n represents an integer corresponding to a hyaluronic acid having a molecular weight ranging from 400 kDa to 1000 kDa;

65



14. Process for manufacturing a X-ray fluorescence probe for imaging of formula I according to claim **12**, comprising:

1) reacting a ligand of formula II



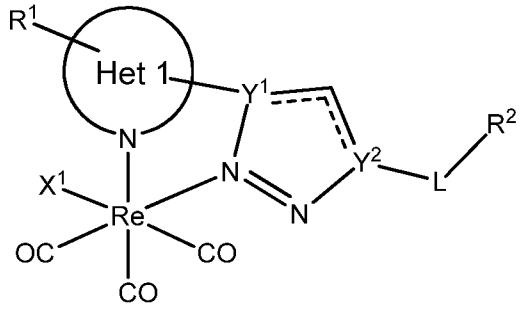
(II)

wherein **Het 1**, $\mathbf{Y^1}$, $\mathbf{Y^2}$, **L** and $\mathbf{R^2}$ are as defined in claims **12**;

with a rhenium-containing reactant of formula III;

(III) $ReX^1(CO)_5$, wherein X^1 represents a halogen atom;

to afford compound of formula I'



(I')

wherein **Het 1**, $\mathbf{Y^1}$, $\mathbf{Y^2}$, **L** and $\mathbf{R^2}$ are as defined in formula I; and $\mathbf{X^1}$ represents halo;

2) and optionally:

- replacing $\mathbf{X^1}$ moiety by **X** as defined in claim **12**; and/or;
- modifying and/or functionalizing –L-R^2;

to form a compound of formula I as defined in claim **12**.

15. Kit for performing X-ray fluorescence imaging, comprising a X-ray fluorescence probe for imaging according to claim **12** or claim **13.**

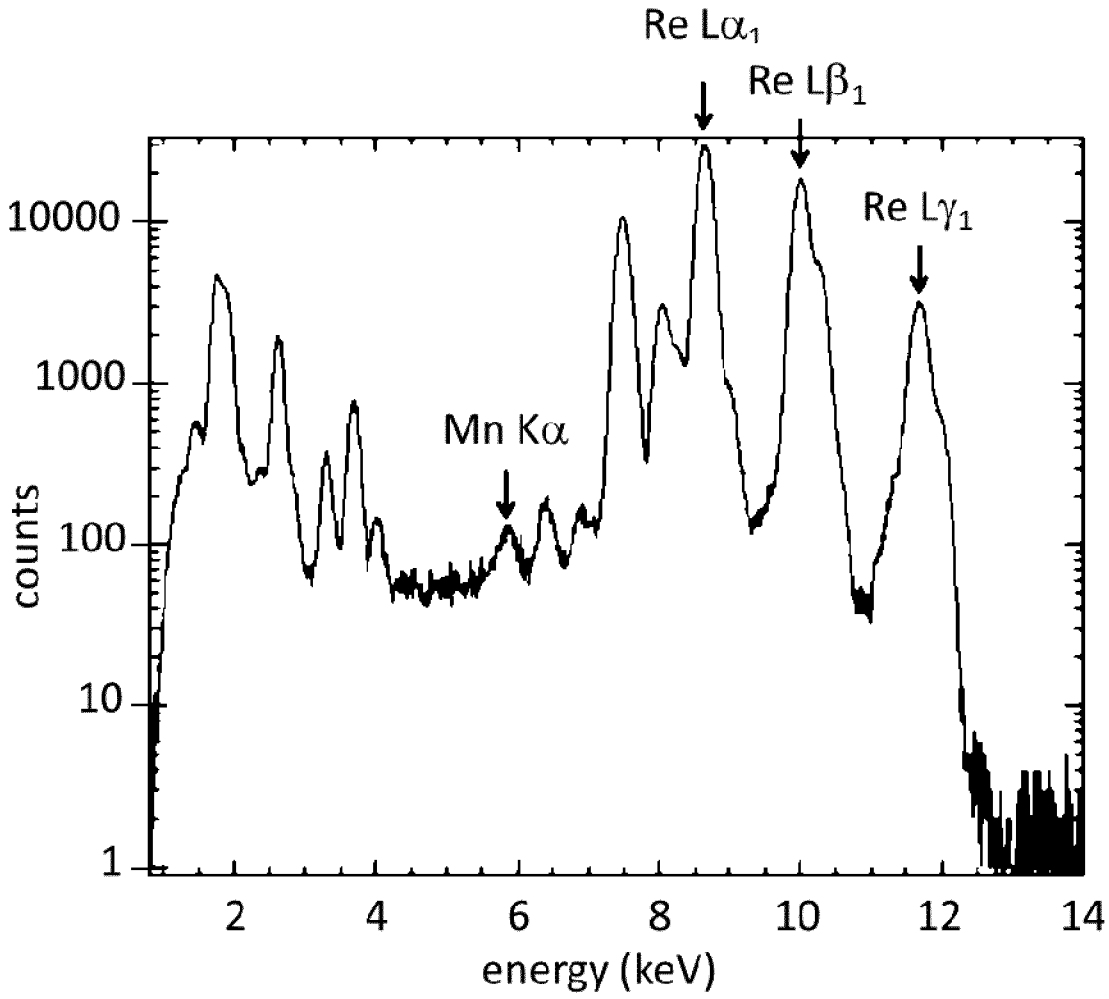

Re Lα1
Re Lβ1
Re Lγ1
Mn Kα
10000
1000
100
10
1
counts
2
4
6
8
10
12
14
energy (keV)

FIG. 1

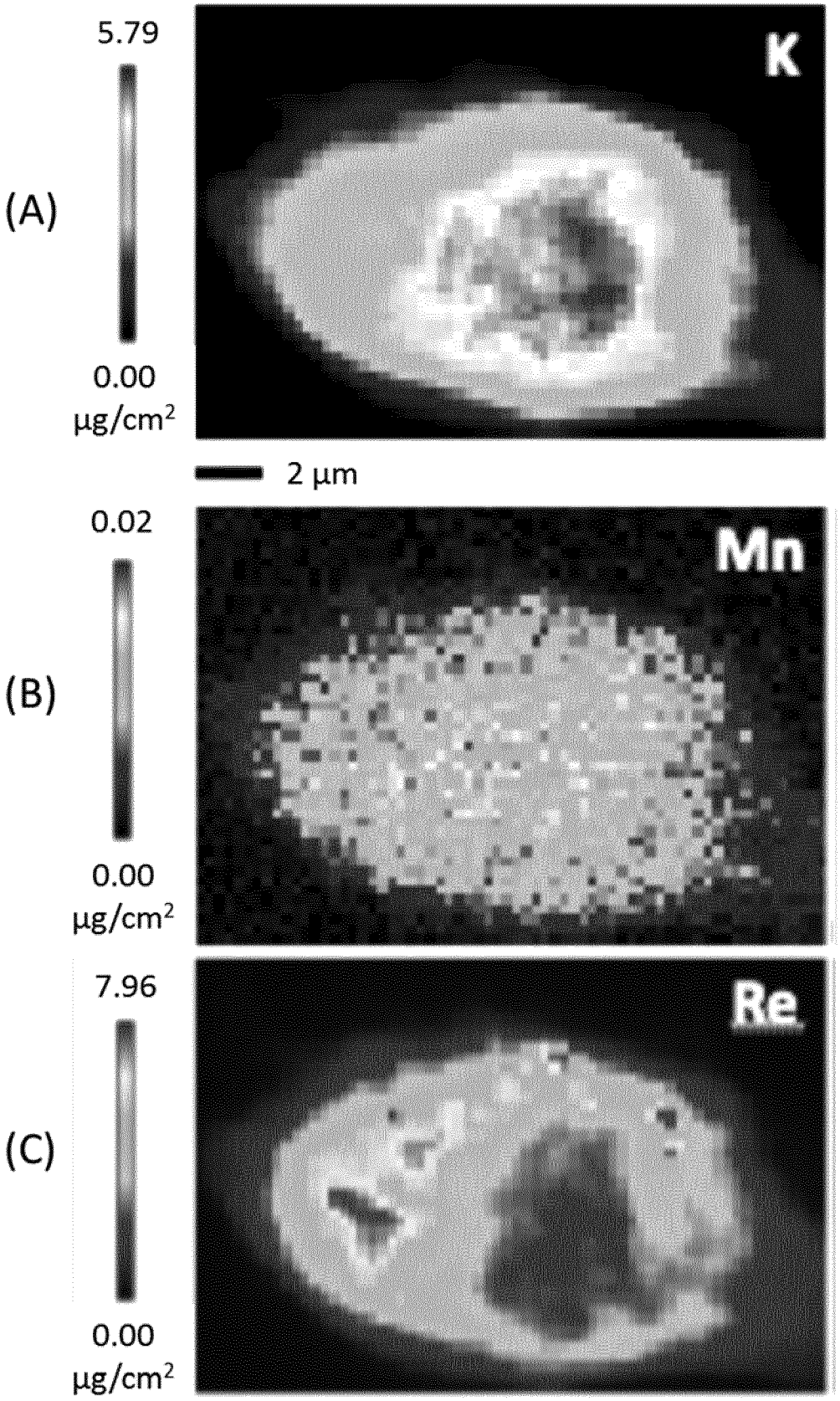


FIG. 2

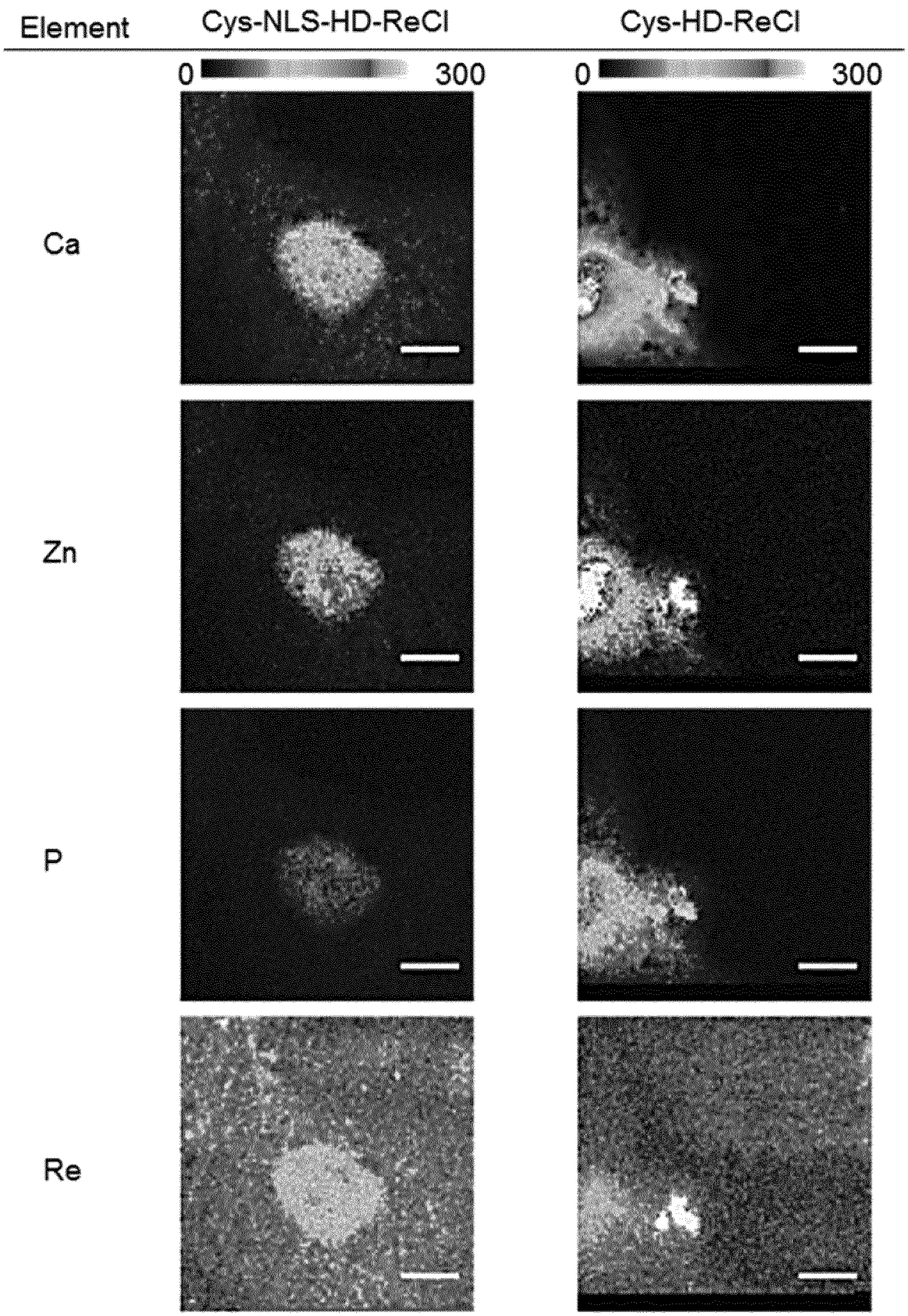


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/066048

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K51/04 C07F13/00
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)
A61K C07F

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, COMPENDEX, CHEM ABS Data, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SYLVAIN CLÈDE ET AL: "An easy-to-detect nona-arginine peptide for epidermal targeting", CHEMICAL COMMUNICATIONS - CHEMCOM, vol. 51, no. 13, 1 January 2015 (2015-01-01), pages 2687-2689, XP055223105, GB ISSN: 1359-7345, DOI: 10.1039/C4CC08737B	12,14,15
Y	page 2688; figures 1,2	1-11,13

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

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

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

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

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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 September 2016	19/09/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pinheiro Vieira, E

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLEDE S ET AL: "Influence of the side-chain length on the cellular uptake and the cytotoxicity of rhenium triscarbonyl derivatives: A bimodal infrared and luminescence quantitative study", CHEMISTRY - A EUROPEAN JOURNAL 20140707 WILEY-VCH VERLAG DEU, vol. 20, no. 28, 7 July 2014 (2014-07-07), pages 8714-8722, XP002748654, DOI: 10.1002/CHEM.201402471	12,14,15
Y	figures 1-4	1-11,13
Y	EVDOKIMOVA O V ET AL: "Up-to-date methods for the determination of rhenium", JOURNAL OF ANALYTICAL CHEMISTRY, KLUWER ACADEMIC PUBLISHERS-PLENUM PUBLISHERS, NE, vol. 67, no. 9, 24 July 2012 (2012-07-24), pages 741-753, XP035089168, ISSN: 1608-3199, DOI: 10.1134/S1061934812090043 the whole document	1-11,13
X	HUANG RACHEL ET AL: "Synthesis, characterization, and biological studies of emissive rhenium-glutamine conjugates", JBIC. JOURNAL OF BIOLOGICAL INORGANIC CHEMISTRY, SPRINGER, DE, vol. 18, no. 7, 8 August 2013 (2013-08-08) , pages 831-844, XP035349331, ISSN: 0949-8257, DOI: 10.1007/S00775-013-1023-3 [retrieved on 2013-08-08]	12,14,15
Y	page 837; figures 2,3	1-11,13
X	MAKOTO OBATA ET AL: "Syntheses, structural characterization and photophysical properties of 4-(2-pyridyl)-1,2,3-triazole rhenium(i) complexes", DALTON TRANSACTIONS: THE INTERNATIONAL JOURNAL FOR INORGANIC, ORGANOMETALLIC AND BIOINORGANIC CHEMISTRY, no. 25, 1 January 2008 (2008-01-01), page 3292, XP055223181, GB ISSN: 1477-9226, DOI: 10.1039/b718538c	12,14,15
Y	page 3296; figures 3-6; table 4	1-11,13

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HÉLÈNE C. BERTRAND ET AL: "Luminescence Modulations of Rhenium Tricarbonyl Complexes Induced by Structural Variations", INORGANIC CHEMISTRY, vol. 53, no. 12, 16 June 2014 (2014-06-16) , pages 6204-6223, XP055223520, EASTON, US ISSN: 0020-1669, DOI: 10.1021/ic5007007	12,14,15
Y	page 6212; figures 10,16	1-11,13

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