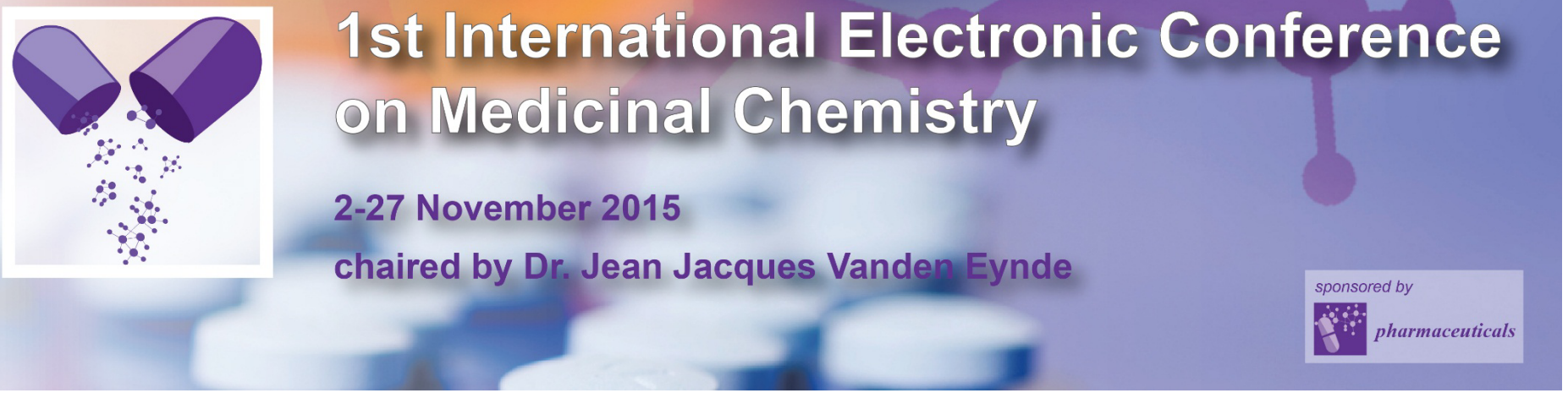

Radiopharmaceuticals radiolabelled with ^{188}Re as potential therapeutic tools for hepatocellular carcinoma targeting

Romain Eychenne [1,*], Jin-Hui Wang [1], Claude Picard [1], Nicolas Lepareur [2], Eric Benoist [1]

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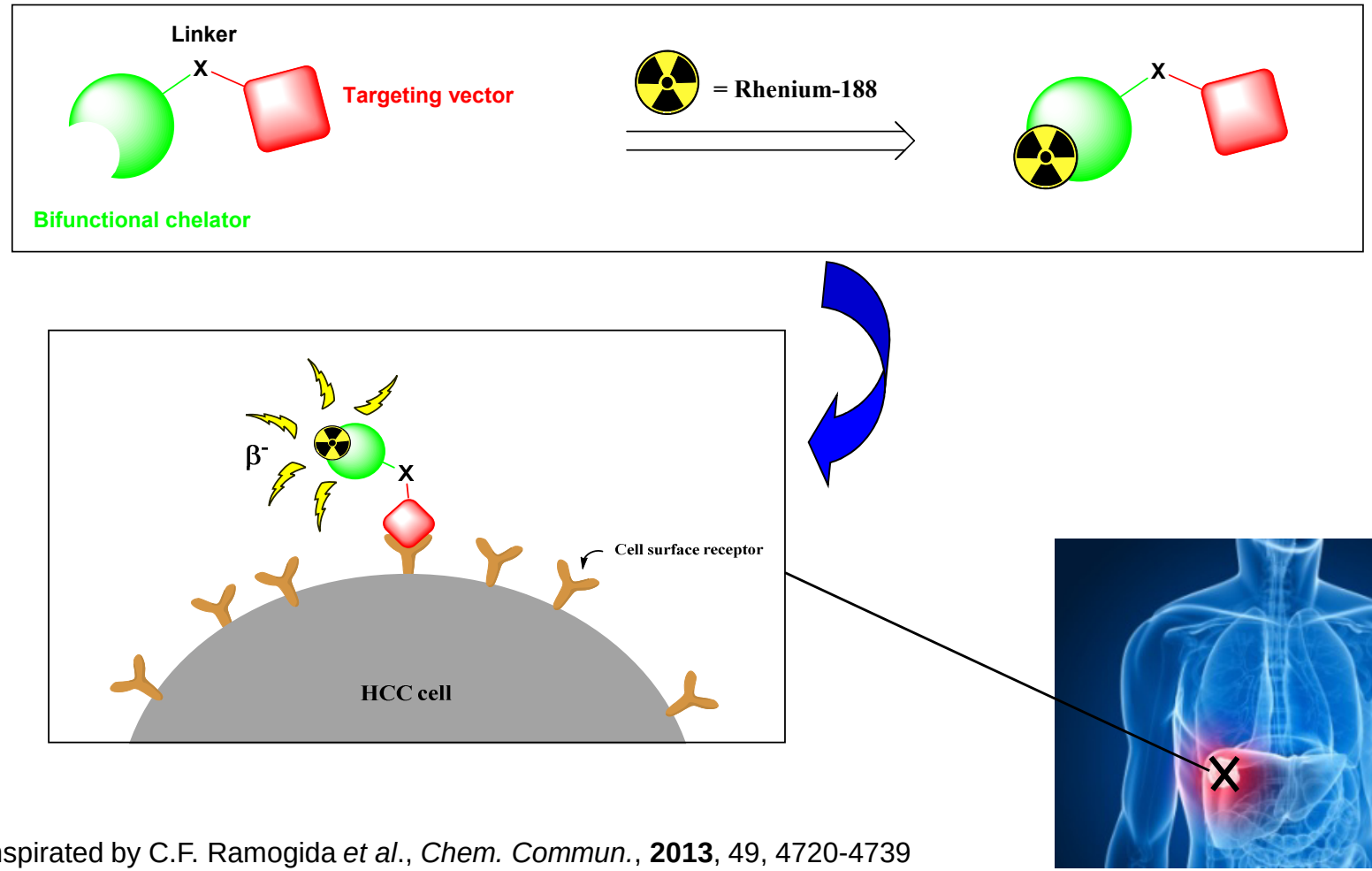
Radiopharmaceuticals radiolabelled with ^{188}Re as potential therapeutic tools for hepatocellular carcinoma targeting



Schemes inspirated by C.F. Ramogida *et al.*, *Chem. Commun.*, **2013**, 49, 4720-4739

Abstract: Hepatocellular carcinoma (HCC), is the second most common cause of death from cancer worldwide (745 000 deaths). Since 2008, HCC is the cancer with the highest mortality rate (0.95). Nowadays, the only systemic treatment that has demonstrated a real benefit in advanced HCC is Sorafenib, but it remains associated with many side effects and this therapy is still very expensive. So, it is desirable to offer a treatment more efficient, and cheaper.
Selective localization or destruction of cancer cells by means of such radiolabelled bioconjugates is a simple and attractive concept, based on the use of the recognition properties of biomolecules towards tumour cells (*magic bullet concept*). The challenge is to develop radiotracers, *so-called* radiopharmaceuticals, which consist in a three-parts system including a biomolecule, a Bifunctional Chelating Agent (BCA) and a radioactive isotope which delivers γ or β^- emission.
In this communication, we reported our first results related to the development of a targeting radiopharmaceutical including: (i) the synthesis of original tripodal N_2O BCAs based on a triazolyl moiety, these chelators being synthesised *via* a click chemistry approach, (ii) a complete structural study of corresponding non-radioactive tricarbonylrhenium complexes (iii) the first trials of coupling and of ^{188}Re-labelling of the tripodal ligand (proof of concept).

Keywords: Targeted r*adiopharmaceuticals; Rhenium-188; Click chemistry; Tricarbonyl complexes*

Introduction (1/5)

- Hepatocellular carcinoma (HCC), major form of primary liver cancers (about 85%) :
  - ***Fifth cancer*** in terms of impact (782 000 cases / per year in the world)
  - ***Second most common cause*** of death from cancer worldwilde (745 000 deaths).

- Since 2008 (according to 2008 [1] and 2012 [2] datas) :

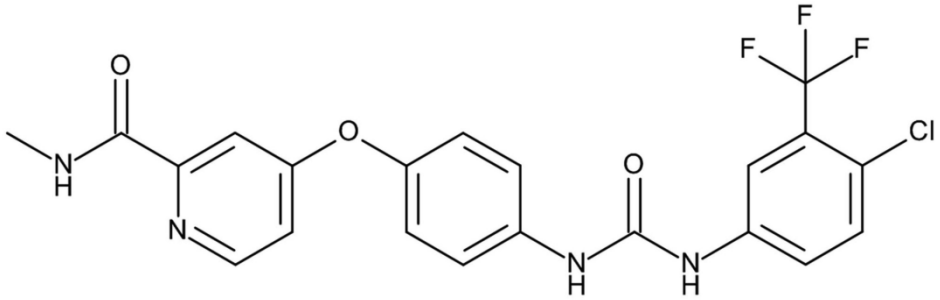
HCC = The highest mortality rate (0.95)

- Management of HCC complicated because of underlying liver diseases
- A curative treatment can be offered in very few cases.

[1] J. Ferlay *et al., Int. J. Cancer*, **2010**, 127, 2893-2917
[2] J. Ferlay *et al., Int. J. Cancer,* **2014**, 136, E359–E386

Introduction (2/5)

The only systemic therapy with a real benefit for metastatic HCC is Sorafenib.



Sorafenib

Advantages :

- Tumor-cell proliferation
- Tumor angiogenesis
- Increases the rate of apoptosis in a wide range of tumor models

Drawbacks :

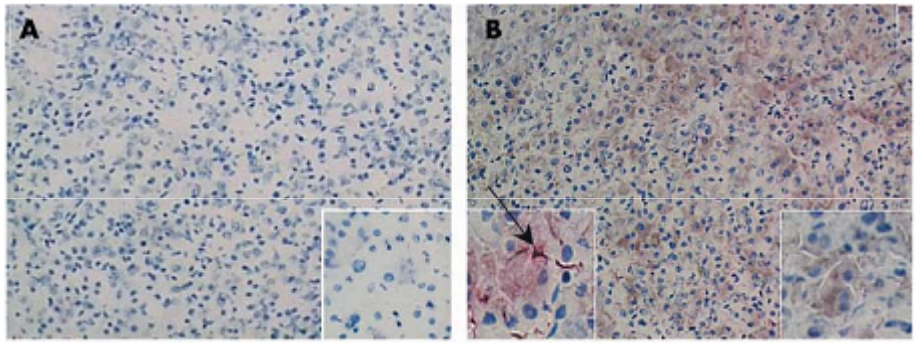
- Many side effects
- Very expensive

Important to find an alternative treatment

Introduction (3/5)

What kind of alternative treatment ?

Some studies have shown that SSTRs (Somatostatin Receptors) are largely overexpressed in HCC cases, and even, in extrahepatic metastasis [3, 4]



Immunochemistry of SSTRs in HCC [4]

(A) Negative control

(B) Immunoreaction showing the presence of these receptors

SSTRs seem to be promising biomarker for targeting HCC metastasis

[3] J.C. Reubi *et al., Gut*, **1999**, 45, 766-774
[4] H. Reynaert *et al., Gut*, **2004**, 53, 1180-1189

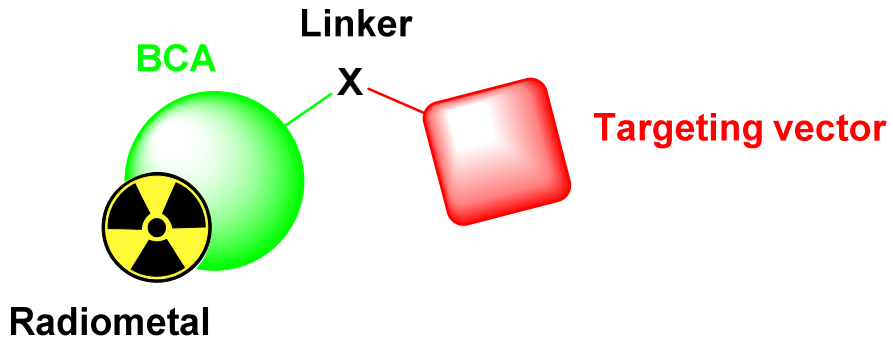
Introduction (4/5)

How to target SSTRs in HCC metastasis ?

Using a targeted radiopharmaceutical

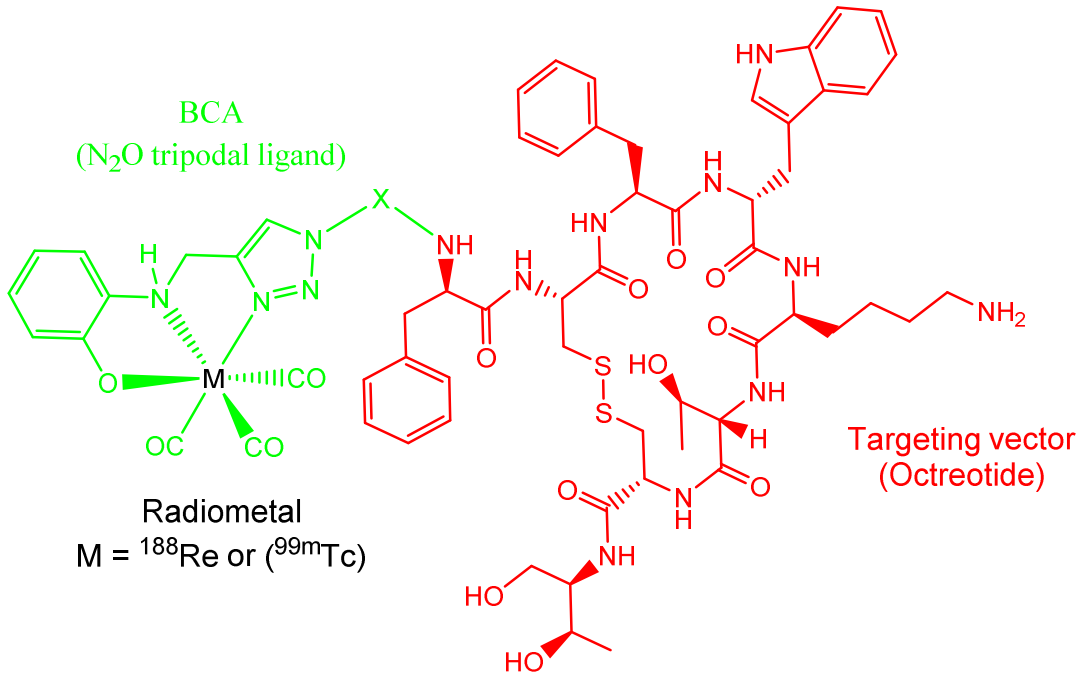
Radiopharmaceutical features :

- ***Radiometal :*** *Localizer (γ or β^+ emitter) or destroyer element (β^- emitter)*
- ***Bifunctional Chelating Agent (BCA) :*** *Chelating cavity + functionalised arm*
- ***Targeting vector :*** *Vectorisation*



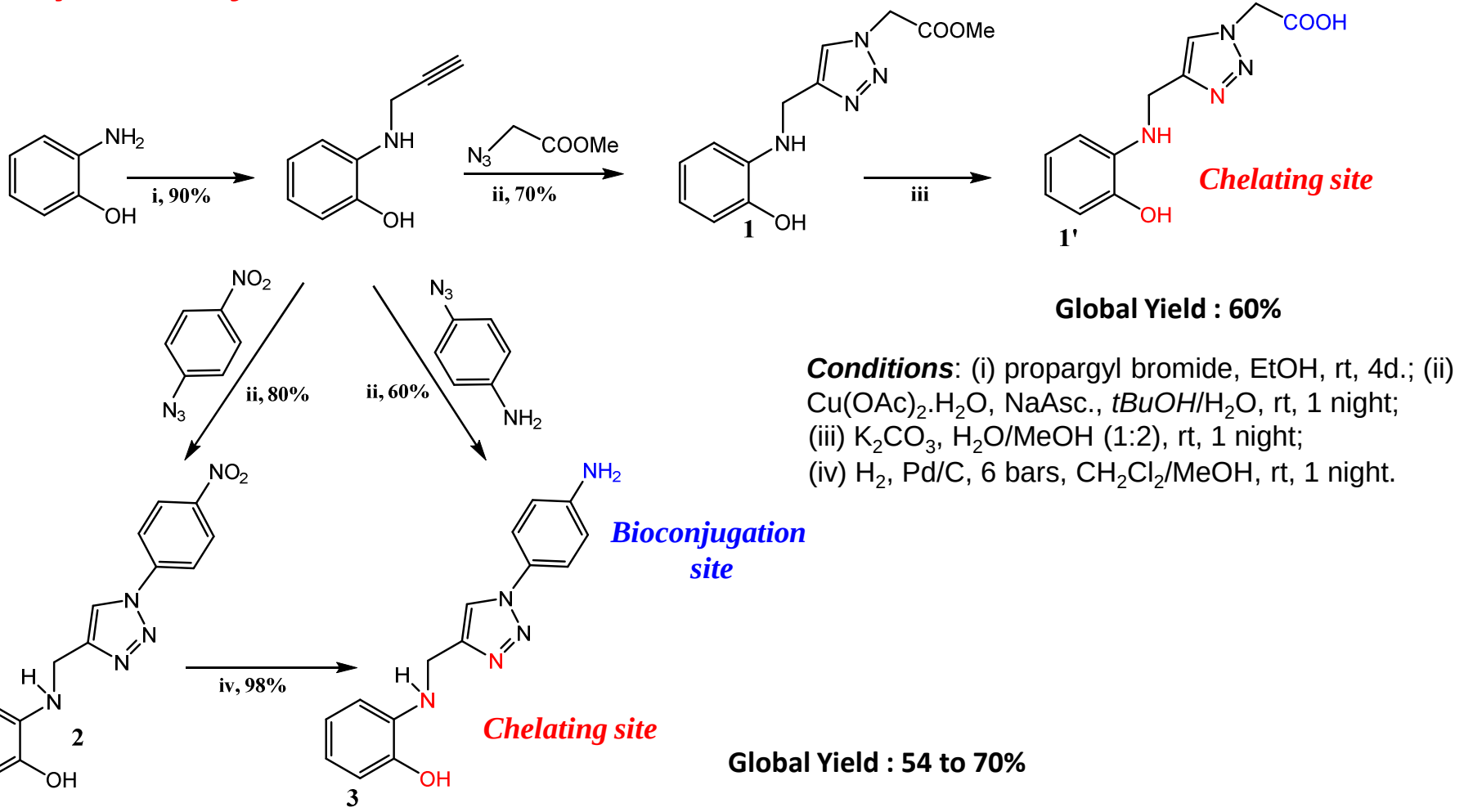
Introduction (5/5)

Our project : Develop a HCC targeting ^{188}Re-radiopharmaceutical



Results and discussion (1/14)

Synthesis of BCAs



Global Yield : 60%

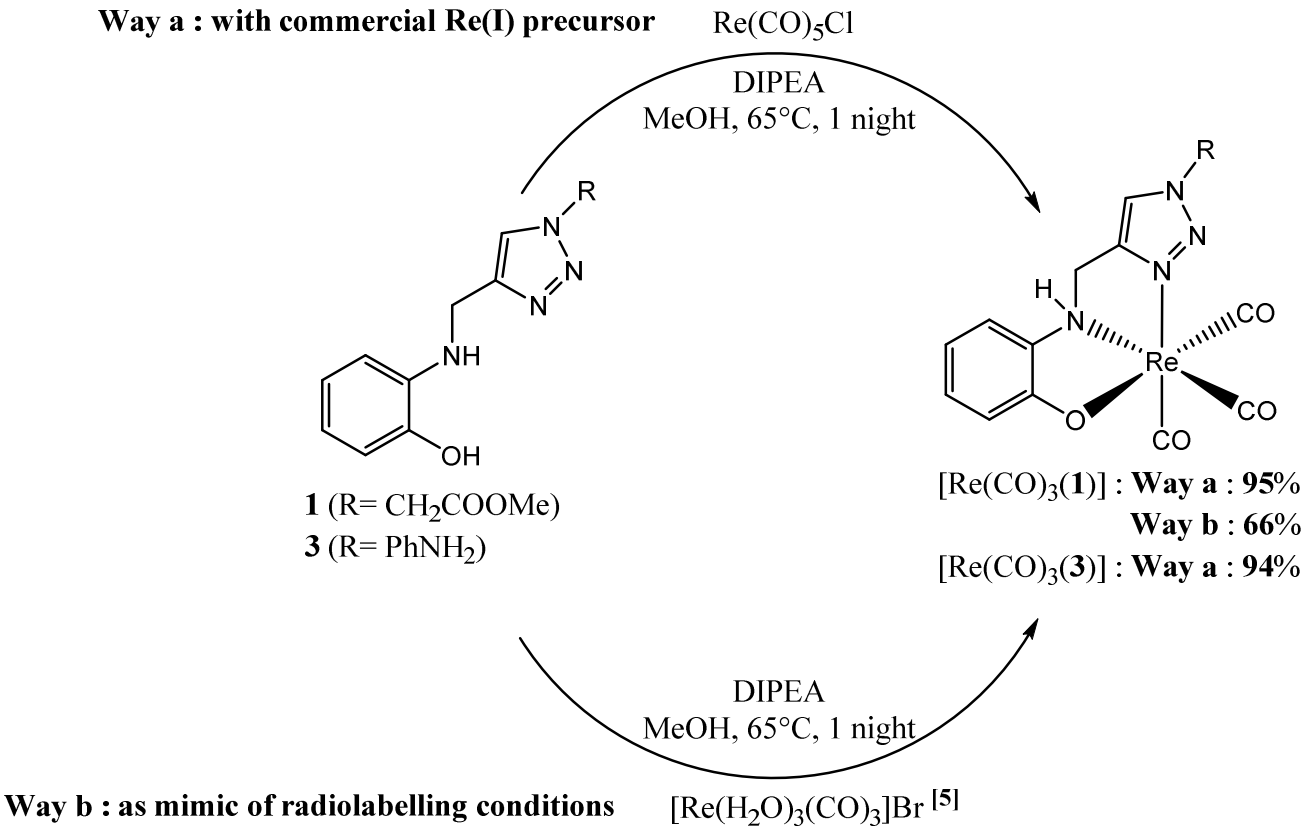
Conditions: (i) propargyl bromide, EtOH, rt, 4d.; (ii) $Cu(OAc)_2.H_2O$, NaAsc., *tBuOH*/H_2O, rt, 1 night; (iii) K_2CO_3, H_2O/MeOH (1:2), rt, 1 night; (iv) H_2, Pd/C, 6 bars, CH_2Cl_2/MeOH, rt, 1 night.

Global Yield : 54 to 70%

Results and discussion (2/14)

(macroscopic study)

Structural study of « cold » rhenium complexes

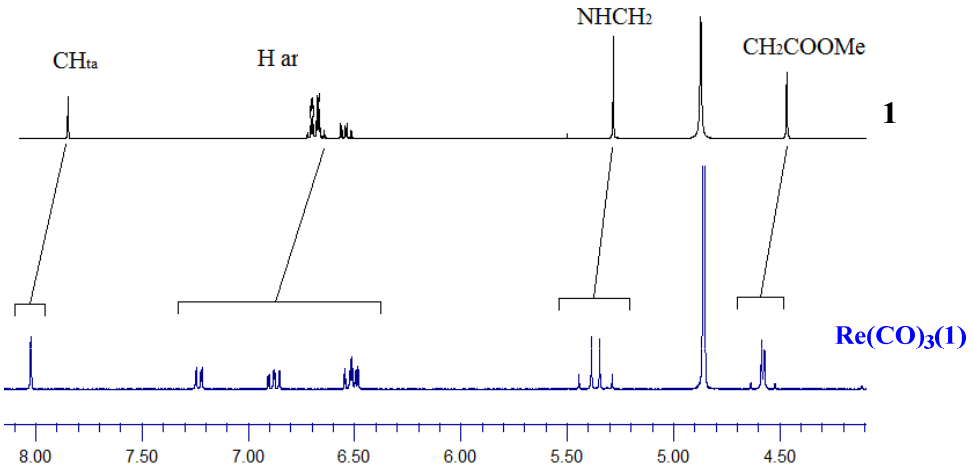


[5] N. Lazarova *et al., Inorg. Chem. Commun.*, **2004**, 7, 1023-1026.

Results and discussion (3/14)

(macroscopic study)

Structural study of « cold » rhenium complexes

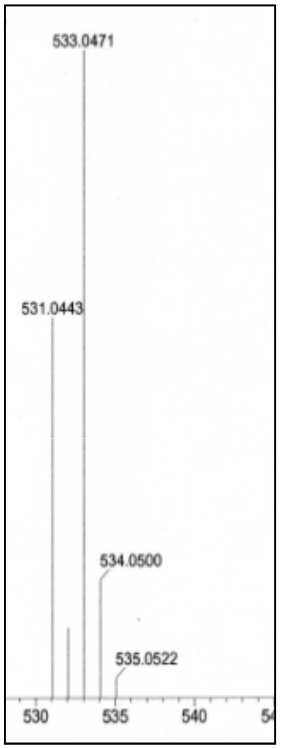


^{1}H NMR shows the effect of complexation

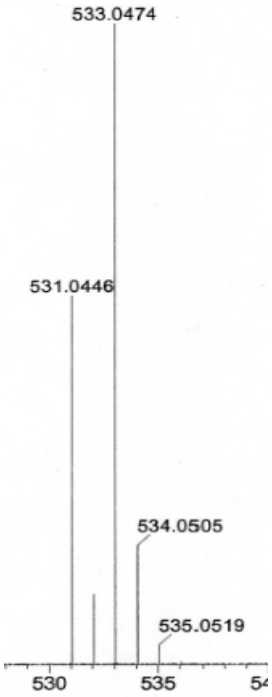
(i) Shift of triazole signal

(ii) Splitting of aromatic signals

(iii) Magnetic inequivalence of CH_2



Theoretical



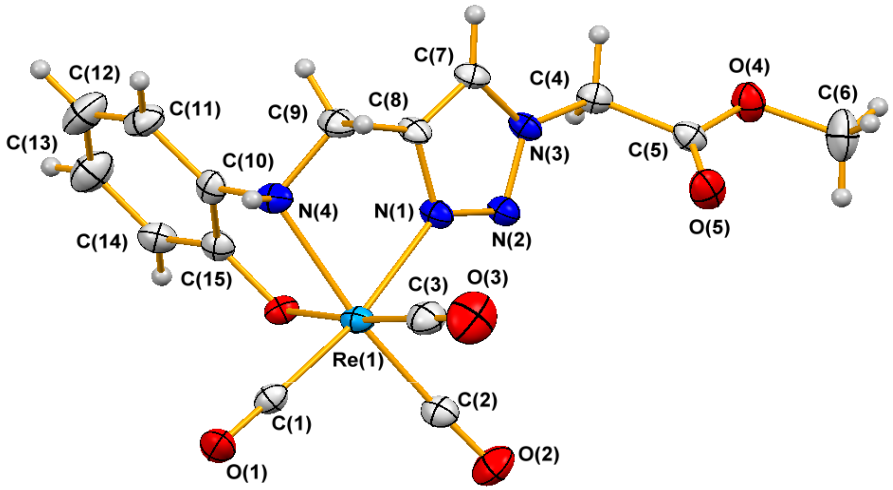
Experimental

Mass spectrum (ESI$^+$) confirms the structure of our complex

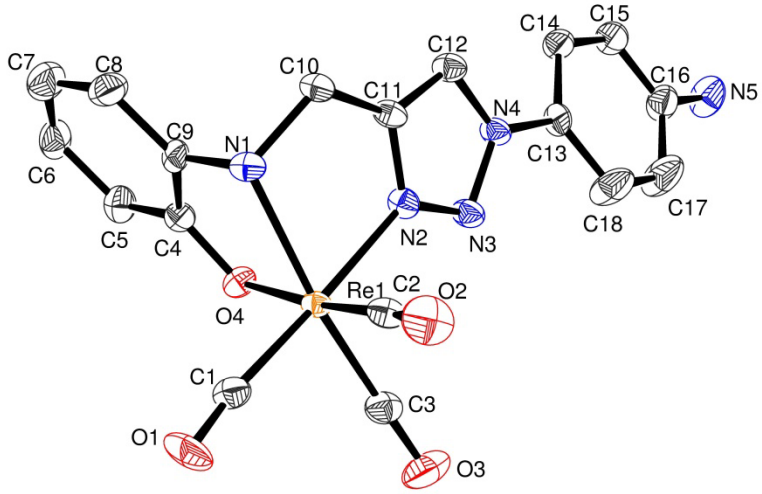
Results and discussion (4/14)

(macroscopic study)

Structural study of « cold » rhenium complexes



[Re(CO)$_3$(**1**)]



[Re(CO)$_3$(**3**)]

X-ray complexes structures :

(i) Classical bond lenghts and bond angles

(ii) Octahedral complex with facial coordination geometry

(iii) Mononuclear and neutral complexes

Results and discussion (5/14)

(macroscopic study)

Structural study of « cold » rhenium complexes

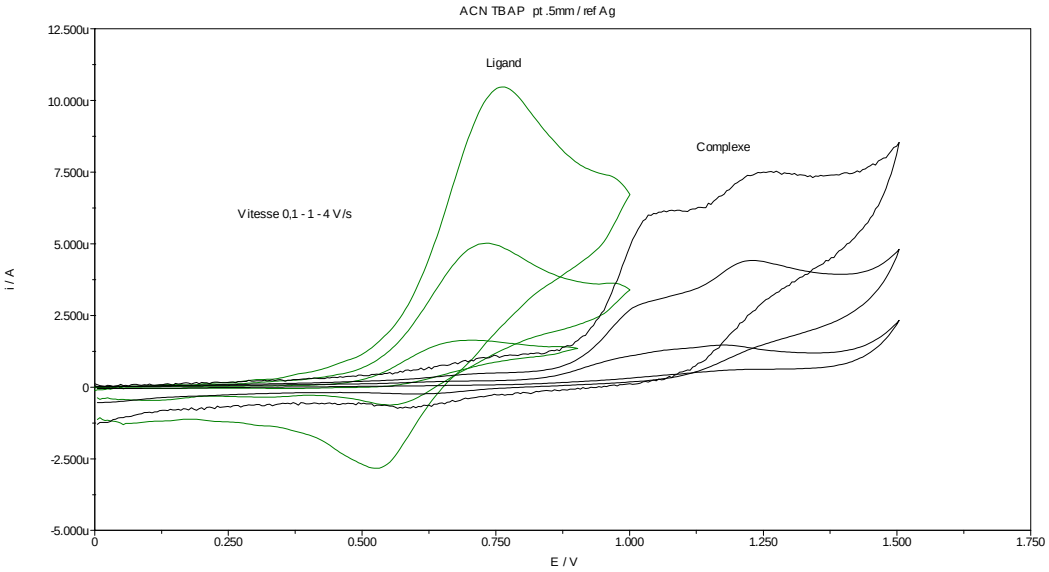


Figure : Selected cyclic voltammograms at a Cv electrode for ligand **1** (in green) and rhenium complex [$Re(CO)_3$(**1**)] (in black), in MeCN, [Bu_4NClO_4] = 0.1 mol.L^{-1} at different potential scan rates 0.1, 1 and 4 V/s; analyte concentration 1 mmol.L^{-1}.

Ligand	Ep_{ox}(V)	Complex	$Ep_{red\ ta}$(V)	$Ep_{ox\ Re(I)}$ (V)	Ep_{ox}(V)
1	0.70	[$Re(CO)_3$(**1**)]	-2.37	1.20	1.02

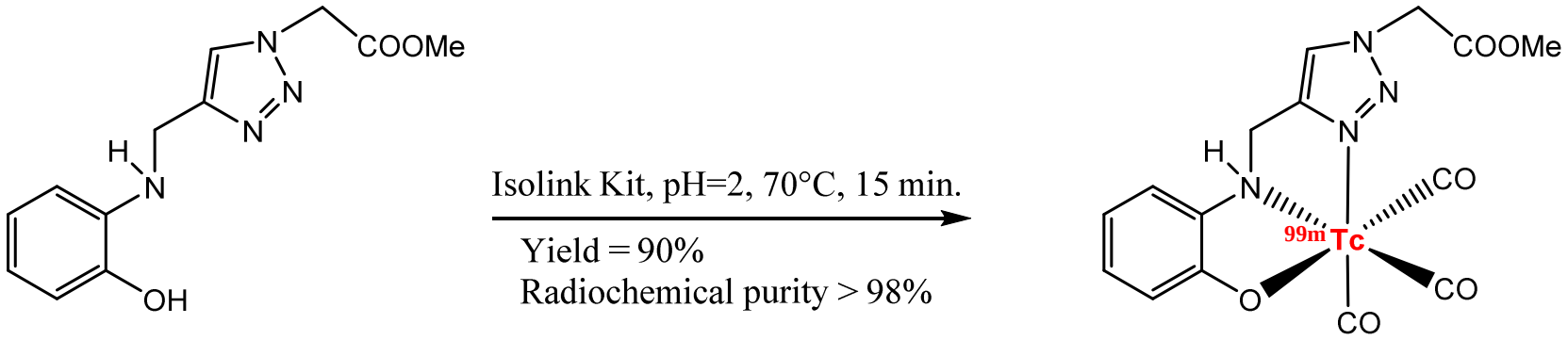
Table : Electrochemical data for ligand **1** and its corresponding rhenium complex

Slight displacement of the oxidation peak between ligand 1 and [$Re(CO)_3$(1)] (Influence of rhenium coordination)

Results and discussion (6/14)

(microscopic study)

Radiolabelling with ^{99m}Tc



Concept of radiolabelling with ^{99m}Tc validated

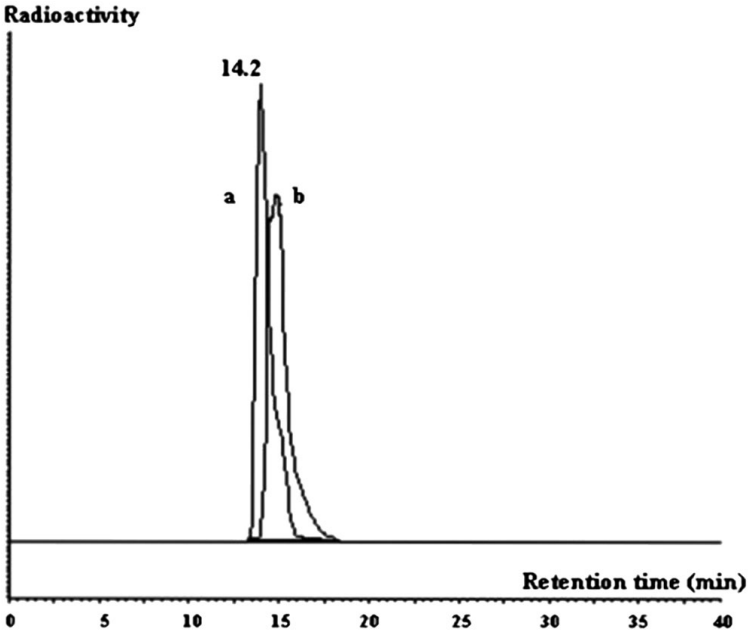
Isolink kit → $[^{99m}Tc(CO)_3(H_2O)_3]$

Results and discussion (7/14)

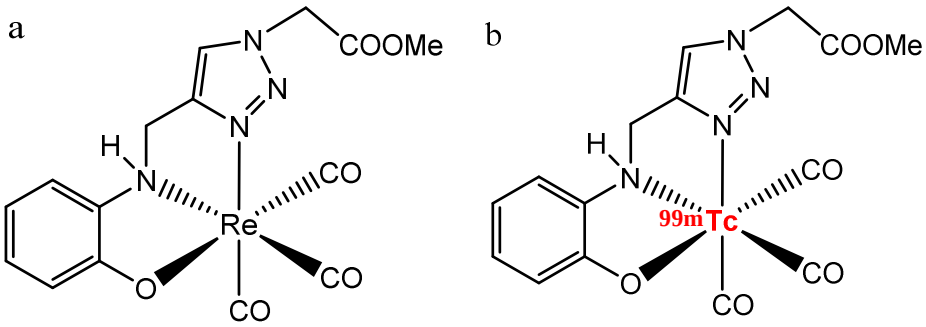
(microscopic study)

Radiolabelling with ^{99m}Tc

- HPLC Comparison [6]



C18 (Shim-pack VP-ODS, SHIMADZU) column (250 × 4.6 mm)
A: MeOH 0.1% TFA; B: H_2O 0.1% TFA; 1 mL/min



Isostructurality of ^{99m}Tc/Re complexes

[6] S. Guizani et al., *J. Label. Compd Radiopharm.*, **2014**, *57,* 158-163.

Results and discussion (8/14)

(microscopic study)

Radiolabelling with ^{99m}Tc

- Biological behavior in healthy mice [6]

Table 1. Tissue distribution data of **^{99m}Tc-3** in normal Swiss mice*

Tissues	2 min	5 min	30 min	60 min
Blood	3.52 ± 0.62	2.21 ± 0.23	1.11 ± 0.07	0.39 ± 0.04
Brain	0.14 ± 0.02	0.07 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
Heart	0.77 ± 0.06	0.58 ± 0.05	0.23 ± 0.04	0.14 ± 0.01
Lungs	2.45 ± 0.81	2.19 ± 0.75	1.84 ± 0.09	0.93 ± 0.03
Liver	13.69 ± 2.15	12.18 ± 1.91	9.89 ± 0.83	5.24 ± 1.22
Spleen	1.64 ± 0.52	1.36 ± 0.42	0.53 ± 0.19	0.36 ± 0.08
Pancreas	1.18 ± 0.19	0.44 ± 0.21	0.15 ± 0.05	0.03 ± 0.01
Kidneys	9.25 ± 1.83	6.69 ± 1.25	4.86 ± 0.87	2.32 ± 0.87
Intestines	7.25 ± 1.45	7.65 ± 0.89	3.68 ± 1.26	3.23 ± 0.75
Muscle	0.42 ± 0.07	0.34 ± 0.03	0.25 ± 0.02	0.11 ± 0.01
Stomach	0.45 ± 0.03	0.42 ± 0.04	0.38 ± 0.03	0.35 ± 0.02

*Values are %ID.g^{-1} ± standard deviation ($n = 3$)

(i) ***Fast clearance*** of the radiotracer from the bloodstream

(ii) ***No specific uptake*** or long-term retention in organs or tissues

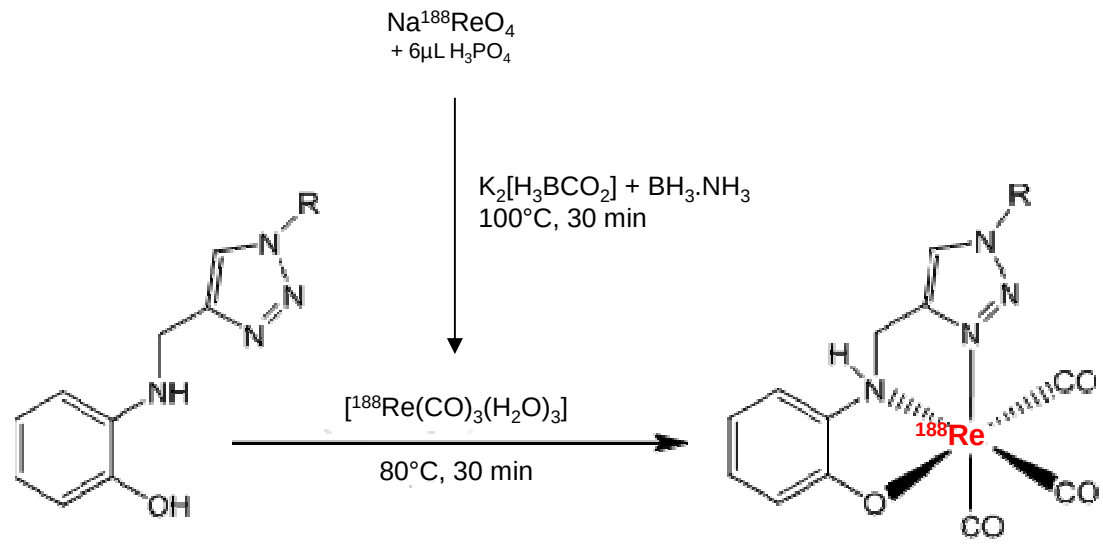
Complex stable « in vivo »

[6] S. Guizani et al., *J. Label. Compd Radiopharm.*, **2014**, *57,* 158-163.

Results and discussion (9/14)

(microscopic study)

Preliminary study of radiolabelling with ^{188}Re



1 (R = CH_2COOMe)

3 (R = $PhNH_2$)

$[^{188}Re(CO)_3(\mathbf{1})]$: **96%**

$[^{188}Re(CO)_3(\mathbf{3})]$: **71%**

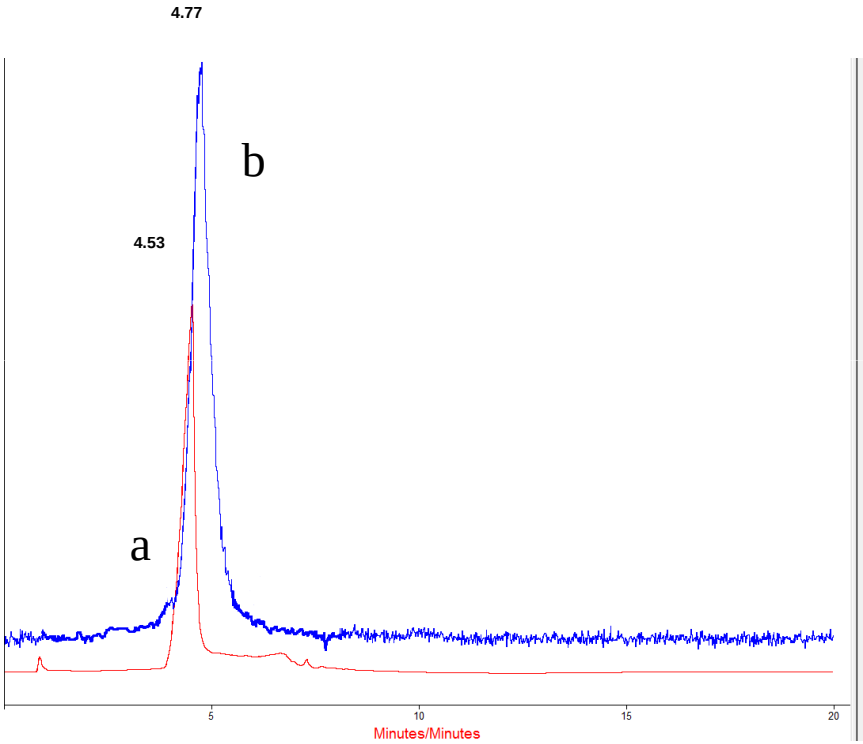
Concept of radiolabelling with ^{188}Re validated

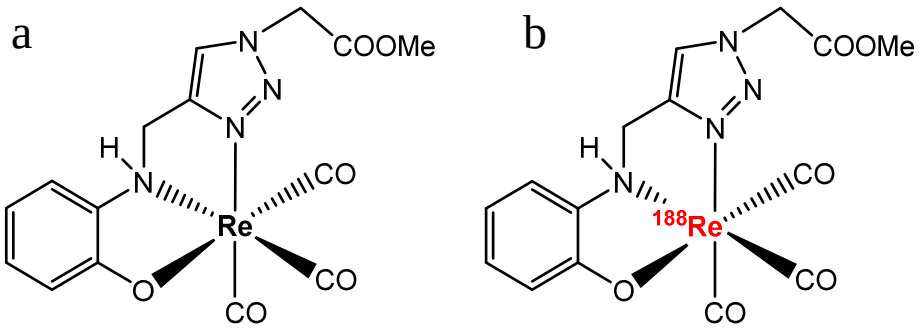
Results and discussion (10/14)

(microscopic study)

Preliminary study of radiolabelling with ^{188}Re

- HPLC Comparison





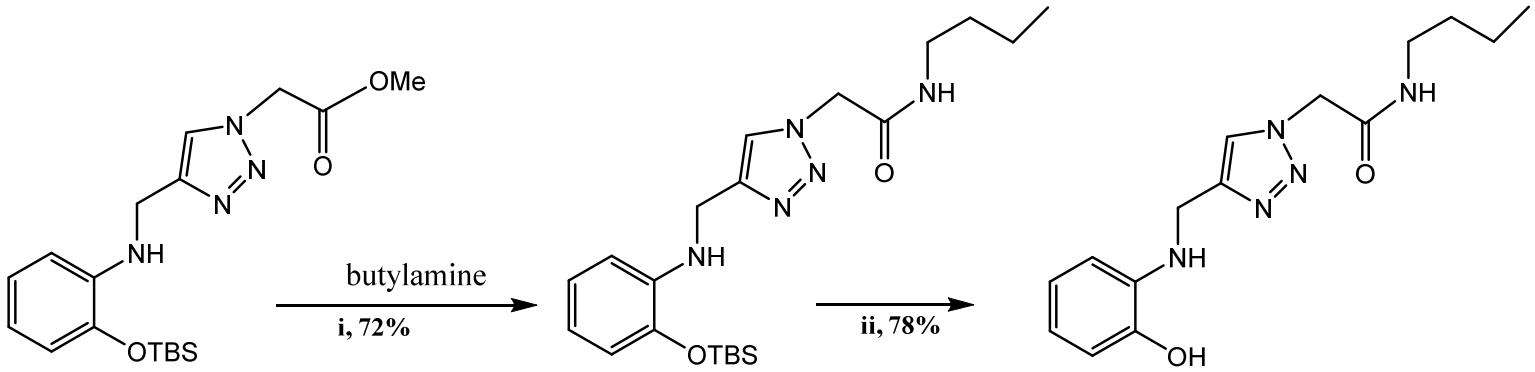
Isostructurality of ^{188}Re/Re complexes

C18 Accucore column (100 × 3 mm);
A: MeOH 0.1% TFA; B: H_2O 0.1% TFA; 0.5 mL/min

Results and discussion (11/14)

First trials of conjugation (proof of concept with amine models)

- *Via* amide bond formation

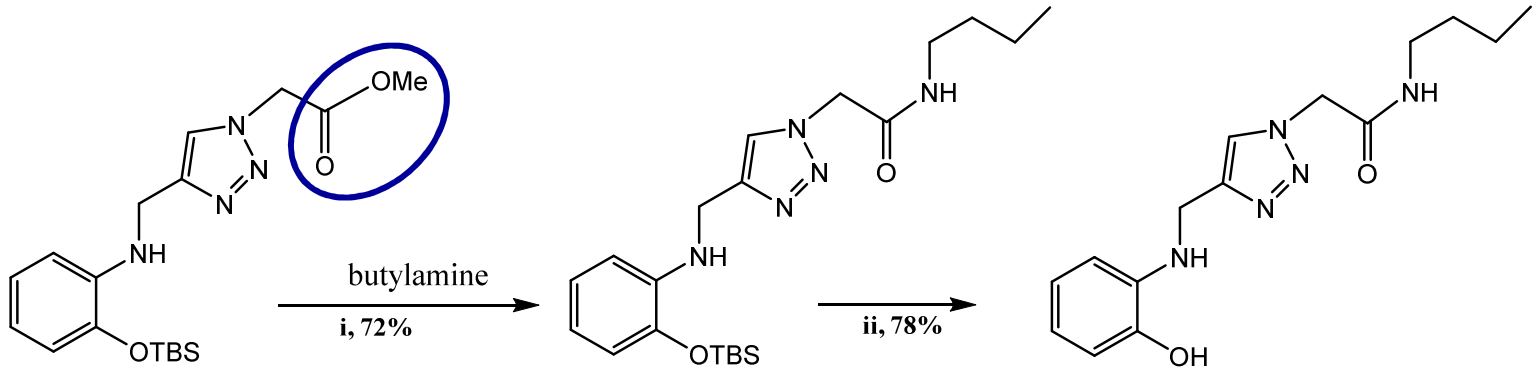


Conditions: (i) DABAL-Me_3, butylamine, THF, 40° C, 1 night; (ii) $NH_4F.HF$, MeOH, r.t., 1 night.

Results and discussion (12/14)

First trials of conjugation (proof of concept with amine models)

- *Via* amide bond formation



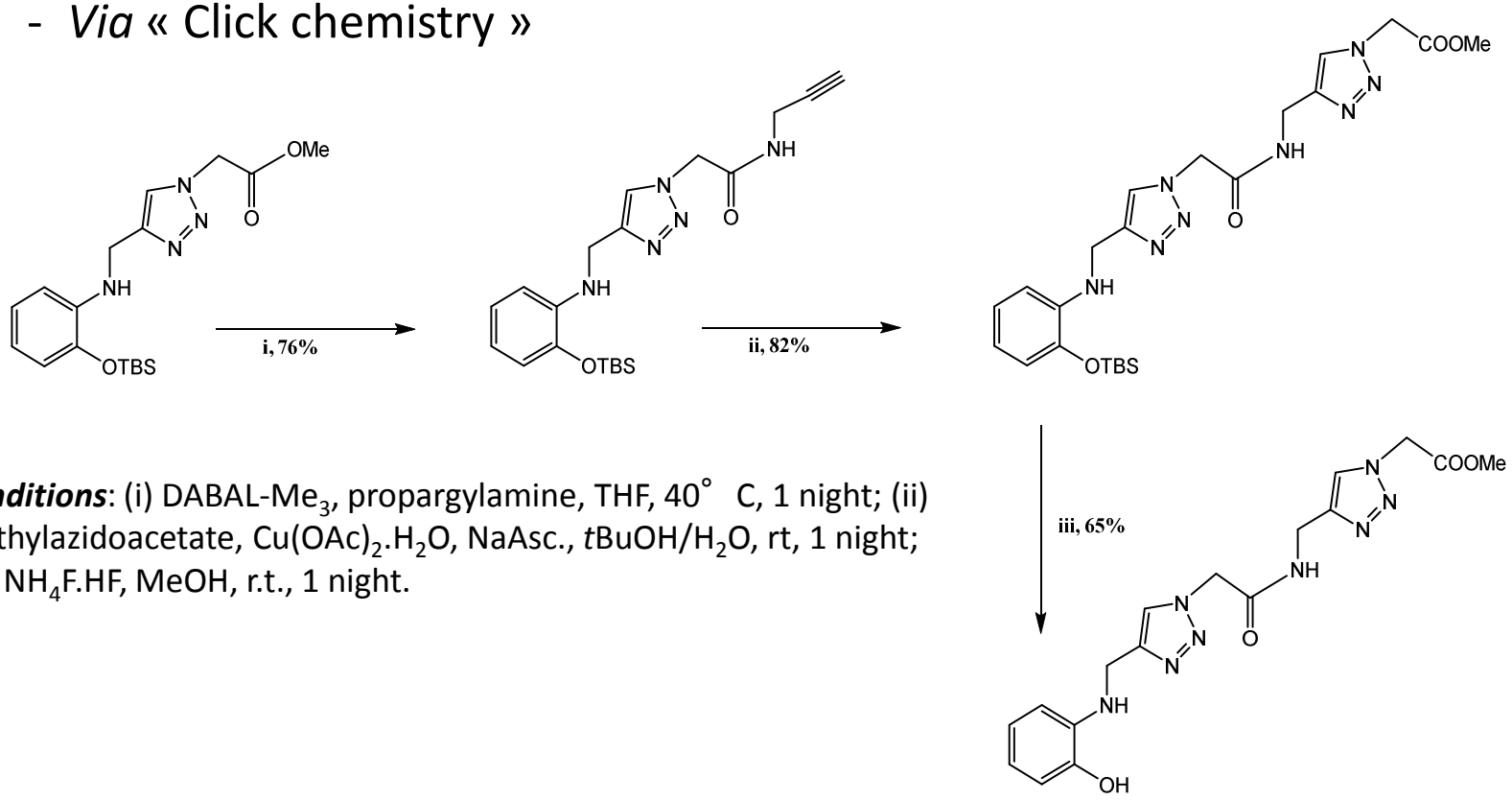
Conditions: (i) DABAL-Me_3, butylamine, THF, 40° C, 1 night; (ii) $NH_4F.HF$, MeOH, r.t., 1 night.

Possibility of bioconjugation with the Phenylalanine amine function of octreotide

Results and discussion (13/14)

First trials of conjugation (proof of concept with amine models)

- *Via* « Click chemistry »

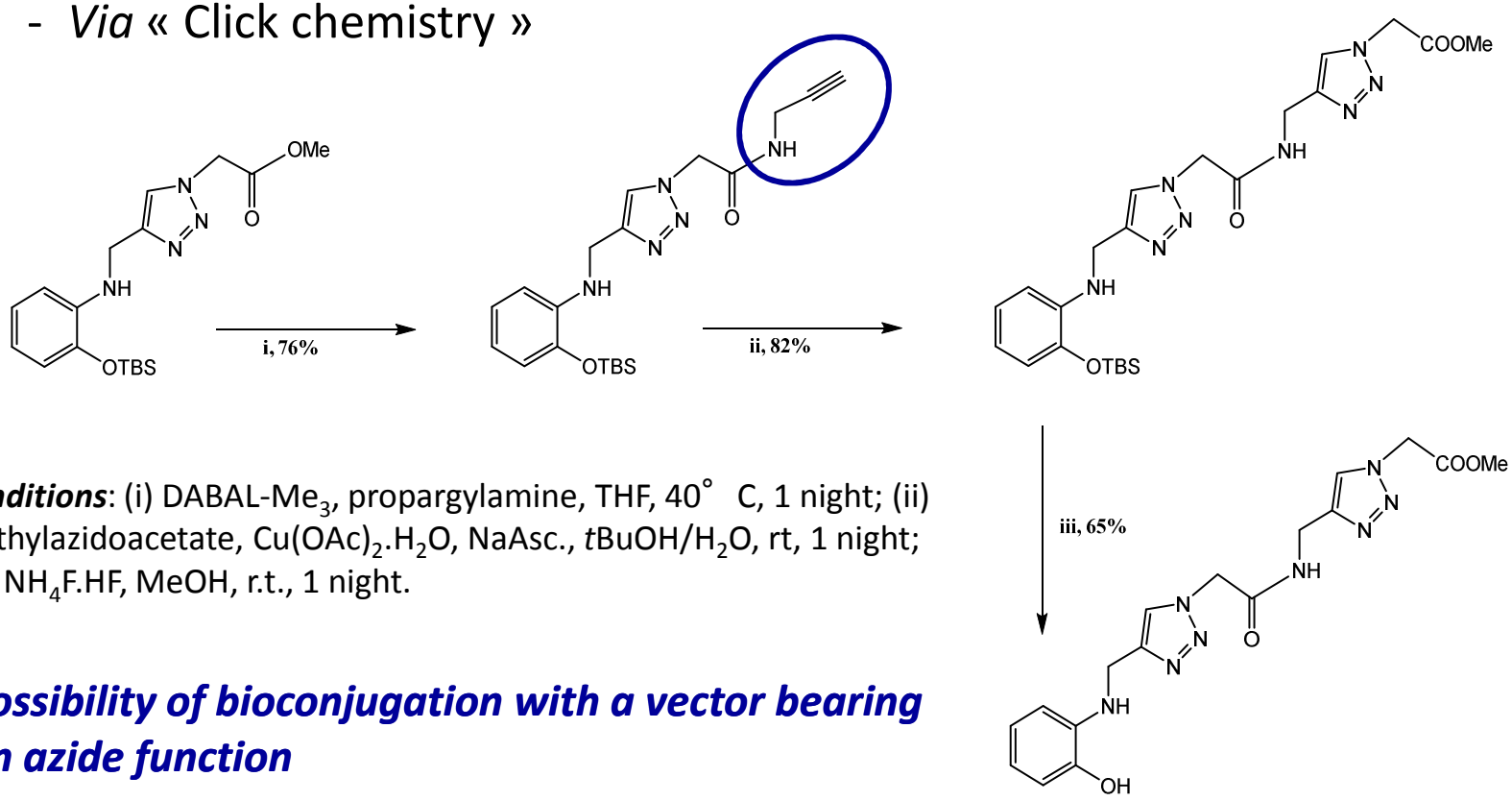


Conditions: (i) DABAL-Me_3, propargylamine, THF, 40° C, 1 night; (ii) methylazidoacetate, $Cu(OAc)_2.H_2O$, NaAsc., *t*BuOH/H_2O, rt, 1 night; (iii) $NH_4F.HF$, MeOH, r.t., 1 night.

Results and discussion (14/14)

First trials of conjugation (proof of concept with amine models)

- *Via* « Click chemistry »

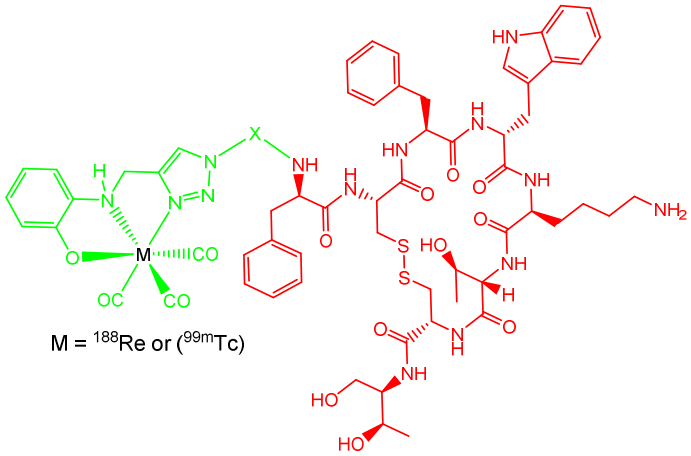


Conditions: (i) DABAL-Me_3, propargylamine, THF, 40° C, 1 night; (ii) methylazidoacetate, $Cu(OAc)_2.H_2O$, NaAsc., *t*BuOH/H_2O, rt, 1 night; (iii) $NH_4F.HF$, MeOH, r.t., 1 night.

Possibility of bioconjugation with a vector bearing an azide function

Conclusions (1/2)

Ultimate goal



Chemistry

Synthesis of BCAs as well as nonradioactive rhenium complexes have been ***performed***

All these « cold » rhenium complexes were ***fully characterised***

Biological study

^{99m}Tc-complex *showed a* ***fast clearance*** *as well as* ***no specific uptake*** *confirming its good* ***in vivo stability***

Radiolabelling

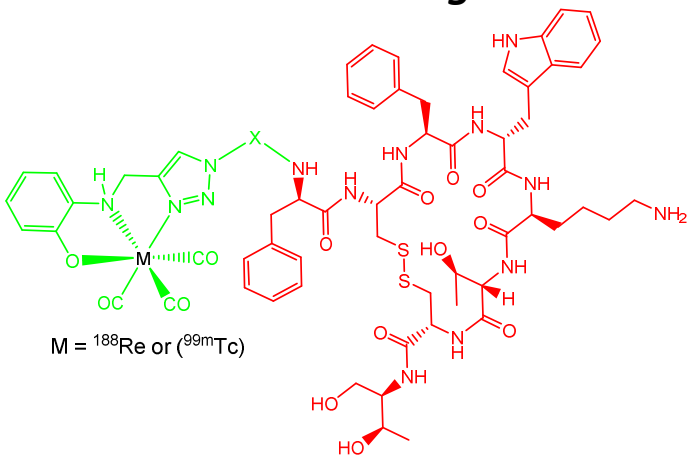
Radiolabelling *with ^{188}Re* ***validated*** *: from good to excellent chemical yield*

➢ ***Chelating cavity adapted for the $M(CO)_3^+$ core***

Conclusions (2/2)

Prospects

Ultimate goal



Chemistry

Conjugation *of ligand* ***1*** *with octreotide via an* ***amine bond formation***

Radiolabelling

Radiolabelling with ^{188}Re of bioconjugate (peptide + ligand 1)

Acknowledgments

- *Laboratory institutions*

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- *Collaborators*

Pr. Paul-Louis FABRE
(Electrochemical studies)

Dr. Mariusz WOLFF
(X-ray structure)