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Synthesis of Tc-99m labeled 1,2,3-triazole-4-yl c-met binding peptide as a potential c-met receptor kinase positive tumor imaging agent

Eun-Mi Kim, Min-Hee Joung, Chang-Moon Lee, Hwan-Jeong Jeong, Seok Tae Lim, Myung-Hee Sohn, Dong Wook Kim *

Department of Nuclear Medicine, Chonbuk National University Medical School, Jeonju, Jeonbuk 561-712, Republic of Korea
Cyclotron Research Center, Chonbuk National University Medical School, Jeonju, Jeonbuk 561-712, Republic of Korea
Research Institute of Clinical Medicine, Chonbuk National University Medical School, Jeonju, Jeonbuk 561-712, Republic of Korea

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ABSTRACT

The mesenchymal-epithelial transition factor (c-Met), which is related to tumor cell growth, angiogenesis and metastases, is known to be overexpressed in several tumor types. In this study, we synthesized technetium-99m labeled 1,2,3-triazole-4-yl c-Met binding peptide (cMBP) derivatives, prepared by solid phase peptide synthesis and the 'click-to-chelate' protocol for the introduction of tricarbonyl technetium-99m, as a potential c-Met receptor kinase positive tumor imaging agent, and evaluated their in vitro c-Met binding affinity, cellular uptake, and stability. The ^{99m}Tc labeled cMBP derivatives ([^{99m}Tc(CO)$_3$]**12**, [^{99m}Tc(CO)$_3$]**13**, and [^{99m}Tc(CO)$_3$]**14**) were prepared in 85-90% radiochemical yields. The cold surrogate cMBP derivatives, [Re(CO)$_3$]**12**, [Re(CO)$_3$]**13**, and [Re(CO)$_3$]**14**, were shown to have high binding affinities (0.13 μM, 0.06 μM, and 0.16 μM, respectively) to a purified cMet/Fc chimeric recombinant protein. In addition, the in vitro cellular uptake and inhibition studies demonstrated the high specific binding of these ^{99m}Tc labeled cMBP derivatives ([^{99m}Tc(CO)$_3$]**12–14**) to c-Met receptor positive U87MG cells.

The hepatocyte growth factor (HGF)–mesenchymal-epithelial transition factor (Met) molecular pathway affects cancer development at different stages, from initiation to metastatic behavior, in many tumor types.[1–4] Since Met was discovered as an oncogene[5], the HGF/c-Met signaling pathway has come into the spotlight as a promising therapeutic target for inhibiting tumor growth and various strategies are currently in development to disrupt the HGF–Met signal transduction pathway.[6–9] Kim et al. previously reported that monoclonal antibody SFN68 inhibits the HGF–c-Met interaction and blocks the biological function of c-Met mediated by HGF.[10] To define the epitope, they screened out the epitope-mimicking peptide, NH_2-Lys^1-Ser^2-Leu^3-Ser^4-Arg^5-His^6-Asp^7-His^8-Ile^9-His^{10}-His^{11}-His^{12}-Ac (c-Met binding peptide; cMBP), from a phage display of a combinatorial peptide library. We recently reported the pharmacokinetic evaluation of ^{125}I-radiolabeled cMBPs and cMBP-X-Cy5.5 (X: linker) for targeting c-Met receptor expression in U87MG tumor models.[11,12] In these previous studies, we found that cMBP could specifically target the c-Met receptor and was a good candidate biomolecule to use a molecular imaging agent.

Nowadays, the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and alkynes, which is commonly called click chemistry, plays a crucial role in a wide range of biomedical applications, such as drug discovery by the combinatorial approach and molecular imaging by the tagging of biological systems, because this reaction is able to proceed selectively and efficiently in aqueous media in the presence of various bioactive molecules such as peptides, proteins, nucleotides, and whole organisms, affording the 1,2,3-triazole in high yield as a stable linker for conjugation between two (bio)molecular components.[13–15] In particular, Mindt and co-workers reported that the 1,2,3-triazole derivatives obtained from this click reaction of azido-biomolecules and propargyl glycine could be regarded as a potent precursor for the preparation of technetium-99m (Tc-99m) labeled radiopharmaceuticals for single photon emission computed tomography (SPECT) because these 1,2,3-triazole chelators can act as efficient ligand systems for the formation of tricarbonyl Tc-99m complexes through the 'click-to-chelate' approach from [M(CO)$_3$(H$_2$O)$_3$]$^+$ (M = ^{99m}Tc, Re).[16–18]

Technetium-99m labeled tracers are particularly useful: (a) SPECT camera is widely used and easily available for noninvasive imaging to detect disease in humans; (b) technetium-99m is readily available at low cost from a ^{99}Mo/^{99m}Tc generator system; (c) finally, technetium-99m has ideal physical properties ($t_{1/2}$ = 6 h, 141 keV).[19,20] In particular, tricarbonyl technetium-99m is regarded as an attractive core for the introduction of technetium-99m into biomolecules due to its small size and high chemical stability.[21,22] In this work, we report the preparation of ^{99m}Tc labeled

* Corresponding author. Tel.: +82 63 250 2396; fax: +82 63 255 1172.
E-mail address: kimdw@chonbuk.ac.kr (D.W. Kim).

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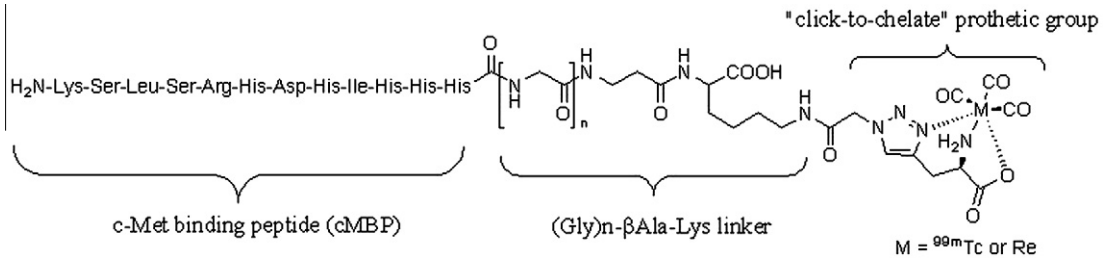
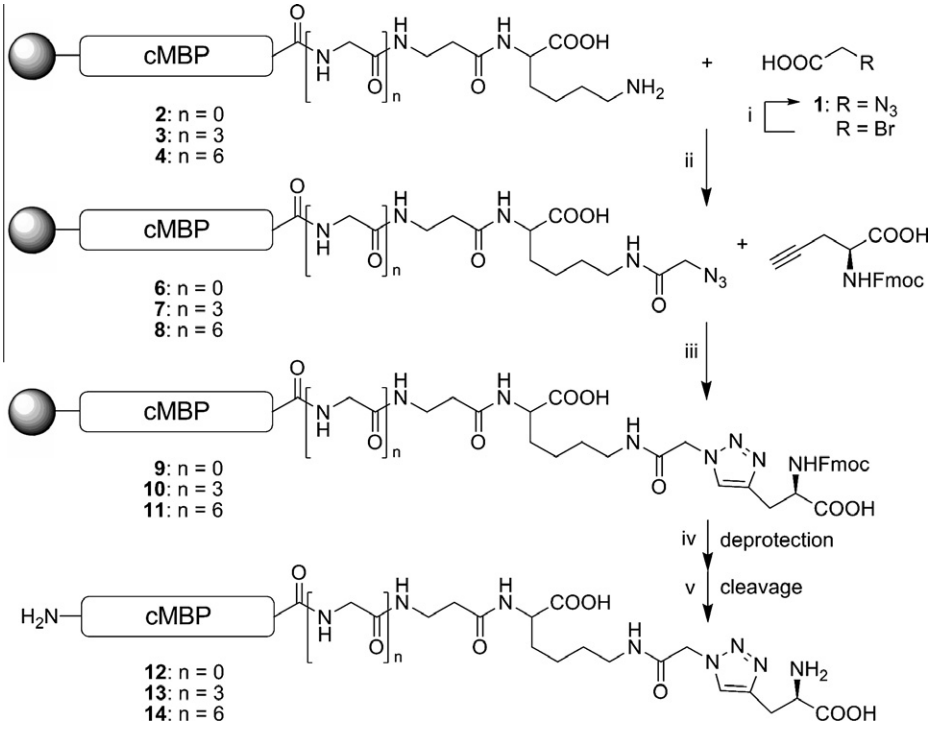


Figure 1. Structures of target molecules. The target molecules are divided into three parts: the 'click-to-chelate' prosthetic group, glycine containing linkage and cMBP moiety.

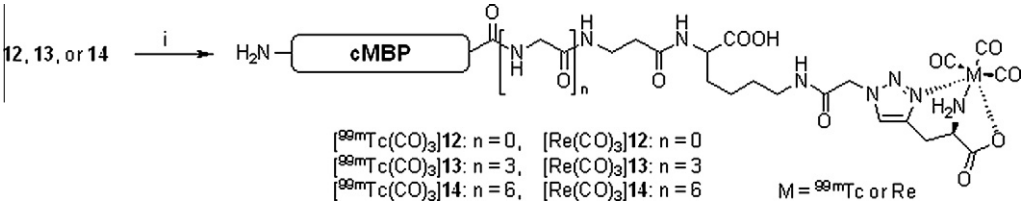
cMBP derivatives as potential c-Met receptor kinase positive tumor SPECT imaging agents using the 'click-to-chelate' protocol for the incorporation of tricarbonyl technetium-99m. As shown in Figure 1, these radiolabeled cMBP analogues were designed with three components: they consist of a cMBP moiety in order to have high target affinity toward the c-Met receptor tyrosine kinase, a $(Gly)_n$-βAla-Lys linker to connect between cMBP and the radiolabeled prosthetic group while taking into consideration their pharmacokinetics (to improve their lipophilicity), and a 'click-to-chelate' prosthetic group to introduce tricarbonyl technetium-99m into the 1,2,3-triazole ligand with minimal steric interference, thereby maintaining the favorable interaction with the target.

Scheme 1 illustrates the synthesis of the precursor for the tricarbonyl ^{99m}Tc labeled cMBP derivatives. The preparation of resins containing cMBP and $(Gly)_n$-βAla-Lys linker (**2**, **3**, and **4**) by solid phase peptide synthesis were followed by the amide condensation between the resins (**2**, **3**, and **4**) and azidoacetic acid (**1**) affording the azido-cMBP resins (**6**, **7**, and **8**), in order to introduce the azide group for the click reaction. The 1,2,3-triazole moiety of the resins (**9**, **10**, and **11**) was prepared by 1,3-dipolar cycloaddition reaction of the azide functionalized cMBP resins and propargyl glycine protected by the 9-fluorenylmethoxycarbonyl (Fmoc) group using Cu(I) as a catalyst. After the removal of the Fmoc protecting group under basic condition, the 1,2,3-triazole-cMBP derivatives (**12**, **13**, and **14**) as precursors for $^{99m}Tc(CO)_3$ labeling were cleaved from the solid phase by treatment with a mixture of triisopropylsilane, thioanisol, water, ethanedithiol, and trifluoroacetic acid (2.5:2.5:2.5:2.5:90), and the precursors were purified by reverse phase HPLC.

Metal coordination reaction of the precursors **12–14** with $[^{99m}Tc(CO)_3(OH_2)_3]^+$, which was readily prepared by a known method,[16–18] in aqueous media for 30 m at 80 °C provided the radioactive cMBP complexes—$[^{99m}Tc(CO)_3]$**12**, $[^{99m}Tc(CO)_3]$**13**, and $[^{99m}Tc(CO)_3]$**14**—in 90%, 89%, and 85% radiochemical yield, respectively (Scheme 2). The reaction was monitored by radio-TLC scanner until the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ peak disappeared. The radiochemical purities of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ was more than 98%. Nonradioactive cMBP complexes—$[Re(CO)_3]$**12**, $[Re(CO)_3]$**13**, and $[Re(CO)_3]$**14** were also prepared using a similar procedure to that employed for the above radioactive cMBP complexes except for the use of $[Re(CO)_3(OH_2)_3]^+$ instead of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ for in vitro assays.



Scheme 1. Synthesis of 1,2,3-triazole precursors. Reagents and conditions: (i) NaN_3, water, 4 °C, 16 h; (ii) DIC, HOBT, DMF, rt, 2 h; (iii) Cu(I)Br, DIPEA, DMF, Fmoc-L-propargylglycine, rt, 4 h; (iv) 20% piperidine in DMF, rt, 10 m (Fmoc deprotection) (v) triisopropylsilane, thioanisol, water, ethanedithiol, trifluoroacetic acid (2.5/2.5/2.5/2.5/90), rt, 3 h (cleavage).



Scheme 2. Reagents and conditions: (i) $[Re(OH_2)_3(CO)_3]^+$, or $[^{99m}Tc(OH_2)_3(CO)_3]^+$, water, 80 °C, 30 m.

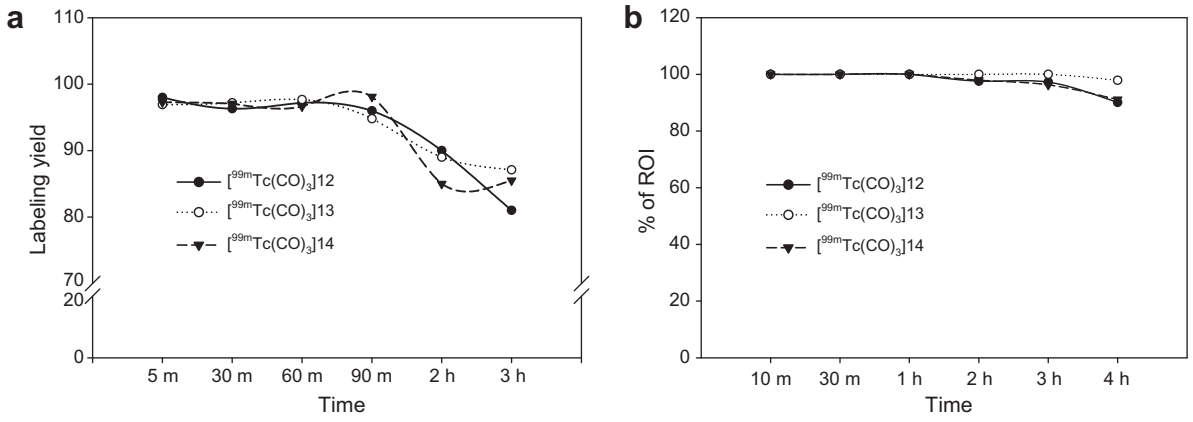


Figure 2. In vitro human serum stability (a) and transchelation challenge test (b) from $[^{99m}Tc(CO)_3]$**12**, $[^{99m}Tc(CO)_3]$**13**, and $[^{99m}Tc(CO)_3]$**14** as a function of the histidine concentration.

Table 1
Competitive binding affinity assay of cold surrogate cMBP derivatives ($[Re(CO)_3]$**12**, $[Re(CO)_3]$**13**, and $[Re(CO)_3]$**14**) using recombinant chimeric c-Met/Fc protein,[a] and the partition coefficient (*P*) of the ^{99m}Tc radiolabeled cMBP complexes[b]

Entry	Compounds	IC_{50} (μM)	Log *P*[c]
1	cMBP	0.12	—
2	$[Re(CO)_3]$**12**	0.13	−2.6 ± 0.02
3	$[Re(CO)_3]$**13**	0.06	−2.0 ± 0.01
4	$[Re(CO)_3]$**14**	0.16	−2.0 ± 0.03

[a] The results of the competitive binding experiment show that the binding of the I-125 labeled cMBP to the HGF receptor was inhibited in a concentration dependent manner by the non-radiolabeled Re complexes.
[b] The log *P* values were determined with the three ^{99m}Tc radiolabeled complexes, $[^{99m}Tc(CO)_3]$**12**, $[^{99m}Tc(CO)_3]$**13**, and $[^{99m}Tc(CO)_3]$**14**.
[c] Data are expressed as means ± SD of duplicate samples.

To investigate their stability, the solutions of these radiolabeled cMBP complexes were adjusted with physiological saline to a concentration of 37 MBq/mL, and aliquots of 100 μL of these solutions were added to 500 μL human plasma and incubated at 37 °C. The percentage of the complexes remaining after 2 h was over 80% (Fig. 2). In the trans-chelation stability study using histidine (concn 0.1 mM) and cystein (concn 0.1 mM), the labeling efficiency of all of the complexes was also over 90% after 3 h (Fig. 2). These results showed that all of the ^{99m}Tc radiolabeled cMBP complexes have sufficient stability for image acquisition.

Table 1 illustrated the competitive c-Met receptor binding assay for the three cold surrogate Re complexes ($[Re(CO)_3]$**12–14**) using a cMet/Fc chimeric recombinant protein, and the partition coefficient (*P*) study with the three ^{99m}Tc radiolabeled complexes ($[^{99m}Tc(CO)_3]$**12–14**). The IC_{50} values of $[Re(CO)_3]$**12–14** (0.13, 0.06, and 0.16 μM, respectively), and free cMBP (0.12 μM) were obtained by competitive displacement studies using ^{125}I-cMBP as a radioligand. The binding affinities of these cold complexes were in the nanomolar range and similar or higher to that of the free cMBP. Thus, this result showed that the 'click-to-chelate' pros-

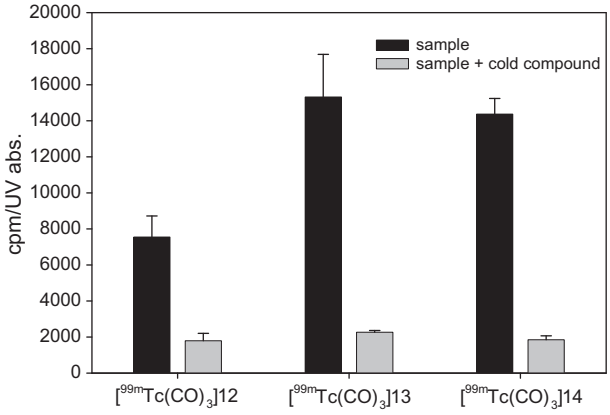


Figure 3. Cellular uptake and blocking study of ^{99m}Tc radiolabeled cMBP complexes ($[^{99m}Tc(CO)_3]$**12**, $[^{99m}Tc(CO)_3]$**13**, and $[^{99m}Tc(CO)_3]$**14**) in U87MG cells. The cells were incubated with ^{99m}Tc radiolabeled cMBP complexes (5.2 nmol) for 1 h. For the blocking studies, ^{99m}Tc radiolabeled cMBP complexes were co-treated with the cold cMBP peptide (86 nmol). The plate was then incubated for 1 h at 37 °C. All of the tests were done in triplicate. The data are presented as the mean ± SD.

thetic group and $(Gly)_n$-βAla-Lys linker in the ^{99m}Tc radiolabeled cMBP complexes have no influence on the binding of the c-Met receptor. The determination of log *P* shows that the insertion of glycine at the linker allowed the lipophilicity to be improved; the log *P* values of $[^{99m}Tc(CO)_3]$**12**, $[^{99m}Tc(CO)_3]$**13**, and $[^{99m}Tc(CO)_3]$**14** were −2.6 ± 0.02, −2.0 ± 0.01, and −2.0 ± 0.03, respectively.

As shown in Figure 3, cellular uptake and blocking studies were performed using a c-Met receptor positive tumor cell line such as U87MG. The $[^{99m}Tc(CO)_3]$**13** complex showed higher cellular uptake than both $[^{99m}Tc(CO)_3]$**12** and $[^{99m}Tc(CO)_3]$**14** in the U87MG cells, with the results being similar to those of the c-Met receptor binding study. Consequently, $[^{99m}Tc(CO)_3]$**13** containing the three glycines (Gly-Gly-Gly) in the linker moiety had the highest c-Met

protein binding affinity as well as cellular uptake, therefore could be used as a radiotracer for the detection of c-Met receptor kinase positive tumor cells. All of the ^{99m}Tc radiolabeled cMBP complexes, [^{99m}Tc(CO)$_3$]**12**, [^{99m}Tc(CO)$_3$]**13**, and [^{99m}Tc(CO)$_3$]**14**, were also significantly inhibited by cold cMBP, which means that they specifically bound to c-Met on the cell surface.

In summary, in our search for SPECT imaging agents for c-Met receptor kinase positive tumors, we have focused on the preparation of ^{99m}Tc radiolabeled cMBP derivatives through solid phase peptide synthesis and the click reaction so that we could explore the introduction of the 'click-to-chelate' prosthetic group linked to (Gly)$_n$-βAla-Lys into the cMBP moiety in a manner that would not affect its c-Met binding. These ^{99m}Tc radiolabeled cMBP complexes were not only efficiently incorporated on to the 1,2,3-triazole ligand in 85–90% radiochemical yield using tricarbonyl ^{99m}Tc, but also showed good stability. In particular, [^{99m}Tc(CO)$_3$]**13** was shown to bind to c-Met receptor positive U87MG cells in a c-Met specific manner and with excellent binding affinities. Further work on the imaging study and in vivo evaluation of some of these ^{99m}Tc radiolabeled cMBP complexes will be reported elsewhere.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.036.

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