ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Heteronuclear Gd-^{99m}Tc Complex of DTPA-Bis(histidylamide) Conjugate as a Bimodal MR/SPECT Imaging Probe

Ji-Ae Park,[†,⊥] Jung Young Kim,[†,⊥] Hee-Kyung Kim,[‡] Wonho Lee,[†] Sang Moo Lim,[§] Yongmin Chang,[‡] Tae-Jeong Kim,*[,∥] and Kyeong Min Kim*[,†]

[†]Molecular Imaging Research Center, Korea Institute of Radiological and Medical Sciences, Seoul 139-706, Korea

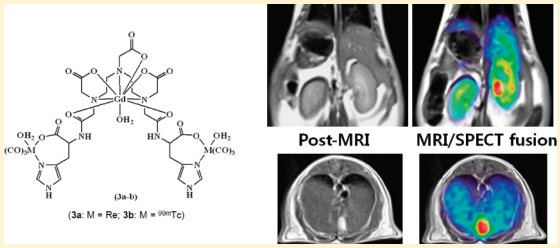
[‡]Department of Medical & Biological Engineering, Kyungpook National University, Daegu 700-422, Korea

[§]Department of Nuclear Medicine, Korea Institute of Radiological and Medical Sciences, Seoul 139-706, Korea

[∥]Department of Applied Chemistry, Kyungpook National University, Daegu 702-701, Korea

Supporting Information

ABSTRACT: The work describes the synthesis and in vivo application of heterotrimetallic complexes of the type {Gd(H_2O)[(M(H_2O)-($CO)_3)_2$(**1**)]} {**1** = DTPA-bis(histidyl-amide); *M* = Re (**3a**); ^{99m}Tc (**3b**)} for dual modality MR/SPECT imaging. Here, the DTPA-bis(histidylamide) conjugate functions as a trinucleating chelate incorporating Gd in the DTPA core with Re or ^{99m}Tc in the pair of histidylamide side arms. The two complexes are chemically equivalent as revealed by HPLC, and their "cocktail mixture" (**3a** + **3b**) has demonstrated itself to be essentially a single bimodal imaging probe. The present system has thus overcome the sensitivity difference problem between MRI and SPECT and paved the way for practical applications.



KEYWORDS: *dual modality, MR/SPECT, imaging probe, cocktail mixture, heterotrimetallic Gd-[^{99m}Tc]$_2$/[Re]$_2$ complexes*

In recent years, there has been a great deal of interest in the design of multimodal imaging probes in an effort to overcome the detection limit of individual imaging modality such as magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), and optical imaging probe. In addition, the marriage of different modalities is expected to provide advantages of individual modality. Consequently, various combinations of different modalities are now known since the first appearance of PET/CT. More recent attempts include MRI/PET, MRI/optical, PET/optical, and MRI/CT, and even greater efforts are under way to develop further exotic multifunctional probes.[1,2] In this regard, single photon emission computed tomography (SPECT) deserves special attention in that it, along with MRI, is one of most popular imaging modalities currently available in clinics. Thus, the combination of MRI and SPECT would put a new entry into a powerful diagnostic probe. MRI is famous for anatomical information and SPECT biological information.

Although quite a few of the dual modal MR/SPECT imaging probes anchored by nanoplatforms are available,[3,4] little endeavor has been made in connection with the small-molecule-based probes for the same purposes. In fact, such a design would be the simplest in concept. Yet, it is challenging to achieve more than a 1:1 ratio of probe types with different sensitivities in a single molecule. For instance, the imaging sensitivity of MRI is typically 6 orders of magnitude lower than that of SPECT. These drawbacks may account for the relative paucity of reports of multimodal small-molecule probes as compared with nanosystems.[5]

One approach to overcome the issue of sensitivity difference would be to use a "cocktail mixture" of two probes with different modalities in varying ratios.[6] However, such a mixture can hardly be considered as truly dual modal unless the component probes in the same mixture exhibit not only the same chemical equivalence but also the same biodistribution. To the best of our knowledge, no such MR/SPECT dual modal probes that meet the criteria mentioned above have been put into practice.

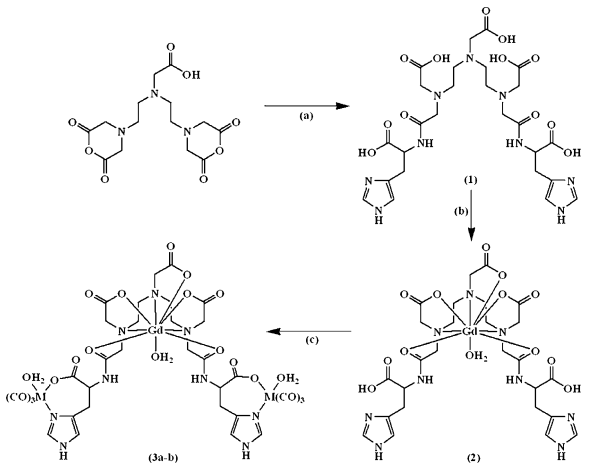
We hereby explore the method of "cocktail mixture" of (cold) rhenium and (hot) technetium-99m in an effort to design a small-molecule-based dual modal MR/SPECT probe. Technetium-99m ($t_{1/2}$ = 6.02 h, E_γ = 140 KeV) is the most commonly used isotope in nuclear medicine because of unique advantages inherent to this isotope.[7] It is also a well-known standard practice, when working with ^{99m}Tc, to use non-radioactive rhenium, to perform larger scale chemistry and characterization. Rhenium exhibits very similar chemistry to technetium.[8] Thus, our goal is to develop a dual probe that consists of a mixture of heterotrimetallic Gd-[^{99m}Tc]$_2$ and Gd-[Re]$_2$ complexes. In each complex, rhenium or technetium is incorporated in the corresponding Gd-complex via a trinucleat-

Received: December 5, 2011
Accepted: February 27, 2012
Published: February 27, 2012

   dx.doi.org/10.1021/ml200285p | *ACS Med. Chem. Lett.* 2012, 3, 299–302

ing chelate such as the diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid (DTPA)-histidine conjugate (Scheme 1).

Scheme 1[a]



[a]Conditions: (a) Histidine, DMF, RT, 6 h. (b) Gd_2O_3, H_2O, 90 °C, 6 h. (c) Compound **3a** (M = Re): $[Re(CO)_3(H_2O)_3]^+$, H_2O, RT, 5 h; compound **3b** (M = ^{99m}Tc): $[^{99m}Tc(CO)_3(H_2O)_3]^+$, H_2O, pH 7–9, RT, 2 h.

The gadolinium complex of DTPA-bis(amide) is a typical T_1 MRI contrast agent (CA) currently used in clinics. This new platform is expected to be a truly single bimodal imaging probe in that the two complexes in the same mixture are chemically equivalent and thus ensure the same pharmacokinetics and colocalization of signal for each modality. The work described here is part of our continuing effort to develop multifunctional MRI CAs.[9,10]

Scheme 1 shows the synthesis of DTPA-bis(histidylamide) (**1**), $[Gd(\mathbf{1})(H_2O)]$ (**2**), and corresponding heterotrimetallic complexes **3a** and **3b**. Detailed procedures for their syntheses are provided in the Supporting Information. In brief, a simple condensation of DTPA-bis(anhydride) with histidine led to the formation of **1** as a new class of heterotrinucleating chelates. Here, it is worth noting that histidine structurally resembles 1,2,3-triazole-4-yl alanine, a well-known monoanionic tridentate chelate, $[NNO]^{1-}$, for coordination to the $Re(CO)_3$ and/or $^{99m}Tc(CO)_3$ cores that are employed for SPECT imaging.[11] In addition, the chemistry of $[M(CO)_3(H_2O)_3]^+$ (M = Re, ^{99m}Tc) for radiopharmaceutical application has been well-established.[12] The formation of **2** from the reaction of **1** with Gd_2O_3 is well-established and thus deserves little attention. The reaction of **2** with $[Re(CO)_3(H_2O)_3]^+$, prepared in situ by stirring $[Et_4N]_2[Re(CO)_3Br_3]$ at RT for 2 h in aqueous media, yielded the corresponding heterotrimetallic complex **3a**, where chelation of the histidylamide side arms to rhenium must take place in a bidentate ($[NO]^{1-}$) manner unlike the parent histidine.

The formation of **3a** was characterized by microanalytical and spectroscopic (NMR, IR, MS) methods (cf. ESI). Namely, the IR spectrum shows two νCO bands at 1998 and 1870 cm^{-1}, a pattern typical for the *fac*-$[Re(CO)_3]$ configuration (Figure S1 in the Supporting Information, ESI).[13] More characteristically, the yttrium analogue of **3a**, $\{Y(H_2O)[(M(H_2O)(CO)_3)_2(\mathbf{1})]\}$, obtained from **1** by replacing $GdCl_3$ with YCl_3 in step b, shows two carbonyl peaks at 198.9 and 198.8 ppm on ^{13}C NMR.[14]

The proton relaxivities, r_1 and r_2, of **3a** are 7.8 ± 0.1 and 8.5 ± 0.1 mM^{-1} s^{-1}, respectively, at 298 K and 128 MHz. The r_1 relaxivity of **3a** is twice as high as that of Magnevist (cf. Table S2 in the Supporting Information). A partial explanation for such an increase in the relaxivity may come from the reduced tumbling rate (τ_R) as a result of the increase in molecular weight through DTPA-histidine conjugation.[15]

The radioactive complex **3b** was also obtained as a single product with a high radiochemical yield (>95%) from the reaction of *fac*-$[^{99\ m}Tc(CO)_3(H_2O)_3]^+$ with **2**. Here, the formation of the equivalent technetium complex with the same *fac*-configuration can be confirmed without ambiguity by the HPLC method. Namely, coinjection of a mixture of **3a** and **3b** would give peaks at the same retention time; the retention times (t_R) were found to be 16.5 and 17 min, respectively (Figure 1), demonstrating their chemical and structural equivalence.

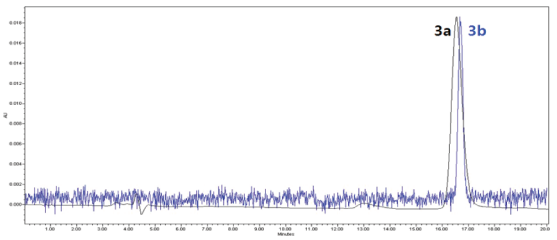


Figure 1. Analytical HPLC chromatograms of **3a** (black, 254 nm) and corresponding **3b** (blue, γ trace).

The bimodal imaging capabilities of **3** were evaluated by MR and SPECT imaging. To overcome the sensitivity difference, the relative amounts of **3a** and **3b** were adjusted as follows: [**3a**] for MRI = 4.08 mg (0.1 mmol Gd/kg); [**3b**] for SPECT = 1.6×10^{-10}–3.19×10^{-10} mg (2 mCi, ^{99m}Tc activity). Figure 2 shows consecutive images of T1-weighted MR and SPECT of mice obtained with the intravenous administration of a mixture of **3a** and **3b**. The figure shows strong enhancement mostly in liver, gallbladder, and kidney. It is to be noted, however, that no enhancement is observed in gallbladder with **2** alone. Thus, the presence of the $Re(CO)_3$ moiety seems to be essential for **3a** to reveal the same biodistribution as **3b**. Excretion is made through renal and bile duct. The fusion images of MR/SPECT (Figure 2c,f) show the same biodistribution, demonstrating once again the chemical equivalence of **3a** and **3b**.

Further Supporting Information about their chemical equivalence can be garnered from the pixel/AUC graphs obtained from the sequential MR and SPECT images shown in Figure 2g,h. Although absolute values in each graph can not be directly compared to each other, the same trends in the graphs for both liver and gallbladder are good enough to provide supportive information in favor of chemical equivalence of **3a** and **3b**. One exception to be noted, however, is a sharp jump in the SPECT signal that is observed during the early period of measurement (<30 min) in the case of liver (Figure 2g). The sharp irregularity may have to do with coordinative unstability of **3b**. We have noted above that the histidylamide side arms in **1** bind to $^{99m}Tc(CO)_3$ in a bidentate fashion leaving a vacant site on ^{99m}Tc for weakly bounding water. In this regard, it is also worth noting that the radiochemical stability of **3b** in human serum decreases for initial 2 h as shown in Figure S2 in the Supporting Information. It is well established in the literature that a tridentate ligand exhibits much higher stability

dx.doi.org/10.1021/ml200285p | *ACS Med. Chem. Lett.* 2012, 3, 299–302

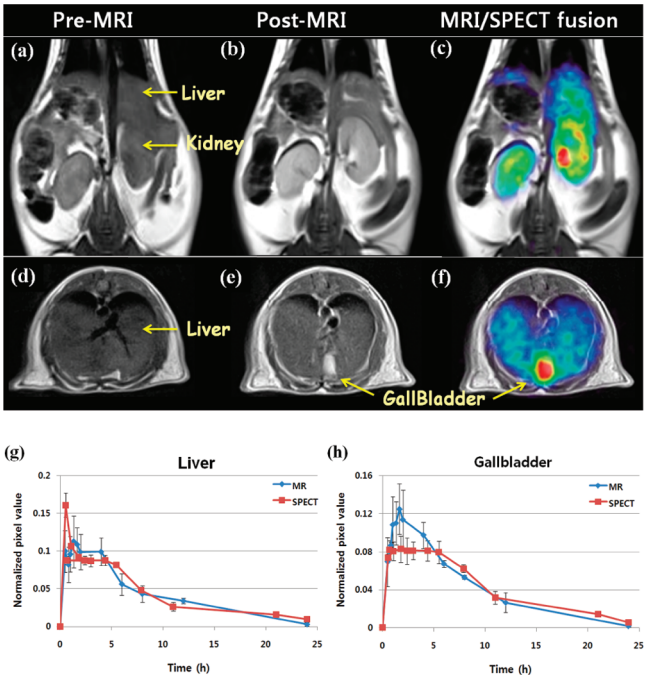


Figure 2. In vivo MR coronal (top) and axial (bottom) images of mice obtained before (a and d) and 1 h after injection of a mixture of **3a** and **3b** (b and e) and overlaid on SPECT images (c and f). Time pixel–intensity curves of liver (g) and gallbladder (h) obtained from sequential MR and SPECT images with normalization using the values of each AUC.

in human plasma than its bidentate counterpart.[16] It is because a tridentate ligand technically blocks any interaction with serum through coordinative saturation on ^{99m}Tc. Thus, it is to be noted that the fusion images were obtained so as to exclude the initial time interval (~30 min) to minimize whatever metabolic differences between **3a** and **3b**.

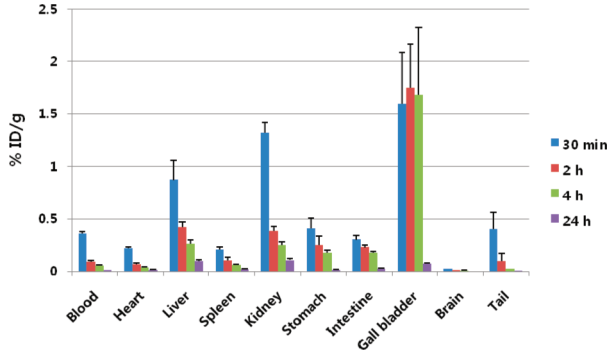


Figure 3. Organ uptake (% ID/g) of **3b** by mice (n = 4) in 30 min and 2, 4, and 24 h.

As stated earlier, one of the unique advantages of small-molecule probe is fast excretion, which is also demonstrated with the present probe. Figure 2g,h, along with Figures S5 and S6 in the Supporting Information, show that excretion is complete within 24 h. In line with these observations is shown in Figure 3 expressed as the percentage injected dose per gram tissue (%ID/g), which is a quantitative representation of the observations shown in Figure S6 in the Supporting Information.

In summary, we have prepared heterotrimetallic complexes **3a** and **3b** incorporating the DTPA-bis(histidylamide) conjugate as a trinucleating chelate for dual modality MR/SPECT imaging. Compounds **3a** and **3b** are chemically equivalent, and therefore, their mixture can rightly be termed as a true and single bimodal imaging probe. The unique and characteristic feature of the present probe is that it enables one to overcome the sensitivity difference problem by regulating the ratio of **3a** to **3b**.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of **1**–**3** and other detailed procedures: NMR spectra, relaxivity measurements, protonation and stability constants, in vivo imaging studies, biodistribution, serum stability assay, and in vitro cell toxicity. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +82-53-950-5587. Fax: +82-53-950-6594. E-mail: tjkim@knu.ac.kr (T.-J.K.). Tel: +82-2-970-1387. Fax: +82-2-970-2416. E-mail: kmkim@kirams.re.kr (K.M.K.).

Author Contributions

⊥These authors contributed equally.

Funding

This work was partially supported by NRF (Grant Nos. 2010-0024143 and 2011-0030162) and MEST through The Programs of Development Research Center of PET Application Technology & The Programs of Clinical Application Research of Radiopharmaceuticals in the National Nuclear Technology Program, ROK.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Louie, A. Multimodality imaging probes: Design and challenges. *Chem. Rev.* **2010**, *110*, 3146–3195.

(2) Choi, J. S.; Park, J. C.; Nah, H.; Woo, S.; Oh, J.; Kim, K. M.; Cheon, G. J.; Chang, Y.; Woo, J.; Cheon, J. A hybrid nanoparticle probe for dual-modality positron emission tomography and magnetic resonance imaging. *Angew. Chem., Int. Ed.* **2008**, *47*, 6259–6262.

(3) Zielhuis, S. W.; Seppenwoolde, J. H.; Mateus, V. A.; Bakker, C. J.; Krijger, G. C.; Storm, G.; Zonnenberg, B. A.; van het Schip, A. D.; Koning, G. A.; Nijsen, J. F. Lanthanide-loaded liposomes for multimodality imaging and therapy. *Cancer Biother. Radiopharm.* **2006**, *21*, 520–527.

(4) Lijowski, M.; Caruthers, S.; Hu, G.; Zhang, H.; Scott, M. J.; Williams, T.; Erpelding, T.; Schmieder, A. H.; Kiefer, G.; Gulyas, G.; Athey, P. S.; Gaffney, P. J.; Wickline, S. A.; Lanza, G. M. High-resolution SPECT-CT/MR molecular imaging of angiogenesis in the Vx2 model. *Invest. Radiol.* **2009**, *44*, 15–22.

(5) Koullourou, T.; Natrajan, L. S.; Bhavsar, H.; Pope, S. J.; Feng, J.; Narvainen, J.; Shaw, R.; Scales, E.; Kauppinen, R.; Kenwright, A. M.; Faulkner, S. Synthesis and spectroscopic properties of a prototype single molecule dual imaging agent comprising a heterobimetallic rhenium-gadolinium complex. *J. Am. Chem. Soc.* **2008**, *130*, 2178–2179.

(6) Manning, H. C.; Goebel, T.; Thompson, R. C.; Price, R. R.; Lee, H.; Bornhop, D. J. Targeted molecular imaging agents for cellular-scale bimodal imaging. *Bioconjugate Chem.* **2004**, *15*, 1448–1495.

(7) Bartholoma, M. D.; Louie, A. S.; Valliant, J. F.; Zubieta, J. Technetium and gallium derived radiopharmaceuticals: Comparing

and contrasting the chemistry of two important radiometals for the molecular imaging era. *Chem. Rev.* **2010**, *110*, 2903−2920.
(8) James, S.; Maresca, K. P.; Babich, J. W.; Valliant, J. F.; Doering, L.; Zubieta, J. Isostructural Re and ^{99m}Tc complexes of biotin derivatives for fluorescence and radioimaging studies. *Bioconjugate Chem.* **2006**, *17*, 590−596.
(9) Jung, K. H.; Kim, H. K.; Lee, G. H.; Kang, D. S.; Park, J. A.; Kim, K. M.; Chang, Y.; Kim, T. J. Gd complexes of macrocyclic diethylenetriaminepentaacetic acid (DTPA) biphenyl-2,2′-bisamides as strong blood-pool magnetic resonance imaging contrast agents. *J. Med. Chem.* **2011**, *54*, 143−152.
(10) Kim, H. K.; Jung, H. Y.; Park, J. A.; Huh, M. I.; Jung, J. C.; Chang, Y.; Kim, T. J. Gold nanoparticles coated with gadolinium-DTPA-bisamide conjugate of penicillamine (Au@GdL) as a T1-weighted blood pool contrast agent. *J. Mater. Chem.* **2010**, *20*, 5411−5417.
(11) Egli, A.; Alberto, R.; Tannahill, L.; Schibli, R.; Abram, U.; Schaffland, A.; Waibel, R.; Tourwe, D.; Jeannin, L.; Iterbeke, K.; Schubiger, P. A. Organometallic ^{99m}Tc-aquaion labels peptide to an unprecedented high specific activity. *J. Nucl. Med.* **1999**, *40*, 1913−1917.
(12) Alberto, R.; Schibli, R.; Waibel, R.; Abram, U.; Schubiger, A. P. Basic aqueous chemistry of $[M(OH_2)_3(CO)_3]^+$ (M = Re, Tc) directed towards radiopharmaceutical application. *Coord. Chem. Rev.* **1999**, *190−192*, 901−919.
(13) He, H.; Lipowska, M.; Christoforou, A. M.; Marzilli, L. G.; Taylor, A. T. Initial evaluation of new $^{99m}Tc(CO)_3$ renal imaging agents having carboxyl-rich thioether ligands and chemical characterization of $Re(CO)_3$ analogues. *Nucl. Med. Biol.* **2007**, *34*, 709−716.
(14) van Straveren, D. R.; Mundwiler, S.; Hoffmanns, U.; Pak, J. K.; Spingler, B.; Metzler-Nolte, N.; Alberto, R. Conjugation of a novel histidine derivative to biomolecules and labeling with $[^{99m}Tc(OH_2)_3(CO)_3]$. *Org. Biomol. Chem.* **2004**, *2*, 2593−2603.
(15) Villaraza, A. J.; Bumb, A.; Brechbiel, M. W. Macromolecules, dendrimers, and nanomaterials in magnetic resonance imaging: the interplay between size, function, and pharmacokinetics. *Chem. Rev.* **2010**, *110*, 2921−2959.
(16) Pietzsch., H.-J.; Gupta, A.; Reisgys, M.; Drews, A.; Seifert, S.; Syhre, R.; Spies, H; Alberto, R.; Abram, U.; Schubiger, P. A.; Johannsen, B. Chemical and biological characterization of technetium(I) and rhenium(I) tricarbonyl complexes with dithioether ligands serving as linkers for coupling the $Tc(CO)_3$ and $Re(CO)_3$ moieties to biologically active molecules. *Bioconjugate Chem.* **2000**, *11*, 414−424.