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Insight into Technetium Amidoxime Complex: Oxo Technetium(V) Complex of *N*-Substituted Benzamidoxime as New Basic Structure for Molecular Imaging

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In search of benzamidoxime (BHam) derivatives that provide a single ^{99m}Tc-labeled compound of high in vivo stability, we synthesized three *N*-alkyl compounds of benzamidoxime (BHam) ligand. They provided a single ^{99m}Tc-labeled compound by ligand exchange reaction of ^{99m}Tc-glucoheptonate in high radiochemical yields (over 95% at MBHam concentration of 1×10^{-5} M). ^{99m}Tc-*N*-methyl benzamidoxime (^{99m}Tc-MBHam) showed higher stability than the parental ^{99m}Tc-BHam. The complex of this compound with $^{99g}TcO^{3+}$ ion was prepared, isolated, and characterized by FT-IR and NMR spectra as well as X-ray diffraction. ^{99g}Tc-MBHam crystallized in an orthorhombic space group $Pna2_1$ with $a = 13.4823(5)$, $b = 15.5410(7)$, $c = 7.7907(3)$ Å, $V = 1632.39(11)$ Å^3, and $Z = 4$. The ^{99g}Tc complex possessed square base pyramid coordination geometry. The equatorial plane was formed by two-amine nitrogen and two-oxime oxygen atoms in trans position, while the oxo core of the technetium(V) occupied the apical position. The ^{99g}Tc-MBHam proved to be identical with the ^{99m}Tc-MBHam prepared at the no-carrier-added level by comparison of their HPLC profiles.

Introduction

Molecular imaging is an emerging field that aims to integrate patient-specific and disease-specific molecular information derived from diagnostic imaging. This technique is also expected to have a major economic impact on the development of new drugs at both preclinical and clinical stages. Among a variety of modalities, single photon emission computed tomography (SPECT) has the advantages of high intrinsic sensitivity, unlimited depth penetration, and wide availability. ^{99m}Tc constitutes one of the most useful radionuclides for the purpose because of its suitable nuclear properties and availability from the ^{99}Mo-^{99m}Tc generator system. Efforts have been and are being made to develop new ^{99m}Tc-labeled probes to promote the application.

Benzamidoxime or benzohydroxamamide (BHam) is a thiol-free bidentate ligand that provides ^{99m}Tc-labeled compounds of high stability with high radiochemical yields even at low BHam concentrations.[1,2] Such properties render BHam attractive as a chelating molecule to prepare a variety of ^{99m}Tc-labeled probes for molecular imaging. However, BHam always provides two ^{99m}Tc-labeled compounds, which hinders the application of BHam to ^{99m}Tc-coordination molecules. On the other hand, tetradentate BHam compounds, *N,N′*-ethylene bis(benzohydroxamamide) [$(C_2(BHam)_2)$] and *N,N′*-propylene bis(benzohydroxamamide) [$(C_3(BHam)_2)$], generated a single ^{99m}Tc-labeled compound of high stability with over 95% radiochemical yields over a wide pH range at ligand concentration as low as 2.5×10^{-6} M.[3] The $C_3(BHam)_2$-based bifunctional chelating agent provided ^{99m}Tc-labeled antibodies of high stability and high specific activity under mild conditions.[4] However, characterization of ^{99m}Tc-labeled compounds with either bidentate or tetradentate BHam derivatives has been limited so far. Our earlier attempts to prepare rhenium-labeled counterparts were abortive.

Previously, we used Hartree−Fock and density functional theory (DFT) calculations to evaluate the structures of BHam ligand and their oxo-technetium(V) complexes.[5]

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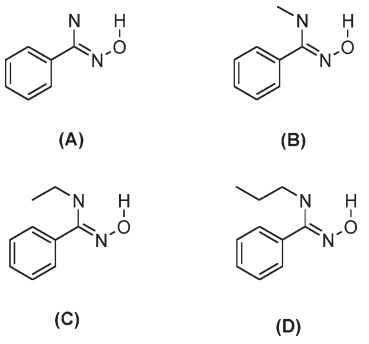

Figure 1. Structures of (A) BHam (M_w. 136, formula $C_7H_8N_2O$), (B) MBHam (M_w. 150, formula $C_8H_{10}N_2O$), (C) EBHam (M_w. 164, formula $C_9H_{12}N_2O$), and (D) PBHam (M_w. 178, formula $C_{10}H_{14}N_2O$).

Two hydroxyimino and ten hydroxyamino conformers of BHam were found as tautomers. The calculation also suggested that $[L_2TcO]^+$ of C_2 conformer would be the most stable. Recent study on the crystal structure of BHam showed that BHam molecules are connected via intermolecular N−H···O and O−H···N hydrogen bonds to form a two-dimensional supramolecular structure[6] as a result of tautomerization.[7−9] The tautomerization through migration of an amine proton to imine leads to a formation of an imino isomer that has lower acidity than the oximino form.[10−12] In the complex formation with technetium, this isomerization of amidoxime may have caused a mixture of ^{99m}Tc-labeled compounds, as was also observed with ^{99m}Tc-racemic D,L penicillamine.[13] The *N,N*-disubstituted aminoxime provides only one oximino isomer.[12,14] In addition, when the fractionated two components of ^{99m}Tc-BHam were reanalyzed by RP-HPLC, each component remained unchanged at pH 7. However, two components reemerged from each component at pH 5.[2] These findings suggested that a decrease in the number of tautomers by decreasing the number of protons in the amino group of BHam would be favorable to provide a single technetium complex. Thus, *N*-substituted amidoxime would constitute an interesting compound to reduce tautomers and generate new class of technetium complexes for molecular imaging.

In the present study, we synthesized three *N*-substituted BHam ligands, *N*-methyl-benzamidoxime (MBHam), *N*-ethyl-benzamidoxime (EBHam), and *N*-propyl-benzamidoxime (PBHam), as shown in Figure 1. The reaction of ^{99m}Tc-glucoheptonate with the three *N*-substituted BHam derivatives was compared to that with BHam. MBHam was selected as ligand for further studies, and the reaction product of $[n\text{BuN}][^{99g}\text{TcOCl}_4]$ with MBHam was characterized by X-ray diffraction (XRD), nuclear magnetic resonance, and infrared spectroscopy. The gathered studies indicated that MBHam provided a single ^{99m}Tc-labeled compound with two MBHam ligands coordinated to one TcO^{3+} core at the trans-position without impairing the unique coordinating ability of BHam.

Experimental Section

General Procedures for Ligand Synthesis and Characterization. All reactions were performed under nitrogen atmosphere unless otherwise noted. Solvents and chemicals obtained from commercial sources were of analytical grade or higher and were used without further purification. BHam was synthesized according to the procedure of Bedford et al. with slight modification.[15] The melting points were measured using a Thomas-Hoover capillary melting point apparatus and were uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded using a JEOL ECP-500 spectrometer (JEOL, Tokyo, Japan). The chemical shifts (δ) were reported in ppm downfield by reference to proton resonances resulting from incomplete deuteration of the NMR solvent. The elemental analyses were performed at the Chemical Analysis Center, Chiba University (Chiba, Japan). The infrared (IR) spectra were recorded using a Perkin-Elmer 2000 FT-IR spectrometer (Perkin-Elmer, MA, U.S.A.). Fast atom bombardment mass spectrometry (FAB-MS) was performed using a JEOL JMS-HX-110A mass spectrometer (JEOL Ltd., Tokyo, Japan). The mass spectrum of $^{99m/99g}$Tc measurements were carried out using an Agilent 6130 Series Quadrupole LC/MS electrospray system equipped with diode array detector (Agilent technologies, Tokyo).

Synthesis of *N*-methyl-benzamidoxime (MBHam). The solution of 2.0 g (13.0 mmol) of benzaldehyde oximinoyl chloride[16] in 20 mL of diethyl ether was cooled to 0 °C in an ice bath. Three milliliters of 40% w/v methylamine (16.3 mmol) dissolved in a mixture of 10 mL of diethyl ether and 2 mL of triethylamine (~13.0 mmol) were added slowly to the benzaldehyde oximinoyl chloride solution at 0 °C.[17] The temperature of the mixture was maintained at 0 °C during the addition. To complete the reaction, the mixture was stirred at room temperature for 3 h and then diluted with 50 mL of H_2O. The aqueous layer was separated and extracted three times with 10 mL of chloroform. The combined organic extracts were washed three times with 10 mL of water and dried over anhydrous magnesium sulfate. After removing the solvent in vacuo, the residue was dissolved in 20 mL of chloroform and triturated with hexane. The solution began to deposit white needle crystals. After standing overnight, the crystal were collected, washed with hexane and then dried under vacuum to yield 1.71 g (79.5%) of *N*-methyl-benzamidoxime. Mp: 162−163 °C (Lit.[18] 163 °C). FAB-MS: *m*/*z* 151 $[MH]^+$. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.73 (s, 3H, $-CH_3$), 5.28 (br s, 1H, OH), 7.36−7.50 (m, 5H, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 157.1 (C═N), 131.2, 129.4, 128.6(2C), 128.3(2C) (Ar), 30.5 ($-CH_3$) IR (KBr, cm^{-1}) 3390(s), 3300−2500(br), 1650(s), 1492(m), 1350(m), 928 and 908(m) Anal. Calcd for $C_8H_{10}N_2O$: C, 63.98; H, 6.71; N, 18.65 found C, 63.62; H, 6.63; N, 18.49.

Synthesis of *N*-ethyl-benzamidoxime (EBHam). EBHam was synthesized following the procedure described above except that ethylamine was used in place of methylamine. A pale yellow viscous oil of EBHam (1.82 g) was obtained after silica gel chromatography using 10% MeOH/$CHCl_3$ as eluent (84.9%). FAB-MS: *m*/*z* 165 $[MH]^+$. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.06 (t, 3H, J = 7.2 Hz, $-CH_3$), 3.03 (d, 2H, J = 6.4 Hz, $-CH_2-$N), 5.22 (br s, 1H, OH), 7.24−7.46 (m, 5H, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.3 (C═N), 131.5, 129.2, 128.3(2C), 128.1(2C)

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(Ar), 38.2 ($-CH_2-$), 16.4 ($-CH_3$) IR (Thin film KBr, cm^{-1}) 3340(s), 3300−2500(br), 1641(s), 1481(m), 1382(m), 933 and 900(m).

Synthesis of *N*-propyl-benzamidoxime (PBHam). PBHam was also synthesized following the procedure described above using propylamine. The product was isolated as 2.19 g of a pale yellow viscous liquid (91.6%). FAB-MS: m/z 179 $[MH]^+$. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.83 (t, 3H, $J = 7.4$ Hz, $-CH_3$), 1.42 (m, 2H, $C-CH_2-C$), 2.95 (s, 2H, $-CH_2-N$), 5.29 (br s, 1H, OH), 7.34−7.46 (m, 5H, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.2 (C=N), 131.4, 129.0, 128.3(2C), 128.0(2C) (Ar), 45.1 ($-CH_2-$), 24.2 ($-CH_2-$), 10.7($-CH_3$) IR (Thin film KBr, cm^{-1}) 3362(s), 3300−2500(br), 1632(s), 1459(m), 1343(m), 940 and 914(m).

General Procedures for Radioactive Material. ***Caution!*** *^{99g}Tc is a weak β^- emitter ($E = 0.292$ MeV; $t_{1/2} = 2.12 \times 10^5$ years) and may only be handled in laboratories approved for use of low-level radioactivity*. Sodium [^{99m}Tc]pertechnetate was daily eluted from a commercial $^{99}Mo/^{99m}Tc$ generator (Nihon Medi-Physics Co. Ltd., Nishinomiya, Japan or Mallinckrodt-Tyco Inc., Petten, Netherlands). Ammonium [^{99g}Tc]pertechnetate was purchased from Oak Ridge National Laboratory (TN, U.S.A.). Tetrabutylammonium tetrachlorooxotechnetate (TBA[$^{99g}TcOCl_4$]) was synthesized following the published procedure.[19] The in-house lyophilized kit containing 4 mg of glucoheptonate (GH) and 1.2 μg of $SnCl_2 \cdot 2H_2O$ was prepared using ultrapure water (Milli Q, Millipore Japan, Tokyo) at pH 9 and used throughout this experiment. The labeling reactions were performed for all solutions under nitrogen atmosphere. The reversed-phase HPLC (RP-HPLC) analysis was carried out with a Cosmosil $5C_{18}$-AR-300 column (4.6×150 mm^2, Nacalai Tesque, Kyoto, Japan) under isocratic conditions using a mixture of 50% solvent A (5 mM phosphate buffer pH 7) and 50% solvent B (methanol) at a flow rate of 1 mL/min. The eluent was monitored with an online UV/visible single beam spectroscopy (Model 100−10, Hitachi Co. Ltd., Tokyo) and a radiodetector (Gibi Star, Raytest, Straubenhardt, Germany). The RP-HPLC was also conducted with a Nucleosil 100−5 C18 column (3×250 mm^2, Macherey-Nagel AGC, Oensingen, Switzerland) at a flow rate of 0.5 mL/min, with a gradient mobile phase starting from 100% A (50 mM triethylammonium phosphate buffer pH 2.5) and 0% B (acetonitrile) to 0% A and 100% B at 30 min. The eluent was monitored with an online ultraviolet detector (250 nm) and a radiodetector (Model LB 508, EG&G Berthold LB, Regensdorf, Switzerland). The ^1H NMR spectra were recorded using a Varian Gemini 300 (Varian, CA, U.S.A.). The infrared (IR) spectra were recorded using a Perkin-Elmer BX II IR spectrometer (Perkin-Elmer, MA, U.S.A.).

Preparation of ^{99m}Tc-Labeled Compound. The lyophilized Sn-GH kit (4 mg) was reconstituted with 100 μL of oxygen-free 0.9% normal saline, sonicated and N_2-purged for 15 min before use. Twenty microliters (0.8 mg Sn-GH) of the solution were mixed with a solution of 1 mL of Na[$^{99m}TcO_4$]. After incubation at 25 °C for 30 min, ^{99m}Tc-glucoheptonate (^{99m}Tc-GH) was obtained. Radiochemical yields were determined by TLC (Silica gel 60 F_{254}, Merck Ltd.) with 100% acetone or 100% saline as the mobile phases. The chemical purity of ^{99m}Tc-GH higher than 99% was use for the subsequent reaction. The ^{99m}Tc-GH solution (100 μL) was mixed with a same volume of oxygen-free MBHam (1×10^{-2} M) in 0.02 M phosphate buffer solution (pH 7), and the reaction mixture was kept for 30 min at room temperature. The radiochemical yields were determined by RP-HPLC under the isocratic conditions mentioned above. To estimate the stability, the eluent of the major radioactive peak was collected and reinjected after 30 min. The similar labeling procedure was employed for ^{99m}Tc-BHam, ^{99m}Tc-EBHam, and ^{99m}Tc-PBHam.

Effects of Ligand Concentration. Similar to the procedure described above, the ^{99m}Tc-GH was added to a series of MBHam and BHam solution of different concentrations ($10^{-7}-10^{-2}$ M). The radiochemical yields were determined by RP- HPLC under the isocratic conditions.

Electrical Properties of ^{99m}Tc-Labeled Compound. Cellulose acetate electrophoresis was performed to determine the net charge of the ^{99m}Tc-labeled compound. Cellulose acetate strips (Separax SP, Johko Co., Tokyo) were soaked in phosphate buffer (pH = 7.4, I = 0.05) for 30 min. The strips were placed in an electrophoresis chamber containing the same buffer. Each sample was spotted on the strip. Each strip was run at a constant current of 1 mA/cm for 25 min. After drying, the strips were cut into 0.5 cm sections, and the radioactivity level of each section was determined by a well-type gamma counter.

Preparation of $^{99m/99g}$Tc-Carrier for Mass Analysis. A lyophilized GH kit containing 0.015 mg of $SnCl_2 \cdot 2H_2O$ and 10 mg of sodium glucoheptonate was reacted with 200 μL (concentrated by MEK extraction of a solution of $^{99m/99g}TcO_4^-$ elute from a 100 mCi ^{99}Mo-^{99m}Tc generator that had not been eluted for a week). The $^{99m/99g}$Tc-GH (100 μL) was then reacted with a same volume of MBHam (1×10^{-2} M), and $^{99m/99g}$Tc-MBHam (20 μL) was purified by RP-HPLC under the isocratic systems. The $^{99m/99g}$Tc-MBHam fractions (4.0 to 5.0 min; 1 mL) were collected. After removing methanol at room temperature under N_2 flow, the 20 μL solution was subjected to LC/MS analysis. The same procedure was conducted to determine $^{99m/99g}$Tc-BHam. The fractions of the two species were collected at 3.5−4.5 and 4.5−5.5 min.

Synthesis of ^{99g}Tc-MBHam. Method A. To a 2 mL solution of TBA[$^{99g}TcOCl_4$] (42.8 mg; 0.086 mmol) dissolved in acetonitrile was dropwise added a 4 mL solution of MBHam (64.4 mg; 0.43 mmol; 5 equiv) in acetonitrile. The reaction mixture was kept stirring for 1.5 h under N_2 atmosphere. The yellow powder of ^{99g}Tc-MBHam (24.8 mg; 69.9%) was collected and washed with water. IR (KBr, cm^{-1}): 3300−2500 (br), 1656 (s), 1348 (m), 1286 (w), 1196 (w), and 957 (m). ^1H NMR (DMSO: ppm) 3.36 (s, 6H, CH_3), 7.59 (m, 10H, Ar).

Method B. TBA[$^{99g}TcOCl_4$] (17.2 mg; 0.034 mmol) and MBHam (26 mg; 0.17 mmol; 5 equiv) were dissolved separately in 2 mL of methanol. The MBHam solution was slowly added to the (TBA)[$^{99g}TcOCl_4$] solution without stirring. Afterward, the mixture was left for a few days, allowing slow diffusion of the two components. This produced the orange crystals suitable for X-ray crystallography.

Synthesis of ^{99g}Tc-BHam. The precursor, ^{99g}Tc-ethyleneglycol was prepared by in situ. Ten drops of triethylamine were added to a mixture of TBA[$^{99g}TcOCl_4$] (160 mg, 0.32 mmol) and ethyleneglycol (10 drops) in THF (7 mL). ^{99g}Tc-ethyleneglycol was obtained as a violet solution. After removing the chloride salt by filtration, a solution of BHam (82 mg, 0.64 mmol, 2 equiv) in THF (15 mL) was added dropwise to the ^{99g}Tc-ethyleneglycol solution. The reaction mixture was continuously stirred for 3 h under N_2 atmosphere. During these times, the color of the solution changed from violet to deep orange. After removal of the solvent in vacuo, the residue was dissolved in methanol (2 mL). Brown solid (65.1 mg, 52.9%) was precipitated by H_2O (2 mL) treatment. IR (KB, cm^{-1}): 3300−2500 (br), 1635 (w), 1480 (m), 1366 (s), and 948 (m). ^1H NMR (DMSO: ppm) 7.43−8.98 (br, 10H, Ar).

X-ray Diffraction (XRD). The XRD data of the complexes were collected at 183(2) K using an Oxford Diffraction Xcalibur system (Oxfordshire, U.K.) equipped with a Ruby detector and graphite-monochromated Mo−K_α radiation (λ = 0.7107 Å). The suitable crystals were covered with oil (Infineum V8512, formerly known as Paratone N), mounted on the top of the glass fiber and immediately transferred to the diffractometer. The Crysalis^{Pro} program was used for the data collection, semiempirical absorption correction, and data reduction.[20] The crystal

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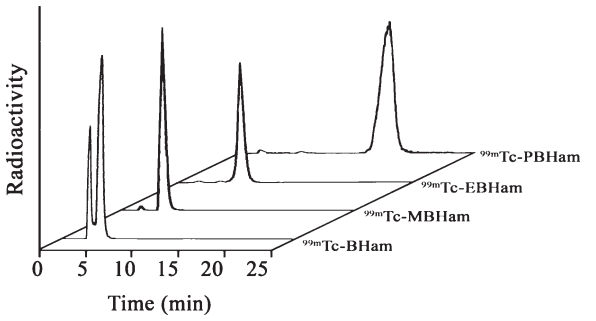
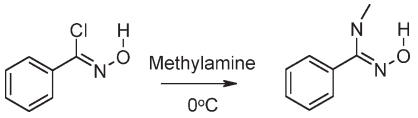


Figure 2. Radiochromatograms of ^{99m}Tc-labeled BHam derivatives: ^{99m}Tc-BHam (retention times = 3.88 and 4.96 min), ^{99m}Tc-MBHam (retention time = 4.67 min), ^{99m}Tc- EBHam (retention times = 7.01 min) and ^{99m}Tc-PBHam (retention times = 16.15 min).

Scheme 1



structures were solved by direct methods using Sir97[21] and refined by full-matrix least-squares methods on F^2 using Shelxl-97.[22] The structures were checked for higher symmetry using Platon[23]

Results and Discussion

Synthesis of *N*-alkyl substituted BHam Ligand. MBHam were prepared by reacting methylamine with phenyl chlorooxime that was obtained from the chlorination of benzaldoxime with *N*-chlorosuccinimide (NCS) in dimethylformamide (DMF).[24] The synthetic route for the MBHam ligand is illustrated in Scheme 1. By replacing the methylamine with ethylamine or *n*-propylamine, EBHam or PBHam was obtained in high yields. MBHam was a white solid, whereas EBHam and PBHam were yellow viscous oils. The ^{1}H and ^{13}C NMR spectra showed that all compounds were in one isomer (*Z*). The IR spectra for all compounds registered a number of characteristic absorption bands. The oxime group showed a broad band at about 3350 cm^{-1}, corresponding to O−H vibrations, and the amine showed a sharp absorption band of N−H stretching at ∼3300−3400 cm^{-1}. The ν C=N energy of 1630 ± 20 cm^{-1} is typical for aryl amidoximes.[25] In FAB-MS analyses, the molecular ion peaks at m/z = 151, 165, and 179 were due to molecular mass of MBHam, EBHam, and PBHam, respectively. The peaks at m/z = 135, 149, and 163 were attributable to loss of the OH moiety from the molecular ion. The other fragment at m/z = 104 and 77 arose from the loss of azomethane and alkyl group (R= −CH_3, −CH_2CH_3, and −$CH_2CH_2CH_3$). For all compounds, the molecular ion peak was also the base ion peak.

^{99m}Tc-Labeled Compounds. Figure 2 shows the RP-HPLC radiochromatograms of ^{99m}Tc-BHam, ^{99m}Tc-MBHam, ^{99m}Tc-EBHam, and ^{99m}Tc-PBHam under the isocratic conditions. Contrary to ^{99m}Tc-BHam, ^{99m}Tc-MBHam, ^{99m}Tc-EBHam, and ^{99m}Tc-PBHam generated a single peak at the retention times of 4.67 min, 7.01 min, and 16.15, respectively. The incorporation of two methyl groups at the amine group of BHam failed to produce ^{99m}Tc-labeled compounds of high stability (data not shown). These results suggested that *N*-monoalkylation of BHam would be useful to produce a single ^{99m}Tc-labeled compound. To further estimate the effect of *N*-alkylation on the chelating ability of BHam and to elucidate the role played by the chemical modification on the formation of a single ^{99m}Tc-labeled compound, further studies were conducted with BHam and MBHam.

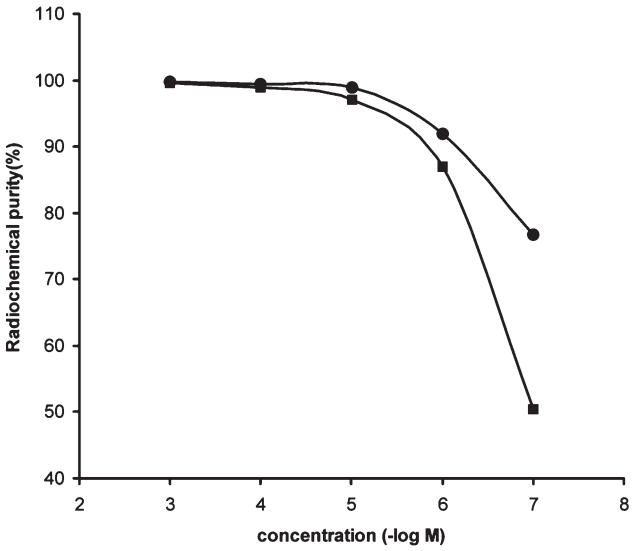


Figure 3. Percent radiochemical yields of ^{99m}Tc-BHam (■) and ^{99m}Tc-MBHam (●) as a function of the logarithmic ligand concentration.

Figure 3 shows the radiochemical yields of ^{99m}Tc-BHam and ^{99m}Tc-MBHam as a function of ligand concentration. For ^{99m}Tc-BHam, the radiochemical yields of the two radiolabeled species were combined and presented as radiochemical yields. MBHam generated a ^{99m}Tc-labeled compound in higher radiochemical yields than BHam at low ligand concentration. Moreover, the radiochemical yields of ^{99m}Tc-MBHam at MBHam concentration below 1 × 10^{-5} M were similar to those of the tetradentate ligand, $C_3(BHam)_2$.[1−3] Figure 4 compares the % intact ^{99m}Tc-labeled species remaining at room temperature as a function of post-labeling time when both ^{99m}Tc-MBHam and ^{99m}Tc-BHam were prepared at ligand concentrations of 10^{-5} M. This indicated that ^{99m}Tc-MBHam possesses higher stability than ^{99m}Tc-BHam. Furthermore, no changes in RP-HPLC radiochromatograms were observed after removing the excess of MBHam ligand from ^{99m}Tc-MBHam by RP-HPLC and reanalyzed under the isocratic conditions (the retention times of ^{99m}Tc-MBHam and MBHam were 4.68 and 2.8 min, respectively under the isocratic conditions). The gathered findings confirmed that *N*-methylation provided BHam an ability to form a single ^{99m}Tc-labeled compound while enhancing the unique chelating properties. The electrophoresis analysis showed that ^{99m}Tc-MBHam possessed net neutral charge, as also observed with ^{99m}Tc-BHam, ^{99m}Tc−$C_3(BHam)_2$, and ^{99m}Tc−$C_2(BHam)_2$.[2,3] The mass spectroscopies of $^{99m/99g}$Tc-MBHam registered the most

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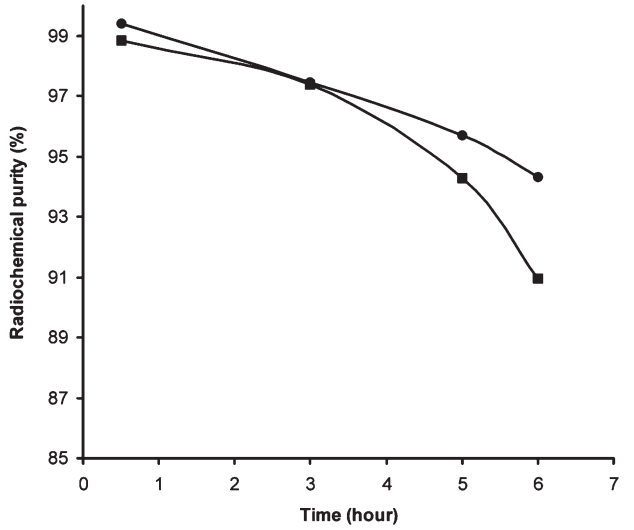

Figure 4. Percent radiochemical purity of ^{99m}Tc-BHam (■) and ^{99m}Tc-MBHam (●) as a function of time when the ligand concentration was fixed at 10^{-5} M.

intensive signal at m/z = 413 while the two $^{99m}/^{99g}$Tc-BHam showed the most intensive signal at m/z = 385. This suggested a formulation of [TcO(L^{-2})(LH^{-1})] for both ^{99m}Tc-MBHam[$C_{16}H_{17}N_4O_3Tc$] and ^{99m}Tc-BHam [$C_{14}H_{13}N_4O_3Tc$], which supported the earlier speculation for the two species of ^{99m}Tc-labeled BHam compounds.[1,2]

Structural Elucidation of ^{99g}Tc-MBHam and ^{99g}Tc-BHam Complexes. All complexes were soluble in dimethyl sulfoxide (DMSO) and 0.1% trifluoroacetic acid (TFA)/methanol, but almost insoluble in other organic solvents such as methanol, ethanol, acetonitrile, THF, ether, and dichloromethane. Therefore, ^{99g}Tc-complexes in 0.1% TFA/methanol solution were prepared and analyzed by the RP-HPLC under the gradient conditions. A mixture of an acidic eluting solvent (50 mM triethylammonium phosphate buffer pH 2.5) and acetonitrile was used to enhance the solubility of the carrier ^{99g}Tc-complexes. The retention time for ^{99g}Tc-MBHam probed by the UV detector was at 19.73 min, and ^{99g}Tc-BHam was at 19.45 and 25.82 min. They were similar to those of ^{99m}Tc-MBHam (20.12 min) and ^{99m}Tc-BHam (21.03 and 26.92 min) monitored by a radioactive detector. This suggested that the structures of ^{99g}Tc complexes were equivalent to those of ^{99m}Tc-labeled compounds prepared at very low technetium concentration. The relative peak areas of the two ^{99g}Tc-BHam were found to be 32.2% for the earlier complex (21.03 min) and 67.8% for the latter complex (26.92 min).

The ^{1}H NMR spectrum of ^{99g}Tc-MBHam prepared from Method A showed a singlet peak at δ = 3.36 due to the *N*-methyl group, suggesting that the two *N*-methyl groups of MBHam were away at the trans-position. Table 1 shows representative IR absorptions of MBHam and its Tc-complex. The IR spectrum of ^{99g}Tc-MBHam showed a vital band at 957 cm^{-1} attributable to Tc=O double bond. This value was comparable to those observed for other oxo complexes of Tc containing σ- and π-donating

Table 1. IR Absorption of Free Ligand and Its ^{99g}Tc-MBHam in the Range of 4000−400 cm^{-1} [a]

IR absorption (ν, cm^{-1})		
MBHam	^{99g}Tc-MBHam	bond
3300−2500 (br)	3300−2500 (br)	O−H stretch
3390 (s)	N.D.	N−H stretch
1650 (s)	1656 (s)	C=N stretch
1350 (m)	1348 (m)	C−N stretch
N.D.	957 (m)	Tc=O stretch

[a] Abbreviations: s = strong, m = medium, w = weak, br = broad.

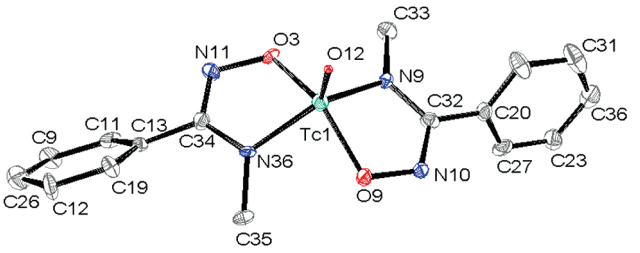

Figure 5. Molecular structure of ^{99g}Tc-MBHam with atom-labeling scheme. Hydrogen atoms are omitted for clarity.

ligands.[26,27] Owing to functional groups in MBHam, the other bands were also observed. The broad and intense band observed in 3300−2500 cm^{-1} as the stretching vibrations of the O−H groups suggested the deposition of solvent (H_2O) on complex to form hydrogen bonds. The strong bands were also observed in the range 1656 and 1348 cm^{-1} resulting from the C=N and C−N groups, respectively. The new bands appearing at 1286 and 1196 cm^{-1} were assigned to Tc−N and Tc−O vibration of a chelate ring.

Crystal Structures. Method B was found to be useful to produce single crystals of ^{99g}Tc-MBHam of XRD quality. The perspective XRD crystal structure of ^{99g}Tc-MBHam is shown in Figure 5. The crystal data and structure refinement parameters for ^{99g}Tc-MBHam are presented in Table 2. The selected bond distances and bond angles are listed in Table 3. ^{99g}Tc-MBHam crystallized in the orthorhombic system in space group $Pna2_1$. The metal atom is coordinated by three oxygen and two nitrogen atoms in a square pyramid, with an oxygen atom at the apex and the other atoms forming a basal plane. The Tc atom is displaced from the basal plane defined by the N(9), N(36), O(3), and O(9) atoms toward O(12) by 0.706 Å. ^{99g}Tc is coordinated by two MBHam ligands with the *N*-hydroxyl oxygen atoms being at the trans position. The Tc=O (oxygen core) bonds (1.610 Å) were shorter than the Tc−O (*N*-hydroxyl oxygen atoms) bonds (1.932−1.940 Å). The C−N single and double bond distances in the coordinated ligands ranged 1.37−1.39 Å and 1.26−1.29, respectively. They were similar to those of C=N (C sp^2−N sp^2) in non chelate BHam[6] and its derivatives.[28,29] The short distance of Tc−N bond on N(36) 1.968 Å and N(9) 2.001 Å indicated deprotonation of the amine donor, as also observed with TcO-amine oxime and

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Table 2. Crystal Data and Structure Refinement for ^{99g}Tc-MBHam

	$C_{16}H_{15}FN_4O_3Tc$
formula mass	428.32
crystal system	orthorhombic
space group	$Pna2_1$
a, Å	13.4823(5)
b, Å	15.5410(7)
c, Å	7.7907(3)
α, deg	90.00
β, deg	90.00
γ, deg	90.00
unit cell volume, Å^3	1632.39(11)
Z	4
temperature, K	183(2)
radiation type	MoKα
absorption coefficient, μ/mm^{-1}	0.917
no. of reflections measured	8075
no. of independent reflections	3173
R_{int}	0.0467
final R_1 values ($I > 2\sigma(I)$)	0.0694
final $wR(F^2)$ values ($I > 2\sigma(I)$)	0.1667
final R_1 values (all data)	0.0992
final $wR(F^2)$ values (all data)	0.1764
goodness of fit on F^2	0.968

Table 3. Selected Bond Distances (Å) and Angles (deg) for Compound ^{99g}Tc-MBHam

	Bond Lengths (Å)		
Tc (1)−N (9)	2.001	C(34)−N(11)	1.261
Tc (1)−O (12)	1.610	C (34)− N (36)	1.395
Tc (1)−O (3)	1.932	C (34)−C (13)	1.488
Tc (1)−O (9)	1.940	C (32)−N (10)	1.295
Tc (1)−N (36)	1.967	C (32)−N (9)	1.371
		C (32)−C (20)	1.449
	Bond Angles (deg)		
O (12)−Tc (1)−O (3)	111.24	O (3)−Tc (1)−N (36)	78.57
O (12)−Tc (1)−O (9)	109.31	O (3)−Tc (1)−N (9)	86.90
O (12)−Tc (1)−N (36)	111.75	O (9)−Tc (1)−N (36)	85.80
O (12)−Tc (1)−N (9)	110.71	O (9)−Tc (1)−N (9)	79.84
O (3)−Tc (1)−O (9)	139.44	N (36)−Tc (1)−N (9)	137.53

TcO-mercaptoacetyltriglycine.[27,30,31] Angles found for the N(36) and N(9) center 111.91°, 120.68°, 126.60° and 126.93°, 113.33°, 119.55, respectively, were typical of a sp^2 hybridized (N-center). The smaller Tc−N−C, 113.33°, 111.91° would be attributable to 5 member ring strain, not to the geometry of a neutral NH (sp^3) donor. These findings suggested a small degree of delocalization of the amidoxime group in ^{99g}Tc-MBHam. This was supported by the delocalization of the π electron over the amidoximato (ONCN) functional as observed in chromium, aluminum and iron amidoxime complex.[32,33] Figure 6 shows intermolecular hydrogen bonds from the C−N−H on oxime group to C=N on neighboring complex. This N−H−N hydrogen bonding in the coordination system was supported by tautomerism of hydrogen on the amidoxime group of LH^{-1}. The formula $[TcO(L^{-2})(LH^{-1})]$ suggested different coordination modes of the two ligands in the coordination sphere. The X-ray studies indicated

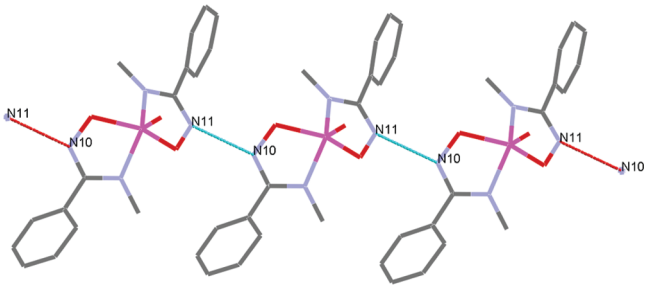
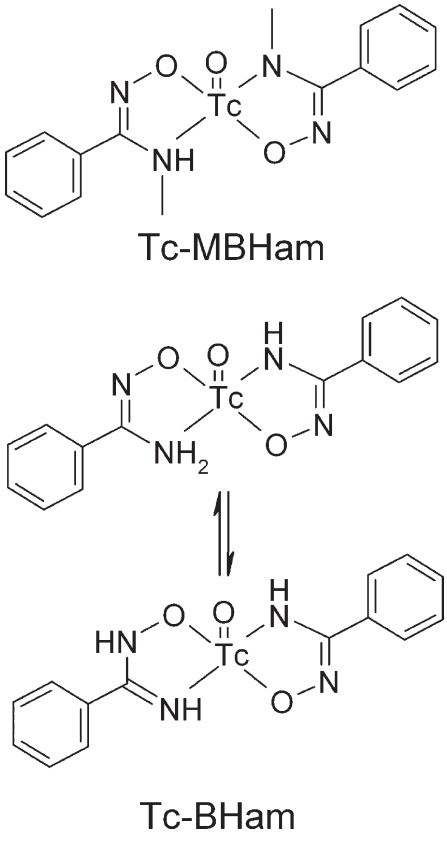


Figure 6. One-dimensional intermolecular hydrogen bond network of ^{99g}Tc-MBHam.

Scheme 2



that only a LH^- provides the migration of a proton from the amine group to the oxime group. On the other side, the unprotonated oxime nitrogen in L^{-2} was a pair to generate the hydrogen bond. The alteration between single and adjacent double bonds accompanied by the migration of a proton would cause the deviation of ligand structure in the coordinated ligand.[34−36] The formation of a single Tc-MBHam complex would be attributable to a proton migration on the coordinated ligand. The isomerization of MBHam by a proton migration is not possible in the L^{-2} form (Scheme 2). The *N*-methyl substitution of BHam not only provided a single ^{99m}Tc-MBHam but also improved stability and radiochemical

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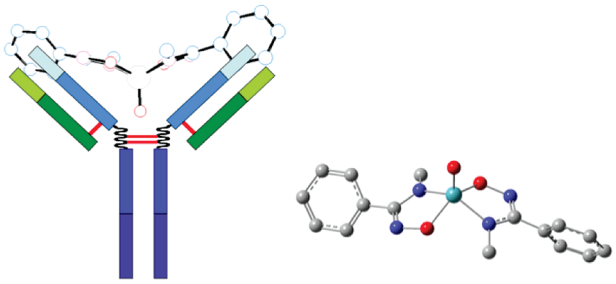

Figure 7. ^{99m}Tc-labeled *N*-substituted BHam as an IgG antibody mimic. The Y-like shaped structure of ^{99g}Tc-MBHam suggests that when a targeting molecule is incorporated via each amine group in BHam, the two targeting molecules would point to each direction of the two heavy chains of an IgG antibody.

yield of the Tc-MBHam complex. This could be attributable in part to an electron-donating effect of the methyl groups,[37,38] or a delocalization degree in the amidoxime group. The steric interference by *N*-alkyl group may also be involved. Unfortunately, owing to low solubility, ^{99m}Tc-BHam mixtures could not be separated to prepare crystals suitable for X-ray analysis. According to RP-HPLC and spectroscopic data of ^{99g}Tc-BHam, one of the ^{99m}Tc-BHam species would be closely related to the structure of ^{99m}Tc-MBHam. In addition, the methyl groups in Tc-MBHam pointed to each direction of the two heavy chains of an IgG antibody (Figure 7). This suggests that when a targeting molecule such as RGD peptide is attached via each amine group in BHam, the two RGD peptides in the resulting ^{99m}Tc-labeled compound would point to each direction of the two heavy chains of an IgG antibody, which might increase the drive toward binding to a target molecule (e.g., $\alpha_v\beta_{III}$ integrin).

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Conclusions

Three *N*-substituted BHam were found to provide a single ^{99m}Tc-labeled compound. MBHam generated a ^{99m}Tc-labeled compound in high radiochemical yields under mild conditions even at low ligand concentration. $^{99m/99g}$Tc-MBHam is the first Tc-amidoxime complex characterized by IR, NMR spectroscopy, and XRD analysis. The complex structure supports the formation of metal to ligand ratio of 1:2. The conformation of the two ligands is at the trans-position upon forming a square-pyramid. In addition, amine function on the bidentate ligands may provide a variety of molecular designs for new ^{99m}Tc-labeled probes.

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Supporting Information Available: Crystallographic data in CIF format. Further details are given in Figures S1−S3. This material is available free of charge via the Internet at http://pubs.acs.org.