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Assessment of Macrocyclic Triamine Ligands As Synthons for Organometallic ^{99m}Tc Radiopharmaceuticals

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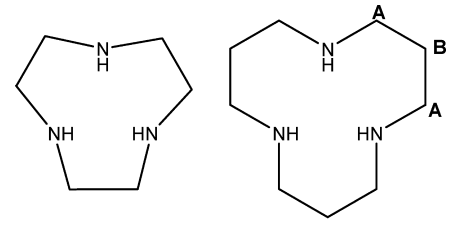
In a search of coordination molecules suitable to the *fac*-{^{99m}Tc(CO)$_3$}$^+$ core as a synthon for ^{99m}Tc-radiopharmaceuticals, nonradioactive rhenium complexes of two macrocyclic triamine compounds with different chelate ring structures, 1,4,7-triazacyclononane (9N3) and 1,5,9-triazacyclododecane (12N3), were synthesized and characterized. ^{99m}Tc-labeled 9N3 and 12N3 compounds were also prepared using [^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ and were characterized by both in vitro and in vivo studies. 9N3 produced a single rhenium complex, whereas 12N3 generated two major complexes. The crystallographic data and infrared absorption wavenumber assigned to the C−O stretch suggested that the coordination geometry of 9N3 would be more suitable to *fac*-{Re(CO)$_3$}$^+$ than that of 12N3. In contrast, both 9N3 and 12N3 provided a single ^{99m}Tc-labeled compound. However ^{99m}Tc-labeled 9N3 exhibited higher stability than ^{99m}Tc-labeled 12N3 in rat plasma and in the presence of histidine at an elevated temperature. In biodistribution studies, both ^{99m}Tc-labeled compounds did not show any specific accumulation of radioactivity in any organs except for the excretory organs such as the liver and kidney. These findings showed that 9N3 would constitute a macrocyclic chelating molecule of choice to prepare ^{99m}Tc radiopharmaceuticals using a *fac*-{^{99m}Tc(CO)$_3$}$^+$ core.

Introduction

The radioisotopes technetium-99m (^{99m}Tc) and rhenium-186,188 ($^{186/188}$Re) play a prominent role in developing radiopharmaceuticals for diagnostic and therapeutic nuclear medicine, respectively. Since ^{99m}Tc and $^{186/188}$Re radiopharmaceuticals of high stability with high radiochemical yields are required for targeted imaging and radiotherapy, the metal oxidation state and ligand environments for complexation should be optimized. Preferably, technetium chelating molecules are of small molecular size, high stability, and rapid excretion and allow versatile modification.

The organometallic complex [M(OH$_2$)$_3$(CO)$_3$]$^+$ (M = Tc or Re)[1,2] has attracted significant attention in radiopharmaceutical chemistry, due to (a) the small size of the *fac*-{M(CO)$_3$}$^+$ core, (b) high affinity for a large variety of donor atoms, and (c) the chemically robust *fac*-{M(CO)$_3$}$^+$ low-spin d$_6$ core. Among various candidates, macrocyclic compounds with saturated tridentate amines may be attractive ligands for the *fac*-{M(CO)$_3$}$^+$ core.[3−7] A macrocyclic triamine compound, 1,4,7-triazacyclononane (9N3, Scheme 1), has a minimum structural requirement as the tridentate chelate ligand, with which is advantageous to prepare ^{99m}Tc radiopharmaceuticals of small molecular sizes. The coordination chemistry of technetium and rhenium with tripodal ligand systems has been well-explored.[8] However, the

Scheme 1



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relationship between chelate ring structures and physicochemical and/or biological characters of the resulting technetium and rhenium complexes remains uncertain.

The tricarbonyl complexes with 1,4,7-trithiacyclononane (9S3) have also been reported.[5,7,9] When compared with 9S3, 9N3 allows versatile molecular design by modifying the secondary nitrogen atoms with appropriate functional groups to prepare multivalent compounds.[10]

In this study, we synthesized and characterized nonradioactive *fac*-$\{Re(CO)_3\}^+$ complexes of 1,4,7-triazacyclononane (9N3) and 1,5,9-triazacyclododecane (12N3) to estimate the effect of chelate ring structure on complexation yields. Rhenium is the third-row transition-metal congener of technetium, usually exhibits complexation chemistry similar to technetium, and is frequently used as a nonradioactive surrogate for characterization of ^{99m}Tc radiopharmaceuticals.[11] ^{99m}Tc-labeled 9N3 and 12N3 were also prepared using $[^{99m}Tc(OH_2)_3(CO)_3]^+$, and their radiochemical yields, stabilities, and in vivo behaviors were compared.

Experimental Details

General Methods. $[Re(CO)_5Br]$, 9N3, and 12N3 were purchased from Sigma-Aldrich Japan Co. Ltd. (Tokyo, Japan). $Na_2[BH_3CO_2]$, $Na_2B_4O_7 \cdot 10H_2O$, sodium tartrate, and Na_2CO_3 were purchased from Wako Pure Chemical Industries Ltd. (Tokyo). All reagents and solvents were of reagent grade and used without further purification. Tribromo tricarbonyl rhenate tetraethylammonium salt ($[Et_4N]_2[Re(CO)_3Br_3]$) was prepared from $[Re(CO)_5Br]$ according to the literature methods.[12] Technetium-99m as $Na^{99m}TcO_4$ was eluted in saline solution from a ^{99}Mo/^{99m}Tc generator (Nihon Medi-Physics Co. Ltd., Nishinomiya, Japan).

^{1}H and ^{13}C NMR spectra were recorded on an ECP-500 (JEOL Ltd., Tokyo). Proton chemical shifts are expressed as parts per million using tetramethylsilane or deuterated solvent as an internal reference. Infrared (IR) spectra were recorded as attenuated total reflection (ATR) method and run on an Excalibur FTS-3000MX (Varian Technologies Japan Ltd., Tokyo). Liquid chromatography–mass spectrometry (LC-MS) was performed on a Quattro Micro API Mass Spectrometer (Nihon Waters K.K., Tokyo) with an ESI source in positive ion mode.

Reversed phase HPLC was performed with a C18 Develosil ODS-UG-5 column (4.6 × 250 mm, Nomura Chemical Ltd., Seto, Japan) at a flow rate of 1 mL/min with a mobile phase starting from 90% A (0.1% aqueous trifluoroacetic acid (TFA)) and 10% B (acetonitrile with 0.1% TFA) for 2 min to 10% A and 90% B at 10 min, and the final composition was kept for an additional 8 min. In HPLC analyses, radioactivity was detected on a Steffi (raytest, Straubenhardt, Germany). Data processing and chromatographic control were conducted by the Empower Software (Nihon Waters K.K.). Thin layer chromatography (TLC) analyses were performed with reversed phase plates (RP18 F_{254}, Merck Ltd., Tokyo) developed with 0.1% TFA H_2O and 0.1% TFA CH_3CN (2:1). The radioactivity of TLC was detected on Rita Star (raytest, Straubenhardt). The radioactivity of each solution and tissue was determined by a dose calibrator (Curiemeter IGC-3, ALOKA Ltd., Tokyo) or an auto well gamma counter (ARC-380M, ALOKA Ltd.).

Synthesis of $[Re(CO)_3(9N3)]Br$, Re-9N3. 9N3 (20 mg, 0.157 mmol) in 5 mL of acetonitrile was added to a stirred solution of $[Et_4N]_2[Re(CO)_3Br_3]$ (120 mg, 0.156 mmol) in 10 mL of acetonitrile. The mixture was then heated at 50 °C for 18 h, whereupon a colorless crystalline precipitate formed. This precipitate was filtered out, and recrystalization of the precipitate from H_2O provided needle crystals (Yield: 70%). ^{1}H NMR, ^{13}C NMR, and MS analysis of the crystals identified the formation of $[Re(CO)_3(9N3)]Br$. ^{1}H NMR (δ (ppm), D_2O): 3.21 (dt, 6H) 2.96 (dt, 6H). ^{13}C NMR (δ (ppm), D_2O): 50.26, 50.16. Mass spectrum (ESIMS): m/z = 400.07, $[C_9H_{15}N_3O_3Re]^+$. IR (ATR, ν/cm^{-1}): 2014, 1881 (*fac*-$Re(CO)_3$). Anal. Calcd (found) for $C_9H_{15}N_3O_3ReBr$: C, 22.6 (22.6); H, 3.15 (3.24); N, 8.77 (8.82); Re, 38.8 (38.9).

Synthesis of $Re(CO)_3$-12N3 Compounds. 12N3 (27 mg, 0.158 mmol) in 10 mL of acetonitrile was added to a stirred solution of $[Et_4N]_2[Re(CO)_3Br_3]$ (120 mg, 0.156 mmol) in 10 mL of deionized H_2O. The mixture was heated at 100 °C for 4 days. There were some products observed, and their composition changed with time. Two major reaction products, **Re-12N3a** and **Re-12N3b,** were isolated by HPLC as stated above and recrystalized from H_2O.

$[Re(CO)_3(12N3)](CF_3COO)$, Re-12N3a. ^{1}H NMR ($\delta$ (ppm), D_2O): 3.06 (dd, 12H), 2.15 (m, 3H), 1.82 (m, 3H). ^{13}C NMR (δ (ppm), D_2O): 50.8, 24.8. Mass spectrum (ESIMS): m/z = 442.1, $[C_{12}H_{21}N_3O_3Re]^+$. IR (ATR, ν/cm^{-1}): 2021, 1888, 1876 (*fac*-$Re(CO)_3$). Anal. Calcd (found) for $C_{14}H_{21}N_3O_5F_3Re$: C, 30.3 (30.4); H, 3.82 (3.82); N, 7.58 (7.61); Re, 33.6 (33.8).

$[Re(CO)_3(12N3)](CF_3COO)$, Re-12N3b. ^{1}H NMR ($\delta$ (ppm), D_2O): 3.30 (m, 2H), 3.19 (m, 2H), 3.11 (m, 2H), 2.96 (m, 6H), 2.12 (m, 4H), 2.00 (m, 1H), 1.69 (m, 1H). ^{13}C NMR (δ (ppm), D_2O): 52.2, 51.5, 25.2. Mass spectrum (ESIMS): m/z = 442.1, $[C_{12}H_{21}N_3O_3Re]^+$. IR (ATR, ν/cm^{-1}): 2020, 1892 (*fac*-$Re(CO)_3$). Anal. Calcd (found) for $C_{14}H_{21}N_3O_5F_3Re$: C, 30.3 (30.3); H, 3.82 (3.92); N, 7.58 (7.54); Re, 33.6 (33.6).

(1) (a) Alberto, R.; Schibli, R.; Angst, D.; Schubiger, P. A.; Abram, U.; Abram, S.; Kaden, T. L. A. *Transition Met. Chem.* **1997**, *22*, 597–601. (b) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, P. A. *J. Am. Chem. Soc.* **1998**, *120*, 7987–7988. (c) Alberto, R.; Schibli, R.; Schubiger, P. A. *J. Am. Chem. Soc.* **1999**, *121*, 6076–6077. (d) Waibei, R.; Alberto, R.; Willuda, J.; Finnern, R.; Schibli, R; Stichelberger, A.; Egli, A.; Abram, U.; Mach, J. P.; Pluckthun, A.; Schubiger, P. A. *Nat. Biotechnol.* **1999**, *17*, 897–901. (e) Drishty, S.; Madhav, M.; Kanchan, K.; Pillai, M. R. A. *J. Labelled Comp. Radiopharm.* **2004**, *47*, 657–668.

(2) (a) Amann, A.; Decristoforo, C.; Ott, I.; Wenger, M.; Bader, D.; Alberto, R.; Putz, G. *Nucl. Med. Biol.* **2001**, *28*, 243–250. (b) Schibli, R.; LaBella, R.; Alberto, R.; Garcia-Garayoa, L.; Ortner, K.; Abram, U.; Schubiger, P. A. *Bioconjugate Chem.* **2000**, *11*, 345–351. (c) Wald, J.; Alberto, R.; Ortner, K.; Candreia, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3062–3066. (d) Petrig, R.; Schibli, R.; Dumas, C.; Alberto, R.; Schubiger, P. A. *Chem.—Eur. J.* **2001**, *7*, 1868–1873.

(3) Gabriele, B.; Willy, H.; Karl, W.; Johannes, W. *Inorg. Chem.* **1985**, *24*, 485–491.

(4) Thomas, B.; Beatriz, S. P. C. D. V.; Karl, W.; Roland, B. *Inorg. Chem.* **1990**, *29*, 1736–1741.

(5) Schibli, R.; Alberto, R.; Abram, U.; Abram, S.; Egli, A.; Schubiger, P. A.; Kaden, T. A. *Inorg. Chem.* **1998**, *37*, 3509–3516.

(6) McGowan, P. C.; Podesta, T. J.; Thornton-Pett, M. *Inorg. Chem.* **2001**, *40*, 1445–1453.

(7) Patrick, M.; Alessandra, M.; Ursula, R. *J. Phys. Chem. A* **2004**, *108*, 11494–11499.

(8) Alberto, R.; Herrmann, W. A.; Kiprof, P.; Baumgaertner, F. *Inorg. Chem.* **1992**, *31*, 895–899.

(9) Pomp, C.; Drücke, S.; Küppers, H. J.; Wieghardt, K. *Z. Naturforsch.* **1988**, *43b*, 299.

(10) Goel, A.; Baranowska-Kortylewicz, J.; Hinrichs, S. H.; Wisecarver, J.; Pavlinkova, G.; Augustine, S.; Colcher, D.; Booth, B. J.; Batra, S. K. *J. Nucl. Med.* **2001**, *42*, 1519–1527.

(11) (a) Melchor, V. C.; Robertha, C. H.; Louis, T.; John, E. C.; Dietmar, B.; Robin, D. R.; Lynn, C. F. *Inorg. Chem.* **2007**, *46*, 7326–7340. (b) Tim, S.; Yuko, S.; Cheri, A. B.; Yuji, M.; Michael, J. A.; Shigenobu, Y.; Chris, O. *Inorg. Chem.* **2005**, *44*, 2698–2705. (c) Ernest, W.; Theresa, F.; Samantha, B.; John, V.; Tam, N.; Frank, L.; Linda, F. L. L.; Alfred, P.; Russell, A. B.; John, R. T. *Inorg. Chem.* **1997**, *36*, 5799–5808.

(12) Roger, A.; André, E.; Ulrich, A.; Kaspar, H.; Volker, G.; P, A. S. *J. Chem. Soc., Dalton Trans.* **1994**, *19*, 2815–2820.

Table 1. Crystal data for *fac*-[Re(CO)$_3$(9N3)]Br (**Re-9N3**) and *fac*-[Re(CO)$_3$(12N3)](CF$_3$COO) (**Re-12N3b**)

formula	$C_9H_{15}N_3O_3BrRe$	$C_{14}H_{21}N_3O_5F_3Re$
formula weight	479.35	554.54
crystal system	orthorhombic	orthorhombic
space group	*Pbca* (no. 61)	*Pnma* (no. 62)
a, Å	14.816(2)	15.383(1)
b, Å	13.776(3)	8.612(1)
c, Å	13.667(3)	13.902(1)
α, deg	89	90
β, deg	89	90
γ, deg	90	90
V, Å^3	2789(1)	1841.8(5)
Z	8	4
T, K	298.0	298.0
D_c, g cm^{-3}	2.283	2.000
F(000)	1792.00	1072.00
λ(Cu Kα), Å	1.54178	1.54178
crystal size, mm	0.40 × 0.30 × 0.30	0.15 × 0.15 × 0.10
μ(Cu Kα), cm^{-1}	206.15	136.03
R_1/R_{W2}	0.057/0.076	0.039/0.055

X-ray Crystal Structure Determinations. Crystal data and experimental details are listed in Table 1. Suitable crystals were mounted on top of a glass fiber. All measurements were made on an AFC7S diffractometer with graphite monochromated Cu Kα radiation ($\lambda = 1.54178$ Å). Data were collected at a temperature of 25 °C using the $\omega-2\theta$ scan technique to a maximum 2θ value of 138.0° with WinAFC.[13] An empirical absorption correction based on azimuthal scans of several reflections was applied which resulted in transmission factors ranging from 0.59 to 1.00. Structures were solved with direct methods using SHELXS-97[14] or SIR97[15] and were refined by full-matrix least-squares methods on F^2 with SHELXL-97.[16]

Synthesis of [^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$. [^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ was prepared according to the literature method,[1] starting from Na^{99m}TcO$_4$. Na^{99}TcO$_4$ was eluted in advance from a ^{99}Mo-^{99m}Tc generator. The time interval from the preliminary elution to the elution in use was controlled in accordance with the intended use, from approximately 24 h to 7 days. The amount of $^{99m/99}$Tc molar quantity was calculated from the time interval of elution and radioactivity in elution by the Bateman equations.[17] Approximately 1.0 mL of generator eluate containing 1−20 GBq of Na^{99m}TcO$_4$ was added to the mixture consisting of Na$_2$[BH$_3$CO$_2$] (4.5 mg, 0.043 mmol), Na$_2$B$_4$O$_7$·10H$_2$O (2.85 mg, 0.00747 mmol), sodium tartrate (8.5 mg, 0.037 mmol), and Na$_2$CO$_3$ (7.15 mg, 0.0675 mmol). [^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ was obtained after 20 min at 100 °C, with the final solution pH of ca. 12. The reaction progress and product identification were determined by HPLC.[1b] After the reaction, the solution pH was adjusted to ca. 7 using a diluted HCl solution.

Synthesis of [^{99m}Tc(CO)$_3$(9N3)]$^+$, ^{99m}Tc-9N3. 9N3 was dissolved in phosphate buffer (100 mmol/L, pH 7.2) to prepare 10 mmol/L solution of 9N3. [^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ (1 mL, ca. 1 GBq) was added to 1 mL of 10 mmol/L 9N3, and the mixture was heated at 100 °C for 30 min. Radiochemical yield of the labeled compound was assessed by TLC and HPLC. Part of the structural information was assessed by LC-MS.

Synthesis of [^{99m}Tc(CO)$_3$(12N3)]$^+$, ^{99m}Tc-12N3. 12N3 was dissolved in phosphate buffer (100 mmol/L, pH7.2) to prepare a 10 mmol/L solution of 12N3. [^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ (1 mL, ca. 1 GBq) was added to 1 mL of 10 mmol/L 12N3, and the mixture was heated at 100 °C for 30 min. The radiochemical yield of the labeled compounds was assessed by TLC and HPLC. Part of the structural information was assessed by LC-MS.

Challenge Experiments. To estimate the stability of the ^{99m}Tc-labeled compounds, a histidine challenge test[18] was performed. The reaction solution of ^{99m}Tc-labeled compounds were subjected to HPLC, and the fractions containing the respective ^{99m}Tc-labeled compound were combined, evaporated to dryness under the stream of N$_2$, and reconstituted in 100 mmol/L phosphate buffer (pH 7.2). The amount of complexes was calculated from the radioactivity and initial amount of NaTcO$_4$ (^{99}Tc and ^{99m}Tc). The ^{99m}Tc compound (0.1 mL, approximately 10 pmol/L) was mixed with 0.1 mL solution of $10 \times 10^{-3}-10$ nmol/L histidine in 100 mmol/L phosphate buffer (pH 7.2). The mixture was incubated at 37 °C for 1 h or 100 °C for 30 min and analyzed by HPLC.

Stability in Rat Plasma. A solution of HPLC-purified each ^{99m}Tc-labeled compound (0.1 mL, approximately 10 MBq), prepared as mentioned above, was added to 0.9 mL of fleshly prepared rat plasma, and the mixture was incubated at 37 °C. At appropriate periods of time (10 and 30 min and 1 and 3 h), 0.1 mL aliquots (in duplicate) were withdrawn and 0.4 mL of ethanol was added to precipitate proteins. Samples were centrifuged at 2000*g* for 10 min. The supernatant was separated from the precipitate, and the sediment was washed twice with ethanol (1 mL, each), and the radioactivity levels of each fraction were counted in a gamma counter to estimate protein-bound radioactivity. The supernatant was analyzed by TLC under conditions similar to those described above.

Biodistribution Studies. Animal studies were conducted in accordance with our institutional guidelines and were approved by the Chiba University Animal Care Committee. The in vivo behavior of the ^{99m}Tc-labeled compounds was evaluated in ddY male mice (Charles River Ltd., Tokyo) weighing 20−25 g. A solution of 100 *μ*L (120 kBq) of respective ^{99m}Tc-labeled compound was administered intravenously from the tail vein, and the animals were maintained on normal diet ad libitum. At 10 min and 1, 3, 6, and 24 h, mice were sacrificed by decapitation. Tissues of interest were removed, weighed, and the radioactivity counts were determined using an auto well gamma counter. Urine and feces were collected by 24 h postinjection, and the radioactivity counts were determined. Biodistribution results were expressed as a percent of injected dose per organ (% I.D./total organ) and as a percent of injected dose per gram (% I.D./g).

To estimate the in vivo stability of respective ^{99m}Tc-labeled compounds, urine samples collected by 24 h postinjection were combined and centrifuged (2000*g*, 5 min) and the supernatant was analyzed by HPLC under the conditions described above.

(13) *WinAFC*, version 1.03. 3-9-12; Diffractometer Control Software. Rigaku Corpoaration: Matsubara, Akishima, Tokyo, Japan, 1999.
(14) Sheldrick, G. M. *Acta Crystallogr., Sect. A* **1990**, *46*, 467–473.
(15) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115–119.
(16) Sheldrick, G. M. *SHELXL-97*; University of Göttingen: Göttingen, Germany, 1997.
(17) (a) Keegan, R. P.; Gehrke, R. J. *Appl. Radiat. Isot.* **2003**, *59*, 137–143. (b) see theSupporting Information.
(18) (a) Hnatowich, D. J.; Virzi, F.; Fogarasi, M.; Rusckowski, M.; Winnard, P. J. *Nucl. Med. Biol.* **1994**, *21*, 1035–1044. (b) Hnatowich, D. J.; Qu, T.; Chang, F.; Ley, A. C.; Ladner, R. C.; Rusckowski, M. *J. Nucl. Med.* **1998**, *39*, 56–64. (c) Qu, T.; Wang, Y.; Zhu, Z.; Rusckowski, M.; Hnatowich, D. J *Nucl. Med. Commun.* **2001**, *22*, 203–215. (d) Michael, L.; Roberto, L. B.; Elisa, G.; Annette, G. B. *Bioconjugate Chem.* **2001**, *12*, 1028–1034. (e) von Guggenberg, E.; Behe, M.; Behr, T. M.; Saurer, M.; Seppi, T.; Decristoforo, C. *Bioconjugate Chem.* **2004**, *15*, 864–871. (f) Neva, L.; John, B.; John, V.; Paul, S.; Shelly, J.; Jon, Z. *Inorg. Chem.* **2005**, *44*, 6763–6770.

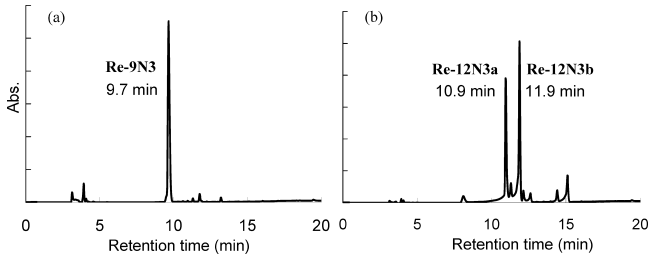

Figure 1. HPLC chromatograms of a reaction solution of 9N3 (a) or 12N3 (b) with $[Et_4N]_2[Re(CO)_3Br_3]$. To avoid precipitation, methanol was used in place of acetonitrile as the solvent.

Results and Discussion

Complexation Reaction of Rhenium Complexes. A single rhenium complex of 9-membered 9N3 (**Re-9N3**) was obtained by reacting 9N3 with $[Et_4N]_2[Re(CO)_3Br_3]$ in acetonitrile in good yields (70%). In contrast, the complexation reaction of 12-membered 12N3 with $[Et_4N]_2[Re(CO)_3Br_3]$ required higher temperature and longer reaction times than those of 9N3. **Re-12N3a** (HPLC retention time 10.9 min in Figure 1 and Table 5) was predominantly generated at an early stage of the reaction, whereas an increase in **Re-12N3b** (11.9 min) was observed with time. As a result, 12N3 produced two major complexes (**Re-12N3a** and **Re-12N3b**). The purified **Re-12N3a** or **Re-12N3b** was stable in acidic or neutral aqueous solution. However, these compounds were rapidly interconverted to each other and reached equilibrium (**Re-12N3a**:**Re-12N3b** = 80:20) in basic aqueous solution (chromatograms are shown in the Supporting Information) although both complexes have the same composition as shown below. This suggests that the interconversion may be mediated by a temporary coordination of the OH^- ion to the rhenium center.

Structure of Rhenium Complexes. In the $^1H/^{13}C$ NMR spectrum of a purified rhenium complex of 9N3, the resonance signals of 9N3 ligand appeared in downfield regions in comparison with those of free 9N3 (Table 2). These downfield shifts would be originated from electron donation from the heteroatoms of 9N3 ligand to metal. The chemical shifts of methylene protons in 9N3 ligand changed from equivalent to nonequivalent upon complexation. Similarly, in the ^{13}C NMR spectrum, two signals assigned to the carbons atoms of the 9N3 ligand appeared from a single signal upon complexation (Table 2), suggesting that the two sets of three carbons adjacent to each other became nonequivalent upon complexation. These results suggest that 9N3 would be coordinated to rhenium via three nitrogen atoms equivalently. Since the ligand, 9N3, can provide only facial coordination, three carbonyl groups in the rhenium complex should be present at facial positions. This was confirmed by the infrared spectra, which consisted of two strong bands assigned to the C−O stretch, 1881 and 2014 cm^{-1}, with the low-frequency band being very broad. These results are compatible with the formation of *fac*-$M(CO)_3L$ derivatives (C_{3v} local symmetry; $A_1 + E$) where all three donor nitrogen atoms are equivalent.

The two major reaction products of 12N3 with $[Et_4N]_2[Re(CO)_3Br_3]$ (**Re-12N3a** and **Re-12N3b**) were purified using HPLC with a mixture of H_2O and acetonitrile containing TFA as a mobile phase. NMR, MS, and elemental analyses of the purified complexes suggested that the two products had chemical structure of $[Re(CO)_3(12N3)](CF_3COO)$. The NMR spectrum of **Re-12N3a** showed profiles similar to those of free 12N3 (Table 2). **Re-12N3a** had magnetically equivalent six methylenes next to nitrogen atoms (**A** in Scheme 1). Three methylenes not connected with nitrogen atoms (**B** in Scheme 1) were magnetically equivalent, but the three sets of two protons bonded to a same carbon (protons on **B**) became nonequivalent upon complexation as also observed with **Re-9N3**. These findings indicated that 12N3 ligand in **Re-12N3a** formed conformation similar to free 12N3 with an exception of the nonequivalent two protons. In contrast, NMR spectrum of **Re-12N3b** was completely different from free 12N3 and **Re-12N3a**. The equivalent methylene carbons next to nitrogen of 12N3 became nonequivalent each other upon complexation. In addition, the ^1H NMR spectrum became complex compared to **Re-12N3a**. These findings suggested 12N3 ligand in **Re-12N3b** lost the C_{3v} local symmetric property.

Description of the Structures. Re-9N3 crystallized in the monoclinic space group *Pbca*. An ORTEP view of the complex along with the corresponding atom-numbering scheme is given in Figure 2. Selected bond lengths, bond angles, and torsion angles are summarized in Table 3. The metal center has distorted octahedral coordination sphere with a facial arrangement of the three carbonyl groups. The coordinated macrocyclic ligand forms five-membered rings with the metal center. The N−Re−N and the C−Re−C angles have average values of 77.2° and 89.9°, respectively. Thus, they only slightly deviate from the expected ideal 90° for an octahedron in the macrocyclic ligand part. The Re−C bond lengths possess a mean distance of 1.91 Å and are a valid distance, compared with reported rhenium and technetium complexes.[5,19,20] The average bond lengths (2.188 Å) between the rhenium and nitrogen of 9N3 are similar to other comparable complexes.[8,21] The bond lengths or angles of carbonyl groups of **Re-9N3** are close to those of the rhenium 9S3 complex.[9] However the N−Re−N angles are narrower than S−Re−S due to the smaller atomic radius of nitrogen than that of sulfur. It would be attributable to the enlarged distortion of the 9N3 complex from an ideal octahedron when compare to the 9S3 complex. In addition, the conformation of 9N3 in **Re-9N3** (synclinal and anticlinal) is similar to that of free 9N3 as estimated by crystallographic measurement[22] in Figure 4. The conformation of 9S3[23] is similar to that of 9N3. Since both *fac*-$[^{99}Tc(CO)_3(9S3)]Br$[5] and free 9S3 ligand contain synclinal and anticlinal confor-

(19) Roger, A.; Roger, S.; André, E. P.; August, S.; Wolfgang, A. H.; Georg, A.; Ulrich, A.; Thomas, A. K. *J. Organomet. Chem.* **1995**, *492*, 217–224.
(20) Pietzsch, H. J.; Gupta, A.; Reisgys, M.; Drews, A.; Seifert, S.; Syhre, R.; Spies, H.; Alberto, R.; Abram, U.; Schubiger, P. A.; Johannsen, B. *Bioconjugate Chem.* **2000**, *11*, 414–424.
(21) Lipowska, M.; Cini, R.; Tamasi, G.; Xu, X.; Taylor, A. T.; Marzilli, L. G. *Inorg. Chem.* **2004**, *43*, 7774–7783.
(22) Andrew, R. B.; Daniel, L. J.; Lisandra, L. M. *Acta Crystallogr., Sect. E* **2005**, *61*, o330–o332.
(23) Glass, R. S.; Wilson, G. S.; Setzer, W. N. *J. Am. Chem. Soc.* **1980**, *102*, 5068–5069.

Table 2. NMR Assignments for 9N3, 12N3, *fac*-[Re(CO)$_3$(9N3)]Br, and *fac*-[Re(CO)$_3$(12N3)](CF$_3$COO)[a]

		free ligand	complex
Re-9N3	^{1}H	2.71 (12H)	2.96 (6H), 3.21 (6H)
	^{13}C	46.1	50.26, 50.16
Re-12N3a	^{1}H	1.58 (6H) 2.66 (12H)	1.82 (3H), 2.15 (3H), 3.06 (12H)
	^{13}C	27.2, 48.9	24.8, 50.8
Re-12N3b	^{1}H	1.58 (6H) 2.66 (12H)	1.69 (1H), 2.00 (1H), 2.12 (4H), 2.96 (6H), 3.11 (2H), 3.19 (2H), 3.30 (2H)
	^{13}C	27.2, 48.9	25.2, 51.5, 52.2

[a] Expressed as δ (ppm) in D_2O.

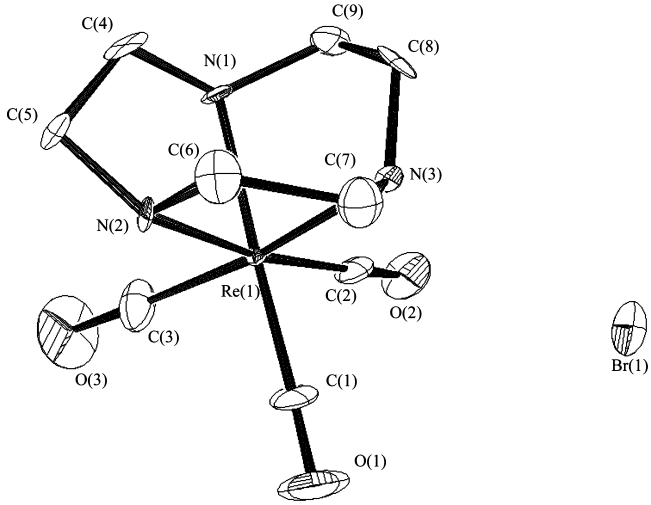

Figure 2. ORTEP plot of [Re(CO)$_3$(9N3)]Br (**Re-9N3**). Hydrogen atoms are omitted for clarity. Ellipsoids are drawn at the 50% probability level.

Table 3. Selected Bond Lengths (Å), Bond Angles (deg), and Torsion Angles (deg) of *fac*-[Re(CO)$_3$(9N3)]Br (**Re-9N3**)

Bond Lengths			
Re(1)−N(1)	2.213	Re(1)−C(1)	1.935
Re(1)−N(2)	2.176	Re(1)−C(2)	1.921
Re(1)−N(3)	2.169	Re(1)−C(3)	1.883
N(1)−C(4)	1.482	N(2)−C(6)	1.460
N(1)−C(9)	1.490	N(3)−C(7)	1.481
N(2)−C(5)	1.509	N(3)−C(8)	1.500
C(4)−C(5)	1.519		
C(6)−C(7)	1.511		
C(8)−C(9)	1.489		

Bond Angles			
N(1)−Re(1)−N(2)	76.85	C(1)−Re(1)−C(2)	89.47
N(2)−Re(1)−N(3)	77.59	C(2)−Re(1)−C(3)	91.14
N(3)−Re(1)−N(1)	77.72	C(3)−Re(1)−C(1)	89.53

Torsion Angles			
N(1)−C(4)−C(5)−N(2)	42.9	C(7)−N(3)−C(8)−C(9)	−133.0
C(4)−C(5)−N(2)−C(6)	74.6	N(3)−C(8)−C(9)−N(1)	42.8
C(5)−N(2)−C(6)−C(7)	−134.3	C(8)−C(9)−N(1)−C(4)	74.0
N(2)−C(6)−C(7)−N(3)	40.9	C(9)−N(1)−C(4)−C(5)	−134.7
C(6)−C(7)−N(3)−C(8)	74.3		

mations,[24] 9N3 ligand may have an equivalent feature to 9S3 ligand in terms of coordination geometry.

Re-12N3b. *fac*-[Re(CO)$_3$(12N3)](CF$_3$COO) crystallized in the monoclinic space group *Pnma*. An ORTEP view of the complex along with the corresponding atom-numbering scheme is given in Figure 3. Selected bond lengths, bond angles, and torsion angles are summarized in Table 4. The metal center has an octahedral coordination sphere with a facial arrangement of the three carbonyl groups. The complex has a plane of mirror symmetry passing through N(1), Re(1), C(1), O(1), and C(7), and does not have C_{3v} symmetric property as notified by NMR studies. The bond lengths are normal in comparison with reported complexes.[5,9,19,20] The

(24) A table summarizing the torsion angles is indicated in the Supporting Information.

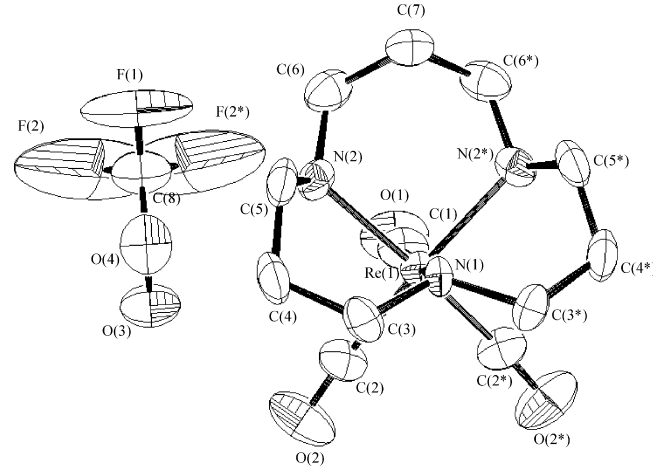

Figure 3. ORTEP plot of [Re(CO)$_3$(12N3)](CF$_3$COO) (**Re-12N3b**). Hydrogen atoms are omitted for clarity. Ellipsoids are drawn at the 50% probability level.

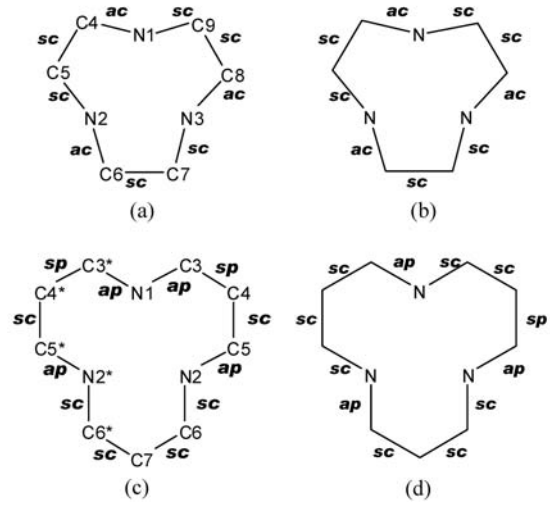

Figure 4. Appropriate description of the geometric relationship between atoms in 9N3 (a and b) and 12N3 (c and d) in coordinated (a and c) and uncoordinated (b and d) conformations. The uncoordinated conformation of 9N3 was determined by crystallographic data from ref 22, and that of 12N3, by a computational simulation from ref 25. The designation *sp* represents synperiplanar conformation with torsion angle between 0° to ±30°, *sc*, synclinal conformation (30−90° and –30° to –90°), *ac*, anticlinal conformation (90−150° and –90° to –150°), and *ap*, antiperiplanar conformation (±150−180°).

Table 4. Selected Bond Lengths (Å), Bond Angles (deg), and Torsion Angles (deg) of *fac*-[Re(CO)$_3$(12N3)](CF$_3$COO) (**Re-12N3b**)

Bond Lengths			
Re(1)−N(1)	2.204	Re(1)−C(1)	1.930
Re(1)−N(2)	2.248	Re(1)−C(2)	1.909
N(1)−C(3)	1.483	C(3)−C(4)	1.510
N(2)−C(5)	1.501	C(4)−C(5)	1.515
N(2)−C(6)	1.497	C(6)−C(7)	1.514

Bond Angles			
N(1)−Re(1)−N(2)	80.30	C(1)−Re(1)−C(2*)	88.65
N(2)−Re(1)−N(2*)	89.94	C(2)−Re(1)−C(2*)	85.15

Torsion Angles			
N(1)−C(3*)−C(4*)−C(5*)	27.7	C(7)−C(6)−N(2)−C(5)	79.3
C(3*)−C(4*)−C(5*)−N(2*)	−79.4	C(6)−N(2)−C(5)−C(4)	−176.0
C(4*)−C(5*)−N(2*)−C(6*)	176.0	N(2)−C(5)−C(4)−C(3)	79.4
C(5*)−N(2*)−C(6*)−C(7)	−79.3	C(5)−C(4)−C(3)−N(1)	−27.7
N(2*)−C(6*)−C(7)−C(6)	−74.6	C(4)−C(3)−N(1)−C(3*)	173.5
C(6*)−C(7)−C(6)−N(2)	74.6	C(3)−N(1)−C(3*)−C(4*)	−173.5

Table 5. HPLC Retention Time of Re Complexes and ^{99m}Tc Compounds

	Re	^{99m}Tc
starting complex	$[Et_4N]_2[Re(CO)_3Br_3]$: 12.2 min	$[^{99m}Tc(OH_2)_3(CO)_3]^+$: 11.3 min
9N3	**Re-9N3**: 9.7 min	**^{99m}Tc-9N3**: 9.2 min
12N3	**Re-12N3a**: 10.9 min **Re-12N3b**: 11.9 min	**^{99m}Tc-12N3**: 10.3 min

ring size of 12N3 is too large to keep ideal ligand conformation and octahedral coordination sphere. N(1) of the 12N3 ligand deviates to the inward side of 12N3 to coordinate the metal center, and this conformation of N(1) is different from the C_{3v} conformation of the free 12N3 ligand, as estimated by theoretical calculations.[25] Furthermore, the N−Re−N angles of **Re-12N3b** (80.3° and 89.9°) are larger than that of **Re-9N3** (76.9−77.7°), presumably due to the larger ring size of 12N3. The larger N−Re−N angle induces narrower C−Re−C angles of **Re-12N3b** (85.2° and 88.7°) compared with **Re-9N3** (89.5−91.1°). The C−Re−C angles back away from the ideal octahedral angle. With respect to the 12N3 ligand conformation, it is to be noted that there are close to eclipsed relationships (synperiplanar) between N(1) and C(5)/C(5*); the torsion angle is 27.6°. The theoretical calculations showed that free 12N3 ligand has mainly synclinal and antiperiplanar conformations (Figure 4) and there was conformational difference between free 12N3 ligand (C_{3v}) and **Re-12N3b** (C_s). The large conformational freedom of 12N3[25] would enable the 12N3 ligand to coordinate rhenium core with changing the propylenediamine bridge conformations.

These findings suggested that 9N3 fit the *fac*-{Re(CO)$_3$}$^+$ core whereas 12N3 was slightly large to the *fac*-{Re(CO)$_3$}$^+$ core. In addition, 12N3 coordinated to *fac*-{Re(CO)$_3$}$^+$ core with changing the conformation of free 12N3 ligand. Infrared spectrum supported the stability advantage of 9N3 over 12N3 as a ligand for the *fac*-{Re(CO)$_3$}$^+$ core. The infrared absorption wavenumber assigned to the C−O stretch of **Re-9N3** was lower than that of the **Re-12N3b** complex. These findings indicated that the contribution from π-backbonding

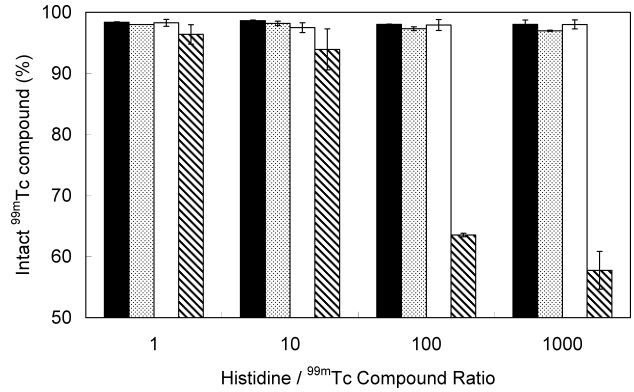

Figure 5. Stability of **^{99m}Tc-9N3** and **^{99m}Tc-12N3** in the presence of excess histidine: (black bar) **^{99m}Tc-9N3** at 37 °C for 1 h; (dotted bar) **^{99m}Tc-9N3** at 100 °C for 30 min; (white bar) **^{99m}Tc-12N3** at 37 °C for 1 h; (striped bar) **^{99m}Tc-12N3** at 100 °C for 30 min.

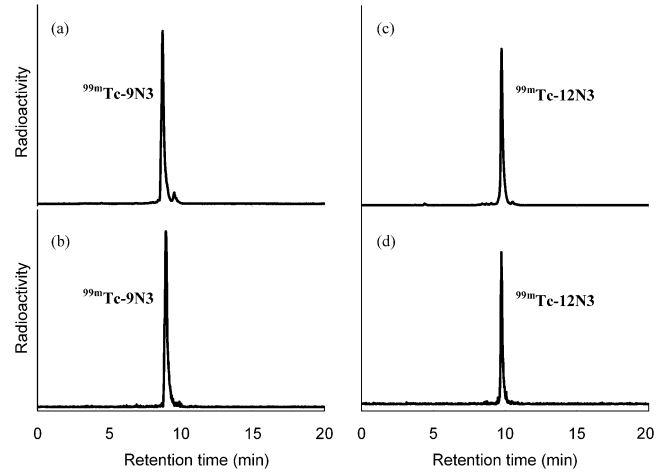

Figure 6. HPLC radiochromatograms of **^{99m}Tc-9N3** and **^{99m}Tc-12N3** (a and c) and the urine samples collected for 24 h postinjection of the respective compound to mice (b and d).

to the synergic Re−CO bonding[26] of the **Re-9N3** complex was stronger than that of **Re-12N3b** and suggested that the coordination geometry of 9N3 would be more suitable to *fac*-{Re(CO)$_3$}$^+$ than that of 12N3.

Technetium-99m Labeling. The ^{99m}Tc-labeled compounds were synthesized according to a procedure established previously.[1] The formation of the $[^{99m}Tc(OH_2)_3(CO)_3]^+$ precursor was verified by HPLC retention time, before reacting with the ligands. The labeled derivatives were then characterized by their associated radioactive HPLC traces and compared with the corresponding rhenium complexes (monitored at 260 nm). While ^{99m}Tc(CO)$_3$-labeled 9N3 (**^{99m}Tc-9N3**) exhibited a HPLC retention time close to that of the rhenium counterpart, the reaction product of ^{99m}Tc(CO)$_3$ and 12N3 (**^{99m}Tc-12N3**) provided a single radioactivity peak at a retention time close to that of **Re-12N3a** (Table 5; the chromatograms are depicted in the Supporting Information). Similar results were observed in our previous study of fatty acid derivatives of cyclopentadienyl tricarbonyl technetium compounds and its rhenium counterparts.[27] For

(25) Paulo, J. A. R.; Ana, M. A.; Paula, M. M.; José, J. C. T. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1161–1167.
(26) (a) Chatt, J.; Duncanson, L. A. *J. Chem. Soc.* **1953**, 2939–2947. (b) Chatt, J.; Wilkins, R. G. *J. Chem. Soc.* **1952**, 2622–2626.

Table 6. Biodistribution of Radioactivity in ddY Mice of **^{99m}Tc-9N3** and **^{99m}Tc-12N3**[a]

	10 min	1 h	3 h	6 h	24 h
			^{99m}Tc-9N3		
blood	0.63 (0.10)	0.13 (0.01)	0.08 (0.01)	0.10 (0.03)	0.02 (0.01)
spleen	0.39 (0.10)	0.21 (0.04)	0.14 (0.03)	0.12 (0.02)	0.01 (0.00)
pancreas	5.63 (1.87)	2.29 (0.19)	0.28 (0.18)	0.14 (0.05)	0.00 (0.00)
stomach[b]	0.83 (0.14)	0.87 (0.18)	0.24 (0.09)	0.53 (0.36)	0.02 (0.02)
intestines[b]	17.14 (1.50)	13.22 (3.37)	11.42 (3.25)	8.53 (3.52)	0.09 (0.03)
liver	10.61 (1.58)	2.35 (0.64)	0.67 (0.07)	0.51 (0.04)	0.03 (0.00)
kidneys	18.11 (7.21)	3.17 (1.34)	1.38 (0.86)	0.56 (0.11)	0.03 (0.00)
heart	0.75 (0.61)	0.27 (0.17)	0.12 (0.06)	0.09 (0.01)	0.00 (0.00)
lung	1.05 (0.38)	0.40 (0.09)	0.29 (0.03)	0.16 (0.09)	0.01 (0.00)
brain	0.03 (0.01)	0.01 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
muscle	0.23 (0.05)	0.09 (0.01)	0.04 (0.02)	0.06 (0.02)	0.00 (0.00)
bone	0.30 (0.03)	0.13 (0.04)	0.14 (0.08)	0.10 (0.04)	0.00 (0.00)
			^{99m}Tc-12N3		
blood	1.23 (0.17)	0.37 (0.07)	0.20 (0.05)	0.21 (0.02)	0.05 (0.00)
spleen	0.73 (0.17)	0.33 (0.05)	0.32 (0.11)	0.24 (0.04)	0.09 (0.03)
pancreas	3.67 (1.18)	1.39 (0.29)	0.48 (0.05)	0.23 (0.03)	0.06 (0.04)
stomach[b]	0.90 (0.17)	0.64 (0.06)	0.51 (0.14)	0.80 (0.56)	0.16 (0.03)
intestines[b]	16.40 (1.25)	17.69 (2.67)	15.40 (2.68)	5.26 (2.14)	1.11 (0.50)
liver	11.42 (1.54)	4.24 (0.25)	2.14 (0.17)	1.63 (0.14)	0.80 (0.07)
kidneys	17.85 (6.07)	4.89 (2.62)	2.77 (0.82)	2.02 (0.21)	0.56 (0.33)
heart	0.95 (0.18)	0.50 (0.15)	0.29 (0.06)	0.21 (0.03)	0.06 (0.02)
lung	1.41 (0.19)	0.63 (0.06)	0.42 (0.05)	0.32 (0.05)	0.11 (0.09)
brain	0.05 (0.01)	0.02 (0.00)	0.01 (0.00)	0.01 (0.00)	0.00 (0.00)
muscle	0.37 (0.07)	0.19 (0.03)	0.17 (0.02)	0.16 (0.02)	0.05 (0.03)
bone	0.55 (0.28)	0.23 (0.03)	0.16 (0.03)	0.21 (0.06)	0.02 (0.02)

[a] Tissue radioactivity is expressed as percent I.D./g for each group ($n = 5$); results are expressed as mean (SD). [b] Expressed as percent I.D.

structural characterization of ^{99m}Tc-labeled compounds, the reaction solutions were analyzed using an LC-MS method. In order to enrich the molar quantity of $^{99m/99}$Tc compounds, $Na^{99m/99}TcO_4$ was eluted from a generator after an interval of 7 days. $^{99m/99}$Tc-labeled compounds were analyzed by electrospray mass spectrometry, and in each case, the observed molecular ions in relation to the peaks of the radiodetector were consistent with the mass of the desired compounds. Both labeled compounds showed their expected (M + 1) molecular ions with $m/z = 311.8$ for **$^{99m/99}$Tc-9N3**($C_9H_{15}N_3O_3{}^{99}Tc^+$, calc. 312.02), and $m/z = 354.1$ for **$^{99m/99}$Tc-12N3** ($C_{12}H_{21}N_3O_3{}^{99}Tc^+$, calc. 354.06). These findings supported the idea that **^{99m}Tc-9N3** possesses a chemical structure similar to that of **Re-9N3**, while **^{99m}Tc-12N3** is similar to **Re-12N3a**. As stated above, Re predominantly formed **Re-12N3a** at an early stage of the reaction. In addition, while the Re and 12N3 reacted at equivalent molar ratio, a large excess of 12N3 reacted with $^{99m/99}$Tc. This may account for the preferable formation of a **^{99m}Tc-12N3** compound that possesses a configuration similar to that of **Re-12N3a**. The gathered results indicated that the ^{99m}Tc complex produced on the tracer level is identical to those of rhenium complexes characterized on the macroscopic scale.

Challenge Experiments. Since histidine constitutes a competing ligand for the $\{^{99m}Tc(CO)_3\}^+$ core,[1d,28] the stability of **^{99m}Tc-9N3** and **^{99m}Tc-12N3** was assessed in the presence of histidine at a concentration (approximately 10 nmol/mL) similar to that in human plasma (87 ± 3 nmol/mL[29]). Both **^{99m}Tc-9N3** and **^{99m}Tc-12N3** remained stable at 37 °C for 3 h (Figure 5). While **^{99m}Tc-9N3** also remained stable when the temperature was brought to 100 °C, generation of ^{99m}Tc-labeled histidine was observed from **^{99m}Tc-12N3** at the elevated temperature (Figure 5). These findings indicated that **^{99m}Tc-9N3** possessed much higher stability than **^{99m}Tc-12N3**.

Stability in Rat Plasma. The stability of **^{99m}Tc-9N3** and **^{99m}Tc-12N3** was also assessed in freshly prepared rat plasma at 37 °C. The plasma protein binding was estimated to be 1.1−1.6% at all time points for both complexes. More than 95% of radioactivity was observed as an intact **^{99m}Tc-9N3** up to 3 h in rat plasma. In contrast, a 20% decrease in an intact **^{99m}Tc-12N3** fraction was observed after 10 min incubation, due to a formation of hydrophilic compound as determined by TLC (uncharacterized). The differences in stability between **^{99m}Tc-9N3** and **^{99m}Tc-12N3** in plasma were well-correlated with the findings of histidine challenge at 100 °C. These results are presented in the Supporting Information.

Biodistribution Studies. The biodistribution of radioactivity after injection of **^{99m}Tc-9N3** and **^{99m}Tc-12N3** to mice is shown in Table 6. Both ^{99m}Tc-labeled compounds showed no specific accumulation of radioactivity in any organs except for the excretory organs such as the liver and kidney. Although no statistical significances were observed, **^{99m}Tc-9N3** tended to eliminate from the liver and kidney at a rate faster than that of **^{99m}Tc-12N3**. At 24 h postinjection, 85 ± 2% and 10 ± 3% of injected radioactivity were recovered

(27) Uehara, T.; Uemura, T.; Hirabayashi, S.; Adachi, S.; Odaka, K.; Akizawa, H.; Magata, Y.; Irie, T.; Arano, Y. *J. Med. Chem.* **2007**, *50*, 543–549.

(28) (a) Kristin, E. B.; Mary, D.; Julie, L. P.; Christina, M. P.; Vijay, S.; David, P. W. *Bioconjugate Chem.* **2002**, *13*, 1226–1237. (b) Jae, K. P.; Paul, B.; Bernhard, S.; Kirstin, O.; Roger, A. *Chem.—Eur. J.* **2003**, *9*, 2053–2061. (c) Sang, H. P.; Sepp, S.; Hans, J. P. *Bioconjugate Chem.* **2006**, *17*, 223–225.

(29) Filho, J. C.; Bergström, J.; Stehle, P.; Fürst, P. *Clin. Nutr.* **1997**, *16*, 299–305.

in the urine and feces for **^{99m}Tc-9N3**, while 77 ± 2% and 18 ± 1% were recovered in the urine and feces for **^{99m}Tc-12N3**. The HPLC analyses of urine samples of mice collected by 24 h postinjction of **^{99m}Tc-9N3** or **^{99m}Tc-12N3** (Figure 6) showed that the urine samples depicted radiochromatograms identical to **^{99m}Tc-9N3** or **^{99m}Tc-12N3**. The discrepancy in the stability of ^{99m}Tc-labeled 12N3 between plasma incubation and biodistribution studies would be attributable to rapid elimination rate of the ^{99m}Tc-labeled compound from the circulation after intravenous injection. These findings reinforced that **^{99m}Tc-9N3** would constitute a more favorable molecule as the synthon for ^{99m}Tc radiopharmaceuticals.

Conclusion

The ring conformation of a macrocyclic ligand can be specified by the torsion angles around the C−C and C−N bonds. When 9N3 coordinates in an endo-type formation, 6 out of 9 bonds were synclinal and the rest of the bonds were anticlinal. In contrast, two C−C bonds were eclipsed conformation and the other were synclinal or antiperiplanar for the 12N3 complex. The distortion of the two eclipsed conformations may affect complexation yields of the Re complex and the stability of the ^{99m}Tc compound. These results reinforce the idea that the five-membered chelate ring is more stable than the six-membered one for a tricarbonyl technetium complex with triazamacrocyclic ligands, as also reported in other metals.[30] Indeed, 9N3 would possess preferable coordination geometry for a *fac*-$\{Re(CO)_3\}^+$ core, which was reflected in the different reactivity between 9N3 and 12N3 to the tricarbonyl rhenium complex, as supported by crystallographic data and the infrared spectrum. 9N3 also provided a ^{99m}Tc-labeled compound of higher stability and faster whole body elimination rates with the *fac*-$\{^{99m}Tc(CO)_3\}^+$ core. In addition, the secondary nitrogen atoms of 9N3 allow versatile modification of up to three functional groups to prepare multivalent compounds for targeting. These findings showed that 9N3 would constitute a useful synthon as a basic chelating molecule for ^{99m}Tc radiopharmaceuticals and $^{186/188}$Re therapeutic radiopharmaceuticals.

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Supporting Information Available: X-ray crystallographic files in CIF format for the crystal structure determinations of complexes **Re-9N3** and **Re-12N3b** and chromatographic data, NMR spectral data, and other data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(30) (a) Helmut, S.; Rene, C.; Bernard, P. *Inorg. Chem.* **1974**, *13*, 462–465. (b) Anna, J.; Göran, H.; Öjvind, D. *Organometallics* **2002**, *21*, 2283–2292. (c) James, H. T.; Ron, H. N.; Abraham, C.; Arthur, E. M. *Inorg. Chem.* **1979**, *18*, 2977–2982.