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Acyclic Chelate with Ideal Properties for ^{68}Ga PET Imaging Agent Elaboration

Eszter Boros,[†,§] Cara L. Ferreira,[‡] Jacqueline F. Cawthray,[†,§] Eric W. Price,[†] Brian O. Patrick,[†] Dennis W. Wester,[‡] Michael J. Adam,*[,§] and Chris Orvig*[,†]

Medicinal Inorganic Chemistry Group, Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia V6T 1Z1, Canada, MDS Nordion, 4004 Wesbrook Mall, Vancouver, British Columbia, Canada V6T 2A3, and TRIUMF, 4004 Wesbrook Mall, Vancouver, British Columbia, Canada V6T 2A3

Received July 27, 2010; E-mail: adam@triumf.ca; orvig@chem.ubc.ca

Abstract: We have investigated novel bifunctional chelate alternatives to the aminocarboxylate macrocycles NOTA (N_3O_3) or DOTA (N_4O_4) for application of radioisotopes of Ga to diagnostic nuclear medicine and have found that the linear N_4O_2 chelate H_2dedpa coordinates ^{67}Ga quantitatively to form $[^{67}Ga(dedpa)]^+$ after 10 min at RT. Concentration-dependent coordination to H_2dedpa of either ^{68}Ga or ^{67}Ga showed quantitative conversion to the desired products with ligand concentrations as low as 10^{-7} M. With ^{68}Ga, specific activities as high as 9.8 mCi nmol^{-1} were obtained without purification. In a 2 h competition experiment against human *apo*-transferrin, $[^{67}Ga(dedpa)]^+$ showed no decomposition. Two bifunctional versions of H_2dedpa are also described, and these both coordinate to ^{67}Ga at RT within 10 min. Complete syntheses, characterizations, labeling studies, and biodistribution profiles of the ^{67}Ga complexes are presented for the new platform chelates. The stability of these platform chelates is higher than that of DOTA.

Introduction

Disruptions in the supply chain of ^{99}Mo, the parent of the clinically important daughter isotope ^{99m}Tc, have worldwide effects on diagnostic nuclear medicine and have turned many researchers' attention to other generator-produced isotopes.[1] Millions of diagnostic procedures annually take advantage of the ^{99}Mo/^{99m}Tc isotope pair for single-photon-emission computed tomography (SPECT); Canada, among other countries, has in recent times been responsible for the interrupted supply chain. Among attractive alternatives to ^{99}Mo/^{99m}Tc is the ^{68}Ge/^{68}Ga generator system,[2] which has the potential to be eluted for up to one year due to the long half-life ($t_{1/2}$ = 271 d) of the parent radionuclide ^{68}Ge. The ^{68}Ga generator has been used to prepare ^{68}Ga radiopharmaceuticals for clinical imaging in Europe for several years,[3] but the development of a generator with regulatory approval for human use would further facilitate the transition of ^{68}Ga based agents into the clinic in North America.

Ga forms stable complexes with many multidentate ligands,[4] and the daughter radionuclide, ^{68}Ga, has suitable properties for high-quality positron-emission tomography (PET) imaging including a short half-life ($t_{1/2}$ = 68 min), decay by 89% positron emission and a maximum positron energy of 1.899 keV.[5−7]

The efficient and strong chelation of this radiometal in its only biologically stable oxidation state 3+ has been investigated for the past 40 years with high hopes for potentially useful complexes in radiopharmacy.[8,9] The sustained efforts of Martell and Welch over several decades[2,10] have been succeeded by the significant progress of Maecke and co-workers in the late 1990s when bifunctional versions of the tri- and tetra-aza-based aminocarboxylate macrocyclic chelators NOTA and DOTA were able to deliver highly promising results not only for the isotopes ^{68}Ga and ^{111}In but also for a wide range of radiolanthanides.[11] Many attempts to find chelators of comparable kinetic and thermodynamic stability for ^{68}Ga have been less successful.[12−14] The macrocyclic chelators themselves are challenging to

[†] University of British Columbia.
[‡] MDS Nordion.
[§] TRIUMF.

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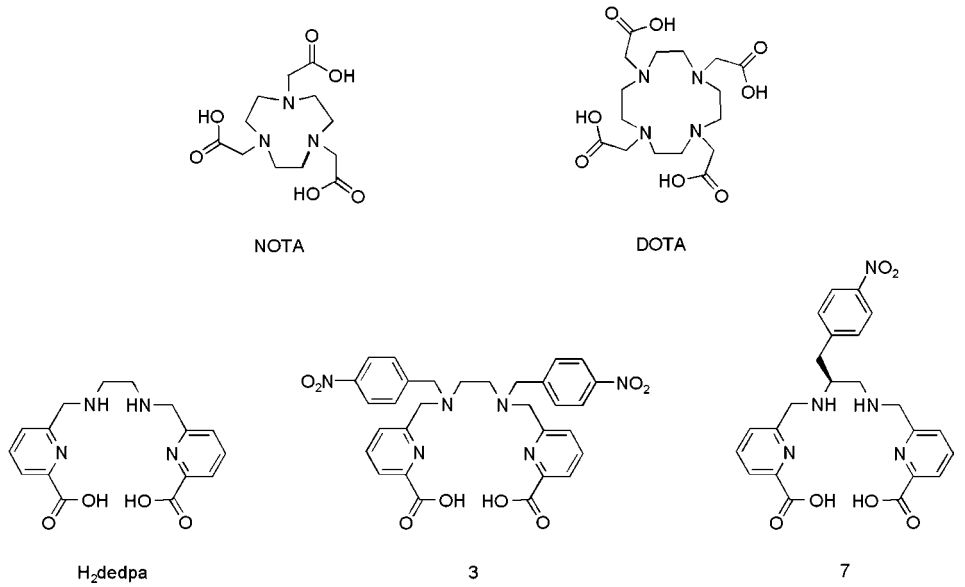
Scheme 1. NOTA, DOTA, H_2dedpa, and dedpa Derivatives, **3** and **7**



synthesize as selectively functionalized analogues, and most research groups in need of bifunctional derivatives of NOTA or DOTA are supplied by a commercial source.

Herein we report a linear chelating ligand with optimal properties for the field of gallium radiopharmaceutical chemistry; H_2dedpa forms stable complexes of Ga quickly and under mild conditions, lending itself well to elaboration with targeting vectors for radionuclide delivery. Initial studies of dedpa^{2-} coordination with Ga and two of its radioisotopes ^{67,68}Ga, as well as the preparation of bioconjugate precursors **3** and **7** (Scheme 1), are reported along with relevant transferrin competition studies and biodistribution results. These most promising results suggest that dedpa^{2-} and its derivatives have great potential to be powerful new tools for the radiopharmaceutical chemistry of gallium.

Experimental Section

Materials and Methods. All solvents and reagents were from commercial sources and were used as received unless otherwise indicated. Human serum *apo*-transferrin was purchased from Sigma-Aldrich (St. Louis, MO). The analytical thin-layer chromatography (TLC) plates were aluminum-backed ultrapure silica gel 60, 250 μm; the flash column silica gel (standard grade, 60 Å, 32−63 mm) was provided by Silicycle. ^{1}H and ^{13}C NMR spectra were recorded at RT on Bruker AV300, AV400, or AV600 instruments; the NMR spectra are expressed on the δ scale and were referenced to residual solvent peaks or internal tetramethylsilane. Electrospray-ionization mass spectrometry (ESI-MS) spectra were recorded on a Micromass LCT instrument at the Department of Chemistry, University of British Columbia. IR spectra were collected neat in the solid state on a Thermo Nicolet 6700 FT-IR spectrometer. The HPLC system used for analysis consisted of a Waters Alliance HT 2795 separation module equipped with a Raytest Gabi Star NaI (Tl) detector and a Waters 996 photodiode array (PDA) detector. ^{67}Ga was obtained as a 0.1 M HCl solution, and ^{68}Ga (5−10 mCi/mL) (both MDS Nordion Inc.) was obtained from a generator constructed of titanium dioxide sorbent that was charged with ^{68}Ge and eluted with aqueous HCl (0.1 M).[15] The generator has been previously used for radiolabeling NOTA- and DOTA-based chelate systems, and the resulting radiochemical yields and specific activities achievable for these chelates using this generator have been reported.[16] Analysis of radiolabeled complexes was done on a Phenomenex Hydrosynergy RP C18 4.6 mm × 150 mm analytical column ([Ga(dedpa)]$^+$), Phenomenex Jupiter 5μ C18 300 A 4.6 mm × 100 mm (transferrin (Tf) challenge with [Ga(dedpa)]$^+$, NOTA *versus* H_2dedpa challenge, H_2dedpa challenge of Ga(NOTA), retention time of ^{67}Ga−Tf: 10.7 min) and Waters XBridge BEH130 4.6 mm × 150 mm ([Ga**3**]$^+$, [Ga**7**]$^+$, as well as the transferrin challenges thereof, retention time of ^{67}Ga−Tf: 2.5 min).

H_2dedpa·2HCl. Protected precursor 1,2-[{6-(methoxycarbonyl)pyridin-2-yl}methylamino]ethane was synthesized according to the literature.[17] Deprotection of 1,2-[{6-(methoxycarbonyl)-pyridin-2-yl}methylamino]ethane was achieved by dissolving (37 mg, 0.1 mmol) in 4 mL of a 1:1 mixture of THF and water. LiOH (10 mg, 0.41 mmol, 4.1 equiv) was added and the reaction mixture was stirred for 2.5 h. Reaction monitoring was performed by TLC (20% CH_3OH in CH_2Cl_2, t_R of starting material: 0.8, t_R of product: 0.0). The solvent was removed *in vacuo*, and 12 M HCl was added to the glass-like solid to precipitate the dihydrochloride salt, which was collected by filtration to afford 24 mg (0.059 mmol, 59%) of a white solid. ^{1}H NMR (d_6-DMSO, 300 MHz) δ: 8.12−8.09 (m, 4H, ortho-/para-H), 7.78 (d, ortho-H), 4.52 (s, 2H, CH_2), 3.51 (d, 2H, CH_2). ^{13}C NMR (d_6-DMSO, 150 MHz) δ: 166.1, 153.5, 148.1, 139.7, 127.2, 124.9, 104.6, 93.8, 50.7, 43.8. IR (cm^{-1}): 2678, 2600, 2427, 1761, 1749, 1599. HR-ESI-MS calcd for $C_{16}H_{19}N_4O_4$: 331.1406; found: 331.1329 [M+H]$^+$. Elemental analysis: calcd % for H_2dedpa·2HCl (402.8): C 47.65, H 5.00, N 13.81; found: C 47.30, H 5.11, N 13.38.

[Ga(dedpa)][ClO_4]. H_2dedpa·2HCl (21 mg, 0.052 mmol) was dissolved in a CH_3OH−water mixture (1:2). $Ga(ClO_4)_3$·$6H_2O$ (24 mg, 0.052 mmol) was added, and the pH was adjusted to 4.5 by addition of 0.1 M NaOH. The reaction mixture was heated for 30 min and then set aside in the fume hood for slow evaporation. After 72 h, rhombic colorless crystals suitable for X-ray diffraction had precipitated in quantitative yield. ^{1}H NMR (300 MHz, d_6-DMSO) δ: 8.59 (t, 2H, para-H), 8.29 (d, 2H, meta-H), 8.09 (d, meta-H), 4.60−4.32 (dd, 4H, py-CH_2−NH), 3.06 (m, 2H, CH_2), 2.4 (m, 2H, CH_2). ^{13}C NMR (75 MHz, d_6-DMSO) δ: 162.0, 150.4, 145.2, 144.1,

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129.2, 126.5, 122.0. HR-ESI-MS calcd for $C_{16}H_{16}{}^{69}GaN_4O_4$: 397.0427; found: 397.0431 $[M]^+$. IR (cm^{-1}): 2360, 2341, 1695, 1664, 1606. Product t_R on HPLC: 5.5 min (gradient: A - NaOAc buffer, pH 4.5; B - MeOH, 0−5% B linear gradient 20 min).

[67/68Ga(dedpa)]+. General Labeling Procedure. $^{67}GaCl_3$ (100 μL, 1 mCi) or $^{68}Ga^{3+}$ in a 0.1 M HCl solution was added to 900 μL of a 10^{-4} M solution of ligand in 10 mM NaOAc solution (pH 4.5) and left for 10 min at RT. The reaction progress was monitored by analytical HPLC which showed that the reaction had proceeded to 99%. Product t_R on HPLC: 6.1 min (gradient: A - NaOAc buffer, pH 4.5; B - CH_3OH, 0−5% B linear gradient 20 min). The high specific activity of 9.837 ± 0.136 mCi/nmol, with yields 99.9 ± 0.1% was achieved with 900 μL of a 10^{-7} M solution of H_2dedpa in 10 mM NaOAc solution (pH 4.5) and 100 μL $^{68}Ga^{3+}$ in a 0.1 M HCl solution (0.98 mCi) under standard labeling conditions (as described above); this experiment was done in triplicate.

Complex Stability against Transferrin. For *apo*-transferrin competition, $^{67}GaCl_3$ was added to a 10^{-4} M solution of ligand in 10 mM NaOAc solution (pH 4.5). Complex formation was checked by HPLC. A 400-μL aliquot was added to 1 mg/mL *apo*-transferrin in a $NaHCO_3$ solution (10 mM, 600 μL) and incubated at 37 °C (water bath). Complex stability was checked at time points 10 min, 1 h, and 2 h via analytical HPLC. No decomposition was detected.

Competition for Chelation Experiment with NOTA. $^{67}GaCl_3$ was added to 10^{-4} M solution of both NOTA and H_2dedpa in 10 mM NaOAc solution (pH 4.5). After a reaction time of 10 min at room temperature the reaction mixture was checked for the formed complex by analytical HPLC. Over 98% of the ^{67}Ga-dedpa complex was detected, opposed to 0.2% Ga-NOTA.

(1,2-[*N*,*N*′-{*p*-Nitrobenzyl}methyl]-*N*,*N*′-[6-{methoxycarbonyl}-pyridin-2-yl]methylamino)ethane (2). 1 was synthesized according to the literature.[17] 4-Nitrobenzyl bromide (135 mg, 0.625 mmol) was dissolved in 20 mL of acetonitrile together with **1** (105 mg, 0.293 mmol). Na_2CO_3 (400 mg) was added into the solution, and the reaction was stirred overnight at 70 °C. Subsequently, the suspension was filtered and the solvent removed *in vacuo*. The resulting orange oil was purified by column chromatography (Silica, CH_2Cl_2); the product was eluted with 5% CH_3OH and isolated as an orange oil (60 mg, 0.095 mmol, 33%, $R_f = 0.6$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01 (d, 2H, benzyl-H), 7.98 (d, 2H, py-H), 7.76 (t, 2H, py-H), 7.59 (d, 2H, py-H), 7.46 (d, 2H, benzyl-H), 4.98 (s, 6H, CH_3) 3.83 (s, 4H, py-CH_2-NH), 3.69 (s, 4H, benzyl-CH_2-NH), 2.71 (s, 4H, -CH_2-CH_2-). ^{13}C NMR: (75 MHz, $CDCl_3$) δ: 165.8, 160.1, 147.6, 147.3, 137.6, 129.3, 125.9, 123.8, 123.7, 60.6, 58.7, 53.1, 52.3. HR-ESI-MS calcd for $C_{32}H_{32}N_6NaO_8$: 651.2179; found: 651.2289 $[M + Na]^+$.

(1,2-[*N*,*N*′-{*p*-Nitrobenzyl}methyl]-*N*,*N*′-bis-[6-carboxy-2-pyridylmethyl]ethylenediamine (3). 1 (27 mg, 0.042 mmol) was dissolved in 4 mL of a 3:1 mixture of THF and water. LiOH (5 mg, 0.21 mmol) was added to the solution, resulting in an immediate color change of the solution. The reaction was monitored by TLC and found to be complete after 45 min. The solvent was removed *in vacuo* to afford a white solid (25 mg, 0.041 mmol, 97%). ^{1}H NMR (400 MHz, MeOD) δ: 8.12 (d, 2H, benzyl-H), 8.10 (d, 2H, py-H), 7.91 (t, 2H, py-H), 7.42 (d, 2H, py-H), 7.36 (d, 2H, benzyl-H), 3.88 (s, 4H, py-CH_2-NH), 3.56 (s, 4H, benzyl-CH_2-NH), 2.41 (s, 4H, -CH_2-CH_2-). ^{13}C NMR (100 MHz, MeOD) δ: 172.5, 159.2, 155.0, 148.78, 145.0, 139.9, 132.2, 125.9, 124.4, 123.4, 61.0, 57.6, 31.1. HR-ESI-MS calcd for $C_{30}H_{27}N_6O_8$: 599.1890; found: 599.1887 $[M - H^+]^-$.

[Ga(3)](NO3). 3 (7 mg, 0.011 mmol) was dissolved in a CH_3OH−water mixture (1:2). $Ga(NO_3)_3 \cdot 6H_2O$ (4 mg, 0.011 mmol) was added, and the pH was adjusted to 4.5 by addition of 0.1 M NaOH. The reaction mixture was stirred at 60 °C for 2 h. The solvent was removed *in vacuo* to afford a white solid in quantitative yield. The solid was redissolved in a mixture of water and methanol (1:2). Colorless plates suitable for X-ray diffraction were obtained by slow evaporation of the solvent mixture. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.71 (t, 2H, py-H), 8.49 (d, 2H, py-H), 8.32 (d, 2H, benzyl-H), 8.18 (d, 2H, py-H), 7.72 (d, 2H, benzyl-H), 5.03−4.34 (dd, 4H, py-CH_2-NH), 4.18−3.87 (dd, 4H, -CH_2-CH_2-), 3.13 (s (br), 4H, benzyl-CH_2−NH). ^{13}C NMR (150 MHz, CD_3OD) δ: 165.1, 152.0, 150.3, 148.3, 145.7, 138.1, 134.5, 129.8, 125.6, 124.8, 57.7, 55.4, 48.1. HR-ESI-MS calcd. for $C_{30}H_{26}N_6O_8{}^{69}Ga$: 667.1068; found: 667.1075 $[^{69}M]^+$.

[67/68Ga(3)]+. $^{67}GaCl_3$ (100 μL, 1 mCi) or $^{68}Ga^{3+}$ (100 μL, 1 mCi) in a 0.1 M HCl solution was added into 10^{-4} M solution of ligand in 10 mM NaOAc solution (pH 4) and left to react for 10 min at room temperature. Reaction control was performed by analytical HPLC which showed that the reaction had proceeded to 98%. Product t_R on HPLC: 10.8 min (gradient: A - NaOAc buffer, pH 4.5; B - CH_3OH, 0−100% B linear gradient 20 min). For the *apo*-transferrin competition, $^{67}GaCl_3$ was added to 10^{-4} M solution of **3** in 10 mM NaOAc solution (pH 4.5). Complex formation was checked on HPLC (peptide column). A 400-μL aliquot was added to 1 mg/mL *apo*-transferrin in a $NaHCO_3$ solution (10 mM, 600 μL) and incubated at 37 °C (water bath). Complex stability was checked at time points 10 min, 1 h, and 2 h via analytical HPLC. Complex was 51% intact after 2 h.

2-(*p*-Nitrobenzyl)-*N*,*N*′-[6-{methoxycarbonyl}pyridin-2-yl]methylamino)ethane (6). 4 and **5** were synthesized according to the literature.[17,18] To a mixture of **4** (0.46 g, 2.36 mmol) in methanol (50 mL), was added **5** (0.78 g, 4.72 mmol). The mixture was refluxed for 2 h and then cooled to 0 °C in an ice bath. After cooling, $NaBH_4$ (0.139 g, 3.67 mmol) was added slowly and stirred at 0 °C for 2 h. Saturated aqueous $NaHCO_3$ was then added (150 mL) and the mixture stirred for 15 min, followed by extraction with dichloromethane (5 × 80 mL). The combined dichloromethane fractions were dried over $MgSO_4$ and evaporated to give 0.96 g of crude yellow oil. Subsequent purification of a 50-mg aliquot with column chromatography (10% CH_3OH in dichloromethane) afforded the product as a colorless oil (5 mg, 0.012 mmol, 8%, $R_f =$ 0.05). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.12 (d, 2H, benzyl-H), 7.99 (d, 2H, py-H), 7.78 (t, 2H, py-H), 7.54 (dd, 2H, py-H), 7.35 (d, benzyl-H), 4.06−4.03 (m, 4H, py-CH_2−NH), 3.97 (s, 6H, CH_3), 3.04−2.60 (m, 5H, CH_2−CH−CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.9, 160.8, 147.7, 147.6, 147.4, 146.8, 137.7, 130.4, 125.9, 123.8, 58.5, 53.1, 52.6, 52.1, 39.6. HR-ESI-MS calcd. for $C_{25}H_{28}N_5O_6$: 494.2040; found 494.2049 $[M + H^+]^+$.

2-(*p*-Nitrobenzyl)-(1,2-[*N*,*N*′-{*p*-nitrobenzyl}methyl]-*N*,*N*′-bis-[6-carboxy-2-pyridylmethyl]ethylenediamine (7). 6 (5 mg, 0.012 mmol) was dissolved in 2 mL of a 3:1 mixture of THF and water. LiOH (1 mg, 0.04 mmol) was added into the solution. The reaction was monitored by TLC and found to be complete after 30 min. The solvent was removed *in vacuo* to afford a light-yellow solid (4 mg, 0.01 mmol, 83%). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.15 (d, 2H, benzyl-H), 7.99 (d, 2H, py-H), 7.88 (t, 2H, py-H), 7.41 (m, 4H, py-H/benzyl-H), 4.12−3.91 (m, 4H, py-CH_2−NH), 2.75−2.17 (m, 5H, CH_2−CH−CH_2). ^{13}C NMR (100 MHz, CD_3OD) δ: 165.0, 160.2, 155.2, 149.0, 148.1, 139.5, 131.6, 125.6, 124.6, 123.4, 59.5, 51.9, 39.9, 39.7. HR-ESI-MS calcd. for $C_{23}H_{22}N_5O_6$: 464.1570; found: 464.1581 $[M - H^+]$.

[Ga(7)](NO3). 7 (2.5 mg, 5.3 μmol) was dissolved in water. $Ga(NO_3)_3 \cdot 6H_2O$ (2 mg, 5.5 μmol) was added, and the pH was adjusted to 5 by addition of 0.1 M NaOH. The reaction mixture was stirred at 60 °C for 2 h. The solvent was removed *in vacuo* to afford an off-white solid in quantitative yield. ^{1}H NMR (300 MHz, CD_3OD) δ: 8.62 (t, 2H, py-H), 8.36 (d, 2H, benzyl-H), 8.20−8.09 (m, 4H, py-H), 7.53 (d, 2H, benzyl-H), 4.81−4.39 (m, 4H, py-CH_2-NH), 3.59−2.21 (m, 5H, CH_2-CH-CH_2). ^{13}C NMR (150 MHz, CD_3OD) δ: 165.5, 165.4, 152.1, 151.6, 148.8, 147.4, 147.3 146.1, 145.9, 145.3, 131.8, 129.1, 129.0, 125.1, 124.7, 124.6, 58.9, 53.7, 51.5, 37.6. HR-ESI-MS calcd for $C_{23}H_{21}{}^{69}GaN_5O_6$: 532.0748.; found: 532.0743 $[M]^+$.

[67/68Ga(7)]+. $^{67}GaCl_3$ or $^{68}Ga^{3+}$ (100 μL, 1 mCi) in a 0.1 M HCl solution was added into 10^{-4} M solution of **7** in 10 mM NaOAc

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solution (pH 4) and left to react for 10 min at room temperature. The reaction was monitored by analytical HPLC which showed that the reaction had proceeded to 98%. Product t_r on HPLC: 7.7 min (gradient: A - NaOAc buffer, pH 4.5; B - CH_3OH, 0−100% B linear gradient 20 min). For the *apo*-transferrin competition, $^{67}GaCl_3$ was added to 10^{-4} M solution of **7** in 10 mM NaOAc solution (pH 4.5). Complex formation was checked on HPLC (peptide column). A 400-μL aliquot was added to a 1 mg/mL *apo*-transferrin $NaHCO_3$ solution (10 mM, 600 μL) and incubated at 37 °C (water bath). Complex stability was checked at time points 10 min, 1 h, and 2 h via analytical HPLC. The complex was 97% intact after 2 h.

Solution Thermodynamics. Carbonate-free solutions of the titrant, NaOH, were prepared by dilution of 50% solution (Acros Organics) with freshly boiled MQ water under a stream of purified nitrogen gas. The solution was standardized with potassium acid phthalate, and the extent of carbonate accumulation was periodically checked by titration with a standard hydrochloric acid solution and determination of the corresponding Gran titration plot.[19] Gallium ion solutions were prepared by dilution of the appropriate atomic absorption (AA) standard. The exact amount of acid present in the gallium standard was determined by titration of an equimolar solution of Ga and Na_2H_2EDTA. The amount of acid present was determined by Gran's method.[19]

Potentiometric titrations were performed using a Metrohm Titrando 809 equipped with a Ross combination pH electrode and a Metrohm Dosino 800. Data were collected in triplicate using PC Control (Version 6.0.91, Metrohm). The titration apparatus consisted of a 10 mL water-jacketed glass vessel maintained at 25.0 ± 0.1 °C (Julabo water bath). Prior to and during the course of the titration, a blanket of nitrogen, passed through 10% NaOH to exclude any CO_2, was maintained over the sample solution. The ionic strength was maintained at 0.16 M using NaCl. Prior to each potentiometric equilibrium study, the electrode was calibrated using standard HCl solutions. Calibration data were analyzed by standard computer treatment provided within the program MacCalib[20] to obtain the calibration parameters E_0 and pK_w.

As the degree of formation of Ga(III) complexes at low pH (<2) was too high for the determination of stability constants by use of direct potentiometry, the ligand−ligand competition method using Na_2H_2EDTA was also performed. Equilibrium was rapidly established (less than 10 min); however, up to 15 min was permitted between each titration point. The four successive proton dissociation constants corresponding to hydrolysis of Ga(III) aqueous ion included in the calculations were taken from Baes and Mesmer.[21] The protonation constants of H_2dedpa and stability constants of Ga(III) were calculated from the experimental data using Hyperquad 2008.

Biodistribution Data. The protocol used in the animal studies was approved by the Institutional Animal Care Committee of the University of British Columbia and was performed in accordance with the Canadian Council on Animal Care Guidelines. A total of 16 female ICR (20−30 g) mice were used for the animal study of each of the three compounds. $[^{67}Ga(dedpa)]^+$, $[^{67}Ga(\mathbf{3})]^+$, or $[^{67}Ga(\mathbf{7})]^+$ was prepared as described above and then diluted in phosphate-buffered saline to a concentration of 100 μCi/mL. Each mouse was i.v. injected with ∼10 μCi (100 μL) of the ^{67}Ga complex and then sacrificed by CO_2 inhalation at 30 min, 1 h, 2 h, or 4 h after injection ($n = 4$ at each time point). Blood was collected by cardiac puncture, and plasma was separated from whole blood by centrifuging (2500 rpm, 15 min). Urine was collected from the bladder. Tissues collected included kidney, liver, spleen, femur, muscle, heart, lung, intestine, and brain. Tissues were weighed and counted on a gamma counter, and the counts were converted to % injected dose/gram (%ID/g).

X-ray Crystallography. Data for compound $[Ga(dedpa)][ClO_4]$ were collected with graphite-monochromated Mo Kα radiation (0.71073 Å) at −17 °C on a Bruker X8 APEX II diffractometer. The structure was solved using direct methods using SIR-97[22] and refined using SHELXL-97.[23] All non-hydrogen atoms were refined anisotropically. All N−H hydrogen atoms were located in a difference map and refined isotropically. All other hydrogen atoms were placed in calculated positions and refined using a riding model. Data for compound $[Ga(\mathbf{7})]ClO_4$ were collected with graphite-monochromated Mo Kα radiation at −183 °C on a Bruker APEX DUO diffractometer. The structure was solved using direct methods using SIR-97[22] and refined using SHELXL-97.[23] The material crystallizes with two crystallographically independent moieties in the asymmetric unit. One perchlorate anion is disordered and was modeled in two orientations, with restraints used to maintain reasonable geometries. Finally, MeOH solvent was found in the lattice. Two molecules of solvent were located and modeled; however, one region within the asymmetric unit had residual electron density that could not be properly modeled. The SQUEEZE[24] program was used to generate a data set free of residual electron density in that region. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in calculated positions and refined using a riding model. A summary of the relevant crystallographic data for both compounds can be found in the Supporting Information.

Results and Discussion

In this contribution we report for the first time our study of a linear platform chelate that has optimal properties in its chelation of gallium. The use and the synthesis of H_2dedpa (originally[25] named H_2bpce) has been previously reported with divalent metals showing reasonable chelation properties.[25] In the 1990s, our research group expended significant effort investigating tripodal, nonmacrocyclic ligands containing amines, pyridines, and carboxylates showing moderate chelation properties for Ga(III),[26] but dedpa^{2-} exceeds them all in its properties of fast chelation with high specific activity at room temperature.

Most recently, we have synthesized a library of linear hexadentate chelate ligands, and screened them for their ability to bind ^{67}Ga (a longer-lived Ga isotope that can serve as a model for ^{68}Ga). Under mild reaction conditions (room temperature, aqueous buffer, pH 4), H_2dedpa coordinated ^{67}Ga quantitatively within 10 min (as does NOTA[27]). DOTA however, requires heating for quantitative reaction yields.[28] Concentration-dependent coordination of H_2dedpa to both ^{68}Ga and ^{67}Ga at concentrations as low as 10^{-7} M showed quantitative conversion to the desired product. When coordinating to ^{68}Ga, high specific activities (as high as 9.8 ± 0.1 mCi nmol^{-1}) were obtainable in 99% radiochemical yield without any purification steps. This is the highest specific activity measured for any chelator with ^{68}Ga, when neither heating nor ^{68}Ga prepurification is used.[29]

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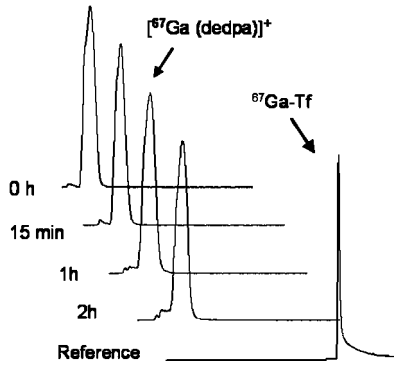


Figure 1. HPLC traces of $[^{67}Ga(dedpa)]^+$ *versus apo*-transferrin in competition; the trace of ^{67}Ga-transferrin is shown for reference (gradient: A - NaOAc buffer, pH 4.5; B - MeOH, 0−100% B linear gradient 20 min).

Table 1. Formation Constants (log K_{ML}) and pM^a of Ga(III) Complexes

ligand	log K_{ML}	pM
$dedpa^{2-}$	28.11(8)	27.4
EDTA[31]	21.7	18.3
DOTA[32]	21.33	18.5
NOTA[33]	30.98	27.9
transferrin[b]	20.3	21.3

[a] Calculated for 10 μM total ligand and 1 μM total metal at pH 7.4 and 25 °C. [b] Conditional constant for log K_{ML} from ref 30.

To investigate the stability of the ^{67}Ga radiochemical complex, a 2-h competition experiment was conducted in the presence of excess human *apo*-transferrin; the iron-sequestering/-transport protein that has very high affinity for Ga(III) (Figure 1).[30] The $[^{67}Ga(dedpa)]^+$ complex was fully intact after 2 h, suggesting that it should have very high *in vivo* stability, similar to that reported for ^{68}Ga NOTA complexes.[16] In a direct competition for chelation of ^{67}Ga with equal concentrations of both NOTA and H_2dedpa, over 96% was coordinated by $(dedpa)^{2-}$, less than 1% by NOTA, demonstrating the expected faster Ga complexation with the acyclic H_2dedpa than with macrocyclic NOTA.

Solution thermodynamic investigations of the corresponding cold complex $[Ga(dedpa)]^+$ have provided a complex stability constant of log K_{ML} = 28.11(8), obtained by ligand−ligand competition with EDTA using potentiometric titration. A more relevant indicator of the extent to which a metal complex is formed in solution is given by pM (−log[free M]) which considers the influence of ligand basicity and chelate hydrolysis. The values of log K_{ML} and pM of the Ga(III) complexes of $dedpa^{2-}$ and other relevant multidentate ligands are shown in Table 1. The high values of log K_{ML} and pM for $[Ga(dedpa)]^+$ confirm the high affinity of $dedpa^{2-}$ for Ga(III) as well as high thermodynamic stability.

The solid-state X-ray crystal structure (Figure 2) provides significant insight into the coordination environment of $[Ga(dedpa)]^+$. In comparison with the crystallized Ga complexes of NOTA[11] and DOTA[34] which have widely dispersed metal-to-ligand bond distances, $[Ga(dedpa)]^+$ has a more equally distributed array of bond lengths, suggesting that the unusually high stability of the complex is due to a near-perfect fit with the Ga^{3+} ion (for further information about structural data see the Supporting Information). Another characteristic that clearly differentiates $[Ga(dedpa)]^+$ from complexes with the macrocyclic chelators is the C_2 rotational axis, which has also been confirmed in solution through ^{13}C NMR spectroscopy.

To investigate different modes of functionalization analogous to the bifunctional versions of the macrocyclic chelators, two model compounds **3** and **7** have been synthesized (Scheme 2). Compound **3** displays derivatization through the two aliphatic nitrogens, affording a scaffold capable of carrying two targeting molecules, while compound **7** is derivatized through the backbone of the ethylenediamine (en) component of the basic ligand structure, retaining the original coordination environment more closely, but only capable of carrying one targeting molecule. Both **3** and **7** incorporate the nitrobenzyl functionality which can be converted easily into the corresponding amino- or isothiocyanato-benzyl, coupling moieties frequently employed for conjugation to target molecules via a free carboxylate or primary amine, respectively.[35,36]

Compound **3** is synthesized from 1,2-[{6-(methoxycarbonyl)pyridin-2-yl}methylamino]ethane,[17] which is then subsequently alkylated with 4-nitrobenzyl bromide (see the Supporting Information). Intermediate **2** is purified and the carboxylates are deprotected under standard conditions to afford the clean product **3** as a white solid. The complex with cold (nonradioactive) gallium(III) is formed within 2 h at pH 4−5 under gentle heating (Figure 3), and again the C_2 rotational axis was con-

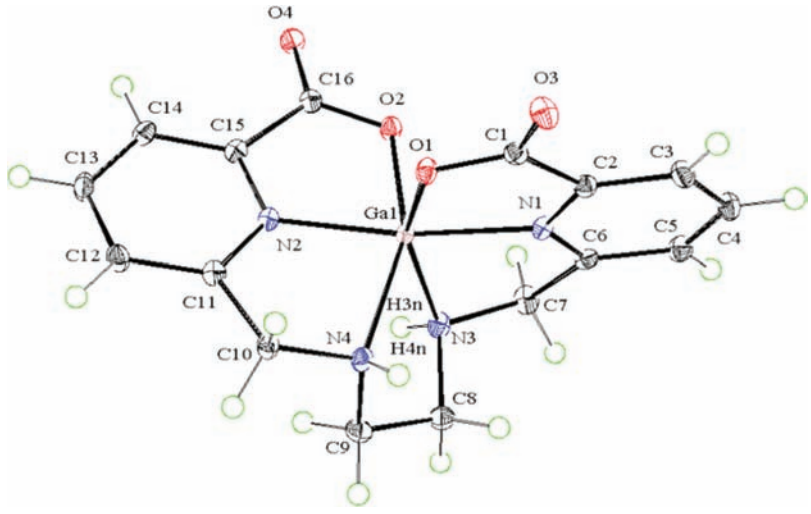
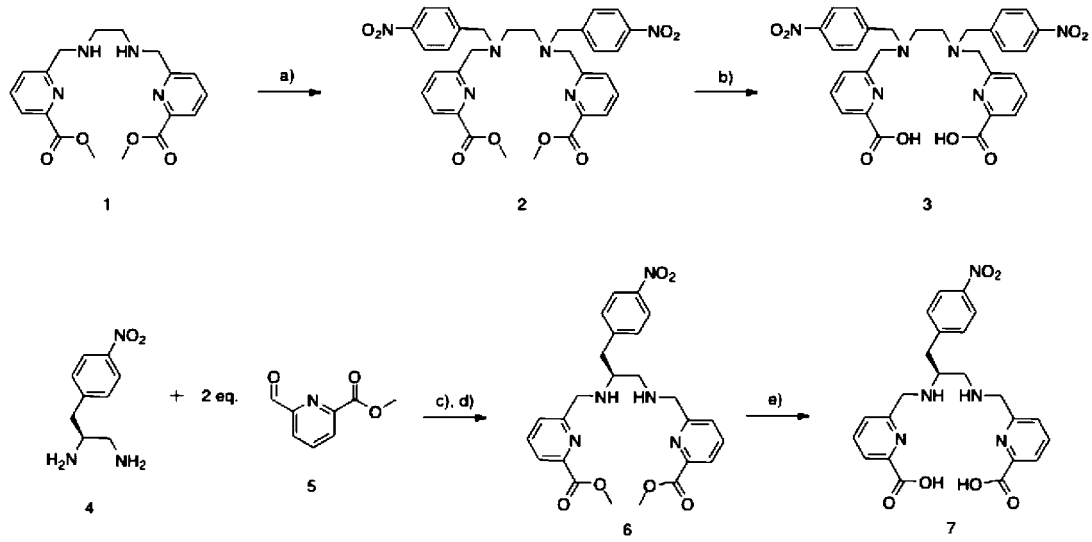


Figure 2. Solid-state structure of the cation in $[Ga(dedpa)][ClO_4]$; relevant bond lengths [Å]: N1−Ga: 1.9866(16); N2−Ga: 1.9902(16); N3−Ga: 2.1115(16); N4−Ga: 2.1132(16); O1−Ga: 1.9708(13); O2−Ga: 1.9828(13).

Scheme 2. Syntheses of Compounds **3** and **7**[a]



[a] Reagents and conditions: a) 4-nitrobenzyl bromine, Na_2CO_3, CH_3CN, 18 h; b) LiOH, THF/water (3:1), 45 min; c) CH_3OH, reflux, 2 h; d) $NaBH_4$, 0°C, 2 h; e) LiOH, THF/water (3:1), 30 min.

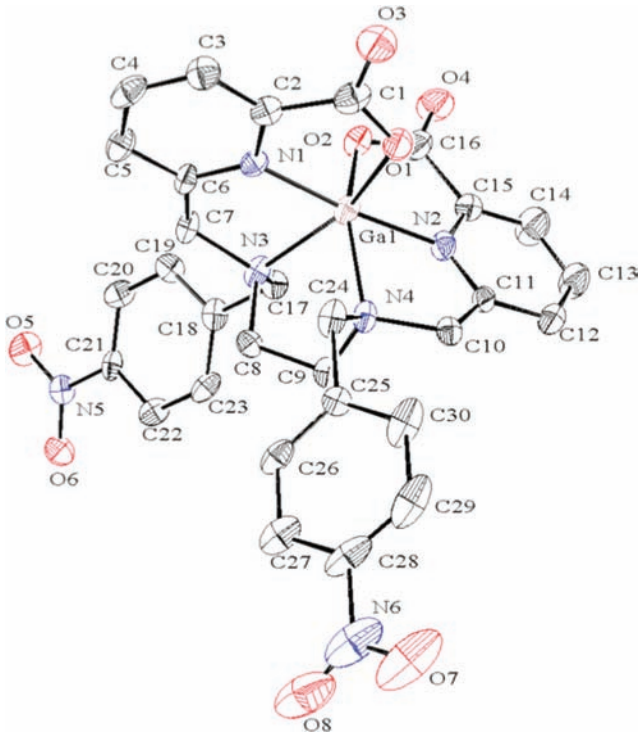


Figure 3. Solid state structure of the cation in [Ga(**3**)][ClO_4]; relevant bond lengths [Å]: N1−Ga: 1.992(5); N2−Ga: 1.981(5); N3−Ga: 2.188(5); N4−Ga: 2.159(5); O1−Ga: 1.967(4); O2−Ga: 1.976(4).

firmed in both the solid-state structure and the solution NMR spectrum (see the Supporting Information). Coordination to ^{67}Ga or ^{68}Ga forms the complex within 10 min at room temperature in 98% radiochemical yield. The subsequent *apo*-transferrin challenge experiment revealed 51% of the radiolabeled complex remained intact after 2 h in the presence of excess *apo*-transferrin; a stability inferior to that of [Ga(dedpa)]$^+$, but comparable to that of Ga-DOTA (for data see the Supporting Information).[16] Concentration-dependent coordination to ^{68}Ga showed that **3** is capable of coordinating under standard, mild conditions at concentrations as low as 10^{-6} M (Table 2).

Table 2. Stability of Investigated Chelators at Various Times in the 2 h Competition Experiment in the Presence of Excess Human *apo*-Transferrin

ligand	10 min (%)	1 h (%)	2 h (%)
H_2dedpa	>99	>99	>99
3	88	69	51
7	98	97	97

Compound **7** is furnished through a different route; **4** is derived from 4-nitro-L-phenylalanine,[18] while **5** is afforded through a four-step synthesis from 2,6-pyridinedicarboxylic acid.[17] A one-pot reductive amination process produces **6** in moderate yields along with a mixture of impurities, which can be separated from the product through column chromatography. The subsequent deprotection leads to **7** as a light-orange solid. The complex with cold gallium(III) is formed within 2 h at pH 4−5 under gentle heating, whereas the ^{67}Ga and ^{68}Ga complexes are formed within 10 min at room temperature in 97% radiochemical yield. The subsequent *apo*-transferrin challenge experiment reveals a stability comparable to that of [Ga(dedpa)]$^+$, with over 97% of the complex remaining intact after 2 h (traces, see Supporting Information). Concentration-dependent coordination to ^{68}Ga showed that **7** is capable of coordinating under standard, mild conditions at concentrations as low as 10^{-6} M much like **3**.

The biodistribution study in mice (Figure 4, raw data in the Supporting Information) indicated that [^{67}Ga(dedpa)]$^+$ cleared from the background tissue, such as muscle, within the first 30 min and was excreted mainly through the kidneys. The *in vivo* stability of [^{67}Ga(dedpa)]$^+$ was supported by the low uptake in bone, which is known to be a site of increasing accumulation for weakly chelated ^{67}Ga.[37] The overall biodistribution profile compares well to that of macrocyclic chelators evaluated in a

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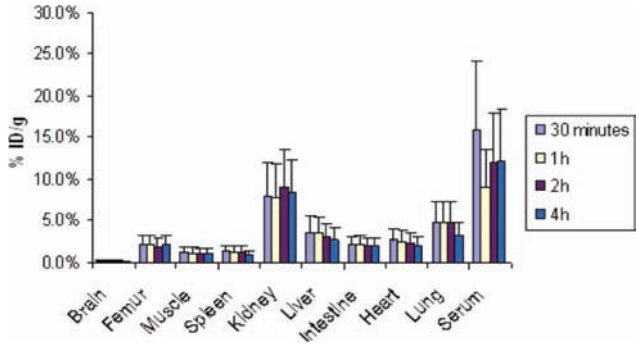

Figure 4. Biodistribution over 4 h of $[^{67}Ga(dedpa)]^+$ in female ICR mice.

similar study,[16] also exhibiting the low uptake in liver and intestines that is characteristic of ionic compounds. The persistent high uptake in the blood serum was not confirmed with the derivatized compounds, suggesting that added functionality influences biodistribution. Serum stability studies done *in vitro* confirmed that $[^{67}Ga(dedpa)]^+$ was stable to transchelation by serum proteins.

In the biodistribution studies for $[^{67}Ga(\mathbf{3})]^+$ and $[^{67}Ga(\mathbf{7})]^+$, whole blood was collected instead of serum, and urine was collected as an additional data point (Figure 5, raw data in the Supporting Information). Both $[^{67}Ga(\mathbf{3})]^+$ and $[^{67}Ga(\mathbf{7})]^+$ exhibit improved clearance from all organs, low bone uptake (indicator for complex stability) and excretion through urine. Despite lower *in vitro* stability, the biodistribution of $[^{67}Ga(\mathbf{3})]^+$ suggests high stability *in vivo* and shows better clearance from blood and kidneys than both $[^{67}Ga(\mathbf{7})]^+$ and $[^{67}Ga(dedpa)]^+$. It is possible that compounds containing secondary amines associate stronger with blood serum proteins and kidney tissue; however, the difference in biodistribution of even $[^{67}Ga(\mathbf{7})]^+$ and $[^{67}Ga(dedpa)]^+$ shows that added functionalities, such as peptides or other targeting vectors, have a great impact on the interaction of these compounds with *in vivo* systems.

Conclusion

H_2dedpa complexes quickly with Ga and forms complexes of very high stability, comparing well to the widely used macrocyclic chelator NOTA and exceeding the properties of DOTA. H_2dedpa and its derivatives can be coordinated to Ga isotopes under mild room-temperature conditions at high specific activities in short reaction times, making it an ideal scaffold for further elaboration and applications such as peptide labeling. The high radiochemical yield and high specific activity of the products could obviate the need for time-consuming HPLC purification, a major advantage for the short-lived isotope, ^{68}Ga. In addition, the biodistributions of $[^{67}Ga(dedpa)]^+$, $[^{67}Ga(\mathbf{3})]^+$,

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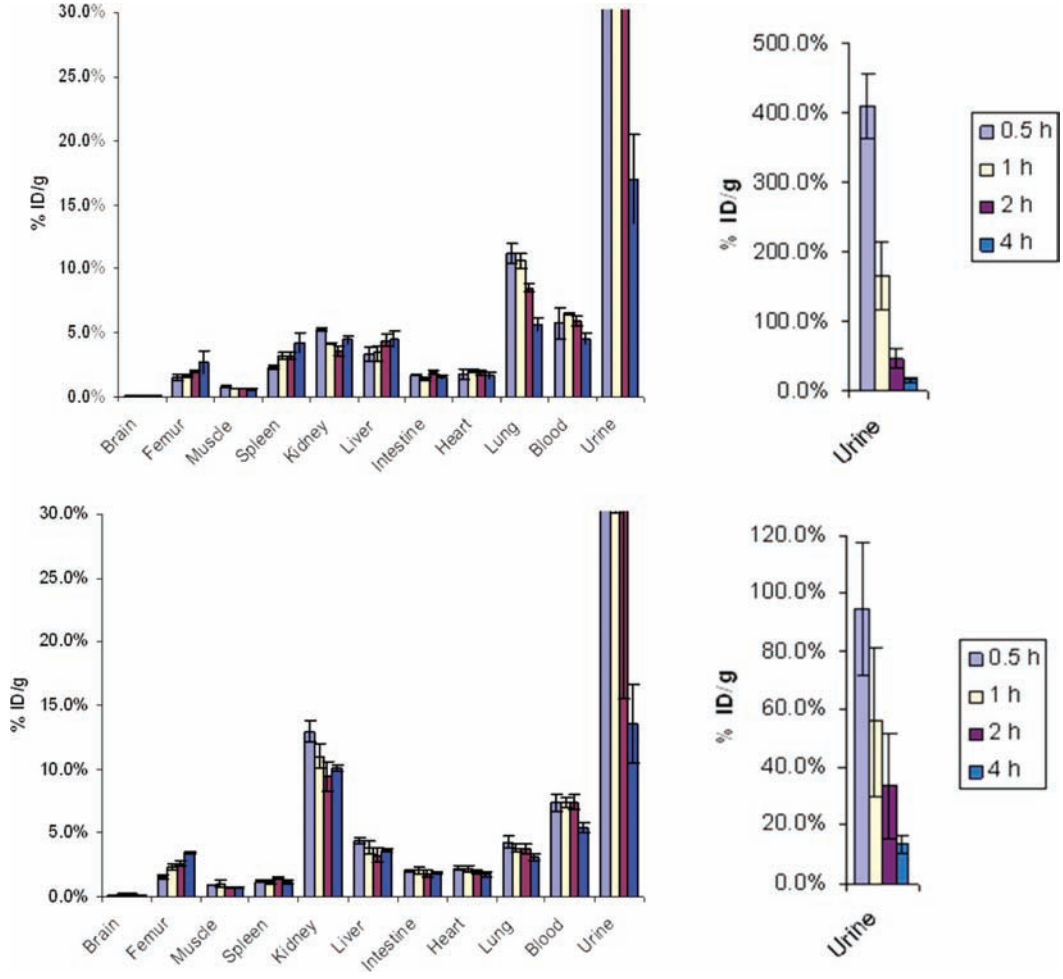

Figure 5. Biodistribution of $[^{67}Ga(\mathbf{3})]^+$ (above) and $[^{67}Ga(\mathbf{7})]^+$ (below) in female ICR mice over 4 h; complete data for urine is also shown in separate diagrams to the right.

and [^{67}Ga(**7**)]$^{+}$ confirm the stability of the complexes measured *in vitro* with general clearance, rendering these frameworks a good basis for elaboration of new Ga bioconjugates.

It is important to note that many of the advantageous properties described have been observed previously with only one macrocyclic chelate (NOTA), while they are unexpected for an acyclic system. We are currently applying these results to small biomolecules and other radiometals to evaluate the true potential of this exciting chelation scaffold.

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Supporting Information Available: Detailed information on potentiometric measurements, X-ray structure determinations (including tables of bond lengths and angles), detailed HPLC radio-traces, ^{1}H NMR spectra of key compounds, and detailed data of all biodistribution studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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