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Formation and Characterization of Gallium(III) Complexes with Monoamide Derivatives of 1,4,7-Triazacyclononane-1,4,7-triacetic Acid: A Study of the Dependency of Structure on Reaction pH

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Two monoamide derivatives of 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) conjugated with methylamine (**4**) or benzylamine (**5**) were synthesized by treating di-*tert*-butyl 1,4,7-triazacyclononane-1,4-diacetate (**1**) with 2-chloro-*N*-benzyl- or -*N*-methylacetamide, followed by an acid cleavage reaction. Complexes of **4** and **5** chelated to Ga^{3+} to give Ga-**4** and Ga-**5**, respectively, in reaction solutions at different pH values (3 and 5). Complexes Ga-**4** and Ga-**5** were characterized by single-crystal X-ray diffraction and multinuclear NMR spectroscopy. In the solid state, these complexes were isostructural, and the coordination spheres of the metal ions exhibited distorted octahedral geometries. In the case of the Ga-**4** complex, which was formed at both pH values, the metal ion is coordinated to the amide nitrogen atom of the modified pendent arm of **4**. However, in the case of Ga-**5**, the metal ion is coordinated to a nitrogen or an oxygen atom of the amide linkage when the pH of the reaction soultion was 5 or 3, respectively. No significant difference was found between the ^{1}H NMR spectra of the complexes formed at pH = 3 and 5. However, ^{71}Ga NMR spectra showed a broad resonance signal and a narrow singlet for the complex synthesized at the lower pH, but only a single narrow singlet for the complex prepared at neutral pH. Variable-temperature ^{1}H NMR spectra showed that complexes Ga-**4** and Ga-**5** are rigid in solution. The stability of these complexes in the physiological pH range and at high temperature suggests that NOTA can be used as a bifunctional chelating agent to label biomolecules with radioactive gallium by direct conjugation of the complex to target molecules. This finding could open up a wide range of applications for NOTA-type bifunctional chelating agents by eliminating the multi-step synthesis routes required to introduce extra linker groups to the labelling agent.

Introduction

Heterocyclic compounds such as NOTA and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) have been used as bifunctional chelating agents to label peptides and proteins with radioactive metals.[1] In the present study, we concentrated on NOTA derivatives because they produce extremely stable complexes with ^{68}Ga,[1b,2] which was recently reported to have a huge potential in medical applications.[3] DOTA contains four amine groups and four carboxylic acid groups that can act as coordination sites, but only six of these (four amine and two carboxylic acid groups) are involved in complex formation to produce a hexacoordinated chelate complex with Ga^{III},[4] which allows for the conjugation of a biomolecule to DOTA by formation of an amide bond between the biomolecule and one of the free DOTA carboxylic acid groups (Figure 1).

Ligand NOTA also forms a hexacoordinated chelate with Ga^{III}.[5] However, metal–NOTA chelates contain no free carboxylic acid groups for conjugation with a biomolecule. Thus, all reported chelating agents based on NOTA include a branched linker such as isothiocyanate or an extra acetic acid group. For example, isothiocyanatobenzyl-1,4,7-triazacyclononane-1,4,7-triacetic acid (SCN-NOTA) has an isothiocyanate residue, and 1,4,7-triazacyclononane-1,4-diacetic-7-succinic acid (NODASA) has a branched acetic acid group (Figure 1).[1c,6] However, it should be noted that these NOTA derivatives require cumbersome, low-yielding, multi-step syntheses, and thus they are intrinsically costly to produce.

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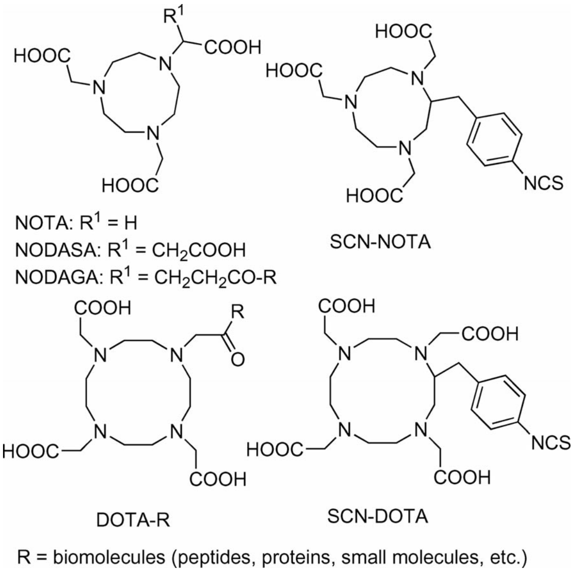


Figure 1. NOTA and DOTA derivatives as bifunctional chelating agents.

The potential benefit to medicine of positron emission tomography (PET) and ^{68}Ga-based radiopharmaceuticals is driving the developments of chelate complexes that can undergo a wide range of chemical modifications.[7] Recent studies have shown that some ^{68}Ga-labelled peptides exhibit distinctly better imaging properties than their ^{111}In-labelled analogues[4a,8] and ^{18}F-based radiotracers.[9] One important characteristic of ^{68}Ga is its cyclotron-independent availability by the ^{68}Ge/^{68}Ga generator system. Isotope ^{68}Ga is an excellent positron emitter, with 89% positron branching accompanied by low photon emission (1077 keV, 3.22%).[10] Unlike other PET radioisotopes such as ^{18}F or ^{11}C, ^{68}Ga cannot bind covalently to target vectors. Instead it must be complexed with a bifunctional chelating agent (BCA) prior to being conjugated to a vector, which has the advantage that labeling can be done straightforwardly, and immediately before diagnostic examinations, thus the loss of radioactivity is minimized.

The purpose of the present study was to determine whether NOTA derivatives, in which one of the carboxy residues is conjugated with an amine, can make stable complexes with gallium. To investigate this possibility, we synthesized gallium complexes of NOTA derivatives that have methylamine or benzylamine conjugated with one of their three carboxylic acid groups. The coordination chemistry was studied by crystallography and multinuclear NMR spectroscopy conducted with sample solutions at different pH values.

Results and Discussion

Synthesis and Characterization

We synthesized monoconjugated NOTA derivatives by treating 1 equiv. of **1** with 1.1 equiv. of 2-chloro-*N*-methyl- or -*N*-benzylacetamide in the presence of K_2CO_3 (Scheme 1). The Ga^{III} complexes were obtained from aqueous solutions of the ligands **4** or **5** and $Ga(NO_3)_3$ by heating the reaction solutions, which were at different pH values (solutions were adjusted to pH = 3 or 5 by using 0.5 M Na_2CO_3), in a boiling water bath for 15 min. The complexes were purified by preparative HPLC and were then dissolved in an ethanol/water mixture. Crystals were obtained by slow evaporation of the solvents.

X-ray crystallography data showed no pH-dependant structural features for Ga-**4** (Figure 2), whereas the structures of Ga-**5** differed when prepared at pH = 3 and 5 (Figure 3). Crystal data collection and refinement details for all compounds are listed in Tables 1 and 2. A gallium ion fits perfectly inside the nitrogen and oxygen coordination cavity of NOTA. When NOTA derivatives with methylamine or benzylamine bound to one of the pendent acetic acid groups were introduced into the structure, one metal coor-

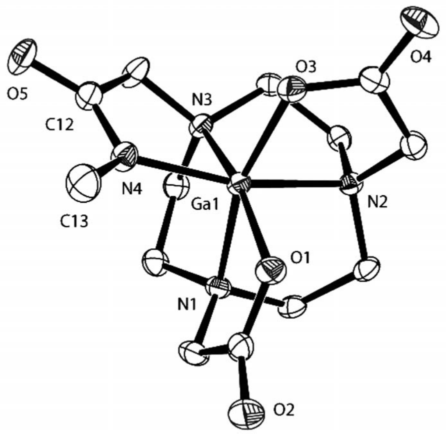
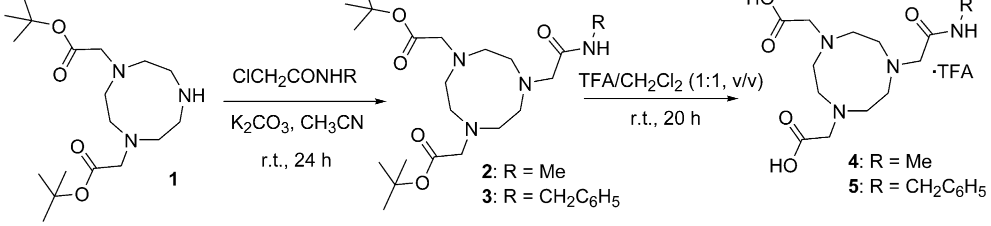


Figure 2. Molecular structure of Ga-**4** chelated at pH = 3 and 5 (complex structures were identical). Hydrogen atoms and counterions have been omitted for clarity.



Scheme 1. Synthesis of ligands **4** and **5**.

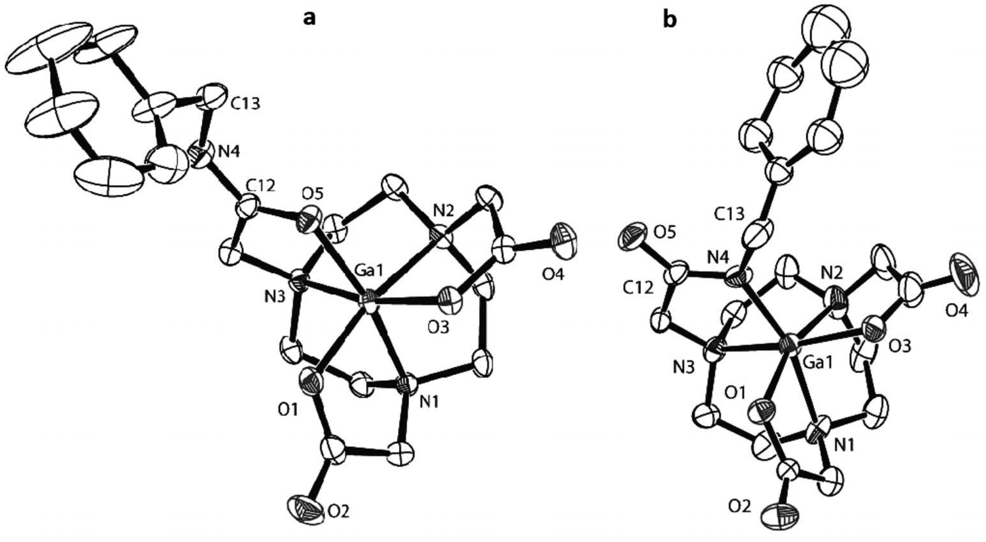


Figure 3. Molecular structures of Ga-**5** chelated at pH = 3 (a) and pH = 5 (b). The gallium atom is coordinated to the oxygen atom (O5) of the amide group when the complex is formed at pH = 3 and to the nitrogen atom (N4) of the amide group when formed at pH = 5. Hydrogen atoms and counterions have been omitted for clarity.

dinating bond should be established between the metal ion and either an amide nitrogen (N4) or an amide oxygen (O5) atom.

When chelation was performed at pH = 3, **4** showed gallium coordination with N4 (Figure 2), whereas **5** showed coordination with O5 (Figure 3a). On the other hand, when prepared at pH = 5, both complexes showed metal binding to N4 along with five other donor atoms to form a hexadentate complex (Figure 3b). The coordination of the amide oxygen atom at low pH might be due to protonation of O5, leading to the formation of an imide bond due to isomerism that also causes the double bond character of the carbon–nitrogen bond. It is noteworthy that the oxygen atom of the amide group always coordinates with gallium in the case of NOTA and [(nitroimidazolyl)ethyl]amine conjugates.[11] The coordination geometries for the metal centers in both Ga-**4** and Ga-**5** formed at both chelating pH values were distorted octahedra with three amine-N atoms occupying one face and the two carboxylate O-atoms and either N4 or O5 occupying the opposite face. Distortion of the coordination spheres from a regular octahedron was evident from the compression of the N–Ga–N angles (average angles are 83.22° for Ga-**4** and 84.35° for Ga-**5**) and expansion of the O1–Ga–O3, N4–Ga–O and O5–Ga–O angles [O1–Ga–O3 94.22(8)°, N4–Ga–O 96.99° (av.) for Ga-**4**; O1–Ga–O3 95.39(8)° and O5–Ga–O 94.65° (av.) for Ga-**5**] from the ideal value of 90°. As a result, in Ga-**4** the *trans* angles of N1–Ga–O3, N2–Ga–N2 and N3–Ga–O1 are 164.23(8)°, 163.76(8)°, and 165.30(9)°, respectively. In Ga-**5** formed at pH = 3 the three *trans* N–Ga–O angles are 165.01(9)°, 168.15(10)°, and 166.71(8)° for N1–Ga–O5, N2–Ga–O1, and N3–Ga–O3, respectively.

The average Ga–N bond length is 2.116 Å in Ga-**4** prepared at pH = 5, which is slightly longer than that in Ga(NOTA) (av. Ga–N 2.090 Å),[5c,12] and in the case of Ga-**5** prepared at pH = 3, the average length was exactly the same as for Ga(NOTA), indicating the structural similarity between Ga-**5** and Ga(NOTA). The average Ga–O bond

Table 1. Selected bond lengths [Å] and bond angles [°] for complexes Ga-**4** and Ga-**5**.[a]

	Ga-**4**·H_2O (pH = 5)	Ga-**5**·CF_3COO·H_2O (pH = 3)
Length [Å]		
Ga–N1	2.103(2)	2.084(2)
Ga–N2	2.123(2)	2.081(2)
Ga–N3	2.122(2)	2.105(2)
Ga–O1	1.9601(16)	1.9135(19)
Ga–O3	1.9313(19)	1.9218(17)
Ga–N4	1.948(2)	–
Ga–O5	–	1.963(2)
N4–C12	1.325(3)	1.294(4)
O5–C12	1.243(3)	1.274(3)
N4–C13	1.464(3)	1.465(4)
Angle [°]		
N1–Ga–N2	83.49(9)	84.71(9)
N2–Ga–N3	82.69(8)	84.40(8)
N3–Ga–N1	83.49(9)	83.95(9)
O1–Ga–N1	81.84(8)	83.92(9)
O1–Ga–N2	96.58(7)	168.15(10)
O1–Ga–N3	165.30(9)	97.72(8)
O1–Ga–N4	99.47(8)	–
O1–Ga–O3	94.22(8)	95.39(8)
O1–Ga–O5	–	93.95(9)
O3–Ga–N1	164.23(8)	99.63(9)
O3–Ga–N2	81.79(8)	83.21(8)
O3–Ga–N3	100.19(8)	166.71(8)
O3–Ga–N4	94.50(9)	–
N4–Ga–N1	101.20(9)	–
N4–Ga–N2	163.76(8)	–
N4–Ga–N3	82.42(8)	–
O5–Ga–N1	–	165.01(9)
O5–Ga–N2	–	97.89(9)
O5–Ga–N3	–	81.63(9)
O5–Ga–O3	–	95.34(8)

[a] Data for Ga-**5**·$2H_2O$ (pH = 5) are not included because of the disorder at the benzyl and solvent positions.

Table 2. Summary of the crystallographic data obtained for complexes Ga-**4** and Ga-**5**.

	Ga-**4**·H_2O (pH = 3)	Ga-**4**·H_2O (pH = 5)	Ga-**5**·CF_3COOH·H_2O (pH = 3)	Ga-**5**·H_2O (pH = 5)
Emprical formula	$C_{13}H_{23}GaN_4O_6$	$C_{13}H_{23}GaN_4O_6$	$C_{21}H_{28}F_3GaN_4O_8$	$C_{19}H_{29}GaN_4O_7$
Formula mass	401.07	401.07	591.19	495.18
Space group	$P2_1/n$	$P2_1/n$	$P2_1/n$	$Pbca$
Crystal size [mm]	0.17 × 0.10 × 0.06	0.27 × 0.21 × 0.18	0.27 × 0.24 × 0.19	0.34 × 0.25 × 0.17
a [Å]	7.5577(4)	7.5581(4)	8.9067(3)	10.3789(3)
b [Å]	15.0490(12)	15.0604(7)	23.1929(11)	14.2489(4)
c [Å]	14.0340(12)	14.0363(7)	11.8265(4)	32.3078(6)
α [°]	90	90	90	90
β [°]	94.992(5)	94.984(3)	93.237(2)	90
γ [°]	90	90	90	90
V [Å^3]	1590.1(2)	1591.68(14)	2439.13(16)	4777.9(2)
Z	4	4	4	8
$\rho_{\text{calcd.}}$ [g/cm^3]	1.675	1.674	1.610	1.377
μ [mm^{-1}]	1.770	1.769	1.206	1.196
F(000)	832	832	1216	2064
T [K]	293(2)	293(2)	293(2)	293(2)
θ range [°]	$3.03 \leq \theta \leq 27.41$	$3.25 \leq \theta \leq 27.47$	$2.45 \leq \theta \leq 27.47$	$1.26 \leq \theta \leq 27.58$
Index range	$-9 \leq h \leq 9$ $-19 \leq k \leq 19$ $-18 \leq l \leq 17$	$-9 \leq h \leq 9$ $-17 \leq k \leq 19$ $-18 \leq l \leq 18$	$-11 \leq h \leq 11$ $-27 \leq k \leq 30$ $-15 \leq l \leq 15$	$-13 \leq h \leq 13$ $-18 \leq k \leq 18$ $-41 \leq l \leq 41$
Total reflections	9314	6548	9861	10163
Unique reflections	3548	3628	5559	5486
R_{int}	0.4353	0.0392	0.0608	0.0469
Transmission factors	0.901–0.753	0.741–0.647	0.803–0.737	0823–0.687
Data/restraints/parameters	3548/0/226	3628/0/226	5559/0/419	5486/0/275
Goodness of fit on F^2	0.986	1.019	0.971	1.963
R indices [$I > 2\sigma(I)$]	R = 0.0568, R_w = 0.0903	R = 0.0382, R_w = 0.0739	R = 0.0469, R_w = 0.0726	R = 0.1495, R_w = 0.4682
R indices (all data)	R = 0.1235, R_w = 0.1073	R = 0.0658, R_w = 0.0817	R = 0.1041, R_w = 0.0848	R = 0.1843, R_w = 0.4838
Largest difference peak/hole [e/Å^3]	0.520/–0.362	0.308/–0.356	0.327/–0.322	1.918/–2.540

lengths in Ga-**4** formed at pH = 5 and in Ga-**5** formed at pH = 3 are 1.946 Å and 1.933 Å, respectively. These values are comparable to that of Ga(NOTA) (av. Ga–O 1.930 Å). The C12–N4 (bond between the amide carbon and nitrogen atoms) bond length is 1.294(4) Å for Ga-**5** synthesized at pH = 3, which was shorter than the length of the same bond in Ga-**4** prepared at pH = 5 [1.325(3) Å]. Also, the C12–O5 bond length in Ga-**5** formed at pH = 3 is 1.274(3) Å, which is slightly longer than the same bond in Ga-**4** formed at pH = 5 [1.243(3) Å]. These findings provide evidence of –N=C–O–M bond formation when the gallium atom coordinates to the oxygen atom of the amide group.

NMR Characterization

The structures of Ga-**4** and Ga-**5** in aqueous solution were elucidated by NMR spectroscopy (^{1}H, ^{13}C, NOESY, HSQC, and ^{71}Ga). Assignments of resonance signals were based on NOESY and HSQC experiments conducted at 25 °C (Figures S6–S9). The proton resonance signals from the macrocyclic backbones were tentatively assigned, due to superimposed axial and equatorial proton signals, in the ^{1}H NMR spectra of Ga-**4** and Ga-**5** in D_2O recorded at 25 °C. No significant differences were observed in the ^{1}H and ^{13}C NMR signals of isolated Ga-**4** compounds synthesized at pH = 3 and at 5 (Figure S2, S3) and redissolved in D_2O for analysis (NMR sample: pH ≈ 7). In the ^{1}H NMR spectrum of Ga-**5**, the methylene hydrogen atoms of the benzyl group showed 2 distinct peaks centered at δ = 3.6 ppm due to the flexibility of the benzyl group when the complex is prepared at pH = 3, which changed to a single peak at δ = 3.63 ppm in the spectrum for Ga-**5** that is formed at pH = 5 (Figure S4). The ^{13}C NMR spectra of Ga-**5** showed a shift of the benzylic methylene carbon signal from δ = 46.45 ppm for Ga-**5** prepared at pH = 5 to δ = 54.48 ppm for Ga-**5** formed at pH = 3 due to differences in the double-bond character of the amide group (Figure S5). NOESY studies showed clearly a coupling interaction between the axial and equatorial hydrogen atoms of the ring methylene groups, along with a coupling interaction between the axial hydrogen atoms of the ring methylene groups and the methylene hydrogen atoms of pendent arms, which was also observed for Ga–NOTA.[13] The ^{1}H NMR spectrum of Ga-**5** showed a doublet at δ = 3.49 ppm, which was assigned to two methylene protons on the modified pendent arm. The different orientations of these protons suggests that the substituted benzyl group is flexible.

^{71}Ga NMR spectroscopic analysis of these complexes was performed in D_2O over the pH range of 3–9 (Figure 4). In the case of Ga-**4**, a small broad signal centered at δ = 175.2 ppm ($\omega_{1/2}$ ≈ 9000 Hz) and a relatively narrow singlet at δ = 171.1 ppm ($\omega_{1/2}$ = 283 Hz) were observed in the spectra recorded at low pH. These broad signals disappeared with increasing pH, and only a narrow singlet remained in the spectrum collected at pH = 7. In the spectrum collected at pH = 9, a singlet corresponding to $[Ga(OH)_4]^-$ (δ = 223 ppm)[14] was observed. Similar findings were obtained for Ga-**5**. At low pH, a broad signal at δ = 173.8 ppm ($\omega_{1/2} \approx$ 4000 Hz) was observed, along with a narrow singlet at δ = 170.1 ppm ($\omega_{1/2} \approx$ 279.1 Hz). This broad signal decreased in intensity as the pH increased, but did not disappear at pH = 7. At high pH values, this broad signal disappeared, and a signal corresponding to $[Ga(OH)_4]^-$ was observed in addition to a narrow signal that was assigned to Ga-**5**. Furthermore, the δ values of these complexes were found to be close to that of the Ga(NOTA) complex (δ = 171 ppm).[13] The ^{71}Ga NMR data indicated the presence of two species in solution, possibly one of which includes bound N_3O_3 (narrow signal), and the other must include a bound N_4O_2 species (broad signal) due to differences in the symmetry of the complexes. The ^{71}Ga NMR study provides a clue to the geometry of the complex, which shows the chemical shift and the symmetry of a complex, since the line width is extremely sensitive to molecular symmetry about the nucleus due to the rapid relaxation of the quadrupolar nuclei, especially when the nucleus is interacting with asymmetric electric field gradients. The widths of the ^{71}Ga NMR signals were determined by quadrapolar relaxation induced by the electric field gradients in the distorted Ga^{3+} coordination polyhedra, and these provide a sensitive measure of the degree of distortion of the complexes in solution when corrected for changes in the rotational correlation time. Based on our findings, the widths of the peaks for Ga-**4** and Ga-**5** are between those for Ga(NOTA) ($\omega_{1/2}$ = 210 Hz) and Ga(NOTP) [NOTP = 1,4,7-triazacyclononane-*N*,*N′*,*N′′*-tris(methylenephosphonic) acid] ($\omega_{1/2}$ = 434 Hz) indicating the presence of stable Ga^{3+} complexes in aqueous solution, possibly with an octahedral or pseudo-octahedral geometry.

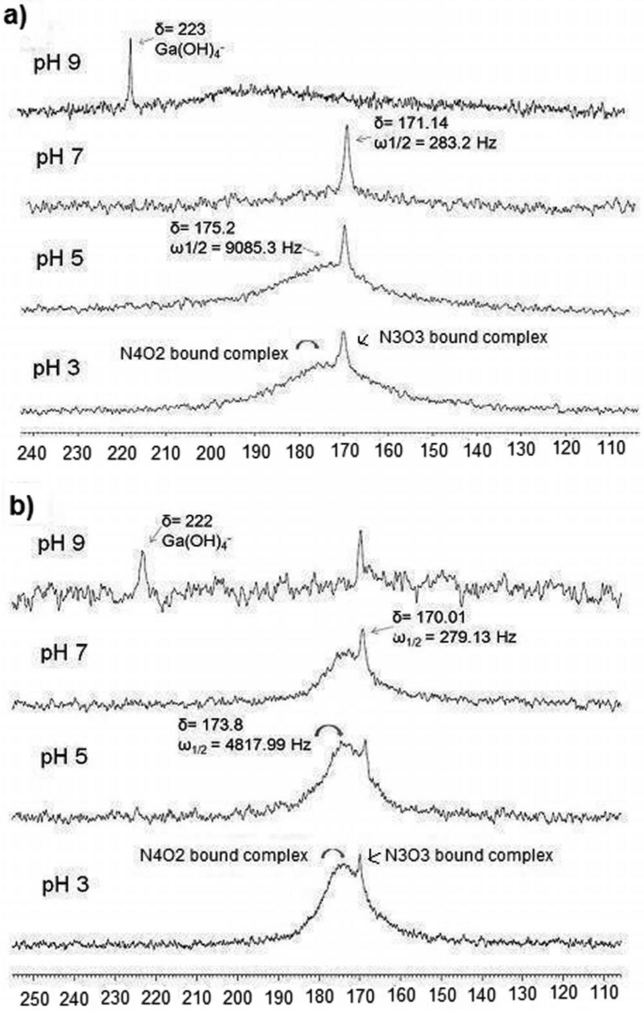


Figure 4. ^{71}Ga NMR spectra of Ga-**4** (a) and Ga-**5** (b) in D_2O at different pH values.

Variable-temperature (VT) ^{1}H NMR spectroscopy of Ga-**4** and Ga-**5** in D_2O (NMR sample solution: pH ≈ 7) showed no significant change in the resonance signals in the spectra recorded within the temperature range of 5–85 °C (Figure 5), indicating that the Ga-**4** and Ga-**5** complexes are stable in solution, and that there is no dissociation of the coordination bond between gallium and the amide nitrogen or oxygen atoms. To investigate the stability of these complexes, ^{1}H NMR spectra were analyzed over the pH range of 3–9 (Figure S10). There were no changes in the resonance signals of these complexes up to pH = 7.6, whereas

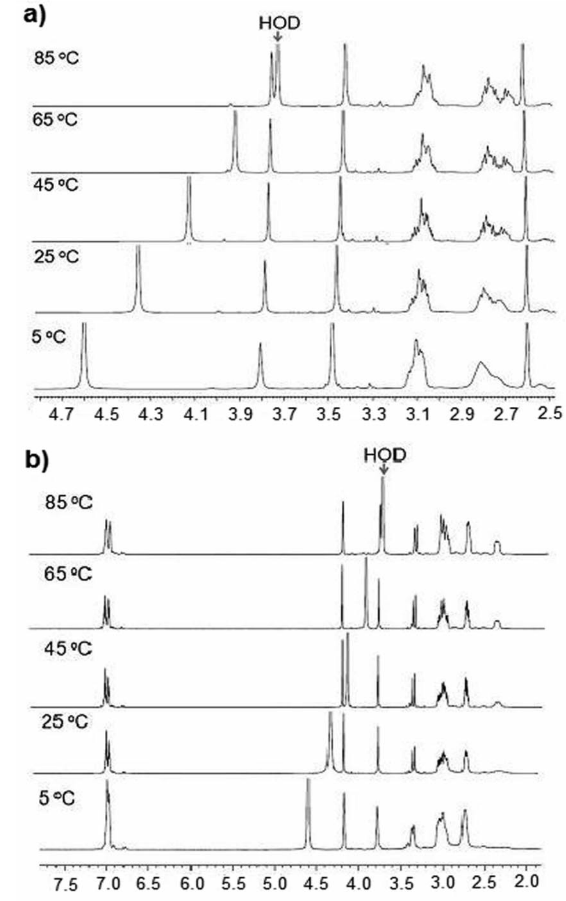


Figure 5. Variable-temperature ^{1}H NMR spectra of Ga-**4** (a) and Ga-**5** (b) in D_2O at pH ≈ 7.

slight signal broadening and a noisy signal appeared at pH = 8, possibly due to a fluxional effect. However, there was no indication of complex decomposition in the range of physiological pH. At pH = 9 both compounds showed decomposition signals that were well supported by ^{71}Ga NMR spectroscopic data recorded at the same pH. In addition, no signal broadening and no coalescence point were noticed in the VT NMR spectra, which suggest that coordinated **4** and **5** remain firmly bound to gallium up to 85 °C.

Conclusions

Ga^{III} complexes of monopendent-arm-modified NOTA derivatives are stable and rigid at physiological pH and at temperatures up to 85 °C. The described direct conjugation of NOTA with amines of biomolecules provides a novel tool for the design of small bioactive molecules, as well as a more convenient means of binding NOTA to biomolecules than using NOTA derivatives containing an extra linker that require multi-step syntheses. This study could lead to the development of a new range of cost-effective and target-specific PET tracers.

Experimental Section

Materials and Physical Methods: Compound di-*tert*-butyl 1,4,7-triazacyclononane-1,4-diacetate (**1**) was purchased from ChemaTech (Dijon, France). Starting material 2-chloro-*N*-methylacetamide was purchased from Frinton Laboratories, Inc. (Vineland, New Jersey, U.S.A.). All other chemicals and solvents were purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.). HPLC-grade solvents were purchased from Fisher (Seoul, Korea). ^{1}H and ^{13}C NMR spectra were recorded with a Jeol 300 JNM spectrometer (300 MHz for ^{1}H, 75 MHz for ^{13}C). In addition, NOESY, HSQC, and ^{71}Ga NMR spectroscopic data were obtained with a Bruker (Germany) Avance 600 NMR spectrometer (600 MHz for correlation NMR, and 183 MHz for ^{71}Ga NMR). ^{1}H resonance shifts were measured relative to tetramethylsilane (TMS), and ^{67}Ga chemical shifts were measured relative to the $[Ga(H_2O)_6]^{3+}$ species present in a 0.1 M $Ga(NO_3)_3$ D_2O solution. In the case of the Ga-**4** and Ga-**5** NMR analysis over a range of pH values, the isolated samples were redissolved in D_2O after synthesis at pH = 3 and 5, and the NMR sample solution pH was controlled by addition of either NaOD or DCl to the solution. Electrospray ionization mass spectra (ESI-MS) were acquired with a Waters ESI ion-trap spectrometer designed to detect positive and negative ions. The samples were diluted 1:100 with water and injected directly into the source. High resolution mass spectra were obtained with a Jeol JMS-AX505WA HP 5890 series II spectrometer with fast atom bombardment (FAB^+) ionization detection.

X-ray Crystallographic Analysis: Data collection and structure analysis were conducted at the Organometallic Laboratory, Seoul National University. Single-crystal diffraction data for all gallium complexes were measured with an Enraf–Nonius CCD single-crystal X-ray diffractometer at room temperature. Unit-cell parameters and intensity-data collections were performed with graphite-monochromated Mo-K_α radiation ($\lambda = 0.71073$ Å). Preliminary orientation matrices and unit-cell parameters were obtained from the peaks on the first 10 data frames, and these were then refined against the complete datasets, which were collected over the angular ranges of $3° < \theta < 27°$ for Ga-**4**·H_2O, $2° < \theta < 27°$ for Ga-**5**·CF_3COOH·H_2O, and $1° < \theta < 27°$ for Ga-**5**·$2H_2O$. A total of ca. 10000 reflections were collected for each complex and about half of these were unique. Data frames were integrated and corrected for Lorentz and polarization effects with DENZO-SMN.[15] Crystallographic drawings were created with the ORTEP[16] program, and the structures were solved by direct methods with SHELXS-97 and refined by full-matrix least squares on F^2 with SHELXL-97.[17] All non-hydrogen atoms were refined anisotropically, except for the benzyl group carbon atoms. All hydrogen atoms were placed at calculated positions, except for those associated with water molecules.

General Synthesis and Characterization of Protected Ligands: To a solution of **1** (0.3 g, 0.84 mmol) and K_2CO_3 (0.231 g, 1.68 mmol) in acetonitrile (4 mL), 2-chloro-*N*-benzylacetamide (0.169 g, 0.92 mmol) or 2-chloro-*N*-methylacetamide (0.099 g, 0.92 mmol) in acetonitrile (2 mL) was added dropwise over the course of 10 min. The reaction mixture was then stirred at room temperature for 24 h. The completion of the reaction was checked by TLC (CH_2Cl_2/methanol, 9:1). The reaction mixture was then filtered, washed with acetonitrile, concentrated in vacuo, and purified by silica gel column chromatography (CH_2Cl_2/MeOH). Products were eluted with 5% methanol to yield compounds **2** (0.187 g, 52%) and **3** (0.254 g, 60%).

***N*-Methylacetamide 2:** ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 1.39 (s, 18 H), 2.61 (d, J = 6 Hz, 3 H, $NHCH_3$), 2.72–2.82 (m, 12 H), 3.21 [s, 2 H, $CH_2C(O)NH$], 3.24 [s, 4 H, $CH_2C(O)O$], 9.09 [br, 1 H, $C(O)NH$] ppm. ^{13}C NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 25.8 (3 C), 28.1 (9 C), 54.1 (2 C), 55.1 (2 C), 55.5 (2 C), 58.3 (2 C), 60.7, 80.9 (2 C), 171.0 (2 C), 173.2 ppm. ESI-MS: calcd. for $[C_{21}H_{41}N_4O_5]^+$ ($[M + H]^+$) 429; found 429.3. HRMS: calcd. for $[M + H]^+$ 429.3077; found 429.3079.

***N*-Benzylacetamide 3:** ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 1.38 (s, 18 H), 2.59 (br. s, 8 H), 2.75 (br. s, 4 H), 2.95 [s, 4 H, $CH_2C(O)O$], 3.30 [s, 2 H, $CH_2C(O)NH$], 4.40 (d, J = 6 Hz, 2 H, benzyl), 7.11–7.28 (m, 5 H, aromatic), 9.31 [br., 1 H, $C(O)NH$] ppm. ^{13}C NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 28.0 (9 C), 43.2 (2 C), 54.3 (2 C), 55.4 (2 C), 56.5 (2 C), 58.3 (2 C), 61.0, 80.7 (2 C), 127.1, 127.9 (2 C), 128.3 (2 C), 138.8, 171.0 (2 C), 172.5 ppm. ESI-MS: calcd. for $[C_{27}H_{45}N_4O_5]^+$ ($[M + H]^+$) 505; found 505.4. HRMS: calcd. for $[M + H]^+$ 505.3390; found 505.3394.

General Procedure for the Synthesis of Ligands 4 and 5: Compound **2** or **3** was dissolved in a mixture of TFA and CH_2Cl_2 (TFA/CH_2Cl_2, 1:1; 12 mL), and the mixture was stirred at room temperature for 20 h. The solvent was then removed to give compounds **4** (0.18 g, 96%) and **5** (0.25 g, 98%) as TFA salts containing 1 equiv. of TFA.

***N*-Methylacetamide 4:** ^{1}H NMR (D_2O, 300 MHz, 25 °C): δ = 2.61 (s, 3 H, $NHCH_3$), 3.13–3.22 (m, 12 H), 3.60 [s, 2 H, $CH_2C(O)NH$], 3.75 [s, 4 H, $CH_2C(O)O$] ppm. ^{13}C NMR (D_2O, 300 MHz, 25 °C): δ = 26.3 (3 C), 51.3 (2 C), 51.5 (2 C), 51.6 (2 C), 57.2 (2 C), 58.5, 169.3, 171.2 (2 C) ppm. ESI-MS: calcd. for $[C_{13}H_{25}N_4O_5]^+$ ($[M + H]^+$) 317; found 317.2. HRMS: calcd. for $[M + H]^+$ 317.1825; found 317.1828.

***N*-Benzylacetamide 5:** ^{1}H NMR (D_2O, 300 MHz, 25 °C): δ = 2.78–2.89 (m, 12 H), 3.42 [s, 2 H, $CH_2C(O)NH$], 3.45 [s, 4 H, $CH_2C(O)O$], 3.81 (s, 2 H, benzyl), 7.73–7.78 (m, 5 H, aromatic) ppm. ^{13}C NMR (D_2O, 300 MHz, 25 °C): δ = 43.6 (2 C), 49.2 (2 C), 51.3 (2 C), 51.4 (2 C), 56.9 (2 C), 58.6, 114.6 (q, CF_3COO^-), 127.8 (2 C), 128.0, 129.1 (2 C), 137.7, 162.7 (q, CF_3COO^-), 168.8, 170.6 (2 C) ppm. ESI-MS: calcd. for $[C_{19}H_{29}N_4O_5]^+$ ($[M + H]^+$) 393; found 393.2. HRMS: calcd. for $[M + H]^+$ 393.2138; found 393.2140.

Synthesis and Characterization of Ga-4 Complexes: A solution of $Ga(NO_3)_3\cdot xH_2O$ (0.030 g, 0.12 mmol) in water (0.5 mL) was added to a solution of **4** (0.05 g, 0.12 mmol) in water (1 mL). The pH of the solution was adjusted to 3 or 5 with Na_2CO_3 solution (0.5 M) and tested with microfine pH paper (Sigma–Aldrich, Hydrion$^{\text{TM}}$ Microfine$^{\text{TM}}$). The mixtures were then heated in a boiling water bath for 15 min. Chelation was taken to completion (the reactions were monitored by evaluating the evolution of the m/z = 383 peak in the mass spectra). Solutions were then filtered through PVDF syringe filters (Whatman, 0.45 µm) and purified by RP-HPLC (XTerra® prep RP18, 19 × 250 mm; water/EtOH eluent: 100% water for 5 min and 0 to 80% EtOH for 25 min). Collected fractions were lyophilized to obtain the complexes as white solids (0.03 g, 68%). Crystals for X-ray crystallography were obtained by slow concentration of EtOH/H_2O (80:10, v/v) solutions of the complexes. ^1H NMR (D_2O, 300 MHz, 25 °C): δ = 2.86 (s, 3 H, NHCH_3), 3.01–3.18 (m, 6 H), 3.30–3.35 (m, 6 H), 3.66 [s, 4 H, CH_2C(O)O], 4.04 [s, 2 H, CH_2C(O)NH] ppm. ^{13}C NMR (D_2O, 300 MHz, 25 °C): δ = 28.4 (3 C), 53.6 (2 C), 53.7 (2 C), 53.9 (2 C), 60.3, 62.5, 173.2, 175.2 (2 C) ppm. ESI-MS: calcd. for $[C_{13}H_{22}GaN_4O_5]^+$ ($[M + H]^+$) 383; found 383.1.

Synthesis and Characterization of Ga-5 Complexes: The above experimental procedure was also applied to produce Ga-**5** complexes with **5** (0.2 g, 0.39 mmol) and $Ga(NO_3)_3\cdot xH_2O$ (0.1 g, 0.39 mmol) in water (1.5 mL) at pH = 3 or 5. The products of the reactions were obtained as light yellow solids (0.12 g, 66%). Single crystals for X-ray diffraction analysis were grown by slow concentration of EtOH solutions of the complexes at room temperature. ^1H NMR (D_2O, 300 MHz, 25 °C): δ = 2.57 (br., 2 H), 2.85–2.98 (m, 4 H), 3.11–3.31 (m, 8 H), 3.58 [d, J = 18 Hz, 2 H, CH_2C(O)NH], 4.0 (s, 2 H), 4.39 (s, 2 H), 7.17–7.22 (m, 5 H) ppm. ^{13}C NMR (D_2O, 300 MHz, 25 °C): δ = 46.5 (2 C), 53.4 (2 C), 53.7 (2 C), 53.9 (2 C), 60.3 (2 C), 62.4, 128.9 (2 C), 129.0, 129.6 (2 C), 136.1, 173.4, 175.0 (2 C) ppm. ESI-MS: calcd. for $[C_{19}H_{26}GaN_4O_5]^+$ ($[M + H]^+$) 459; found 459.1.

Supporting Information (see footnote on the first page of this article): NMR spectra of ligands **4** and **5** and their Ga^{III} complexes.

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