**ABSTRACT:** The chelator Bn$_2$DT3A was used to produce a novel $^{68}$Ga complex for positron emission tomography (PET). Unusually, this system is stabilized by a coordinated hydroxide in aqueous solutions above pH 5, which confers sufficient stability for it to be used for PET. Bn$_2$DT3A complexes Ga$^{3+}$ in a hexadentate manner, forming a mer-mer complex with log $K$([Ga(Bn$_2$DT3A)])$^-$ = 18.25. Above pH 5, the hydroxide ion coordinates the Ga$^{3+}$ ion following dissociation of a coordinated amine. Bn$_2$DT3A radio-labeling displayed a pH-dependent speciation, with $[^{68}\text{Ga}](\text{Bn}_2\text{DT3A})(\text{OH})^-$ being formed above pH 5 and efficiently radiolabeled at pH 7.4. Surprisingly, $[^{68}\text{Ga}](\text{Bn}_2\text{DT3A})(\text{OH})^-$ was found to show an increased stability in vitro (for over 2 h in fetal bovine serum) compared to $[^{68}\text{Ga}](\text{Bn}_2\text{DT3A})$. The biodistribution of $[^{68}\text{Ga}](\text{Bn}_2\text{DT3A})(\text{OH})^-$ in healthy rats showed rapid clearance and excretion via the kidneys, with no uptake seen in the lungs or bones.

**INTRODUCTION**

Positron emission tomography (PET) is a highly sensitive technique that can be used to image molecular processes. While the resolution is not as high as other imaging modalities (typically in the mm range), the high sensitivity allows for target-specific imaging of cellular receptors using peptides and antibodies.

A range of radioactive nuclei can be used for PET; gallium-68 ($^{68}$Ga) is a PET isotope that has favorable physical decay properties for diagnostic imaging, with a high positron branching ratio ($\beta^0 = 89\%$) and a half-life ($t_{1/2} = 67.71$ min) suitable for use with small peptide targeting units. $^{68}$Ga is also available from a radionuclide generator. This is a more accessible route to on-site isotope production than the more conventional cyclotron production, although the activities produced are lower than those achievable by cyclotron production of $^{68}$Ga.

While weakly coordinated Ga$^{3+}$ salts such as gallium citrate or nitrate have been used in clinical nuclear imaging, $^{68}$Ga is typically incorporated into a radiotracer through the use of a chelator. These radiotracers have found significant success in recent years, in particular the somatostatin targeting $[^{68}\text{Ga}](\text{DOTATATE})$, which has been approved for diagnostic imaging of neuroendocrine tumors and prostate specific membrane antigen targeting $^{68}$Ga probes, which are being utilized clinically for identification of prostate cancer metastases. A range of chelators have been applied to $^{68}$Ga complexation, the most widely used is the macrocycle 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). DOTA is a versatile chelator, capable of complexing a variety of metals. However, this versatility also means that it is not the ideal chelator for $^{68}$Ga. This is reflected in the forcing radiolabeling conditions required for radiochemical yields (RCYs) $>$95% (elevated temperatures of $80\,^\circ\text{C}$ and acidic conditions of pH 4) and reduced stability of the resulting complex (80% intact after 2 h incubation in serum). A more suitable macrocyclic chelator, 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), demonstrates the improved radiolabeling efficiency (no heating required) and stability (>98% stable to serum over 2 h) that can be obtained by using specifically designed chelators for $^{68}$Ga.

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An area of growing interest in the development of chelators for $^{68}$Ga is the ability to radiolabel the chelator at higher pH values. This would allow for a simpler radiolabeling process and for the use of targeting moieties that may degrade under acidic conditions, expanding the breadth of diagnostic agents possible using $^{68}$Ga. Some key examples of chelators that have achieved this goal are 4-acetamido-N,N′-bis[(3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl]-4-[(3-hydroxy-1,6-dimethyl-4-oxo-1,4-dihydropyridin-2-yl)methyl]amino]-3-oxopropyl]heptanediamide (THP, Figure S1), 2,2′-6-[(1-carboxyethyl)amino]-6-phenyl-1,4-diazepane-1,4-diyl]dipropionic acid (DATA, Figure S1), and 2,2′-[(ethane-1,2-diylbis[(2-hydroxybenzyl)azanediyl]]diacetic acid (HBED, Figure S1). These chelators have an acyclic or semicyclic design that improves the coordination kinetics.

Substitution of chelators can impact upon the biodistribution of PET radiotracers as such, having a range of suitable chelators will aid in the rapid development of a radiotracer with optimized biodistribution and target uptake. Further development of chelators for $^{68}$Ga will also aid in the understanding of the design of systems capable of producing highly stable chelates under mild conditions. This would allow for the radiolabeling of sensitive biomolecules possessing an appropriate biological half-life.

In this manuscript, we report the synthesis of a novel hexadentate acyclic chelator, 2,2′-[[[(carboxymethyl)azanediyl]bis(ethane-2,1-diyl)]bis[bis(benzylazanediyl)]diacetic acid (Bn$_2$DT3A, Scheme 1), characterize its Ga$^{3+}$ complex, and explore the radiolabeling efficiency of this system with $^{68}$Ga. Bn$_2$DT3A resembles the well-studied diethylentriamine-N,N,N′,N″-pentaaetic acid (DTPA, Figure S1) chelator; however, benzyl units have been substituted in place of two of the acetic acid arms. While DTPA has been applied to $^{68}$Ga complexation, the radiolabeling efficiency was not sufficiently high and the resulting complex was unstable under relevant biological conditions. A more rigid derivative, 2,2′-[[2-{2-[bis(carboxymethyl)amino]-cyclohexyl}[carboxymethyl]]amino]ethyl]azanediyl]diacetic acid (CHX-A$^+$-DTPA, Figure S1), has been demonstrated to produce a stable complex with $^{68}$Ga and applied to imaging in humans. The substitution of the acetate arms of DTPA for benzyl units to give Bn$_2$DT3A results in a chelator with a coordination number that matches the ideal octahedral Ga$^{3+}$ coordination sphere (coordination number = 6), increases the ligand rigidity, and offers sites distant from the coordination sites for future functionalization. Benzyl units were chosen to increase steric bulk and lipophilicity and therefore reduce access of competitors to the Ga$^{3+}$ ion. The benzyl units also afforded a UV-active tag to aid in monitoring synthesis and purification.

Upon investigation of this system, we demonstrated that a species with a hydroxide anion coordinated to the Ga$^{3+}$ center was present when the complex was formed under neutral conditions. We have shown via computational studies that the coordination of a hydroxide anion to the Ga$^{3+}$ center of Ga-Bn$_2$DT3A results in a system with a larger energy barrier to dissociation than the equivalent water complex. This is reflected in the in vitro stability to FBS where the hydroxide complex is stable for over 2 h.

## RESULTS AND DISCUSSION

**Ligand Synthesis and Ga$^{3+}$ Complexation.** Bn$_2$DT3A was prepared in a three-step synthesis (Scheme 1), with an overall yield of 23%. Diethylentriamine was selectively protected at the terminal amine sites through a reductive amination with benzaldehyde. This selective protection is confirmed by the symmetry of the benzyl arms and alky backbone in the $^1$H NMR (Figure S2). Alkylation with tert-butyl bromoacetate introduced protected carboxylic acid moieties to yield the proligand, 3. The incorporation of the acetate arms in two different environments can be seen in the $^1$H NMR, reflecting the central and terminal amine functionalities (Figure S6). The proligand was then deprotected using trifluoroacetic acid to yield the ligand Bn$_2$DT3A as a white powder. The benzyl units are retained, and the two acetate arm environments are distinguishable in the $^1$H NMR with the central arm being more shielded than the terminal arms (Figure S10).

![Scheme 1. Synthesis of Bn$_2$DT3A and Subsequent Complexation of Ga$^{3+}$. (a) (i) EtOH, Benzaldehyde, Reflux. (ii) NaBH$_4$, 0 °C. (b) MeCN, Na$_2$CO$_3$, tert-Butyl Bromoacetate, 60 °C. (c) DCM, TFA, 0 °C to RT. (d) H$_2$O, GaCl$_3$, pH 4, Reflux](https://doi.org/10.1021/acs.inorgchem.2c01992)
Complexation of Ga\(^{3+}\) by Bn\(_2\)DT3A was achieved at room temperature and confirmed by HRMS (m/z = 524.1734, calculated m/z = 524.1307). The \(^1\)H NMR indicates a high level of asymmetry with multiple overlapping peaks with a high degree of \(^1\)H–\(^1\)H coupling, limiting the analysis (Figure S14). Regardless, the spectra confirm the suggested model as well-resolved spectra were obtained in the pH region in which [M(L)] is the dominant species present in solution. At pD 3.3, the presence of two sharp peaks, at 3.56 and 3.85 ppm, seem to correspond to the formation of the protonated species, [Ga(HL)]\(^{3+}\), although this could not be confirmed due to overlap with the surrounding peaks. While there are clearly changes in the spectra between pD 4.0 and pD 7.3, such as the broadening of the signal between 3.45 and 3.33 ppm and the change in spectral form at 3.06 ppm, these are difficult to quantify due to the large number of overlapping signals, making precise analysis unsuitable. Hydroxide coordination leads to significant signal broadening in spectra collected above pD 6.8, which could be ascribed to intermediate ligand flexibility of the partly coordinated ligand molecule. In addition, decomposition can be seen at high pH by the improved resolution of the \(^1\)H NMR spectrum reflecting the free ligand being produced, increasing symmetry and flexibility resulting in sharp, well-defined peaks being observed at pD 8.8 and 10.0 (Figure S18).

Crystal Structure. A crystal of suitable quality for single-crystal X-ray diffraction was obtained from an acidic aqueous solution of [Ga(Bn\(_2\)DT3A)]. The obtained structure (Figure 1) shows a hexadentate ligand, fully satisfying the coordination sphere of Ga\(^{3+}\). The nitrogen atoms coordinate Ga\(^{3+}\) in a mer fashion, as do the oxygen atoms of the carboxylate arms. The Ga\(^{3+}\) ion lies 0.220(5) Å above the plane of the three nitrogen atoms. The bite angle of each chelating unit is between 108.8(1)° and 108.8(1)° for [Ga(DOTA)]\(^{3+}\), [Ga(DOTA)]\(^{3+}\), and [Ga(EDTA)]\(^{3+}\). The Ga\(^{3+}\) complex does not form any classical hydrogen bonds, but within the solid-state structure, there are many C–H···O interactions. Further details of the crystal structure determination are given in the SI (Figure S24 and Table S2). Crystals were grown at two further pH levels (5.3 and 6.8): the crystal structure obtained from these two preparations was the same; the same molecule, [Ga(Bn\(_2\)DT3A)], was present as a more complicated hydrate. This is likely due to the low solubility of the neutral complex in comparison to other species in solution. Further details are given in the SI (Figures S25 and S26, Table S3).

Thermodynamic Stability. Potentiometry was performed to obtain protonation constants of Bn\(_2\)DT3A and on the system with Ga\(^{3+}\), Cu\(^{2+}\), and Zn\(^{2+}\) to obtain thermodynamic stability constants and an understanding of the effect of pH on speciation in solution. Five protonation constants were determined for Bn\(_2\)DT3A (Table 1). By comparison to similar ligands (Table 1), the first three protonation constants were assigned to the amines of Bn\(_2\)DT3A. The remaining two protonation constants correspond to the carboxylic acid arms, with the final arm being too acidic to detect the corresponding constant. The amine sites are more acidic than those reported for DTPA — this is due to the stabilizing effect of the additional negatively charged carboxylate arms in DTPA, making amine deprotonation more difficult. This can be seen by comparing the reported values for the protonation constants of glycine (log \(K_a = 9.8\)) and benzylamine (log \(K_a = 9.36\)) benzylamine has a more acidic amine than glycine as it lacks the internal hydrogen bonding provided by the carboxylate arm. The two carboxylate protonation constants obtained for Bn\(_2\)DT3A have similar values — this contrasts with those reported for NOTA, which have significantly differing values. This is likely due to the flexibility of the linear ligand Bn\(_2\)DT3A allowing for independent protonation of the arms, whereas in the rigid macrocyclic system of NOTA, the arms will likely interact, forming internal hydrogen bonds where a deprotonated arm stabilizes a protonated arm at low pH. It is surprising that the carboxylate arms of Bn\(_2\)DT3A are approximately one log \(K_a\) unit more acidic than those of DTPA, although this may again be due to hydrogen bonding between the additional carboxylate arms of DTPA, stabilizing the partially deprotonated ligand at low pH.

A 1:1 metal ligand complex is formed between Ga\(^{3+}\) and Bn\(_2\)DT3A between pH 2 and 8. Above this pH, the formation of [Ga(OH)\(_4\)]\(^{−}\) dominates the speciation of Ga\(^{3+}\) in solution. The ligand Bn\(_2\)DT3A has a slightly greater affinity for Cu\(^{2+}\) than Ga\(^{3+}\) (Table 2); the affinity for both ions is greater than

![Figure 1. Molecular structure of [Ga(Bn\(_2\)DT3A)] determined by X-ray crystallography. Hydrogen atoms have been omitted for clarity. Colors: gallium (pale brown); carbon (gray); nitrogen (blue); oxygen (red).](https://doi.org/10.1021/acs.inorgchem.2c01992)

| Table 1. Protonation Constants of the Discussed Ligands |
|-----------------|----------|----------|----------|
| constant        | Bn\(_2\)DT3A\(^a\) | DTPA \(^{40,41}\) | NOTA \(^{44,45}\) |
| log \(K_1\)     | 9.70     | 9.84     | 10.22    |
| log \(K_2\)     | 7.48     | 9.02     | 8.56     |
| log \(K_3\)     | 3.34     | 4.25     | 4.31     |
| log \(K_4\)     | 1.50     | 2.8      | 1.96     |
| log \(K_5\)     | 1.40     | 2.22     | 0.7      |

\(^{a}\)T = 25 °C, I = 0.1 M NMe\(_2\)Cl.
Table 2. Stability Constants and Dissociation Constants (log $\beta_{HL,M}$) of Bn$_2$DT3A Complexes$^{44}$

| equilibrium                                      | Ga$^{3+}$ | Cu$^{2+}$ | Zn$^{2+}$ |
|--------------------------------------------------|-----------|-----------|-----------|
| $M + L \leftrightarrow [M(L)]$                   | 18.25     | 18.9      | 14.12     |
| $[M(HL)] \leftrightarrow [M(L)] + H$            | 2.73      | 2.8       | 4.16      |
| $[M(L)] + H_2O \leftrightarrow [M(L)(OH)] + H$  | 5.32      |           | 12.06     |
| $[M(L)(OH)] + H_2O \leftrightarrow [M(L)(OH)_2] + H$ | 8.21 |

Charges are omitted. ($T = 25$ °C, $I = 0.1$ M NMe$_2$Cl). The stability constants corresponding to the formation of [Cu(HL)] were determined without ionic strength control.

Table 2. Stability Constants and Dissociation Constants (log $\beta_{HL,M}$) of Bn$_2$DT3A Complexes$^{44}$

![Figure 2](image1.png)

**Figure 2.** Speciation of Ga$^{3+}$ in solution with Bn$_2$DT3A. ($T = 25$ °C, $I = 0.1$ M NMe$_2$Cl, [Bn$_2$DT3A] = 4 mM, [Ga$^{3+}$] = 2 mM).

![Figure 3](image2.png)

**Figure 3.** Calculated energy of water molecule (blue) and hydroxide anion (green) interacting with [Ga(Bn$_2$DT3A)] at various Ga–O distances.
dissociation of one of the ligand coordinating atoms, either an amine or a carboxylate.

The distribution diagram clearly shows that the hydroxide species is the major species at physiological pH and is relevant for in vitro and in vivo investigations (see below).

**Molecular Modeling.** HF-3c calculations show that coordination of a water molecule to the \([\text{Ga(Bn}_2\text{DT3A)}]^{-}\) complex is not thermodynamically favorable (Figure 3). An initial energy barrier of 20 kJ mol\(^{-1}\) prevents the water molecule from approaching the \(\text{Ga}^{3+}\) ion, and, if it were to coordinate to the metal ion, the resulting species is 40 kJ mol\(^{-1}\) less stable than the dissociated system. In contrast, a hydroxide ion is shown to be able to approach the \(\text{Ga}^{3+}\) center, with an overall stabilization of 240 kJ mol\(^{-1}\) as it approaches from 3.0 to 1.8 Å. The hydroxide complex is calculated to be 160 kJ mol\(^{-1}\) more stable than the dissociated system. The calculations suggest that one of the terminal amine groups is replaced by a hydroxide anion. As the hydroxide coordination can only proceed at sufficient hydroxide anion concentration, the reaction takes place in solution with neutral pH.

**Radiolabeling with \(^{68}\text{Ga}\).** When incubated with \(^{68}\text{Ga}, \text{Bn}_2\text{DT3A}\) was found to be capable of achieving high radiochemical yields at both pH 4 and pH 7.4; however, multiple products were formed with pH-dependent abundance. The two radiolabeled products were isolated by semi-preparative HPLC (Figure 4) and assessed independently for their stability to fetal bovine serum (FBS, Figure 4). The major product at pH 4, \([^{68}\text{Ga}][\text{Ga(Bn}_2\text{DT3A)}]^{-}\), was found to be poorly stable to competition by FBS, with none of the complex remaining after 30 min (Figure 4D). In contrast, the major product at pH 7.4, attributed to \([^{68}\text{Ga}][\text{Ga(Bn}_2\text{DT3A)}-(OH)]^{-}\), was shown to be stable to FBS for over 2 h with no decomplexation seen (Figure 4B and Figure S20). Thus, this radiolabeled product is suitable for further PET applications.

The effect of pH on the population of the products was further investigated (Figure 5A). At low pH, a negligible amount of the desired hydroxido species was formed; above pH 5, the FBS stable product became more populous. According to the distribution diagram, this stable product corresponds to the species \([\text{Ga(Bn}_2\text{DT3A)}-(OH)]^{-}\) (Figure 5A). This is also supported by its shorter retention time when analyzed by HPLC (Figure 4A), suggesting an increased hydrophilicity due to its charge. The partition coefficient for

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**Figure 4.** (A) Radio-HPLC of \([^{68}\text{Ga}][\text{Ga(Bn}_2\text{DT3A)}-(OH)]^{-}\) following semipreparative HPLC purification. (B) Stability of \([^{68}\text{Ga}][\text{Ga(Bn}_2\text{DT3A)}-(OH)]^{-}\) to FBS assessed by radio-TLC. (i) Isolated species. (ii) After 30 min incubation in FBS. (iii) After 120 min incubation. (C) Radio-HPLC of \([^{68}\text{Ga}][\text{Ga(Bn}_2\text{DT3A)}]^{-}\) following semipreparative HPLC purification. (D) Stability of \([^{68}\text{Ga}][\text{Ga(Bn}_2\text{DT3A)}]^{-}\) to FBS assessed by radio-TLC. (i) Isolated species. (ii) After 30 min incubation in FBS. (E) Radio-TLC of \([^{68}\text{Ga}][\text{GaCl}_3]^{-}\) incubated with FBS.
this species was determined to be log $D_{\text{octanol/PBS}}(pH 7.4) = -2.91 +/- 0.07$, this fulfills drug development requirements, which is advantageous to future uses as a radiotracer.

The temperature of the radiolabelling reaction and the concentration of the chelator have previously been shown to affect the ratio of diastereomers formed when radiolabelling HBED with $[^{68}\text{Ga}]$ as such, these parameters were also investigated, along with the radiolabelling incubation time. The ligand concentration has a significant impact on the radiochemical yield (RCY); ligand concentrations of at least 100 $\mu$M are required to achieve RCYs >90% at pH 7.4 at room temperature in 15 min. The use of a higher ligand concentration in the radiolabelling reaction also promoted the formation of $[^{68}\text{Ga}][\text{Ga(Bn}_{2}\text{DT3A})(\text{OH})]^{-}$ (Figure 5B).

The temperature of the radiolabelling reaction has a profound impact upon the ratio of the species formed (Figure 5C); elevated temperatures favor the formation of the $[^{68}\text{Ga}][\text{Ga(Bn}_{2}\text{DT3A})(\text{OH})]^{-}$ product with ratios of 20:1 achievable at pH 7.4 and 60 $^\circ$C after 5 min (Figure S21).

The reaction time has a modest effect on the RCY and the ratio of the species (Figure 5D). The increase in the ratio of species formed with increasing reaction time suggests that there is some slow exchange between the two species; this was not observed when isolating the species by semipreparative HPLC so it may require excess ligand to be present.

The obtained thermodynamic stability constants, HF-3c calculations, and kinetic inertness toward FBS all support the formation of a stable complex in which $\text{Ga}^{3+}$ is coordinated by $\text{Bn}_{2}\text{DT3A}$ in a five-coordinate manner with the additional coordination site occupied by a hydroxide anion. The formation of this species, $[^{68}\text{Ga}][\text{Ga(Bn}_{2}\text{DT3A})(\text{OH})]^{-}$, occurs only in a significant proportion at pH $> 5$.

These optimized conditions for the production of $[^{68}\text{Ga}][\text{Ga(Bn}_{2}\text{DT3A})(\text{OH})]^{-}$ ($[L] > 100 \mu$M, $T > 60^\circ$C, $t > 15$ min, pH 7.4) are similar to typical radiolabelling conditions for DTPA ($[L] = 155 \mu$M, $T = 25^\circ$C, $t = 20$ min, pH = 3.5) or $[^{68}\text{Ga}][\text{Ga(Cl}_{2}\text{Benzimidazole})(\text{OH})]^{-}$ and CHX-A$^-$-DTPA ($[L] = 74 \mu$M, $T = 95^\circ$C, $t = 5$ min, pH = 3.6–4).$^{32}$ The most noticeable difference is the increased pH of radiolabelling, potentially allowing for the radiolabelling of pH-sensitive motifs with $[^{68}\text{Ga}]$ by using $\text{Bn}_{2}\text{DT3A}$ as a chelator instead of DTPA or CHX-A$^-$-DTPA. In terms of stability, less than 60% $[^{68}\text{Ga}][\text{Ga(DTPA)}]$ remained intact after 2 h incubation in serum,$^{31}$ and $[^{68}\text{Ga}][\text{Ga(CHX-A$^-$/DTPA)}]$ was approximately 85% intact after 2 h—-in this comparison, $[^{68}\text{Ga}][\text{Ga(Bn}_{2}\text{DT3A})(\text{OH})]^{-}$ shows a significant improvement with no decomplexation seen after 2 h incubation with serum. Despite these differences, $\text{Bn}_{2}\text{DT3A}$ is still outperformed by the macrocyclic NOTA, which is typically radiolabelled at room temperature at much lower ligand concentrations, albeit at acidic pH ($[L] = 10 \mu$M, $T = 25^\circ$C, $t = 10$ min, pH = 3.5).$^{20}$

**In Vivo Assessment.** Following optimization of the radiolabelling conditions, the $[^{68}\text{Ga}][\text{Ga(Bn}_{2}\text{DT3A})(\text{OH})]^{-}$ complex was investigated in vivo. Following semipreparative HPLC purification, the isolated species was reformulated into phosphate buffered saline (PBS) and administered into healthy male Sprague-Dawley rats via tail-vein injection. The biodistribution was monitored by sequential PET scans (Figure 6 and Figures S35–S34) followed by a computed tomography (CT) scan to allow for co-registration of the images. The activity rapidly accumulated within the kidneys before passing through the bladder, indicating a renal clearance. No uptake in the liver, lungs, or bones could be observed. When $[^{68}\text{Ga}][\text{Ga(citrate)}]^{-}$, a weakly coordinated system in which release of $[^{68}\text{Ga}][\text{Ga}^{3+}]$ is expected,$^{31}$ was studied in the same manner, some minor uptake in the lungs and transient localization in the prostate gland of the rats was observed (Figure 6 and Figures S27–S34). Uptake was also observed in the leg joints following injection of $[^{68}\text{Ga}][\text{Ga(citrate)}]^{-}$ (Figure 6), which was not observed following injection of $[^{68}\text{Ga}][\text{Ga(Bn}_{2}\text{DT3A})(\text{OH})]^{-}$. When nonchelated $[^{68}\text{Ga}][\text{Ga}^{3+}]$ is injected, high initial uptake is reported in the heart and blood followed by renal clearance with a
prolonged heart and blood uptake along with liver and joint uptake. In comparison to these two systems, it is clear that 
$[^{68}\text{Ga}][\text{Ga(Bn}_2\text{DT3A})(\text{OH})]^{-}$ is rapidly excreted via the kidneys with minimal uptake outside of this pathway, suggesting that the $^{68}\text{Ga}^{3+}$ ion remains complexed by Bn$_2$DT3A.

The rapid clearance of $[^{68}\text{Ga}][\text{Ga(Bn}_2\text{DT3A})(\text{OH})]^{-}$ suggests that it will be a good choice of chelate for uptake.

Inorganic Chemistry

While this versatility is often undesired in the design of bifunctional derivatives could produce a system that can be efficiently labeled at pH 7.4 without heating, resulting in a serum stable product for in vivo application.

## CONCLUSIONS

A novel hexadentate chelator, Bn$_2$DT3A, has been prepared and applied to the coordination of Ga$^{3+}$ and to radiochemistry with $^{68}$Ga.

Bn$_2$DT3A forms a distorted octahedral mer-mer 1:1 complex with Ga$^{3+}$ under acidic conditions with a thermodynamic stability of $\log K[\text{Ga(Bn}_2\text{DT3A)}] = 18.25$. Hydroxide anion coordination occurs with a $pK_a$ of 5.32. HF-3c calculations attribute the species structure to the dissociation of one of the amines and insertion of a hydroxide anion.

Bn$_2$DT3A is capable of complexing other metal ions, as evidenced by its acceptable Cu$^{2+}$ and Zn$^{2+}$ thermodynamic stability constants. This gives the system a greater versatility, and this is being explored through $^{68}$Ga$^{3+}$ labeling experiments. While this versatility is often undesired in the design of chelators for radiometals due to the potential for complexation of other metal ions that may be present in the radiolabeling solution, the design of the ligand Bn$_2$DT3A, with benzylic units that can be substituted to increase or decrease steric hindrance and electronic properties, will allow for optimization of the system to improve selectivity for $^{68}$Ga$^{3+}$ in the future.

When Bn$_2$DT3A is radiolabeled with $^{68}$Ga, two species are formed in a pH-dependent manner. The radiolabeling conditions can be tuned to vary the ratio of these products, and they can be isolated by semipreparative HPLC. The product that is formed above pH 5 and promoted by elevated temperatures and high ligand concentrations was attributed to the deprotonated species $[^{68}\text{Ga}][\text{Ga(Bn}_2\text{DT3A})(\text{OH})]^{-}$. This species was stable to biological competitors for over 2 h in contrast to the neutral species $[^{68}\text{Ga}][\text{Ga(Bn}_2\text{DT3A})(\text{OH})]^{-}$. It was administered to healthy rats and found to have a rapid renal clearance with negligible uptake outside of the clearance pathway.

$[\text{Ga(Bn}_2\text{DT3A})]$ shows an increased in vitro stability upon hydroxide coordination, which allows it to be used for PET applications. This system is promising for further development of chelators for the complexation of $^{68}$Ga under mild conditions.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.inorgchem.2c01992.

Experimental details, spectra, speciation diagrams, in vivo data, and crystallographic data (CCDC 1864389, 2125953) (PDF)

Accession Codes

CCDC 1864389 and 2125953 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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### Author Contributions

T.W.P. performed the synthesis, radiolabeling, analysis of in vivo data, and manuscript preparation. I.R. contributed to acquisition of in vivo data. S.J.A. and A.-M.S. contributed to the planning and performing of in vivo experiments. T.J.P. contributed to the crystal structure data collection and analysis. V.K. and P.H. contributed to the potentiometric data collection and analysis. D.M.B. contributed to the molecular modeling. G.J.S. contributed to the conceptualization, super-
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