

# Chelating Rare-Earth Metals ( $\text{Ln}^{3+}$ ) and $^{225}\text{Ac}^{3+}$ with the Dual-Size-Selective Macrocyclic Ligand $\text{Py}_2$ -MacroDipa

Aohan Hu,<sup>†</sup> Megan E. Simms,<sup>‡</sup> Vilmos Kertesz,<sup>§</sup> Justin J. Wilson,<sup>\*,†</sup> and Nikki A. Thiele<sup>\*,‡</sup>

<sup>†</sup>Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853, United States

<sup>‡</sup>Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, United States

<sup>§</sup>Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, United States

## ABSTRACT

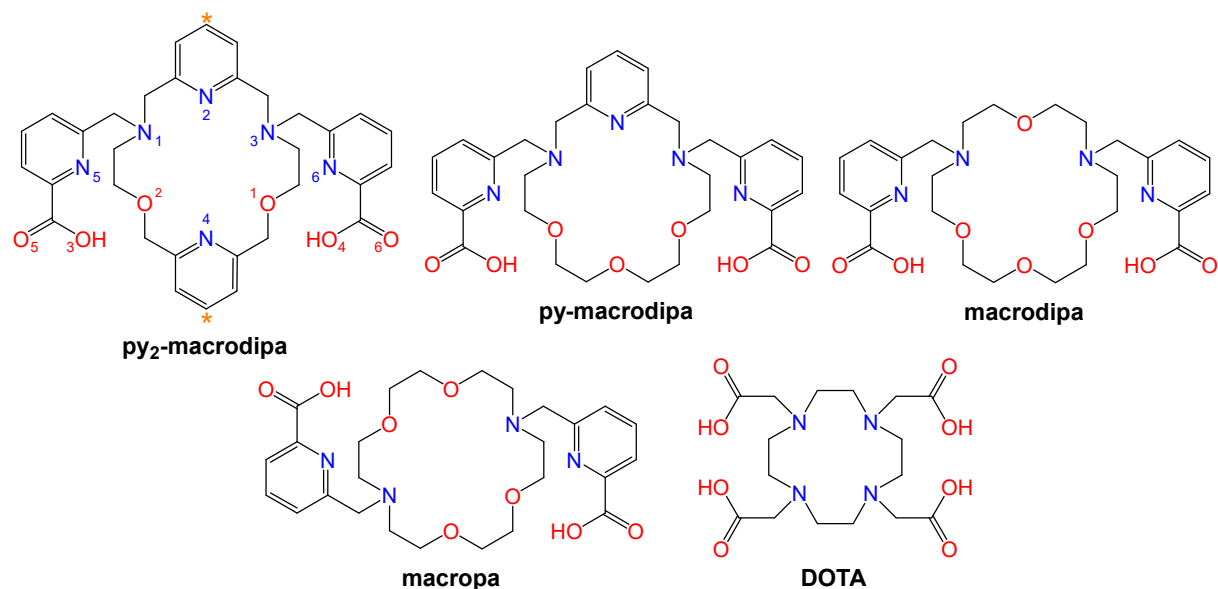
Radioisotopes of metallic elements, or radiometals, are widely employed in both therapeutic and diagnostic nuclear medicine. For this application, chelators that efficiently bind the radiometal of interest and form a stable metal–ligand complex with it are required. Towards the development of new chelators for nuclear medicine, we recently reported a novel class of 18-membered macrocyclic chelators that is characterized by their ability to form stable complexes with both large and small rare-earth metals ( $\text{Ln}^{3+}$ ), a property referred to as dual size selectivity. A specific chelator in this class called py-macrodipa, which contains one pyridyl group within its macrocyclic core, was established as a promising candidate for  $^{135}\text{La}^{3+}$ ,  $^{213}\text{Bi}^{3+}$ , and  $^{44}\text{Sc}^{3+}$  chelation. Building upon this prior work, here we report the synthesis and characterization of a new chelator called  $\text{py}_2$ -macrodipa with two pyridyl units fused into the macrocyclic backbone. Its coordination chemistry with the  $\text{Ln}^{3+}$  series was investigated by NMR spectroscopy, X-ray crystallography, DFT calculations, analytical titrations, and transchelation assays. These studies reveal that  $\text{py}_2$ -macrodipa retains the expected dual size selectivity and possesses an enhanced thermodynamic affinity for all  $\text{Ln}^{3+}$  compared to py-macrodipa. By contrast, the kinetic stability of  $\text{Ln}^{3+}$  complexes with  $\text{py}_2$ -macrodipa is only improved for the light, large  $\text{Ln}^{3+}$  ions. Based upon these observations, we further assessed the suitability of  $\text{py}_2$ -macrodipa for use with  $^{225}\text{Ac}^{3+}$ , a large radiometal with valuable properties for targeted alpha therapy. Radiolabeling and stability studies revealed  $\text{py}_2$ -macrodipa to efficiently incorporate  $^{225}\text{Ac}^{3+}$ , and to form a complex that is inert in human serum over 3 weeks. Although  $\text{py}_2$ -macrodipa does not surpass the state-of-the-art chelator macropa for  $^{225}\text{Ac}^{3+}$  chelation, it does provide another effective  $^{225}\text{Ac}^{3+}$  chelator. These studies shed light on the fundamental coordination chemistry of the  $\text{Ln}^{3+}$  and may inspire future chelator design efforts.

## INTRODUCTION

Radiopharmaceutical agents, which leverage radionuclides for medicinal therapy and diagnosis, have been receiving increasing attention as new modalities progress to the clinic.<sup>1-5</sup> Across the entire periodic table, many metallic elements have been identified to possess radioisotopes suitable for this application.<sup>6-8</sup> In order to harness these radiometals for clinical use, a chelator that can rapidly form thermodynamically and kinetically stable complexes is usually required. Facile complex formation minimizes radioactivity loss during handling before in vivo administration, and high complex stability prevents in vivo release of the potentially harmful radiometal. A significant body of research has been devoted to chelator design for radiometals by addressing their distinct chemical properties and coordination chemistry preferences.<sup>7-10</sup>

As a recent contribution to this area, we developed a class of ligands that exhibit “dual size selectivity”, a property characterized by their ability to preferentially bind both the large and small rare-earth metal ions ( $\text{Ln}^{3+}$ ). This dual-size-selective property is attributed to a significant conformational toggle that occurs in this ligand class as they bind metal ions of different sizes (Scheme 1) to optimize complex stability. For large  $\text{Ln}^{3+}$ , a 10-coordinate, nearly  $C_2$ -symmetric complex is formed (Conformation A), whereas for small  $\text{Ln}^{3+}$ , an 8-coordinate asymmetric complex arises (Conformation B).<sup>10-12</sup> The first member of this ligand class identified was the macrocyclic chelator macrodipa (Chart 1).<sup>11</sup> Despite the unique selectivity profile of macrodipa, the kinetic lability of the corresponding  $\text{Ln}^{3+}$ -macrodipa complexes limited its use for biomedical applications.<sup>12</sup>

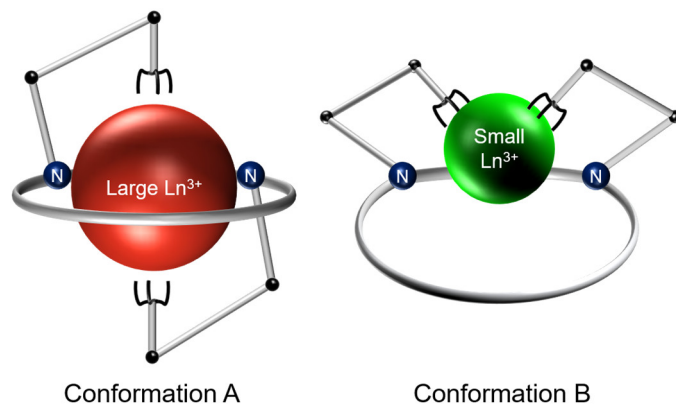
**Chart 1. Structures of Ligands Discussed in This Work.**



To overcome this limitation, we modified the structure of macrodipa by incorporating a stronger pyridyl donor in place of an ethereal oxygen within the macrocycle to afford the second-generation ligand py-macrodipa (Chart 1). This change led to a significant enhancement of both the thermodynamic and kinetic stability of its  $\text{Ln}^{3+}$  complexes, while maintaining its dual size selectivity. Based on these improvements of macrodipa, we showed that py-macrodipa efficiently formed stable complexes with  $^{135}\text{La}^{3+}$  and  $^{44}\text{Sc}^{3+}$ , two valuable radionuclides for nuclear medicine with significantly disparate ionic radii.<sup>12</sup> To further validate the potential of py-macrodipa for nuclear medicine applications, we assessed its suitability for chelating  $^{225}\text{Ac}^{3+}$  and  $^{213}\text{Bi}^{3+}$ , two therapeutically valuable  $\alpha$ -emitters.<sup>13,14</sup> These studies showed that py-macrodipa is a promising candidate for  $^{213}\text{Bi}^{3+}$  chelation, but does not form a complex of sufficient kinetic stability for  $^{225}\text{Ac}^{3+}$  nuclear medicine applications.<sup>15</sup>

The inefficacy of py-macrodipa for the stable chelation of  $^{225}\text{Ac}^{3+}$  motivated us to target a third-generation dual-size-selective chelator that forms complexes with even greater thermodynamic and kinetic stability. Based on the improvements that were observed upon the incorporation of a single pyridyl group into the macrocycle of macrodipa, we targeted the novel chelator  $\text{py}_2$ -macrodipa (Chart 1), which contains an additional pyridyl donor. In this work, we discuss the synthesis of this new ligand, its coordination chemistry with  $\text{Ln}^{3+}$  ions, and its potential application for  $^{225}\text{Ac}^{3+}$  radiotherapy.

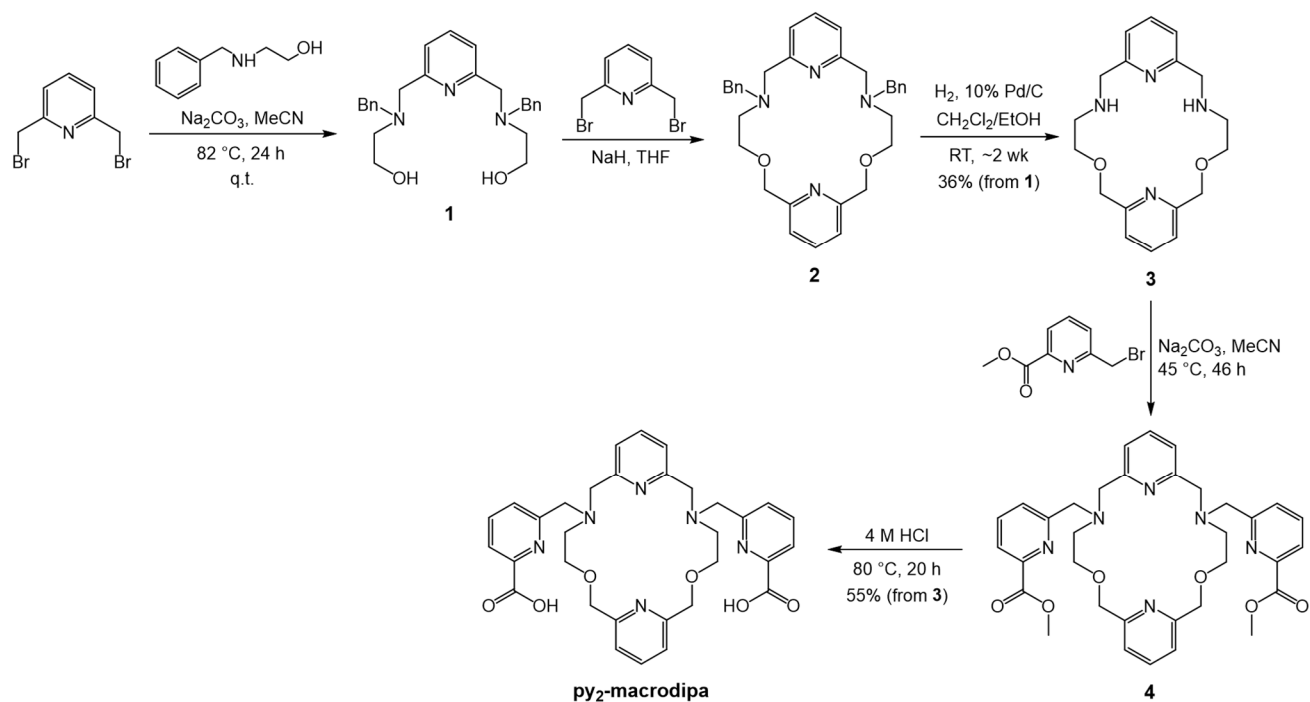
**Scheme 1. Depiction of the Conformational Toggle Present in Dual-Size-Selective Chelators when Binding  $\text{Ln}^{3+}$  Ions with Different Sizes.<sup>a</sup>**



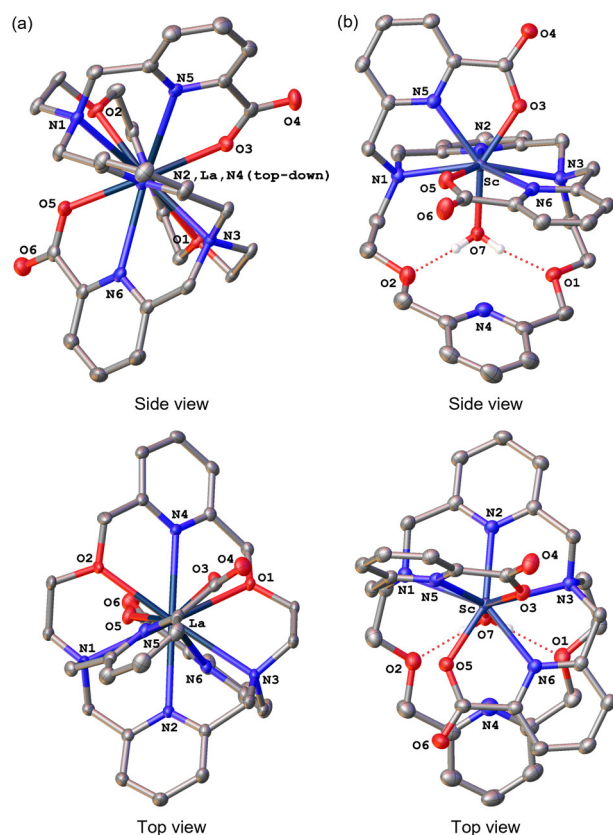
<sup>a</sup>Reproduced from *J. Am. Chem. Soc.* **2020**, *142*, 13500. Copyright 2020 American Chemical Society.

## RESULTS AND DISCUSSION

**Scheme 2. Synthetic Route to py<sub>2</sub>-macrodipa.**

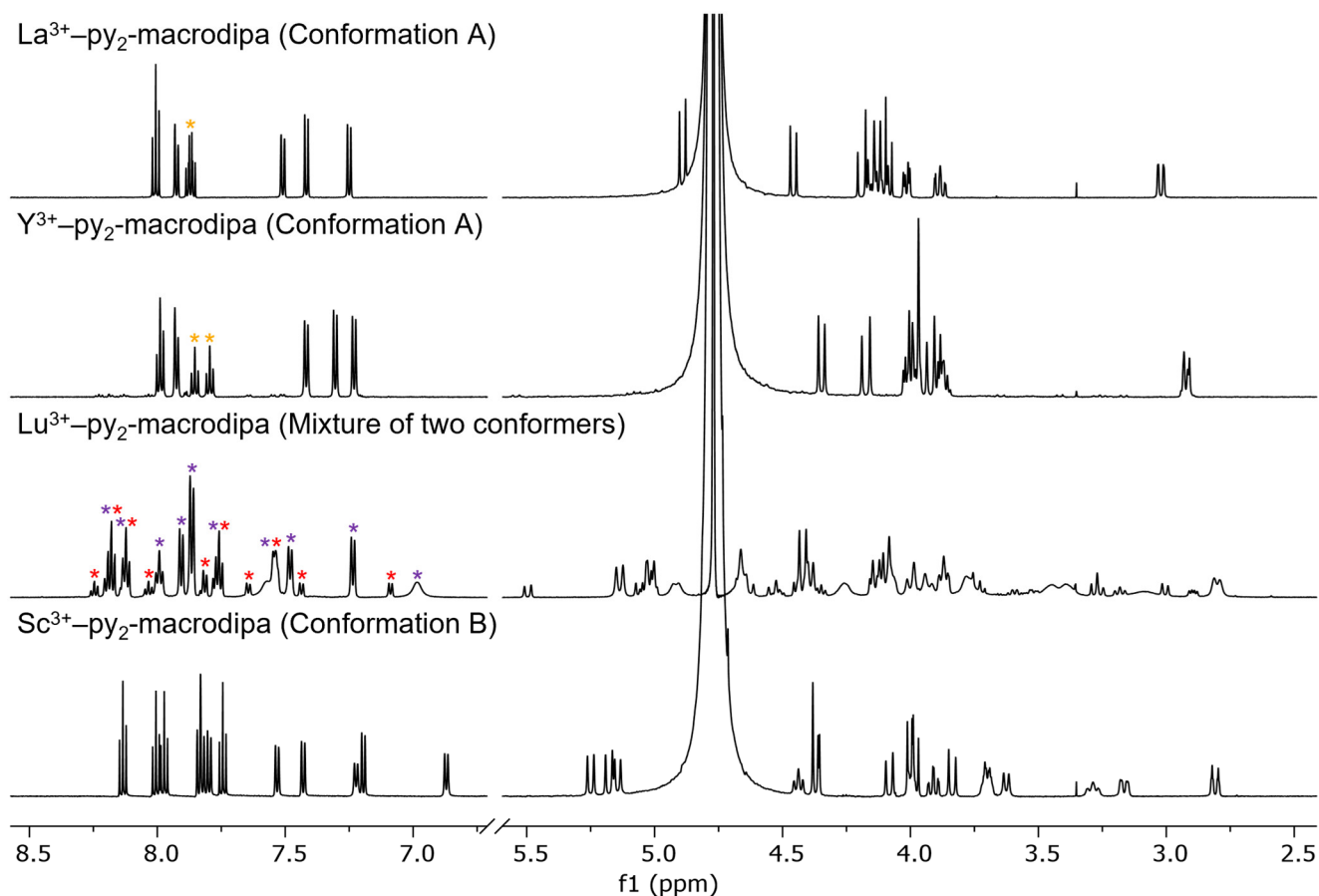


The chelator py<sub>2</sub>-macrodipa was synthesized via the five-step procedure shown in Scheme 2, with an overall yield of 20%. The benzyl-protected macrocyclic backbone **2** was constructed in two steps commencing from commercially available 2,6-bis(bromomethyl)pyridine and 2-benzylaminoethanol. The benzyl groups were then removed by reduction with H<sub>2</sub> over a Pd/C catalyst to yield the desired dipyridyl macrocycle **3**. Subsequent installation of the two picolinate pendent arms via alkylation and acidic deprotection afforded py<sub>2</sub>-macrodipa. The identity and purity of the intermediates and final product were verified by NMR spectroscopy, mass spectrometry, and analytical HPLC (Figures S1–S13).



**Figure 1.** Crystal structures of (a)  $[La(py_2\text{-macrodipa})]^+$  and (b)  $[Sc(py_2\text{-macrodipa})(OH_2)]^+$  complexes. Thermal ellipsoids are drawn at the 50% probability level. Solvent, counterions, and nonacidic hydrogen atoms are omitted for clarity.

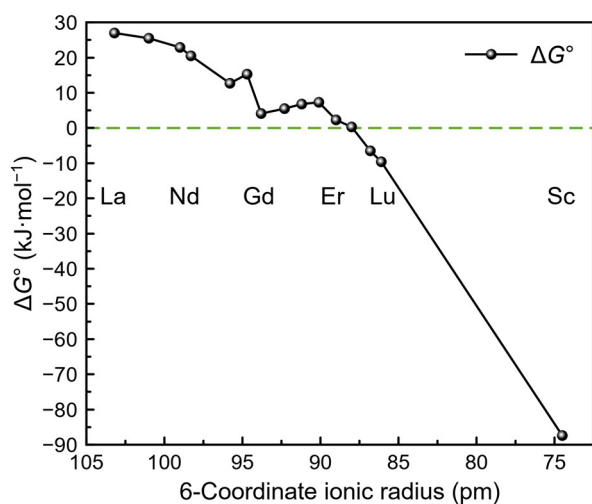
To understand the influence of the second pyridyl unit on the coordination chemistry of  $py_2\text{-macrodipa}$ , the crystal structures of its complexes with two representative  $Ln^{3+}$  ions,  $La^{3+}$  and  $Sc^{3+}$ , were determined by X-ray crystallography (Figure 1).  $La^{3+}$ , the largest  $Ln^{3+}$ , forms a complex with  $py_2\text{-macrodipa}$  that attains the 10-coordinate, distorted  $C_2$ -symmetric Conformation A, with all nitrogen and oxygen donor atoms on the ligand engaged in metal binding. By contrast, the  $py_2\text{-macrodipa}$  complex of  $Sc^{3+}$ , the smallest  $Ln^{3+}$ , adopts the 8-coordinate, asymmetric Conformation B. In this crystal structure, three of the macrocycle-based ligand donor atoms (O1, O2, N4) do not directly interact with the  $Sc^{3+}$  center, whose coordination sphere is completed by an inner-sphere water molecule. The conformations observed for the  $La^{3+}$  and  $Sc^{3+}$  crystal structures are in line with our expectations, based on the well characterized conformational toggle (Scheme 1) observed for the earlier generation analogues  $macrodipa$  and  $py\text{-macrodipa}$  upon binding to  $Ln^{3+}$  of different sizes.



**Figure 2.**  $^1\text{H}$  NMR spectra of  $\text{La}^{3+}$ -,  $\text{Y}^{3+}$ -,  $\text{Lu}^{3+}$ -, and  $\text{Sc}^{3+}$ - $\text{py}_2$ -macrodipa complexes (600 MHz,  $\text{D}_2\text{O}$ , pD 7, 25 °C). The yellow asterisks in the spectra of the  $\text{La}^{3+}$  and  $\text{Y}^{3+}$  complexes highlight the decrease in symmetry of Conformation A in moving from larger to smaller ions. The purple (Conformation A) and red (Conformation B) asterisks in the spectrum of the  $\text{Lu}^{3+}$  complex indicate the two conformers present in solution.

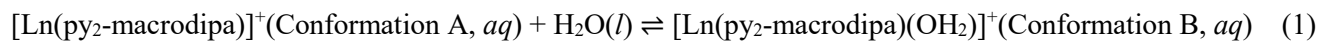
Having verified that this conformational change occurs in the solid state, we next leveraged NMR spectroscopy to characterize the solution structures of  $\text{Ln}^{3+}$ - $\text{py}_2$ -macrodipa complexes with the diamagnetic  $\text{La}^{3+}$ ,  $\text{Y}^{3+}$ ,  $\text{Lu}^{3+}$ , and  $\text{Sc}^{3+}$  ions, for which the ionic radius decreases from 103.2 pm to 74.5 pm.<sup>16</sup> The  $^1\text{H}$  and  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of these four complexes were acquired in  $\text{D}_2\text{O}$  at pD 7 (Figures 2 and S17–S24). Figure 2 shows a stacked arrangement of the  $^1\text{H}$  NMR spectra of these four complexes for comparison. The  $^1\text{H}$  NMR spectra of the complexes of  $\text{La}^{3+}$  and  $\text{Y}^{3+}$  show equivalency in their resonances, indicating that the symmetric Conformation A predominates for the  $\text{py}_2$ -macrodipa complexes of these larger ions. By contrast,  $\text{Sc}^{3+}$ , the smallest  $\text{Ln}^{3+}$ , yields an NMR spectrum of lower symmetry, revealing it to attain the asymmetric Conformation B in aqueous solution. The  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra of these complexes are also consistent with these conformational assignments (Figures S18, S20, and S24). These NMR observations indicate that the solid-state crystallographic studies of the  $\text{La}^{3+}$  and  $\text{Sc}^{3+}$  complexes (Figure 1) are a good representation of their solution structures.

The  $^1\text{H}$  NMR spectrum of the  $\text{py}_2$ -macrodipa complex of the intermediately-sized  $\text{Lu}^{3+}$  is more complicated than those of  $\text{La}^{3+}$ ,  $\text{Y}^{3+}$ , and  $\text{Sc}^{3+}$ . Two different species, in a molar ratio of 4:1, are observed. The  $^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of this complex likewise reveals the presence of two species (Figure S22). These two species are assigned to be a mixture of Conformation A and B in equilibrium. Close inspection of the aromatic region of the  $^1\text{H}$  NMR spectrum (Figures 2 and S21), reveals an asymmetry in both conformers, despite our prior observations that Conformation A typically gives rise to spectra with symmetry. Our tentative explanation for this observation is that the Conformation A gradually descends in symmetry as the  $\text{Ln}^{3+}$  gets smaller, and the size-matching becomes poorer. This argument is supported by comparing the  $^1\text{H}$  NMR spectra of the large  $\text{La}^{3+}$  and smaller  $\text{Y}^{3+}$  complexes (Figure 2), where a decrease in symmetry of Conformation A begins to appear. Most apparent are the two triplets in the aromatic region (labeled with orange asterisks), which can be assigned as the geminal hydrogens on the carbon atoms labeled with orange asterisks in Chart 1. For the large  $\text{La}^{3+}$ , the chemical shifts of these two triplets are nearly identical, whereas for the smaller  $\text{Y}^{3+}$ , their chemical shifts begin to diverge more substantially and indicate different chemical environments for these two H atoms, presumably due to a decrease in symmetry of Conformation A.



**Figure 3.** DFT-computed standard free energy differences ( $\Delta G^\circ$ ) between Conformations A and B for  $\text{Ln}^{3+}$ - $\text{py}_2$ -macrodipa complexes, plotted versus ionic radii.

To further understand the conformational switch of  $\text{py}_2$ -macrodipa across the  $\text{Ln}^{3+}$  series, we carried out density functional theory (DFT) calculations. All  $\text{Ln}^{3+}$ - $\text{py}_2$ -macrodipa complexes in both Conformations A and B were optimized, using the same level of theory ( $\omega\text{B97XD}/6\text{-31G(d,p)}/\text{LCRECP}$ ) that we previously applied to study  $\text{Ln}^{3+}$ -macrodipa and  $\text{Ln}^{3+}$ - $\text{py}$ -macrodipa.<sup>11,12,17-24</sup> From these geometry optimizations and thermochemical calculations, the standard free energy difference ( $\Delta G^\circ$ ) between Conformations A and B for each  $\text{Ln}^{3+}$  complex was determined (eq 1), as plotted in Figure 3.



As the  $\text{Ln}^{3+}$  series is traversed,  $\Delta G^\circ$  gradually shifts from positive to negative values, consistent with our experimental observations that Conformation A is favored for large  $\text{Ln}^{3+}$ , whereas Conformation B is preferred for small  $\text{Ln}^{3+}$ . A similar computational analysis was applied in our studies of macrodipa and py-macrodipa, which also showed that Conformation A was lower in free energy for large  $\text{Ln}^{3+}$  and vice versa. The crossover point where  $\Delta G^\circ$  switches its sign occurs roughly at  $\text{Tm}^{3+}$ ,  $\text{Er}^{3+}$ , and  $\text{Tb}^{3+}$  for  $\text{py}_2\text{-macrodipa}$ ,  $\text{py-macrodipa}$ ,<sup>12</sup> and  $\text{macrodipa}$ <sup>11</sup> systems, respectively. Thus, modifications of the macrocycle can alter the conformational dynamics and preferences of this ligand class.

Having confirmed by X-ray crystallography and NMR spectroscopy that  $\text{Ln}^{3+}$  complexes of  $\text{py}_2\text{-macrodipa}$  undergo a  $\text{Ln}^{3+}$ -size-dependent conformational toggle like its earlier analogues  $\text{macrodipa}$  and  $\text{py-macrodipa}$ , we next set out to verify our hypothesis that the additional macrocyclic pyridyl moiety would lead to enhanced complex stability. In this regard, we first evaluated the thermodynamic stability of  $\text{Ln}^{3+}$ - $\text{py}_2\text{-macrodipa}$  complexes by determining their stability constants. The magnitude of stability constant measures the thermodynamic affinity of a ligand for a metal ion and is valuable for assessing its potential in different applications.<sup>10,25,26</sup> The protonation constants ( $K_i$ ) of  $\text{py}_2\text{-macrodipa}$ , as well as the stability constants ( $K_{\text{LnL}}$ ) of its  $\text{Ln}^{3+}$  complexes, measured via either potentiometric titrations or UV-Vis spectrophotometric titrations,<sup>27-29</sup> are collected in Table 1. These quantities are defined in eqs 2–3, where the concentration terms represent those at chemical equilibrium, and L signifies the uncomplexed ligand in its fully deprotonated state.

$$K_i = [\text{H}_i\text{L}] / [\text{H}^+][\text{H}_{i-1}\text{L}] \quad (2)$$

$$K_{\text{LnL}} = [\text{LnL}] / [\text{Ln}^{3+}][\text{L}] \quad (3)$$

The  $\log K_{\text{LnL}}$  values of  $\text{py}_2\text{-macrodipa}$ , and those of the related structures  $\text{macrodipa}$ ,  $\text{py-macrodipa}$ , and  $\text{macrodipa}$ , are plotted against  $\text{Ln}^{3+}$  6-coordinate ionic radius<sup>16</sup> in Figure 4. Like  $\text{macrodipa}$  and  $\text{py-macrodipa}$ ,  $\text{py}_2\text{-macrodipa}$  exhibits a dual-size-selective pattern that is not altered via the introduction of an additional pyridyl group in the macrocycle. Importantly, the  $\log K_{\text{LnL}}$  values of  $\text{py}_2\text{-macrodipa}$  are systematically greater compared to those of  $\text{py-macrodipa}$ , indicating that the additional macrocyclic pyridyl group of  $\text{py}_2\text{-macrodipa}$  has a pronounced effect on enhancing the overall  $\text{Ln}^{3+}$ -binding affinity. The  $\log K_{\text{LnL}}$  values of  $\text{py}_2\text{-macrodipa}$  are also significantly higher across the entire  $\text{Ln}^{3+}$  series than those of  $\text{macrodipa}$ , a chelator established as a promising candidate for several large radiometals including  $^{225}\text{Ac}^{3+}$ ,  $^{132/135}\text{La}^{3+}$ ,  $^{131}\text{Ba}^{2+}$ ,  $^{223}\text{Ra}^{2+}$ , and  $^{213}\text{Bi}^{3+}$ .<sup>30-34</sup> This observation highlights the potential of  $\text{py}_2\text{-macrodipa}$  for large radiometal chelation. Although these  $\log K_{\text{LnL}}$  values are smaller than those observed

for the Ln<sup>3+</sup> complexes of the commonly applied chelator DOTA (Chart 1), for which log K<sub>LnL</sub> spans 22.9–25.4 from La<sup>3+</sup> to Lu<sup>3+</sup>, the established efficacy of macropa, which gives rise to substantially lower log K<sub>LnL</sub> values, for these radiometals demonstrate that such high K<sub>LnL</sub> values are not strictly needed for practical use in nuclear medicine. However, future ligand design efforts within this ligand class to further increase these stability constants may further improve value for use in nuclear medicine.

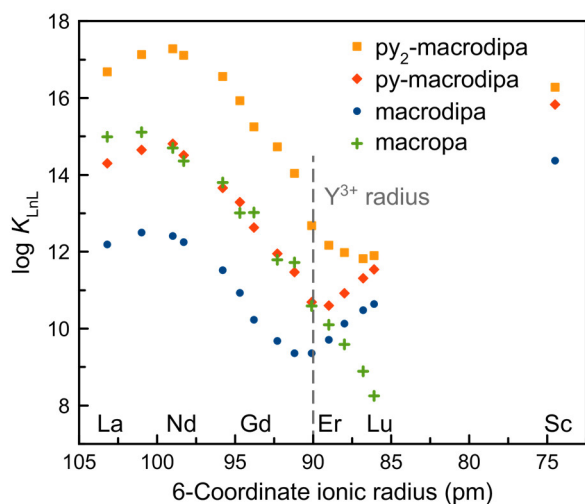
**Table 1. Protonation Constants of py<sub>2</sub>-macrodipa, py-macrodipa, macrodipa, macropa, and Stability Constants of Their Ln<sup>3+</sup> Complexes.**

	py <sub>2</sub> -macrodipa <sup>a</sup>	py-macrodipa	macrodipa	macropa
log K <sub>1</sub>	7.58(4)	7.20 <sup>b</sup>	7.79 <sup>c</sup>	7.41 <sup>d</sup> , 7.41 <sup>e</sup>
log K <sub>2</sub>	6.48(1)	6.54 <sup>b</sup>	7.04 <sup>c</sup>	6.85 <sup>d</sup> , 6.90 <sup>e</sup>
log K <sub>3</sub>	3.52(3)	3.17 <sup>b</sup>	3.18 <sup>c</sup>	3.32 <sup>d</sup> , 3.23 <sup>e</sup>
log K <sub>4</sub>	2.60(5)	2.31 <sup>b</sup>	2.41 <sup>c</sup>	2.36 <sup>d</sup> , 2.45 <sup>e</sup>
log K <sub>5</sub>	2.10(11)			1.69 <sup>d</sup>
log K <sub>LaL</sub>	16.68(8)	14.31 <sup>b</sup>	12.19 <sup>c</sup>	14.99 <sup>d</sup>
log K <sub>CeL</sub>	17.13(7)	14.65 <sup>b</sup>	12.50 <sup>c</sup>	15.11 <sup>d</sup>
log K <sub>PrL</sub>	17.28(6)	14.81 <sup>b</sup>	12.41 <sup>c</sup>	14.70 <sup>d</sup>
log K <sub>NdL</sub>	17.11(3)	14.51 <sup>b</sup>	12.25 <sup>c</sup>	14.36 <sup>d</sup>
log K <sub>SmL</sub>	16.56(4)	13.66 <sup>b</sup>	11.52 <sup>c</sup>	13.80 <sup>d</sup>
log K <sub>EuL</sub>	15.93(4)	13.29 <sup>b</sup>	10.93 <sup>c</sup>	13.01 <sup>d</sup>
log K <sub>GdL</sub>	15.25(7)	12.63 <sup>b</sup>	10.23 <sup>c</sup>	13.02 <sup>d</sup>
log K <sub>TbL</sub>	14.76(6)	11.95 <sup>b</sup>	9.68 <sup>c</sup>	11.79 <sup>d</sup>
log K <sub>DyL</sub>	14.04(2)	11.47 <sup>b</sup>	9.36 <sup>c</sup>	11.72 <sup>d</sup>
log K <sub>HoL</sub>	12.68(5)	10.69 <sup>b</sup>	9.36 <sup>c</sup>	10.59 <sup>d</sup>
log K <sub>ErL</sub>	12.17(3)	10.60 <sup>b</sup>	9.71 <sup>c</sup>	10.10 <sup>d</sup>
log K <sub>TmL</sub>	11.98(2)	10.92 <sup>b</sup>	10.13 <sup>c</sup>	9.59 <sup>d</sup>
log K <sub>YbL</sub>	11.82(5)	11.31 <sup>b</sup>	10.48 <sup>c</sup>	8.89 <sup>d</sup>
log K <sub>LuL</sub>	11.90(3)	11.54 <sup>b</sup>	10.64 <sup>c</sup>	8.25 <sup>c</sup>
log K <sub>ScL</sub>	16.28(4)	15.83 <sup>b</sup>	14.37 <sup>b</sup>	

<sup>a</sup>0.1 M KCl, this work. The values in the parentheses are one standard deviation of the last significant figure. <sup>b</sup>0.1 M KCl, ref 12. <sup>c</sup>0.1 M KCl, ref 11. <sup>d</sup>0.1 M KCl, ref <sup>37</sup>. <sup>e</sup>0.1 M KCl, ref <sup>38</sup>.

Notably, the increase in log K<sub>LnL</sub> in moving from py-macrodipa to py<sub>2</sub>-macrodipa is much greater for the large Ln<sup>3+</sup> than for the small Ln<sup>3+</sup>. This observation can be rationalized by the crystal structures. The newly introduced second pyridyl unit directly interacts and stabilizes the metal center in Conformation A (Figure 1a), whereas it is not bound to the metal in Conformation B (Figure 1b). As such, Conformation A benefits more significantly from the presence of the additional macrocyclic pyridyl donor present in py<sub>2</sub>-macrodipa. Thus, for large Ln<sup>3+</sup> complexes, for which Conformation A prevails, a substantial log K<sub>LnL</sub> enhancement is observed, but diminished impact is found for small

$\text{Ln}^{3+}$  complexes, where Conformation B is dominant. Another noteworthy characteristic discerned from our potentiometric titrations is the identification of the protonated complex species ( $\text{LnHL}$ ) for late  $\text{Ln}^{3+}$ - $\text{py}_2$ -macrodiapa systems (Figures S28–S32), which was not observed for any  $\text{Ln}^{3+}$  complexes of  $\text{py}$ -macrodiapa.<sup>12</sup> Based on this information, the most likely protonation site to form the  $\text{LnHL}$  species is the second pyridyl group (N4, Chart 1), which is substantially basic and not engaged with the  $\text{Ln}^{3+}$  center (Figure 1b) in Conformation B.



**Figure 4.** Stability constants of  $\text{Ln}^{3+}$  complexes formed with  $\text{py}_2$ -macrodiapa,  $\text{py}$ -macrodiapa, macrodiapa, and macropa plotted versus ionic radii.

After demonstrating an enhancement of  $\text{Ln}^{3+}$  complex thermodynamic stability afforded by the second pyridyl group of  $\text{py}_2$ -macrodiapa, we next evaluated its impact on complex kinetic stability, a property of critical importance for chelators applied in radiopharmaceutical settings.<sup>1,9</sup> For comparison to our previous studies, an identical DTPA transchelation challenge<sup>12,39</sup> was applied to assess the  $\text{Ln}^{3+}$ - $\text{py}_2$ -macrodiapa complex kinetic stability. Specifically, the  $\text{Ln}^{3+}$ - $\text{py}_2$ -macrodiapa complexes were treated with 100 equiv of DTPA at pH 7.4 and RT (22 °C), a condition that thermodynamically favors the formation of the  $\text{Ln}^{3+}$ -DTPA complexes.<sup>40–42</sup> This transchelation process, which follows pseudo-first-order kinetics with the large excess of DTPA, was monitored by UV–Vis spectroscopy. The half-lives ( $t_{1/2}$ ) afforded from these pseudo-first-order processes (Table 2) provide a quantitative comparative measure of the complex kinetic stability.

**Table 2 Half-lives of Ln<sup>3+</sup>-py<sub>2</sub>-macrodipa, Ln<sup>3+</sup>-py-macrodipa, and Ln<sup>3+</sup>-macrodipa Complexes when Challenged with 100 Equivalents of DTPA.<sup>a</sup>**

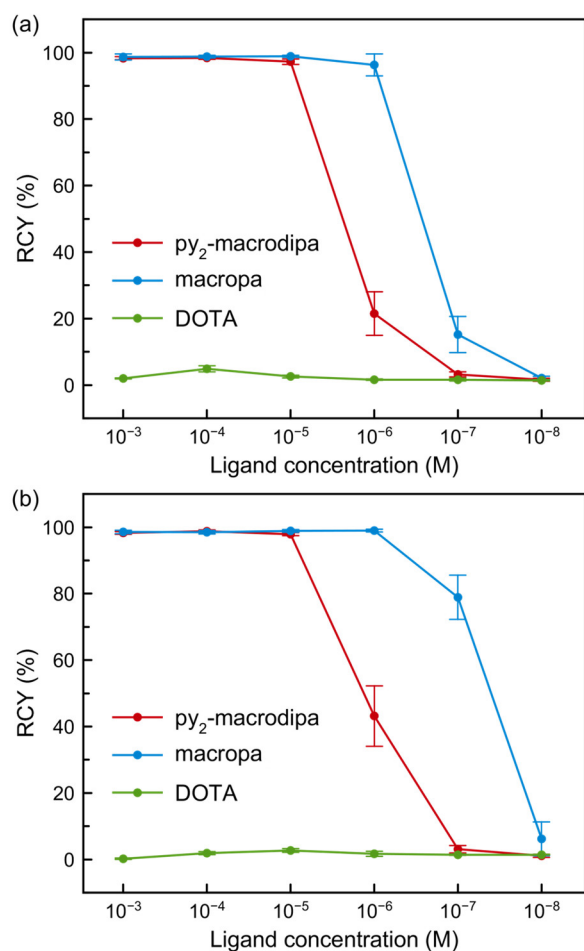
	Ln <sup>3+</sup> -py <sub>2</sub> -macrodipa	Ln <sup>3+</sup> -py-macrodipa <sup>b</sup>	Ln <sup>3+</sup> -macrodipa <sup>b</sup>
La <sup>3+</sup>	>> 5 weeks	6.3 d	1678 s
Gd <sup>3+</sup>	4.5 ± 0.2 d	5524 s	54 s
Lu <sup>3+</sup>	253 ± 6 s	853 s	65 s
Sc <sup>3+</sup>	5.9 ± 0.4 h	16.6 h	782 s

<sup>a</sup>[LnL] = 100 μM, pH 7.4 in MOPS, 22 °C. <sup>b</sup>Ref 12.

The trend in kinetic stability of the Ln<sup>3+</sup>-py<sub>2</sub>-macrodipa complexes follows closely with that observed for their thermodynamic stability, with the light and heavy Ln<sup>3+</sup> complexes undergoing slower transchelation than those in the middle of the series. In comparison to those of macrodipa and py-macrodipa, the early Ln<sup>3+</sup> complexes of py<sub>2</sub>-macrodipa exhibit a remarkable kinetic stability, with <10% dissociation of the La<sup>3+</sup> complex observed after 5 weeks. However, for the smallest Sc<sup>3+</sup>, the kinetic stability of its py<sub>2</sub>-macrodipa complex is significantly lower than that of py-macrodipa, indicating that the second macrocyclic pyridyl is detrimental in this regard. The enhancement of kinetic stability of the La<sup>3+</sup> complex is easily rationalized from the X-ray crystallographic data (Figure 1a), where both pyridyl groups strongly engage the La<sup>3+</sup> center. By contrast, the non-coordinated pyridyl group present in the Sc<sup>3+</sup>-py<sub>2</sub>-macrodipa complex is positioned closely to the inner-sphere water molecule (N4–O7 distance of 2.91 Å), within a range that is consistent with hydrogen bonding, for which the N···O distances typically span from 2.8–3.0 Å.<sup>43</sup> Thus, the pendent pyridyl group is oriented appropriately to facilitate proton-assisted metal-ion dissociation, which will substantially labilize the complex in aqueous solution. The increasing kinetic lability by protonation was also illustrated in other metal complex systems.<sup>44–46</sup> Collectively, the inclusion of this second pyridyl unit of py<sub>2</sub>-macrodipa enhances the thermodynamic stability for all Ln<sup>3+</sup> complexes, but only improves the kinetic stability of complexes with the large Ln<sup>3+</sup>. Thus, py<sub>2</sub>-macrodipa is a promising candidate for large Ln<sup>3+</sup> chelation.

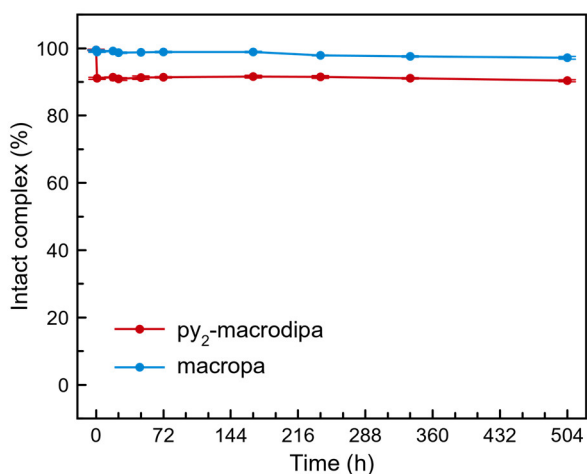
On account of the remarkable thermodynamic and kinetic stability of the La<sup>3+</sup>-py<sub>2</sub>-macrodipa complex, we next sought to investigate the capability of py<sub>2</sub>-macrodipa to chelate the largest trivalent cation Ac<sup>3+</sup>, as both ions possess similar coordination chemistry.<sup>47</sup> Furthermore, the radioisotope <sup>225</sup>Ac<sup>3+</sup> (*t*<sub>1/2</sub> = 9.9 d)<sup>48</sup> emits four α particles through its decay chain, making it valuable for use in targeted internal radiotherapy.<sup>49–51</sup> To assess the suitability of py<sub>2</sub>-macrodipa as a chelator for this radiometal, we carried out <sup>225</sup>Ac<sup>3+</sup> radiolabeling studies with py<sub>2</sub>-macrodipa and benchmarked the results to macropa and DOTA (Chart 1), two macrocyclic chelators that have established precedence for <sup>225</sup>Ac<sup>3+</sup> chelation.<sup>30,52</sup>

Concentration-dependent radiolabeling studies were carried out by incubating different concentrations of py<sub>2</sub>-macrodirpa, macrodirpa, and DOTA with 9.5–11.1 kBq of <sup>225</sup>Ac<sup>3+</sup> at pH 6.0 and 25 °C. The radiochemical yields (RCYs), determined by radio-TLC, are summarized in Figure 5 and Table S3. Notably, py<sub>2</sub>-macrodirpa quantitatively incorporates <sup>225</sup>Ac<sup>3+</sup> at a low concentration of 10<sup>-5</sup> M within 5 min, revealing it to be an efficient chelator for <sup>225</sup>Ac<sup>3+</sup>. This property is nearly on par with that of macrodirpa, the current gold standard, which quantitatively radiolabels <sup>225</sup>Ac<sup>3+</sup> at micromolar concentration, an observation consistent with previously reported results.<sup>15,30</sup> By contrast, DOTA did not significantly undergo <sup>225</sup>Ac<sup>3+</sup> radiolabeling even at a millimolar concentration. Increasing the reaction time to 60 min had only a slight effect on the RCYs for py<sub>2</sub>-macrodirpa and macrodirpa, indicating that the radiolabeling process is nearly complete within 5 min under these mild conditions for both chelators. In addition, the radiolabeling efficiency of py<sub>2</sub>-macrodirpa is comparable to that of py-macrodirpa and significantly better than macrodirpa.<sup>15</sup>



**Figure 5.** Radiochemical yields (RCYs) of <sup>225</sup>Ac<sup>3+</sup> radiolabeling with py<sub>2</sub>-macrodirpa, macrodirpa, and DOTA at different ligand concentrations (pH 6.0, 25 °C). (a) 5-min reaction time; (b) 60-min reaction time.

After establishing effective radiolabeling of py<sub>2</sub>-macrodipa with <sup>225</sup>Ac<sup>3+</sup>, the kinetic stability of its <sup>225</sup>Ac<sup>3+</sup> complex was assessed. Specifically, the <sup>225</sup>Ac<sup>3+</sup> complexes of py<sub>2</sub>-macrodipa and macropa were incubated in human serum at 37 °C over 3 weeks to model the conditions that would be encountered when a radiopharmaceutical agent is administered in vivo. Figure 6 shows the percentage of intact complex remaining throughout the course of this experiment. Both radiocomplexes are sufficiently stable in human serum, as reflected by the fact that they remain >90% intact after 3 weeks. The resistance to human serum for [<sup>225</sup>Ac]Ac<sup>3+</sup>-macropa observed here is also consistent with prior studies.<sup>15,30,53</sup> Although the radiolabeling efficiency of py<sub>2</sub>-macrodipa is comparable to that of py-macrodipa, the kinetic stability of [<sup>225</sup>Ac]Ac<sup>3+</sup>-py<sub>2</sub>-macrodipa is substantially enhanced relative to that of [<sup>225</sup>Ac]Ac<sup>3+</sup>-py-macrodipa, which showed a ~90% complex dissociation in human serum at 37 °C after 24 h.<sup>15</sup> This observation is also consistent with the DTPA transchelation challenge results (Table 2), which also revealed an overall higher Ln<sup>3+</sup> complex kinetic stability for py<sub>2</sub>-macrodipa. Collectively, py<sub>2</sub>-macrodipa shows promise for use with <sup>225</sup>Ac<sup>3+</sup> based on its efficient radiolabeling and high complex stability. It exhibits the best performance for <sup>225</sup>Ac<sup>3+</sup> chelation among the dual-size-selective chelators.



**Figure 6.** The stability of [<sup>225</sup>Ac]Ac<sup>3+</sup>-py<sub>2</sub>-macrodipa and [<sup>225</sup>Ac]Ac<sup>3+</sup>-macropa in human serum at 37 °C.

## CONCLUSION

Building upon macrodipa and py-macrodipa, we designed and prepared the third-generation dual-size-selective chelator py<sub>2</sub>-macrodipa. Its coordination chemistry with Ln<sup>3+</sup> ions was characterized by multiple techniques including X-ray crystallography, NMR spectroscopy, DFT calculations, analytical titrations, and transchelation assays. These three chelators all exhibit dual-size selective properties and are based on the same 18-membered macrocyclic core structures. They differ by the presence of 0, 1, or 2 pyridyl groups within the macrocyclic core. Without an integrated pyridyl group, macrodipa is a relatively poor chelator with respect to both the thermodynamic and kinetic stability of its Ln<sup>3+</sup> complexes. The single pyridyl group in py-macrodipa serves to enhance both properties. In this study, we showed that the introduction of a second pyridyl group in py<sub>2</sub>-macrodipa further enhanced the thermodynamic stability for all Ln<sup>3+</sup> complexes, but the kinetic stability was only improved for the large Ln<sup>3+</sup> complexes. As a general conclusion, it appears that the inclusion of pyridyl groups in place of ethereal donors does benefit complex stability, but the conformations of the resulting complexes also need to be considered when trying to anticipate the magnitudes of these effects. Despite the weaker performance of py<sub>2</sub>-macrodipa for small Ln<sup>3+</sup>, its promising chelation properties for large Ln<sup>3+</sup> prompted us to investigate its use with the therapeutic radiometal <sup>225</sup>Ac<sup>3+</sup>. Radiolabeling and serum stability studies with <sup>225</sup>Ac<sup>3+</sup> revealed py<sub>2</sub>-macrodipa to perform comparably to the state-of-the-art chelator macropa. Although the dual-size-selective properties were demonstrated both thermodynamically and kinetically, efforts need to be undertaken to improve the stability of its complexes with small metal ions for practical nuclear medicine applications. Additionally, ongoing work is also directed toward preparation of a bifunctional analogue of py<sub>2</sub>-macrodipa that can be used to conjugate with biomolecules. Both the macrocyclic backbone and the picolinate pendant arms provide potential opportunities for this functionalization. In any case, this work affords a new potential candidate for <sup>225</sup>Ac<sup>3+</sup> chelation as well as provides new insights on the fundamental Ln<sup>3+</sup> coordination chemistry of and <sup>225</sup>Ac<sup>3+</sup> radiochemistry for future chelator development endeavors.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/xxxxxxx>.

Experimental procedures and supplementary data for ligand synthesis, X-ray crystallography, NMR spectroscopy, DFT calculations, potentiometric titrations, UV–Vis spectrophotometric titrations, DTPA transchelation studies, radiolabeling studies, and serum challenge assays (PDF)

Crystallographic data for  $\text{La}^{3+}$ - and  $\text{Sc}^{3+}$ -py<sub>2</sub>-macrodi<sub>2</sub>a complexes (CIF)

Geometry outputs for all DFT-optimized structures (ZIP)

### Accession Codes

CCDC 2178140–2178141 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](http://www.ccdc.cam.ac.uk/data_request/cif), or by emailing [data\\_request@ccdc.cam.ac.uk](mailto: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.

## AUTHOR INFORMATION

### Corresponding Authors

Nikki A. Thiele – Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, United States; [orcid.org/0000-0003-3301-0849](https://orcid.org/0000-0003-3301-0849); Email: [thielena@ornl.gov](mailto:thielena@ornl.gov)

Justin J. Wilson – Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853, United States; [orcid.org/0000-0002-4086-7982](https://orcid.org/0000-0002-4086-7982); Email: [jjw275@cornell.edu](mailto:jjw275@cornell.edu)

### Author

Aohan Hu – Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853, United States; [orcid.org/0000-0002-9720-3159](https://orcid.org/0000-0002-9720-3159)

Megan E. Simms – Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, United States

Vilmos Kertesz – Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, United States

### Notes

A.H, J.J.W., and N.A.T. are co-inventors on a patent application filed on the use of py<sub>2</sub>-macrodipa and related analogues for nuclear medicine applications.

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## Synopsis.

A novel macrocyclic chelator called  $\text{py}_2\text{-macrodipa}$  is reported, and its coordination chemistry with the rare-earth metals is described. These studies show it to have promising properties for the chelation of large radiometal ions for nuclear medicine. Thus, its ability to chelate the therapeutic radionuclide  $^{225}\text{Ac}^{3+}$  was assessed, demonstrating efficient formation of inert  $^{225}\text{Ac}^{3+}$  complexes.

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