Published in final edited form as:

Inorg Chem. 2020 February 03; 59(3): 1985–1995. doi:10.1021/acs.inorgchem.9b03347.

[^{nat}/⁴⁴Sc(pypa)][−]: Thermodynamic Stability, Radiolabeling, and Biodistribution of a Prostate-Specific-Membrane-Antigen-Targeting Conjugate

Lily Li,

Medicinal Inorganic Chemistry Group, Department of Chemistry, University of British Columbia, Vancouver V6T 1Z1, Canada; Life Sciences Division, TRIUMF, Vancouver V6T 2A3, Canada;

María de Guadalupe Jaraquemada-Peláez,

Medicinal Inorganic Chemistry Group, Department of Chemistry, University of British Columbia, Vancouver V6T 1Z1, Canada;

Eduardo Aluicio-Sarduy,

Department of Medical Physics, University of Wisconsin—Madison, Madison, Wisconsin 53705, United States

Xiaozhu Wang,

Medicinal Inorganic Chemistry Group, Department of Chemistry, University of British Columbia, Vancouver V6T 1Z1, Canada;

Dawei Jiang,

Department of Medical Physics, University of Wisconsin—Madison, Madison, Wisconsin 53705, United States;

Meelad Sakheie,

Life Sciences Division, TRIUMF, Vancouver V6T 2A3, Canada

Hsiou-Ting Kuo

Department of Molecular Oncology, BC Cancer, Vancouver V5Z 1L3, Canada

Todd E. Barnhart, Weibo Cai

Department of Medical Physics, University of Wisconsin—Madison, Madison, Wisconsin 53705, United States;

Valery Radchenko,

Department of Chemistry, University of British Columbia, Vancouver V6T 1Z1, Canada; Life Sciences Division, TRIUMF, Vancouver V6T 2A3, Canada

---

Corresponding Author: Chris Orvig – orvig@chem.ubc.ca.

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.inorgchem.9b03347>.

Representative spectra of the in-batch UV-titration of the Sc³⁺-pypa system as the pH is raised; stepwise protonation constants (log *K*_{HqL}) of [Sc(pypa)][−]; NMR spectra (¹H, ¹³C, HSQC, HMBC, COSY, NOESY) and high resolution mass spectra of [Sc(pypa)][−] complex; radiochemical-yield data and radio-HPLC spectra of [⁴⁴Sc][Sc(pypa)][−] and [⁴⁴Sc][Sc(pypa-C7-PSMA617)][−] complexes; biodistribution studies data (%ID/g) and binding affinity graphs of [⁴⁴Sc][Sc(pypa-C7-PSMA617)][−] complex (PDF)

Complete contact information is available at: <https://pubs.acs.org/doi/10.1021/acs.inorgchem.9b03347>

The authors declare no competing financial interest.

Paul Schaffer,

Life Sciences Division, TRIUMF, Vancouver V6T 2A3, Canada

Kuo-Shyan Lin,

Department of Molecular Oncology, BC Cancer, Vancouver V5Z 1L3, Canada;

Jonathan W. Engle,

Department of Medical Physics, University of Wisconsin—Madison, Madison, Wisconsin 53705, United States

François Bénard,

Department of Molecular Oncology, BC Cancer, Vancouver V5Z 1L3, Canada;

Chris Orvig

Medicinal Inorganic Chemistry Group, Department of Chemistry, University of British Columbia, Vancouver V6T 1Z1, Canada;

Abstract

^{44}Sc is an attractive positron-emitting radionuclide for PET imaging; herein, a new complex of the Sc^{3+} ion with nonmacrocyclic chelator H_4pypa was synthesized and characterized with high-resolution electrospray-ionization mass spectrometry (HR-ESI-MS), as well as different nuclear magnetic resonance (NMR) spectroscopic techniques (^1H , ^{13}C , ^1H – ^{13}C HSQC, ^1H – ^{13}C HMBC, COSY, and NOESY). In aqueous solution (pH = 7), $[\text{Sc}(\text{pypa})]^-$ presented two isomeric forms, the structures of which were predicted using density functional theory (DFT) calculation with a small energy difference of 22.4 kJ/mol, explaining their coexistence. $[\text{Sc}(\text{pypa})]^-$ was found to have superior thermodynamic stability ($\text{pM} = 27.1$) compared to $[\text{Sc}(\text{AAZTA})]^-$ (24.7) and $[\text{Sc}(\text{DOTA})]^-$ (23.9). In radiolabeling, $[\text{Sc}(\text{pypa})]^-$ formed efficiently at RT in 15 min over a range of pH (2–5.5), resulting in a complex that is highly stable (>99%) in mouse serum over at least six half-lives of scandium-44. Similar labeling efficiency was observed with the PSMA (prostate-specific membrane antigen)-targeting H_4pypa -C7-PSMA617 at pH = 5.5 (RT, 15 min), confirming negligible disturbance from the bifunctionalization on scandium-44 scavenging. Moreover, the kinetic inertness of the radiocomplex was proved *in vivo*. Surprisingly, the molar activity was found to have profound influence on the pharmacokinetics of the radiotracers where lower molar activity drastically reduced the background accumulations, particularly, kidney, and thus, yielded a much higher tumor-to-background contrast.

Graphical Abstract

INTRODUCTION

Decay properties of radionuclides can be harnessed for cancer diagnosis and therapy. In nuclear medicine, there are two principal imaging techniques—Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET). Compared to SPECT imaging, PET generally has higher sensitivity and spatial resolution (2–4 mm vs 6–8 mm).¹ In PET imaging, the location of the radiotracer in the body is identified through the coincident detection of two back-to-back ($\sim 180^\circ$) γ -rays (511 keV each) produced upon the annihilation between the positron (β^+) emitted and the electron (e^-) encountered.^{2–4} The travel distance of the positron before the annihilation event correlates to the positron energy, thereby determining the image resolution.⁴

An ideal PET isotope should possess low positron energy, a high positron branching ratio, and a long enough half-life for radiolabeling and probing biological events.⁵ Unlike the more “organic radionuclides” (carbon-11, nitrogen-13, oxygen-15, fluorine-18), which are mostly limited by short half-lives,⁵ a much broader decay spectrum can be found in radiometals such as yttrium-86 ($t_{1/2} = 14.7$ h), copper-61/64 ($t_{1/2} = 12.7$ h/3.33 h), scandium-44 ($t_{1/2} = 3.97$ h), zirconium-89 ($t_{1/2} = 78.5$ h), gallium-68 ($t_{1/2} = 1.13$ h), etc.^{6,7} Their much longer half-lives allow for production in distant cyclotrons, enabling the expansion of PET diagnosis beyond large medical centers. Gallium-68 is one of the most used clinical positron emitters due to its high positron branching ratio ($I_{\beta^+} = 89\%$) and the widely available germanium-68/gallium-68 generator,^{8–10} but the application is restricted by its rather short 68 min half-life, plus the breakthrough of germanium-68 and other metal-ion impurities present in the eluate complicates the separation and radiolabeling procedures.^{5,11,12} In this regard, scandium-44 offers several advantages, including its almost quadrupled half-life, higher positron fraction ($I_{\beta^+} = 94.3\%$) with lower energy ($E_{\beta^+ \text{ avg}} = 632$ keV, compared to gallium-68 ($E_{\beta^+ \text{ avg}} = 830$ keV)), thereby favoring spatial resolution.^{11,13} Furthermore, a human PET/CT imaging study conducted by Singh et al. using [^{44}Sc] [Sc(DOTATOC)] proved prolonged imaging up to 23.5 h postinjection feasible,¹⁴ while ^{68}Ga -labeled tracers are generally limited beyond 4 h.^{15,16} Hence, scandium-44 is

considered a better surrogate marker for long-lived therapeutic isotopes such as lutetium-177 and scandium-47 in a pretherapeutic dosimetry study.^{17–19} Currently, scandium-44 can be produced via either a titanium-44/scandium-44 generator or cyclotron irradiation of a calcium-44 target ($^{44}\text{Ca}(\text{p},\text{n})^{44}\text{Sc}$).^{20,21}

Incorporating a metallic radionuclide into a radiopharmaceutical entails a bifunctional chelator that can secure the radiometal ion. To date, macrocyclic DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; Chart 1) and its derivatives are the workhorses for complexation of many radiometal ions including scandium-44, but the harsh radiolabeling conditions ($>95\text{ }^{\circ}\text{C}$) are a drawback.^{7,11} Therefore, alternatives are sought (Chart 1), including acyclic DTPA (diethylenetriamine-pentaacetic acid) and mesocyclic AAZTA (6-[bis(hydroxycarbonyl-methyl)amino]-1,4-bis-(hydroxycarbonyl methyl)-6-methylperhydro-1,4-diaza-pine).^{18,22} AAZTA offers radiolabeling at room temperature, but it is heavily time-dependent ($\sim 80\%$ radiochemical yield in 30 min).²²

Considering the above-mentioned factors, we set out to explore the potential of H_4pypa for scandium-44 as a chelating agent for pharmaceutical purposes. H_4pypa previously displayed excellent affinity for lutetium-177 (β^- , γ , $t_{1/2} \sim 6.64$ days);²³ therefore, its chelation with scandium-44 can pave the way for this “matched pair” in the theranostic applications. Herein, studies of H_4pypa and the conjugate of glutamate-urea-lysine-based PSMA-targeting pharmacophore, $\text{H}_4\text{pypa-C7-PSMA617}$ (Chart 1), for prostate-cancer (PCa) targeting are reported with respect to its suitability for Sc^{3+} ion. The targeting vector was selected for its excellent affinity and selectivity to PSMA, which overexpresses in nearly all stages of PCa, the most common cancer in men in the United States.^{24–28} Syntheses of H_4pypa and $\text{H}_4\text{pypa-C7-PSMA617}$ were reported in our first report.²³ Herein, the nonradioactive $[\text{Sc}(\text{pypa})]^-$ complex is reported and characterized with HR-ESI-MS and different NMR spectroscopic techniques. Variable-temperature ^1H NMR spectroscopy revealed the coexistence of two structural isomers, the geometries of which were estimated with density functional theory (DFT) calculation. Spectrophotometric and potentiometric titrations were conducted to determine the thermodynamic stability of the complex while radiolabeling experiments were performed to evaluate the complexation efficiency in extremely dilute solutions. To assess the biological stability of the complex and the impacts of injected molar activity on the pharmacokinetics, biodistribution studies were performed on PSMA-xenograft-bearing mice using tracers with two different apparent molar activities.

RESULTS AND DISCUSSION

Metal Complexation and Characterization.

The complex of H_4pypa (Figure 1) with Sc^{3+} was fully characterized with NMR spectroscopy and HR-ESI-MS. ^1H NMR spectra revealed the coexistence of two geometric isomers of the complex (major and minor) which interconverted in a temperature-dependent manner (Figure 2). As shown in the VT-NMR spectra, from $25\text{ }^{\circ}\text{C}$ to $85\text{ }^{\circ}\text{C}$, some minor isomers converted to the major one (Figure 2), and the conversion was reversible as the temperature cooled down. The interconversion could be seen from the significantly reduced signal intensity of the minor ^1H peaks, and therefore, the corresponding correlations in both ^1H – ^{13}C HSQC (Figures S3 and S10) and COSY (Figures S6 and S11) spectra became

invisible at higher temperatures. Although the characterization of the minor isomer by NMR was impossible due to excessive overlapping of the ^1H signals, the major isomer could be identified via ^1H – ^{13}C HSQC and HMBC, as well as COSY and NOESY at 70 °C, at which temperature the intervening cross-peaks from the minor isomer were minimized (Figures S1–S7). The assignment of ^1H and ^{13}C signals is depicted in Figure 1, and the labels can be referred to in Figure 1 (^1H , a–i) and Figures S2B,C (^{13}C , 1–23).

The asymmetry in the Sc-pypa complex could be seen from the twelve aliphatic and nine aromatic ^1H – ^{13}C couplings in the HSQC (Figure S3), as well as the six pairs of diastereotropic methylene-H atoms (Figure S6). The ^3J coupling between the *ortho*-H (g (g'), i (i'), h (h')) and the neighboring *para*-H (g'', i'', h'') (Figure S6) confirmed the three distinct pyridyl groups in the complex. With reference to the chemical shift, g'' (triplet) was assigned to the *para*-H in the central pyridine while two overlapping triplets (i'' and h'') belonged to those in the picolinate arms (Figure S1C). $\text{H}_{\text{a,a'}}$ and $\text{H}_{\text{b,b'}}$ were assigned to the methylene groups in the two acetate arms due to the strong interaction with the adjacent $\text{C}_{\text{C=O}}$ (C_{22} and C_{23} , respectively, Figure S4D). $\text{C}_{\text{H}_{\text{a,a'}}}$ (C_1) correlated to $\text{H}_{\text{e'}}$ and $\text{H}_{\text{d'}}$ (Figure S5B), while $\text{H}_{\text{d'}}$ also coupled to $\text{C}_{\text{H}_{\text{g'}}}$ (C_8 ; Figure S5C) and the quaternary C_{18} (Figure S5E). Therefore, $\text{H}_{\text{d,d'}}$ and $\text{H}_{\text{e,e'}}$ were close to $\text{H}_{\text{a,a'}}$ and were assigned to the methylene groups adjacent to the central pyridine and the pyridine in the picolinate arm, respectively (Figure 1). Similarly, $\text{H}_{\text{c,c'}}$ correlated to both $\text{C}_{\text{H}_{\text{b,b'}}}$ (C_4) and $\text{C}_{\text{H}_{\text{f,f'}}}$ (C_5 ; Figure S5B), while H_{f} spatially coupled to H_{i} (Figure S7B) and $\text{H}_{\text{c,c'}}$ correlated to $\text{C}_{\text{H}_{\text{g}}}$ (C_6 ; Figure S5C). Thus, $\text{H}_{\text{c,c'}}$ and $\text{H}_{\text{f,f'}}$ were assigned to the methylene groups neighboring the middle pyridine and that in the second picolinate arm, respectively (Figure 1). The assignment could be further confirmed by the ^1H – ^{13}C (quaternary) interactions (i.e., $\text{H}_{\text{c,c'}}$ and $\text{H}_{\text{g,g''}}$ with C_{19} ; $\text{H}_{\text{f'}}$ and $\text{H}_{\text{i,i''}}$ with C_{16} ; $\text{H}_{\text{d'}}$ and $\text{H}_{\text{g',g''}}$ with C_{18} ; $\text{H}_{\text{e'}}$ and $\text{H}_{\text{h''}}$ with C_{17} ; Figure S5D,E). The only missing assignments are the carbonyl carbons (C_{20} and C_{21}) in both picolinate arms due to the absence of observable correlation with the adjacent H (Figure S5A). Additionally, the fluxionality in the $[\text{Sc}(\text{pypa})]^-$ complex was reflected by the exchange of the pendent arms in space (between two acetate arms and between two picolinate arms), indicated by the strong exchange cross-peaks (blue) in the NOESY spectrum at 70 °C (a and b', a' and b, c and d', f and e', f' and e, g and g', h'' and i'', h' and i', Figure S7). The spatial exchange of the chelating arms suggested that the fluxionality possibly involved the chelate ring opening and reclosing. The broader ^1H peaks at room temperature, particularly in the aliphatic region, indicated a slower chelating-arm exchange, which was further confirmed by the absence of the exchange cross-peaks (blue) in the NOESY spectrum at room temperature (Figure S12). The intrinsic fluxional behavior of the $[\text{Sc}(\text{pypa})]^-$ complex can be justified by the small size of the Sc^{3+} ion (ionic radii = 0.75–0.87 Å, coordination number = 6–8) relative to the 9-coordinating binding cavity of H_4pypa .²⁹ Nonetheless, the splitting of the methylene- ^1H signals upon coordination implied a 9-coordinated $[\text{Sc}(\text{pypa})]^-$.

Coexisting in equilibrium, two $[\text{Sc}(\text{pypa})]^-$ isomers could not be distinguished with HPLC (Figure S14), perhaps due to the similar chemical properties. Admittedly, the isomerism could be a concern for radiopharmaceutical applications; however, it should not be a limitation since more important considerations in this regard would be the biological stability and properties of the $[\text{Sc}(\text{pypa})]^-$ complex in both isomeric forms, which can only be evaluated by biodistribution studies. Furthermore, the biological properties of a metal

complex are heavily dependent on the conjugated targeting vector. For example, when conjugated to a larger targeting vector such as an antibody or antibody fragment, the influence of the metal-chelate on the biodistributions would be significantly reduced, as long as the complex is stable *in vivo*. As a result, despite the coexistence of both isomers at room temperature, it is still important to evaluate the *in vivo* behavior of the $[\text{Sc}(\text{pypa})]^-$ complex as a bioconjugate.

DFT Calculations.

DFT calculations were carried out to study the structure of the $[\text{Sc}(\text{pypa})]^-$ anion in solution. Two stable isomeric structures, A and B (Figure 3), were identified, featuring different bond conformations around the pyridyl backbone. In structure B, both acetate arms were on the same side of the plane with respect to the central pyridyl moiety, while they were opposite in structure A, similar to the geometry of the $[\text{Lu}(\text{pypa})]^-$ complex, which was previously identified as nine-coordinated by crystallography.²³ Lu^{3+} is slightly larger than Sc^{3+} (ionic radii = 0.87 vs 0.98 Å, coordination number = 8),²⁹ and the common coordination number for Sc^{3+} is between 6 and 8 vs 8 and 9 for Lu^{3+} . However, upon comparing the bond lengths between the structure A and the $[\text{Lu}(\text{pypa})]^-$ complex, the average M–N bonds were similar while the Sc–O bonds were shorter than the Lu–O bonds, suggesting that the $[\text{Sc}(\text{pypa})]^-$ complex in this geometry was also nine-coordinated. In fact, this conformation was also energetically more favorable than structure B. With longer average bond distances between the donor atoms and the Sc^{3+} ion (Table 1), structure B was about 22.4 kJ/mol less stable than was structure A. Nonetheless, the small energy gap reasonably justifies the coexistence of two conformations, with structure A dominating. As seen in the VT-NMR spectra (Figure 2), the isomeric equilibrium shifted toward the major one as the temperature elevated but reversed to the initial position as soon as the temperature cooled down. On the basis of the DFT calculation, the transformation could possibly happen by switching the positions of O3 and N4 in structure B. One possible mechanism could involve breaking the Sc–O3 bond, followed by rearrangement of the Sc–N4 bond and finally reformation of the Sc–O3 bond on the opposite side. Due to the particularly long bond distance between N4 and the Sc^{3+} ion (2.8345 Å, 0.2684 Å longer than that in the structure A), it is also possible that both the Sc–O3 and Sc–N4 bonds were broken, rearranged, and then reformed. Either way, the transformation to structure A resulted in significantly stronger interactions between the metal center and the backbone nitrogen atoms (i.e., N2–N4), and thus a more stable complex.

Solution Thermodynamics.

Due to the competition between the metal ion and protons for the binding sites of a chelator, the basicity of different ionizable and nonionizable protons is an essential consideration when evaluating the metal complexation. In our previous publication, we reported that H_4pypa possessed a total of nine protonation sites, and all the protonation constants were determined using combined potentiometric-spectrophotometric titrations and UV in-batch spectrophotometric titrations.²³ H_4pypa was found to be a moderately basic chelator with the highest $\text{p}K_a$ being 7.78, assigned to one of the protonated tertiary amines in the pyridyl backbone,²³ suggesting that at physiological pH (~7.4) the competition from protons is not a significant concern, as compared to more basic chelators, such as H_4octox (Chart 1, highest

$pK_a = 10.65$).³⁰ Furthermore, the complex formation equilibria of H₄pypa with In³⁺, Lu³⁺, and La³⁺ were studied,¹⁹ and the complex stability (pM) followed the order of In³⁺ (30.5) > Lu³⁺ (22.6) > La³⁺ (19.9), opposite their ionic radii (In³⁺ < Lu³⁺ < La³⁺).²⁹ From this observation, we concluded that the binding cavity of H₄pypa was best suited to fit the smaller In³⁺ ion.

In this paper, Sc³⁺ is the subject metal ion of interest with an even smaller ionic radius compared to that of In³⁺ (0.87 Å vs 0.92 Å, 8-coordinated).²⁹ To investigate the complexation equilibria of the Sc³⁺-H₄pypa system, similar studies were conducted to determine the thermodynamic stability constant (log $K_{Sc(pypa)}$). Because the complexation between Sc³⁺ and H₄pypa was complete at pH 2, direct determination of log $K_{Sc(pypa)}$ by potentiometric titration was not feasible. Alternative approaches using a ligand–ligand competition method with an EDTA competitor (Chart 1), as well as acidic in-batch UV spectrophotometric titrations (Figure 4A), were required to first determine the stability constant of the protonated species ([Sc(Hpypa)]). Once log $K_{Sc(Hpypa)}$ was known, direct potentiometric methods were used to determine the stability constants of the [Sc(pypa)][−] and [Sc(OH)-(pypa)] species. Potentiometric and spectrophotometric experimental data were refined using the Hyperquad2013³¹ and HypSpec2014³² programs. The thermodynamic stability constant of [Sc(pypa)][−] (log $K_{[Sc(pypa)]^-}$) was finally calculated to be around 26.98(1) (0.16 M NaCl, 25 °C; Table S1), which is similar to that of [Sc(AAZTA)][−] (log $K_{Sc(AAZTA)} = 27.69(4)$, 0.1 M KCl, 25 °C)²² and around 3.8 units lower than that of [Sc(DOTA)][−] (log $K_{Sc(DOTA)} = 30.79$, 0.1 M Me₄NCl, 25 °C).³³ In order to accurately compare the stability with other complexes involving different chelators, a parameter that accounts for not only the stability constant but also the basicity and the denticity of the chelator is necessary. Therefore, the pSc value (−log [M]_{free} at [L] = 10 μM, [M] = 1 μM and pH = 7.4) was adopted for a comprehensive measurement of the metal sequestering ability. The calculated pSc value of the Sc³⁺-H₄pypa system was 27.1 (Table S1), which is more than two units higher than that of AAZTA (24.7) and more than three units higher than that of DOTA (23.9).¹⁸ The exceptionally high log $K_{Sc(pypa)}$ and pSc values unequivocally manifest the superiority of H₄pypa over both DOTA and AAZTA to form more thermodynamically stable complexes with the Sc³⁺ ion at physiological pH (7.4). Unexpectedly, as depicted in Figure 4B, [Sc(pypa)][−] was an exception in the otherwise size-dependent pM value of the M³⁺-H₄pypa systems (M³⁺ = Sc³⁺, In³⁺, Lu³⁺, and La³⁺). It could be interpreted that the size of the In³⁺ ion is the most suitable for the binding cavity of H₄pypa among the four metal ions studied. Nonetheless, all of them, once again, exhibited superior metal ion scavenging ability over complexes with the comparison chelators.

Radiolabeling and Mouse Serum Challenge Experiment.

All the radiolabeling experiments with H₄pypa and H₄pypa-C7-PSMA617 presented here were performed at room temperature, and the samples were agitated during incubation. The radiochemical yield was determined with instant thin layer chromatography plates impregnated with silicic acid (iTLC_{SA}), developed in sodium citrate (0.4 M, pH = 4.5), and the radiocomplexes were confirmed with radioactive high-performance liquid chromatography (radio-HPLC; Figure S15). Initial concentration-dependent radiolabeling at room temperature demonstrated that both H₄pypa and H₄pypa-C7-PSMA617 radiolabeled

scandium-44 (2.9 MBq) efficiently in 5 min (0.5 M NH_4OAc buffer, pH = 5.5; Figure 5A), with apparent molar activities (end-of-bombardment (EoB)-corrected) of 45 GBq/ μmol and 44 GBq/ μmol , respectively. The calculated apparent molar activity increased when the radiolabeling mixtures were incubated longer (48 GBq/ μmol (30 min) and 53 GBq/ μmol (60 min) for H_4pypa ; 47 GBq/ μmol (30 min) and 52 GBq/ μmol (60 min) for $\text{H}_4\text{pypa-C7-PSMA617}$; Tables S2 and S4). In order to investigate the effects of pH on the radiolabeling efficiency, pH-dependent radiolabeling experiments with the same amount of radioactivity (i.e., 2.9 MBq) were carried out, showing that the radiolabeling of H_4pypa with scandium-44 was the most efficient at pH = 2 ($98 \pm 0\%$ at 10^{-5} M, $94 \pm 1\%$ at 10^{-6} M, 15 min) and gradually deteriorated as the pH increased ($77 \pm 7\%$ at 10^{-5} M, $10 \pm 5\%$ at 10^{-6} M, pH = 7, 15 min; Figure 5B and Table S3). This phenomenon could be explained by metal ion hydrolysis, supported by studies which proved that different scandium(III) hydrolysis products or the dimeric hydrolysis complex ($[\text{Sc}_2(\mu\text{-OH})_2(\text{H}_2\text{O})_{10}]^{4+}$) could form at pH 1, contingent on the concentration and ionic strength.^{34,35} Nonetheless, in our radiolabeling study, decent yields were still attained at pH = 4 and 5.5 ($95 \pm 2\%$ and $96 \pm 2\%$ at 10^{-5} M; $82 \pm 1\%$ and $89 \pm 2\%$ at 10^{-6} M, respectively, in 15 min).

These radiolabeling results are in sharp contrast with that of the “gold standard” DOTA, reported in the recent, $^{44}\text{Sc}[\text{Sc}(\text{-AAZTA})]$ study ($\sim 0\%$ RCY in 15 min, 0.2 M NH_4OAc buffer pH = 4, 0.1 MBq ^{44}Sc , $[\text{DOTA}] = 0.1 \mu\text{M}$).²² Under the same conditions as DOTA, $\sim 60\%$ RCY was obtained for AAZTA,²² which is similar to $^{44}\text{Sc}[\text{Sc}(\text{pypa})]^-$ ($50 \pm 8\%$ RCY, RT, 15 min, pH = 4, $0.1 \mu\text{M}$; Table S3); however, more ^{44}Sc was used in labeling H_4pypa (~ 2.9 MBq). Also, similar pH-dependent radiolabeling was observed with AAZTA, which possessed the highest affinity for scandium-44 in the pH range 3–4, and declined as the pH was raised ($0.3 \mu\text{M}$, 0.1 MBq, 95°C , 5 min); DOTA, however, yielded only $\sim 15\%$ RCY under the same labeling conditions even at its optimal pH of 3.²² Another study with DOTATOC reported by Pruszyński et al. demonstrated that a temperature as high as 115°C was necessary for $>90\%$ radiolabeling (15 min, pH = 4).³⁶

After the successful radiolabeling studies, both $^{44}\text{Sc}[\text{Sc}(\text{L})]$ (L = pypa and pypa-C7-PSMA617) were challenged with mouse serum. The complexes remained intact in the presence of the mouse serum proteins for at least six half-lives of scandium-44 with $<1\%$ transchelation (Figure 5C), which is extremely encouraging, considering the efficient radiolabeling at room temperature that H_4pypa offers.

PET/CT Imaging, Biodistribution Studies, and Binding Affinity.

The binding affinities of $\text{H}_4\text{pypa-C7-PSMA617}$ to PC3-PIP (PSMA+) and PC3-Flu (PSMA-) cell lines were compared using a series of diluted $\text{H}_4\text{pypa-C7-PSMA617}$ solutions in the presence of a small amount of $^{44}\text{Sc}[\text{Sc}(\text{pypa-C7-PSMA617})]$. The study was performed under the assumptions that the radiolabeling would not affect the binding of the PSMA ligands to the cell surface receptors, and due to the small portions of the radiolabeled tracers, its influence on overall binding was omitted. The results revealed the PSMA-specific binding of $\text{H}_4\text{pypa-C7-PSMA617}$ with high affinity ($K_D = 2.96 \pm 0.81$ nM and $B_{\text{max}} = 7.84 \pm 0.37$ fmol/mg; Figure S18).

For *in vivo* study, two batches of radiotracers were prepared with two different apparent molar activities (7.4 GBq/ μ mol and 74 GBq/ μ mol) and then injected into nude mice ($n = 3$) bearing both PSMA-positive (PC3-PIP, left shoulder) and PSMA-negative (PC3-Flu, right shoulder) tumors to investigate the targeting specificity of the tracer, as well as the effects on pharmacokinetics associated with the injected molar activity. Both PET/CT images (Figure 6) and *ex vivo* biodistribution study (Figures 7, 8) showed that the ^{44}Sc -labeled $\text{H}_4\text{pypa-C7-PSMA617}$ was excreted renally and collected through the bladder, while the tumor accumulation was PSMA-specific. In both experiments, the biodistributions of the radiotracers were largely similar, and the tumor uptake was rapid. After 30 min, the tumor radioactivity had already reached $15.1 \pm 2.4\%$ ID/g (% injected dose/g; 7.4 GBq/ μ mol) and $22.1 \pm 1.9\%$ ID/g (74 GBq/ μ mol). In both cases, the accumulation peaked at 4 h postinjection (p.i.; $20.9 \pm 3.4\%$ and $24.0 \pm 2.8\%$ ID/g, respectively) and gradually reduced over time. Nonetheless, the tumor retention was effective at $14.9 \pm 1.7\%$ ID/g (7.4 GBq/ μ mol) and $20.0 \pm 0.6\%$ ID/g (74 GBq/ μ mol) after about four half-lives of scandium-44. Unexpectedly, comparing the two profiles, although lower apparent molar activity contributed to slightly reduced tumor uptake, it significantly brought down the kidney accumulation by more than half (Figure 8). Consequently, with 7.4 GBq/ μ mol, the tumor-to-kidney ratio increased considerably from 0.39 (30 min p.i.) to 0.57 (16 h p.i.), as opposed to around 0.28 throughout the study when using 74 GBq/ μ mol (Figure S19). Besides the kidney, the background radioactivity generally decreased in tandem with the injected molar activity, leading to much higher tumor-to-background ratios. The contrasts were particularly prominent in blood (113 vs 63), bone (91 vs 61), heart (194 vs 83), lung (85 vs 18), liver (113 vs 37), spleen (34 vs 17), and pancreas (194 vs 83) (16 h p.i.; Table S5). This phenomenon, however, should not be generalized to all PSMA-targeting radiotracers. The results reported by Fendler et al. showed that [^{177}Lu][Lu(DOTAPSA617)] with high molar activity was related to a higher tumor-to-background ratio.³⁷ Therefore, it is crucial to evaluate the effect associated with the molar activity independently for each radiotracer to attain an optimal tumor-to-background contrast.

Despite different tumor models being used, the pharmacokinetics of [^{44}Sc][Sc(pypa-C7-PSMA617)] resembled those for the published ^{111}In -analog, especially the strong kidney retention, but were drastically different from the ^{177}Lu -counterpart where the clearance from the background organs/tissues was much faster, including the kidney ($\sim 2\%$ ID/g at 24 h p.i.), leaving the tumor with major radioactivity accumulations ($\sim 14\%$ ID/g at 24 h p.i.).²³ The observed differences in the biological behavior of ^{44}Sc - and ^{177}Lu -labeled $\text{H}_4\text{pypa-C7-PSMA617}$ were unexpected since it was contrary to that of ^{44}Sc - and ^{177}Lu -labeled DOTAPSA617, which appeared to have similar biodistributions.¹³ In the same tumor models (PC-3 PIP/Flu), both ^{44}Sc - and ^{177}Lu -labeled DOTAPSA617 were rapidly cleared from the background tissues/ organs, including the kidney ($\sim 2\text{--}3\%$ ID/g at 6 h p.i.), while their tumor (PSMA+) uptakes and the tumor-to-kidney ratios were comparable ($\sim 52\text{--}55\%$ ID/g and $17\text{--}23$, respectively, at 6 h p.i.).¹³ The similarity renders [^{44}Sc][Sc(DOTAPSA617)] a promising pretherapeutic imaging agent for the ^{177}Lu counterpart, while the discrepancies in [^{44}Sc][Sc(pypa-C7-PSMA617)] and [^{177}Lu][Lu(pypa-C7-PSMA617)] suggested that the biochemical properties of ^{44}Sc - and ^{177}Lu -labeled pypa complexes are different. Nonetheless, the biodistribution of a radiotracer is heavily dependent on the targeting

molecule, and therefore, the differences observed in [^{44}Sc][Sc(pypa-C7-PSMA617)] and [^{177}Lu][Lu(pypa-C7-PSMA617)] cannot be generalized to other bioconjugates with different targeting vectors. What is important from this animal study is the demonstration of the exceptional *in vivo* stability of the [Sc(pypa)][−] complex, which can be radiolabeled with a simple and robust procedure in 5 min at room temperature, compatible with the temperature-sensitive bio-molecules such as the antibody and antibody fragment. These advantages would be particularly valuable for imaging at late time points and could be exploited when using scandium-47 ($t_{1/2} = 3.35$ days) or lutetium-177 as the therapeutic agents for targeted radioligand/radioimmunotherapy.

CONCLUSION

The potentially nonadentate nonmacrocyclic chelator, H₄pypa, which previously demonstrated excellent affinities for both In³⁺ and Lu³⁺ ions,²³ has also presented highly stable chelation with the Sc³⁺ ion. The complex was synthesized and characterized by HR-ESI-MS and a range of NMR spectroscopic techniques. On the basis of the ¹H NMR spectrum, [Sc(pypa)][−] in aqueous solution (pH = 7) existed as two isomers (major and minor) in an equilibrium further shifted toward the major conformation as the temperature was raised (>25°C). The structures of both isomers were predicted by DFT calculation with a small energy difference of 22.4 kJ/mol, rendering their coexistence reasonable. In addition, the formation constant (log $K_{\text{Sc(pypa)}}$) and pSc value of the [Sc(pypa)][−] complex were calculated to be 26.98(1) and 27.1 (Table S1), respectively, significantly higher than those of [Sc(DOTA)][−] and [Sc(AAZTA)][−]. Furthermore, radiolabeling experiments with scandium-44 confirmed efficient radio-metalation at room temperature in 5–15 min at a chelator concentration as low as 10^{−6} M (pH = 2–5.5), resulting in a highly stable complex upon mouse serum challenge over at least six decay half-lives. Lower pH also led to better radiolabeling (i.e., higher radiochemical yield at lower concentration), possibly due to the facile Sc(III) hydrolysis. To further evaluate the biological applicability of [Sc(pypa)][−], PSMA-targeting [^{44}Sc][Sc(pypa-C7-PSMA617)] was injected into tumor-bearing nude mice in two molar activities, demonstrating PSMA-dependent accumulation and excellent long-term stability. To our surprise, considerable influences from the apparent molar activity on the pharmacokinetic profile were observed where lower apparent molar activity (7.4 GBq/ μmol) substantially increased the tumor-to-background ratio. This finding is important for radiotracer formulation to optimize the biodistribution profile. Most importantly, the long-term *in vivo* stability presented H₄pypa as a promising chelator for scandium-44, and even scandium-47, as a theranostic radiopharmaceutical which can be exploited for immuno-PET imaging and targeted radio-therapy as well.

EXPERIMENTAL SECTION

Materials and Methods.

All solvents and reagents were purchased from commercial suppliers (TCI America, Alfa Aesar, AK Scientific, Sigma-Aldrich, Fisher Scientific, Fluka) and were used as received. ¹H, ¹³C, ¹H–¹³C HSQC, ¹H–¹³C HMBC, COSY, and NOESY NMR spectra were recorded at ambient and elevated temperature on Bruker AV400 instruments, as specified; the NMR

spectra are expressed on the δ scale and were referenced to residual solvent peaks. Low-resolution (LR) mass spectrometry was performed using a Waters ZG spectrometer with an ESCI electrospray/chemical-ionization source, and high-resolution electrospray-ionization mass spectrometry (HR-ESI-MS) was performed on a Micromass LCT time-of-flight instrument at the Department of Chemistry, University of British Columbia. Analyses of radiolabeled compounds were performed with both iTLC-silicic acid impregnated plates (iTLC-SA) purchased from Agilent Technologies and radio-HPLC. The TLC scanner model was a BIOSCAN (system 200 imaging scanner), and the HPLC system was from Agilent Technologies (1200 series). A DIONEX Acclaim C18 5 μ m 120 Å column (250 mm $\times$ 4.60 mm) was used for separation of free radioactivity and the radio-complex. ^{44}Sc was provided by the UW—Madison Cyclotron Lab in 0.1 M HCl solution ($\sim$ 259 MBq). Deionized water was filtered through the PURELAB Ultra Mk2 system.

Na[$^{\text{nat}}\text{Sc}(\text{pypa})$].

$\text{H}_4\text{pypa} \cdot 2\text{TFA} \cdot 2\text{H}_2\text{O}$ (10.5 mg, 1.27×10^{-5} mol, 1 equiv) was dissolved in H_2O (0.5 mL) in a scintillation vial, and 0.1 M NaOH (aq) was added to adjust the pH to 7. $\text{ScCl}_3 \cdot 6\text{H}_2\text{O}$ (4.93 mg, 1.91×10^{-5} mol, 1.5 equiv) was added. The mixture was stirred at room temperature for 1 h, and the complexation was confirmed by LR-ESI-MS. The reaction mixture was dried *in vacuo* and then redissolved in D_2O for NMR spectroscopic characterizations. ^1H NMR (400 MHz, 343 K, D_2O): δ 8.73–8.66 (m, 2H), 8.49 (d, $J = 7.8$ Hz, 1H), 8.42 (t, $J = 7.8$ Hz, 1H), 8.38 (d, $J = 7.7$ Hz, 1H), 8.36–8.31 (m, 2H), 7.98 (d, $J = 7.8$ Hz, 1H), 7.82 (d, $J = 7.8$ Hz, 1H), 5.66 (d, $J = 15.1$ Hz, 1H), 5.47 (d, $J = 15.3$ Hz, 1H), 5.39 (d, $J = 14.9$ Hz, 1H), 5.32 (d, $J = 15.4$ Hz, 1H), 5.21 (d, $J = 15.3$ Hz, 1H), 5.16–5.11 (m, 2H), 4.64 (d, $J = 17.5$ Hz, 1H), 4.46 (d, $J = 16.7$ Hz, 1H), 4.34 (d, $J = 16.9$ Hz, 1H), 4.03 (d, $J = 17.6$ Hz, 1H), 3.82 (d, $J = 17.6$ Hz, 1H). ^{13}C NMR (100 MHz, 343 K, D_2O): δ 179.0, 178.2, 176.8, 173.0, 159.2, 158.6, 158.2, 157.8, 150.0, 149.5, 142.9, 142.6, 142.3, 127.1, 126.8, 123.5, 123.0, 122.4, 63.1, 62.9, 62.2, 61.4, 60.1 HR-ESI-MS calcd for $[\text{C}_{25}\text{H}_{21}\text{N}_5\text{O}_8^{45}\text{Sc} + 2\text{H}]^+$: 566.1106. Found $[\text{M} + 2\text{H}]^+$: 566.1102.

DFT Calculations.

All DFT simulations were performed as implemented in the Gaussian 09 revision D.01 suite of *ab initio* quantum chemistry programs (Gaussian Inc., Wallingford, CT). The B3LYP functional^{38,39} and the effective core potential LanL2DZ basis sets for Sc^{40–42} were applied to optimize the structural geometry in the presence of water solvent (IEF PCM as implemented in G09) without the use of symmetry constraints. Normal self-consistent field (SCF) and geometry convergence criteria were conducted for all the calculations. The calculated structures were visualized using Mercury 4.1.

Solution Thermodynamics.

All potentiometric titrations were carried out with a Metrohm Titrando 809 and a Metrohm Dosino 800 with a Ross combined electrode. A 20 mL and 25 °C thermostated glass cell with an inlet–outlet tube for nitrogen gas (purified through a 10% NaOH solution to exclude any CO_2 prior to and during the course of the titration) was used as a titration cell. The electrode was daily calibrated in hydrogen ion concentration by direct titration of HCl with freshly prepared NaOH solution, and the results were analyzed with the Gran procedure⁴³ in

order to obtain the standard potential E° and the ionic product of water pK_w at 25 °C and 0.16 M NaCl used as a supporting electrolyte. Solutions were titrated with carbonate-free NaOH (0.16 M) that was standardized against freshly recrystallized potassium hydrogen phthalate. In the study of complex formation equilibria, the determination of the stability constants of the Sc(Hpypa) species was carried out by two different methods. The first method used UV–vis spectrophotometric measurements on a set of solutions containing a 1:1 metal to ligand molar ratio ($[H_4pypa] = [M]^{3+} = 1.33 \times 10^{-4}$ M) and different amounts of standardized HCl and NaCl to set the ionic strength constant at 0.16 M when possible. The equilibrium H^+ concentration in this UV in batch titration procedure at low pH solutions (2 < pH < 0) was calculated from solution stoichiometry, not measured with a glass electrode. For the solutions of high acidity, the correct acidity scale H^0 was used.⁴⁴ The spectral range was 200–400 nm at 25°C and a 1 cm path length. The molar absorptivities of all the protonated species of H_4pypa calculated with HypSpec2014³² from the protonation constant experiments²³ were included in the calculations. The second method used competition pH-potentiometric titrations with EDTA as a ligand competitor, and the composition of the solutions was $[M]^{3+} = Sc^{3+} \sim 1.54 \times 10^{-3}$ M, $[H_4pypa] \sim 7.07 \times 10^{-4}$ M, and $[EDTA] \sim 1.55 \times 10^{-3}$ M at 25 °C and $I = 0.16$ M NaCl. The stability constants for the complexes formed by EDTA and Sc^{3+} were from the literature.⁴⁵ Direct pH-potentiometric titrations of the Sc^{3+} - H_4pypa systems were also carried out. Sc^{3+} metal ion solution was prepared by adding the atomic absorption (AA) standard solution to a H_4pypa solution of known concentration in the 1:1 metal to ligand molar ratio. Ligand and metal concentrations were in the range of $(8.40\text{--}8.53) \times 10^{-4}$ M. The exact amount of acid present in the AA standard solution was determined by Gran's method⁴³ of titrating equimolar solutions of Sc(III) and $Na_2H_2\text{--}EDTA$. Each titration consisted of 100–150 equilibrium points in the pH range 1.6–11.5; equilibration time for titrations was up to 5 min for metal complex titrations. Three replicates of each titration were performed. Relying on the stability constants for the species Sc(Hpypa) obtained by the two different methods, the fitting of the direct potentiometric titrations was possible and yielded the stability constants in Table S1. All the potentiometric measurements were processed using the Hyperquad2013 software,³¹ while the obtained spectrophotometric data were processed with the HypSpec2014³² program. Proton dissociation constants corresponding to hydrolysis of Sc(III) aqueous ions included in the calculations were taken from Baes and Mesmer.³⁴ The overall equilibrium (formation) constants $\log \beta$ referred to the overall equilibria $pM + qH + rL \rightleftharpoons M_pH_qL_r$ (the charges are omitted), where p might also be 0 in the case of protonation equilibria and q can be negative for hydroxide species. Stepwise equilibrium constants $\log K$ correspond to the difference in log units between the overall constants of sequentially protonated (or hydroxide) species. The parameter used to calculate the metal scavenging ability of a ligand toward a metal ion, pM , is defined as $-\log [M^{n+}]_{\text{free}}$ at $[ligand] = 10$ mM and $[M^{n+}] = 1 \mu\text{M}$ at pH = 7.4.⁴⁶

Production and Radiochemical Isolation of Scandium-44.

Scandium-44 was cyclotron-produced using $^{nat}\text{Ca}[p,n]^{44}\text{Sc}$ nuclear reactions on pressed targets of metallic calcium (300–350 mg). Target preparation was performed in the air and rapidly mounted in the cyclotron to reduce calcium oxidation. Irradiations were performed at 20 μA for 1 h with direct water cooling, and a 12.7 μm Nb foil was used to degrade the beam

energy from the nominal 16 MeV to 14.1 MeV. Under these conditions, a scandium-44 production yield of 0.4 mCi/ μ Ah was obtained through the reaction of $^{nat}\text{Ca}(\text{p},\text{n})^{44}\text{Sc}$. Isolation of the produced scandium-44 was carried out by single column extraction chromatography using an N,N,N',N'-tetrakis-2-ethylhexyldiglycolamide functionalized resin (DGA-branched, Eichrom).⁴⁰ The target was dissolved in 9 M HCl (10 mL) and passed through a 1 mL fritted solid phase extraction (SPE) tube filled with the DGA resin (~120 mg), loading the scandium-44 and eluting bulk Ca^{2+} . Remaining Ca^{2+} was removed by rinsing the column with 4 M HCl (20 mL). Next, a 12 mL wash with 1 M HNO_3 was performed to elute possible trace metal contaminants such as Zn, Fe, and Cu. Finally, scandium-44 was eluted in a small volume using 0.1 M HCl ($4 \times 500 \mu\text{L}$ fractions). The radionuclidic and chemical purity were confirmed by high purity germanium (HPGe) gamma spectrometry and microwave plasma atomic emission spectroscopy (MP-AES), respectively.

Radiolabeling Studies.

An aliquot of ligand solution (H_4pypa or $\text{H}_4\text{pypa-C7-PSMA617}$) ($10 \mu\text{L}$) in NH_4OAc solution (88 μL , 0.5 M, pH = 2, 4, 5.5, 7) was mixed with around 2.9 MBq of scandium-44 (2 μL). The reactions were agitated at ambient temperature over the desired period. The mixture (3 μL) was spotted on an iTLC-SA plate and then developed in sodium citrate buffer (0.4 M, pH = 4.5). The TLC plate was read by a TLC reader, showing the free metal ion migrated to the solvent front while the complex stayed at the baseline. The areas of both peaks were used to calculate RCY %. The complex was also confirmed with radio-HPLC (A, $\text{H}_2\text{O}/0.1\%$ TFA; B, ACN/ 0.1% TFA, 5% - 65% B over 32 min, 1 mL/min): [^{44}Sc] [$\text{Sc}(\text{pypa})$] (t_{R} = 13.41 min); [^{44}Sc] [$\text{Sc}(\text{pypa-C7-PSMA617})$] (t_{R} = 23.67 min); free ^{44}Sc (t_{R} = 3.50 min).

In Vitro Mouse Serum Challenge.

To the radiolabeled sample, an equal volume (100 μL) of mouse serum was added. The mixture was incubated at 37 °C, and 5 μL aliquots were collected at desired time points (0.5 h, 4 h, 8 h, 24 h). The aliquot was spotted onto the iTLC-SA plate next to the control spot (free scandium-44 in serum without chelator) and developed in sodium citrate solution (0.4 M, pH = 4.5). The TLC plate was read by a TLC reader. The free metal ion migrated to the solvent front while the complex stayed at the baseline. The areas of both peaks were used to calculate RCY %.

In Vitro Competition Binding Assays.

To determine the binding affinity of $\text{H}_4\text{pypa-C7-PSMA617}$ (in the presence of small amount of [^{44}Sc] [$\text{Sc}(\text{pypa-C7-PSMA617})$]) for prostate cancer cells, a receptor saturation assay was performed as previously described.⁴⁷ Briefly, 1×10^5 cells (either PC3-PIP or PC3-FLU) were plated into filter-bottom 96-well plates (Corning), and various concentrations of PSMA ligand solutions (0.03–100 nM) were incubated with the cells for 2 h. The amount of $\text{H}_4\text{pypa-C7-PSMA617}$ ligands in nanomoles and corresponding radioactivity in μCi can be calculated by using the labeling efficiency ($\mu\text{Ci}/\text{nmol}$) to allow accurate measurement of binding affinity. After the incubation period, the wells were rinsed and dried, and the filter papers were counted in an automated gamma counter to determine the amount of bound

tracer. GraphPad Prism was utilized to plot saturation binding isotherms, and the affinity constant (K_D) and maximum specific binding (B_{max}) were determined.

Radiolabeling of Conjugates for *in Vivo* Study.

For the *in vivo* study, radiolabeling with scandium-44 was performed by mixing 111–185 MBq (3–5 mCi) of radiometal ions with 15–25 nmol of H₄pypa-C7-PSMA617 (7.4 or 74 GBq/ μ mol) in 0.5 M NaOAc buffer solution (pH = 5.5). The reactions were incubated for 30 min at room temperature under agitation (300 rpm). Next, the radio-conjugate was purified by reverse phase chromatography using Sep Pak C18 cartridges (Waters), eluted in absolute ethanol, dried at 70 °C in a N₂ stream, and reconstituted in PBS. Quantitative radiochemical yields (>95%) and high radiochemical purities (>95%) were confirmed by radio-TLC using EDTA solution (50 mM, pH = 5.5) as a mobile phase and radio-HPLC.

PET/CT Imaging Studies.

All animal experiments were performed under the approval of the University of Wisconsin Institutional Animal Care and Use Committee. Male nude mice (6–8 weeks) were xenografted with $(1.5\text{--}2.0) \times 10^6$ PC3-PIP (PSMA+) cells (left shoulder) and $(1.5\text{--}2.0) \times 10^6$ PC3-FLU (PSMA-) cells (right shoulder) suspended in Matrigel (1:1). Tumor bearing mice were employed for *in vivo* imaging, when tumors reached a volume of 400–500 mm³. For noninvasive *in vivo* PET/CT imaging, mice ($n = 3$) were administered 5.5–11.1 MBq (150–300 μ Ci) of [⁴⁴Sc][Sc(pypa-C7-PSMA617)] via tail vein injection. Following isoflurane anesthesia, mice were placed into a microPET/microCT scanner (Inveon, Siemens), and 40 million coincidence event static PET scans were acquired at different time points (0.5, 2, 4, 8, and 16 h postinjection, p.i.). CT scans were taken prior to each PET acquisition for attenuation correction and anatomical reference. Quantitative analyses of images were performed by manually drawing volumes of interest over the tumor and other organs of interest, and data were reported as percent injected dose per gram of tissue (%ID/g), mean $\pm$ SD.

Ex Vivo Biodistribution Studies.

Ex vivo tissue distribution studies were performed to validate the *in vivo* imaging quantification results. After the last PET/CT scan, mice were sacrificed, and select organs were harvested, weighed, and counted in an automatic gamma counter (Wizard 2, PerkinElmer). Uptake in each tissue is reported as % ID/g (mean $\pm$ SD).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank Dr. Maria Ezhova for her useful advice on NMR experiments at UBC. We gratefully acknowledge funding from NSERC CREATE IsoSiM at TRIUMF for a Ph.D. research stipend (L.L.), both NSERC and CIHR for financial support via a Collaborative Health Research Project (CHRP to F.B., C.O., and P.S.), NSERC Discovery (C.O.), and financial support from the National Cancer Institute of the National Institutes of Health under Award Number T32CA009206 (E.A.-S.). TRIUMF receives federal funding via a contribution agreement with the National Research Council of Canada.

REFERENCES

- (1). Zeglis BM; Lewis JS A Practical Guide to the Construction of Radiometallated Bioconjugates for Positron Emission Tomography. Dalton Trans. 2011, 40, 6168–6195. [PubMed: 21442098]
- (2). Ramogida CF; Orvig C Tumour Targeting with Radiometals for Diagnosis and Therapy. Chem. Commun. (Cambridge, U. K.) 2013, 49, 4720–4739.
- (3). Kostelnik TI; Orvig C Radioactive Main Group and Rare Earth Metals for Imaging and Therapy. Chem. Rev 2019, 119, 902–956. [PubMed: 30379537]
- (4). Boros E; Packard AB Radioactive Transition Metals for Imaging and Therapy. Chem. Rev 2019, 119, 870–901. [PubMed: 30299088]
- (5). Hao G; N Singh A; Liu W; Sun X PET with Non-Standard Nuclides. Curr. Top. Med. Chem 2010, 10, 1096–1112. [PubMed: 20388114]
- (6). Müller C; Bunka M; Haller S; Köster U; Groehn V; Bernhardt P; van der Meulen N; Türlér A; Schibli R Promising Prospects for ^{44}Sc -/ ^{47}Sc -Based Theragnostics: Application of ^{47}Sc for Radionuclide Tumor Therapy in Mice. J. Nucl. Med 2014, 55, 1658–1664. [PubMed: 25034091]
- (7). Price EW; Orvig C Matching Chelators to Radiometals for Radiopharmaceuticals. Chem. Soc. Rev 2014, 43, 260–290. [PubMed: 24173525]
- (8). Boros E; Ferreira CL; Cawthray JF; Price EW; Patrick BO; Wester DW; Adam MJ; Orvig C Acyclic Chelate with Ideal Properties for ^{68}Ga PET Imaging Agent Elaboration. J. Am. Chem. Soc 2010, 132, 15726–15733. [PubMed: 20958034]
- (9). Fani M; André JP; Maecke HR ^{68}Ga -PET: A Powerful Generator-Based Alternative to Cyclotron-Based PET Radiopharmaceuticals. Contrast Media Mol. Imaging 2008, 3, 53–63. [PubMed: 18383455]
- (10). Maecke HR; Hofmann M; Haberkorn U ^{68}Ga -Labeled Peptides in Tumor Imaging. J. Nucl. Med 2005, 46, 172S–178S. [PubMed: 15653666]
- (11). Hernandez R; Valdovinos HF; Yang Y; Chakravarty R; Hong H; Barnhart TE; Cai W ^{44}Sc : An Attractive Isotope for Peptide-Based PET Imaging. Mol. Pharmaceutics 2014, 11, 2954–2961.
- (12). Breeman WAP; de Blois E; Sze Chan H; Konijnenberg M; Kwekkeboom DJ; Krenning EP ^{68}Ga -labeled DOTA-Peptides and ^{68}Ga -Labeled Radiopharmaceuticals for Positron Emission Tomography: Current Status of Research, Clinical Applications, and Future Perspectives. Semin. Nucl. Med 2011, 41, 314–321. [PubMed: 21624565]
- (13). Umbricht CA; Benesova M; Schmid RM; Turler A; Schibli R; van der Meulen NP; Muller C ^{44}Sc -PSMA-617 for Radiotheragnostics in Tandem with ^{177}Lu -PSMA-617—Preclinical Investigations in Comparison with ^{68}Ga -PSMA-11 and ^{68}Ga -PSMA-617. EJNMMI Res. 2017, 7, 9. [PubMed: 28102507]
- (14). Singh A; van der Meulen NP; Müller C; Klette I; Kulkarni HR; Türlér A; Schibli R; Baum RP First-in-Human PET/CT Imaging of Metastatic Neuroendocrine Neoplasms with Cyclotron-Produced ^{44}Sc -DOTATOC: A Proof-of-Concept Study. Cancer Biother.Radiopharm 2017, 32, 124–132. [PubMed: 28514206]
- (15). Eder M; Schäfer M; Bauder-Wüst U; Hull W-E; Wängler C; Mier W; Haberkorn U; Eisenhut M ^{68}Ga -Complex Lipophilicity and the Targeting Property of a Urea-Based PSMA Inhibitor for PET Imaging. Bioconjugate Chem. 2012, 23, 688–697.
- (16). Gabriel M; Decristoforo C; Kendler D; Dobrozemsky G; Heute D; Uprimny C; Kovacs P; Von Guggenberg E; Bale R; Virgolini IJ ^{68}Ga -DOTA-Tyr3-Octreotide PET in Neuroendocrine Tumors: Comparison with Somatostatin Receptor Scintigraphy and CT. J. Nucl. Med 2007, 48, 508–518. [PubMed: 17401086]
- (17). Domnanich KA; Müller C; Benešová M; Dressler R; Haller S; Köster U; Ponsard B; Schibli R; Türlér A; van der Meulen NP ^{47}Sc as Useful β -Emitter for the Radiotheragnostic Paradigm: a Comparative Study of Feasible Production Routes. EJNMMI Radiopharm. Chem 2017, 2, 5. [PubMed: 29503846]
- (18). Połosak M; Piotrowska A; Krajewski S; Bilewicz A Stability of ^{47}Sc -complexes with Acyclic Polyamino-Polycarboxylate Ligands. J. Radioanal. Nucl. Chem 2013, 295, 1867–1872. [PubMed: 26224932]

- (19). Khawar A; Eppard E; Sinnes JP; Roesch F; Ahmadzadehfar H; Kürpig S; Meisenheimer M; Gaertner FC; Essler M; Bundschuh RA [44Sc]Sc-PSMA-617 Biodistribution and Dosimetry in Patients With Metastatic Castration-Resistant Prostate Carcinoma. *Clin. Nucl. Med* 2018, 43, 323–330. [PubMed: 29485430]
- (20). Severin GW; Engle JW; Valdovinos HF; Barnhart TE; Nickles RJ Cyclotron Produced ^{44}gSc from Natural Calcium. *Appl. Radiat. Isot* 2012, 70, 1526–1530. [PubMed: 22728844]
- (21). Filosofov DV; Loktionova NS; Rösch FA $^{44}\text{Ti}/^{44}\text{Sc}$ Radionuclide Generator for Potential Application of ^{44}Sc -Based PETRadiopharmaceuticals. *Radiochim. Acta* 2010, 98, 149–156.
- (22). Nagy G; Szikra D; Trencsényi G; Fekete A; Garai I; Giani AM; Negri R; Masciocchi N; Maiocchi A; Uggeri F; Tóth I; Aime S; Giovenzana GB; Baranyai Z AAZTA: An Ideal Chelating Agent for the Development of ^{44}Sc PET Imaging Agents. *Angew. Chem., Int. Ed* 2017, 56, 2118–2122.
- (23). Li L; Jaraquemada-Peláez M. d. G.; Kuo H-T; Merckens H; Choudhary N; Gitschtaler K; Jermilova U; Colpo N; Uribe-Munoz C; Radchenko V; Schaffer P; Lin KS; Bénard F; Orvig C Functionally Versatile and Highly Stable Chelator for ^{111}In and ^{177}Lu : Proof-of-Principle Prostate-Specific Membrane Antigen Targeting. *Bioconjugate Chem.* 2019, 30, 1539–1553.
- (24). Haberkorn U; Eder M; Kopka K; Babich JW; Eisenhut M New Strategies in Prostate Cancer: Prostate-Specific Membrane Antigen (PSMA) Ligands for Diagnosis and Therapy. *Clin. Cancer Res* 2016, 22, 9. [PubMed: 26728408]
- (25). Maresca KP; Hillier SM; Femia FJ; Keith D; Barone C; Joyal JL; Zimmerman CN; Kozikowski AP; Barrett JA; Eckelman WC; Babich JW A Series of Halogenated Heterodimeric Inhibitors of Prostate Specific Membrane Antigen (PSMA) as Radiolabeled Probes for Targeting Prostate Cancer. *J. Med. Chem* 2009, 52, 347–357. [PubMed: 19111054]
- (26). Benešová M; Bauder-Wüst U; Schäfer M; Klika KD; Mier W; Haberkorn U; Kopka K; Eder M Linker Modification Strategies To Control the Prostate-Specific Membrane Antigen (PSMA)-Targeting and Pharmacokinetic Properties of DOTA-Conjugated PSMA Inhibitors. *J. Med. Chem* 2016, 59, 1761–1775. [PubMed: 26878194]
- (27). Benešová M; Schäfer M; Bauder-Wüst U; Afshar-Oromieh A; Kratochwil C; Mier W; Haberkorn U; Kopka K; Eder M Preclinical Evaluation of a Tailor-Made DOTA-Conjugated PSMA Inhibitor with Optimized Linker Moiety for Imaging and Endoradiotherapy of Prostate Cancer. *J. Nucl. Med* 2015, 56, 914–920. [PubMed: 25883127]
- (28). Kratochwil C; Bruchertseifer F; Giesel FL; Weis M; Verburg FA; Mottaghy F; Kopka K; Apostolidis C; Haberkorn U; Morgenstern A ^{225}Ac -PSMA-617 for PSMA-Targeted α -Radiation Therapy of Metastatic Castration-Resistant Prostate Cancer. *J. Nucl. Med* 2016, 57, 1941–1944. [PubMed: 27390158]
- (29). Shannon RD Revised Effective Ionic Radii and Systematic Studies of Interatomic Distances in Halides and Chalcogenides. *Acta Crystallogr., Sect. A: Cryst. Phys., Diff., Theor. Gen. Crystallogr* 1976, 32, 751–767.
- (30). Wang X; Jaraquemada-Peláez M. d. G.; Rodríguez-Rodríguez C; Cao Y; Buchwalder C; Choudhary N; Jermilova U; Ramogida CF; Saatchi K; Häfeli UO; Patrick BO; Orvig C H_4octox : Versatile Bimodal Octadentate Acyclic Chelating Ligand for Medicinal Inorganic Chemistry. *J. Am. Chem. Soc* 2018, 140, 15487–15500. [PubMed: 30394734]
- (31). Gans P; Sabatini A; Vacca A Investigation of Equilibria in Solution. Determination of Equilibrium Constants with the HYPER-QUAD Suite of Programs. *Talanta* 1996, 43, 1739–1753. [PubMed: 18966661]
- (32). Gans P; Sabatini A; Vacca A Determination of equilibrium constants from spectrophotometric data obtained from solutions of known pH: The program pHab. *Anal. Chim* 1999, 89, 45–49.
- (33). Pniok M; Kubí ek V; Havlí ková J; Kotek J; Sabatie- Gogová A; Plutnar J; Huclier-Markai S; Hermann P Thermodynamic and Kinetic Study of Scandium(III) Complexes of DTPA and DOTA: A Step Toward Scandium Radiopharmaceuticals. *Chem. - Eur. J* 2014, 20, 7944–7955. [PubMed: 24838869]
- (34). Baes CF Jr.; Mesmer RE *The Hydrolysis of Cations*; John Wiley & Sons: New York, 1986.

- (35). Lindqvist-Reis P; Persson I; Sandström M The Hydration of the Scandium(III) Ion in Aqueous Solution and Crystalline Hydrates Studied by XAFS Spectroscopy, Large-Angle X-Ray Scattering and Crystallography. *Dalton Trans.* 2006, 32, 3868–3878.
- (36). Pruszy ski M; Majkowska-Pilip A; Loktionova NS; Eppard E; Roesch F Radiolabeling of DOTATOC with the Long-Lived Positron Emitter ^{44}Sc . *Appl. Radiat. Isot* 2012, 70, 974–979. [PubMed: 22464928]
- (37). Fendler WP; Stuparu AD; Evans-Axelsson S; Lückerrath K; Wei L; Kim W; Poddar S; Said J; Radu CG; Eiber M; Czernin J; Slavik R; Herrmann K Establishing ^{177}Lu -PSMA-617 Radioligand Therapy in a Syngeneic Model of Murine Prostate Cancer. *J. Nucl. Med* 2017, 58, 1786–1792. [PubMed: 28546332]
- (38). Lee C; Yang W; Parr RG Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B: Condens. Matter Mater. Phys* 1988, 37, 785–789.
- (39). Becke AD Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys. Rev. A: At., Mol., Opt. Phys* 1988, 38, 3098–3100.
- (40). Alliot C; Kerdjoudj R; Michel N; Haddad F; Huclier-Markai S Cyclotron Production of High Purity $^{44}\text{m},^{44}\text{Sc}$ with Deuterons from $^{44}\text{CaCO}_3$ Targets. *Nucl. Med. Biol* 2015, 42, 524–529. [PubMed: 25794463]
- (41). Hay PJ; Wadt WR Ab Initio Effective Core Potentials for Molecular Calculations. Potentials for K to Au Including the Outermost Core Orbitals. *J. Chem. Phys* 1985, 82, 299–310.
- (42). Hay PJ; Wadt WR Ab Initio Effective Core Potentials for Molecular Calculations. Potentials for the Transition Metal Atoms Sc to Hg. *J. Chem. Phys* 1985, 82, 270–283.
- (43). Gran G Determination of the Equivalence Point in Potentiometric Titrations. Part II. *Analyst* 1952, 77, 661–671.
- (44). Paul MA; Long FA H_0 And Related Indicator Acidity Function. *Chem. Rev* 1957, 57, 1–45.
- (45). Vickery RC 348. Lanthanone Complexes with Ethylenediamine-NNN'-N-tetra-acetic Acid. Part III. *J. Chem. Soc* 1952, 0, 1895–1898.
- (46). Harris WR; Carrano CJ; Raymond KN Spectrophotometric Determination of the Proton-Dependent Stability Constant of Ferric Enterobactin. *J. Am. Chem. Soc* 1979, 101, 2213–2214.
- (47). Hernandez R; Sun H; England CG; Valdovinos HF; Ehlerding EB; Barnhart TE; Yang Y; Cai W CD146-Targeted ImmunoPET and NIRF Imaging of Hepatocellular Carcinoma with a Dual-Labeled Monoclonal Antibody. *Theranostics* 2016, 6, 1918– 1933. [PubMed: 27570560]

Figure 2. Sc-pypa variable temperature ^1H NMR downfield (left) and upfield (right) spectra (400 MHz, D_2O , pH 7; 25, 35, 45, 65, 85 $^\circ\text{C}$ from bottom to top). Please refer to Figure S1A for the full ^1H NMR spectrum.

Structure A

Structure B

Figure 3.
Two isomeric species of the $[\text{Sc}(\text{pypa})]^-$ anion simulated using DFT calculations.

Figure 4.

(A) Representative spectra of the in-batch UV-titration of the Sc^{3+} - H_4pypa system as the pH is raised. $[\text{L}] = [\text{Sc}^{3+}] = 1.33 \times 10^{-4} \text{ M}$ at 25°C , $l = 1 \text{ cm}$. The ionic strength was maintained constant ($I = 0.16 \text{ M}$) when possible by the addition of different amounts of NaCl. (B) pM vs ionic radius²⁹ for M^{3+} ions of interest and ligands discussed.

Figure 5.

(A) Concentration-dependent radiolabeling studies of H_4pypa and H_4pypa -C7-PSMA617 with ^{44}Sc at room temperature in NH_4OAc buffer (0.5 M, pH = 5.5) over 5 min. (B) Concentration-dependent radiolabeling studies of H_4pypa with ^{44}Sc at room temperature in NH_4OAc solution (0.5 M, pH = 2, 4, 5.5, 7) over 15 min. (C) Mouse serum stability of the corresponding complexes over 24 h at 37 °C.

Figure 6. Representative PET/CT images (MIP, coronal) of $[^{44}\text{Sc}][\text{Sc}(\text{pypa-C7-PSMA617})]$ (7.4 (top) and 74 (bottom) GBq/ μmol) in PC3-PIP (left shoulder) and PC3-Flu (right shoulder) tumor-bearing mice at different p.i. time points.

Figure 7.
Biodistribution data of $[^{44}\text{Sc}][\text{Sc}(\text{pypa-C7-PSMA617})]$ (7.4 (left) and 74 (right) GBq/ μmol) in PC3-PIP-tumor-bearing mice at selected p.i. time points ($n = 3$ per time point).

Figure 8.

Ex vivo biodistribution data of $[^{44}\text{Sc}][\text{Sc}(\text{pypa-C7-PSMA617})]$ (7.4 and 74 GBq/ μmol) in PC3-PIP-tumor-bearing mice at selected p.i. time points ($n = 3$ per time point).

R = H, R' = H (H_4pypa)

R' = 'Bu ($tBu_4pypa-C7-NHS$)

DOTA

DTPA

EDTA

AAZTA

H_4octox

Chart 1.
Chemical Structures of the Discussed Chelators

Table 1.

Comparison of DFT Calculated Sc–O and Sc–N Bond Lengths in Structures A and B

structure A			structure B		
atom 1	atom 2	length (Å)	atom 1	atom 2	length (Å)
Sc	N1	2.3500	Sc	N1	2.4174
Sc	N2	2.5647	Sc	N2	2.6676
Sc	N3	2.4960	Sc	N3	2.5960
Sc	N4	2.5661	Sc	N4	2.8345
Sc	N5	2.3495	Sc	N5	2.4198
Sc	O1	2.1925	Sc	O1	2.1915
Sc	O2	2.2149	Sc	O2	2.2190
Sc	O3	2.2115	Sc	O3	2.1436
Sc	O4	2.1872	Sc	O4	2.2279