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High-denticity ligands based on picolinic acid for ^{111}In radiochemistry

Eric W. Price, Cara L. Ferreira, Michael J. Adam, and Chris Orvig

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Abstract: Four new acyclic ligands, Bn-H_3nonapa (**3**), H_3nonapa (**4**), *p*-NO_2-Bn-H_3nonapa (**10**), and Bn-H_3trenpa (**7**), were synthesized and studied with nonradioactive In^{3+} and with radioactive $^{111}In^{3+}$. The coordination of these ligands to In^{3+} was confirmed by high-resolution mass spectrometry and nuclear magnetic resonance spectroscopy. Radiolabeling experiments were performed with $^{111}In^{3+}$; these demonstrated H_3nonapa (**4**) to be the best indium ligand of those studied herein, achieving radiochemical yields of ~97% in 10 min at ambient temperature, and stability to transchelation in mouse serum of 44.5% ± 25.9% after 24 h. Although the radiolabeling kinetics of H_3nonapa (**4**) were excellent, serum stability results were inferior to the previously studied ligands DOTA, DTPA, and H_4octapa, suggesting that the presented ligands may find their optimum radiopharmaceutical applications with isotopes other than ^{111}In. Owing to the high denticity of these ligands (9–10 coordinate), they may realize their potential with large ion isotopes such as ^{177}Lu, $^{86/90}$Y, and ^{225}Ac.

Key words: radiopharmaceuticals, coordination chemistry, radiometals, multidentate ligands, picolinates.

Résumé : Quatre nouveaux ligands acycliques Bn-H_3nonapa (**3**), H_3nonapa (**4**), *p*-NO_2-Bn-H_3nonapa (**10**) et Bn-H_3trenpa (**7**) ont été synthétisés et étudiés à l'aide de l'ion In^{3+}, non radioactif, et de l'ion $^{111}In^{3+}$, radioactif. La coordination de ces ligands avec l'ion In^{3+} a été confirmée par spectrométrie de masse de haute résolution et spectroscopie de résonance magnétique nucléaire. Des expériences de radiomarquage avec l'ion $^{111}In^{3+}$ ont été réalisées et ont montré que H_3nonapa (**4**) était le plus performant des quatre ligands étudiés, de par son rendement chimique (environ 97%, après 10 minutes et à la température ambiante) et sa stabilité à l'égard de la transchélation dans du sérum de souris (44,5% ± 25,9% des ligands intacts après 24 h). Bien que la cinétique du radiomarquage de H_3nonapa (**4**) fût excellente, les résultats de la stabilité de ce dernier dans le sérum ont été inférieurs à ceux obtenus pour les ligands précédemment étudiés (DOTA, DTPA et H_4octapa), ce qui signifie que les ligands étudiés ici pourraient être utilisés de façon optimale dans le domaine pharmaceutique avec des isotopes autres que l'indium ^{111}In. En raison de la denticité élevée de ces ligands (coordinence de 9 à 10), ils pourraient atteindre tout leur potentiel avec des isotopes ioniques lourds tels que ^{177}Lu, $^{86/90}$Y et ^{225}Ac. [Traduit par la Rédaction]

Mots-clés : radiopharmaceutiques, chimie de coordination, radiométaux, ligands multidentates, picolinates.

Introduction

The radiometal ^{111}In is a versatile isotope that has been commonly used for single photon emission computed tomography (SPECT) and Auger electron/β^- therapy, typically withtargeting vectors such as peptides, antibodies, and nanoparticles.[1–6] Imaging agents based on ^{111}In are often used for pretherapy scouting scans to obtain dosimetry information, which is then followed by injection of the same agent prepared with a therapeutic isotope such as ^{177}Lu (half-life ($t_{1/2}$) ~ 6.6 d, β^- therapy) or ^{90}Y ($t_{1/2}$ ~ 2.7 d, β^- therapy).[1–6] The fundamental core of any radiometal-based radiopharmaceutical is the ligand, which serves as an anchor for chelating and sequestering radiometals, and must be chosen so that it forms extremely stable complexes that resist transchelation and demetallation in vivo.

There are a number of ligands that perform adequately for radiolabeling with ^{111}In, such as DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid),[7–16] CHX-A″-DTPA (derivative of acyclic chelator diethylenetriaminepentaacetic acid),[8,16–19] and the recent discovery H_4octapa (*N*,*N*-bis(6-carboxyl-2-pyridylmethyl)-ethylenediamene-*N*,*N*-diacetic acid).[20–24] A newly published acyclic bipyridine-chelator (BPCA) has also shown promise for fast ambient temperature radiolabeling of ^{111}In and strong in vivo stability.[3,25,26] Other than H_4octapa and BPCA, not many new ligands have been published for use with ^{111}In in recent years, with most efforts being focused on newer positron emission computed tomography (PET) isotopes like ^{68}Ga.[1–6,27] The macrocyclic ligand DOTA (Chart 1) has been the main workhorse for radiochemical research with the radiometals ^{111}In, ^{177}Lu, and $^{86/90}$Y. Macrocycles have traditionally been favored over acyclic ligands because they are generally more kinetically inert than acyclic chelators, largely as a result of their constrained geometries and partially preorganized binding cavities.[8,9,28–33] The major drawbacks with macrocycles such as DOTA are the requisite heating (40–90 °C) and extended reaction times (30–180 min) needed for quantitative

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This article is part of a Special Issue dedicated to Professor Barry Lever in recognition of his contributions to inorganic chemistry across Canada and beyond. Dedicated, with the greatest respect, affection, and admiration, to Barry Lever, guru to C.O.

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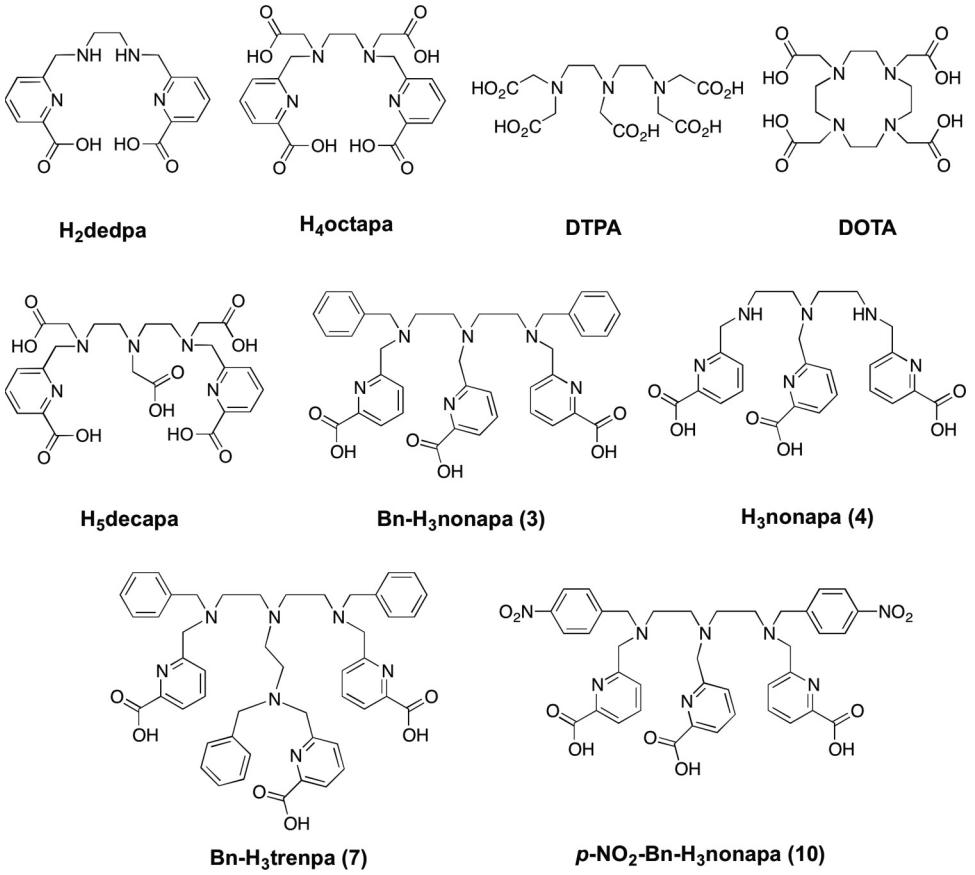
Chart 1. The previously studied chelating ligands H_2dedpa, H_4octapa, H_5decapa, DOTA, and DTPA, and the new compounds Bn-H_3nonapa, H_3nonapa, *p*-NO_2-Bn-H_3nonapa, and Bn-H_3trenpa.



radiolabeling; these conditions are unsuitable for sensitive vectors such as antibodies.[8,9,28–40] The acyclic chelator diethylenetriaminepentaacetic acid (DTPA) (as with most acyclic ligands) exhibits much faster reaction kinetics than DOTA and can radiolabel quantitatively in a matter of minutes at ambient temperature, but the resulting complexes are not nearly as stable in vivo as those of DOTA (Chart 1).[30,31,41–43] New acyclic ligands with rapid ambient temperature radiolabeling kinetics, robust in vivo stability and inertness (compared to macrocycles such as DOTA), and a variety of physical properties (e.g., charge, denticity, and donor atoms) are especially important for antibody vectors; these highly successful vectors are used for targeting a variety of cancers and cannot withstand the elevated reaction temperatures ideally required for macrocycles like DOTA.[30,31,41–43]

In this work, we report the synthesis of four new acyclic ligands based on picolinic acid. These ligands have denticities ranging from 9 to 10, and are based on either a diethylenetriamine (dien) or tris(2-aminoethyl)amine (tren) backbone. The ligands Bn-H_3nonapa (*N*,*N*″-[(benzyl)-*N*,*N*′,*N*″-[(6-carboxy)pyridine-2-yl]methyl]-diethylenetriamine; **3**), H_3nonapa (*N*,*N*′,*N*″-[(6-carboxy)pyridine-2-yl)-methyl]diethylenetriamine; **4**), *p*-NO_2-Bn-H_3nonapa (*N*,*N*″-[(*p*-nitrobenzyl)-*N*,*N*′,*N*″-[(6-carboxy)pyridine-2-yl]methyl]diethylenetriamine; **10**), and Bn-H_3trenpa (*N*,*N*′,*N*″-tris[benzyl]tris[(6-carboxy)pyridine-2-yl)methyl]ethylamine; **7**) have been synthesized and characterized for the first time (Chart 1). The nonradioactive In^{3+} complexes of ligands **3**, **4**, and **10** were synthesized and characterized. These ligands were also studied with the radiometal ion $^{111}In^{3+}$, and assessed by radiolabeling experiments and mouse serum stability assays.

Results and discussion

The four novel ligands Bn-H_3nonapa (**3**), H_3nonapa (**4**), *p*-NO_2-Bn-H_3nonapa (**10**), and Bn-H_3trenpa (**7**) were synthesized using the same general methods used for the original synthesis of the ligand H_2depda.[23,44–46] The amine backbones diethylenetriamine (dien) and tris(2-aminoethyl)amine (tren) were chosen as platforms for assembling high-denticity derivatives of H_2dedpa and H_4octapa (*N*,*N*–bis(6-carboxyl-2-pyridylmethyl)ethylenediamine), although any permutation of these backbones could be theoretically used. Dien and tren were benzyl-protected via reductive amination with benzaldehyde to allow for controlled alkylation of bromopicolinic acid in subsequent steps (Schemes 1, 2, and 3). After alkylation of bromopicolinic acid, benzyl groups could be removed via hydrogenation in glacial acetic acid, or benzyl groups could be retained by bypassing this step (Schemes 1–3). For the ligand H_3nonapa (**4**, Scheme 1), hydrogenation was performed to remove benzyl-protecting groups, followed by deprotection by refluxing in HCl (12 mol/L) or stirring with LiOH at ambient temperature. Benzyl groups were used as placeholders for *p*-NO_2-benzyl groups (e.g., *p*-NO_2-Bn-H_3nonapa (**10**), Scheme 3), which have the potential to be transformed into *p*-SCN-benzyl groups to yield active bifunctional derivatives. Benzyl-isothiocyanate groups are commonly used for facile conjugation reactions to free primary amine groups (e.g., antibodies and peptides), forming stable thiourea bioconjugates. Although excess benzyl groups can result in undesirably high lipophilicity, this is only an issue for peptide conjugates, and will not be an issue when conjugated to large targeting vectors like antibodies and nanoparticles. The cumulative yield obtained for H_3nonapa (**4**) was ~24% in four steps, which was significantly lower than that for Bn-H_3nonapa, which was synthesized in a cumulative yield of

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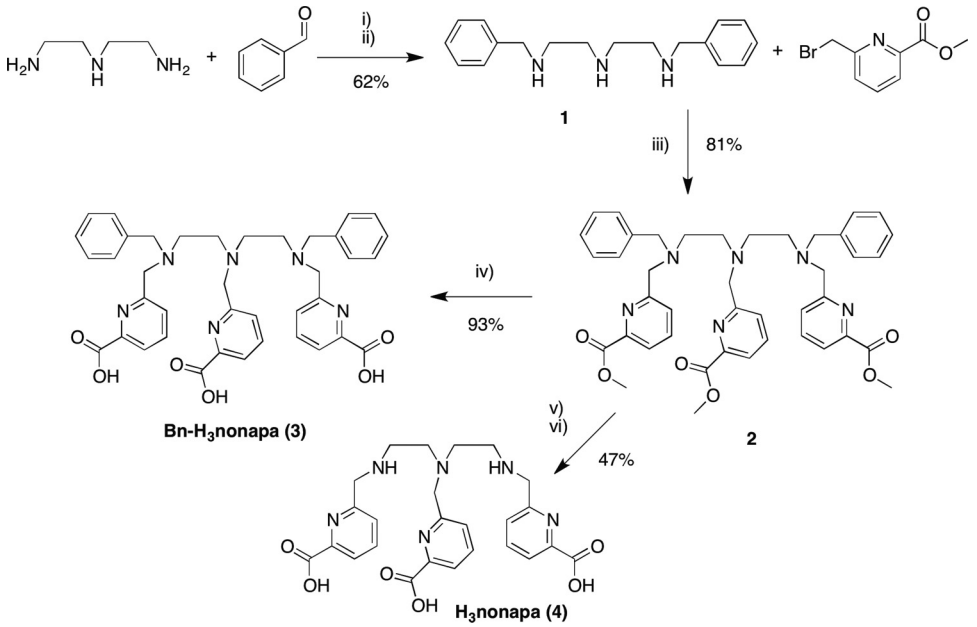
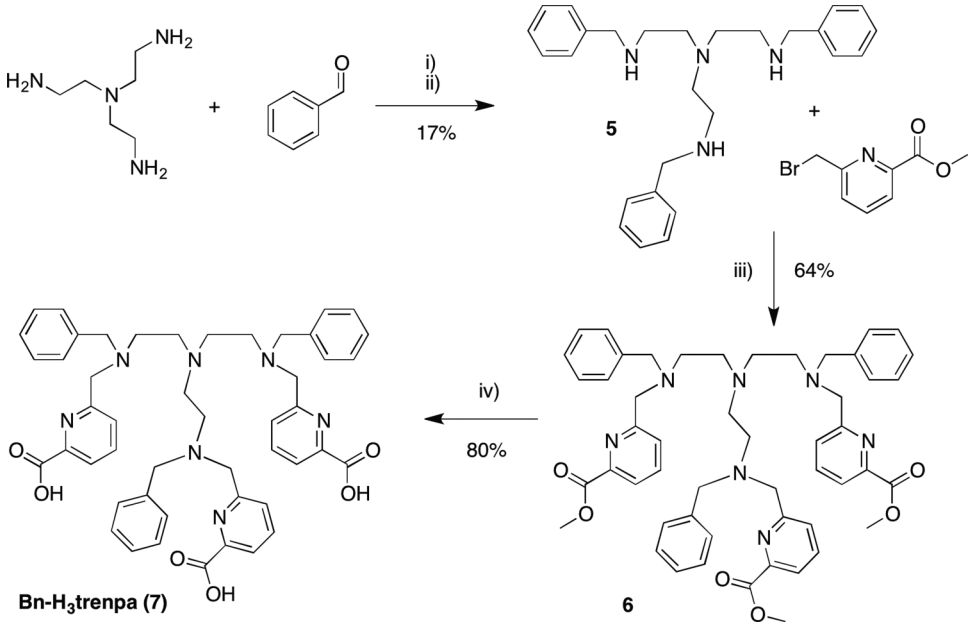
Scheme 1. Synthesis of Bn-H_3nonapa (**3**) and H_3nonapa (**4**). (*i*) Diethylenetriamine (dien), benzaldehyde, MeOH, Ar (g), Δ, 2 h; (*ii*) MeOH, 0 °C, $NaBH_4$, 16 h, 62%; (*iii*) methyl-6-bromomethylpicolinate, CH_3CN, 60 °C, 20 h, 81%; (*iv*) tetrahydrofuran (THF)/H_2O (3:1), LiOH, RT, 2 h, Bn-H_3nonapa (**3**) 93%; (*v*) AcOH, Pd/C (10 wt%), H_2 (g), RT, 16 h; (*vi*) HCl (12 mol/L), Δ, 16 h, H_3nonapa (**4**) 47%.



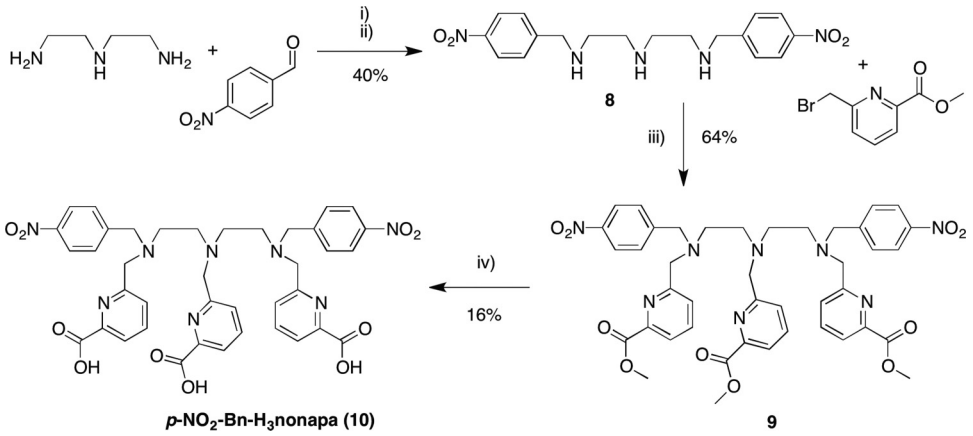
Scheme 2. Synthesis of Bn-H_3trenpa (**7**). (*i*) Tris(2-aminoethyl)amine (tren), benzaldehyde, MeOH, Ar (g), Δ, 2 h; (*ii*) MeOH, 0 °C, $NaBH_4$, 16 h, 17%; (*iii*) methyl-6-bromomethylpicolinate, CH_3CN, 60 °C, 20 h, 64%; (*iv*) THF/H_2O (3:1), LiOH, RT, 2 h, Bn-H_3trenpa (**7**) 80%.



~47% in three steps. This decreased yield is largely a result of a problem previously reported for the synthesis of H_4octapa, where it was determined that hydrogenation conditions resulted in the cleavage of both benzyl and picolinic acid moieties, producing significant quantities of byproduct and substantially decreasing yields and complicating purification of final products.[22] This inefficiency was largely obviated in the synthesis of H_4octapa by not performing the hydrogenation reaction in the presence of the picolinic acid moiety; however, for the synthesis of the currently discussed ligands this was not possible.[22]

The synthesis of Bn-H_3trenpa (**7**) was more challenging than that of H_3nonapa (**4**)/Bn-H_3nonapa (**3**), as the hydrogenation reaction was found to completely decompose the Bn-$(Me)_3$trenpa precursor (compound **6**, Scheme 2). In addition to cleaving benzyl and picolinic acid moieties from the tren backbone, it was also observed by mass spectrometry that hydrogenation conditions cleaved the ethylene bridges of tren. For this reason, the tren-based ligand, H_3trenpa, analogous to the dien-based ligand, H_3nonapa, could not be produced. Additionally, *p*-NO_2-Bn-H_3trenpa could not be synthesized, as the target ligand was found to be unstable and decomposed during synthesis. This instability was also observed in the synthesis of *p*-NO_2-Bn-H_3nonapa (**10**), although to a lesser degree (Scheme 3). In light of the synthetic issues plaguing Bn-H_3trenpa (**7**), it was determined to be the least robust ligand of those presented in this work, and synthesis of a *p*-NO_2-Bn bearing a bifunctional precursor was attempted but

Scheme 3. Synthesis of *p*-NO_2-Bn-H_3nonapa (**10**). (*i*) Diethylenetriamine (dien), *p*-4-nitrobenzaldehyde, MeOH, Ar (g), Δ, 2 h; (*ii*) MeOH, 0 °C, $NaBH_4$, 16 h, 40%; (*iii*) methyl-6-bromomethylpicolinate, CH_3CN, 60 °C, 20 h, 64%; (*iv*) THF/H_2O (3:1), LiOH, RT, 2 h, *p*-NO_2-Bn-H_3nonapa (**10**) 16%.



could not be completed, suggesting little potential of this tren-based ligand system for use in ^{111}In radiopharmaceuticals.

Ligand **10** (*p*-NO_2-Bn-H_3nonapa) was synthesized in the same manner as Bn-H_3nonapa, with the only modification to the procedure being the substitution of *p*-NO_2-benzaldehyde for benzaldehyde in the initial reductive amination reaction. Despite the synthetic steps being identical, the *p*-NO_2-Bn functionality appeared to be more labile and sensitive to decomposition than the regular benzyl groups, decreasing yields and complicating purifications. Ligand **10** was produced in three synthetic steps with a low cumulative yield of ~4%, demonstrating the poor stability of the *p*-NO_2-Bn functionality because the analogous synthesis of Bn-H_3nonapa (**3**) was also completed in three steps, albeit in yields of ~47%. The bifunctional precursor *p*-NO_2-Bn-H_3nonapa (**10**) was not transformed into the active isothiocyanate, although it can be expected that the requisite hydrogenation conditions would effect further decomposition and decreased yields. A synthetic methodology to circumvent the destructive effects of hydrogenation reactions on this system was previously determined, where instead of the *p*-NO_2-Bn groups, *tert*-butyl ester protected *p*-NH_2-Bn functionality was used successfully.[47] Should the radiolabeling efficiency and blood serum stability of these ligands with ^{111}In have been promising enough (vide infra), this synthetic revision could be applied to the presented ligands to more efficiently produce bifunctional derivatives.[47] If these ligands were to find purpose in the future with other radiometals (e.g., ^{177}Lu, $^{86/90}$Y, or ^{225}Ac), a combination of this *tert*-butyl ester protected *p*-NH_2-Bn functionality and the recently devised nosyl-protection strategy should provide reasonable synthetic access to fully bifunctional derivatives.[20,21,47]

The ligands Bn-H_3nonapa (**3**), H_3nonapa (**4**), and Bn-H_3trenpa (**7**) were synthesized and obtained as their HCl salts, and were reacted with indium perchlorate ($In(ClO_4)_3 \cdot 6H_2O$) under aqueous conditions (pH 4–5). The coordination complexes were confirmed via high-resolution mass spectrometry (HR-MS) and nuclear magnetic resonance (NMR) spectroscopy. Coordination to In^{3+} was confirmed by significant changes in chemical shifts and coupling patterns in their NMR spectra (Figs. 1, 2, and 3). The NMR spectra of the indium complexes were obtained in deuterated dimethyl sulfoxide (DMSO-d_6), as the solubility of these neutral indium complexes was very poor in water and methanol. Because these ligands are designed with in vivo radiopharmaceutical applications in mind, their partial insolubility in water is not ideal; however, under the extremely dilute conditions used in radiochemistry, and when coupled to highly soluble targeting vectors such as peptides and antibodies, such insolubility can be overcome.[47] Additionally, highly stable but lipophilic compounds can sometimes be applied to cardiac perfusion imaging or passing the blood–brain barrier, and so investigation into their stability is still warranted.[48]

The complex In(nonapa) (**12**) was the most soluble in water and provided the highest radiochemical yields with ^{111}In, and additionally demonstrated the highest stability in mouse serum (vide infra). It can be observed that the NMR spectra of the free ligands displayed sharp and well-resolved peaks, but upon coordination to In^{3+}, the ^{1}H NMR spectra became much more complicated and broad (Figs. 1–3). In(Bn-nonapa) (**11**) displayed the most significant broadening of peaks, suggesting rapid fluxional isomerization in solution (Fig. 2). The NMR spectrum of In(nonapa) (**12**, Fig. 1) became much more complicated with many new signals being observed compared to the free ligand, but the NMR peaks still appeared sharp and well resolved, suggesting little fluxional isomerization and a decrease in symmetry and (or) the presence of multiple static isomers giving rise to a more complex spectrum. In(Bn-trenpa) (**13**, Fig. 3) showed an NMR spectrum somewhere between those of In(nonapa) (**12**) and In(Bn-nonapa) (**11**), displaying many new signals and broadening of signals to a lesser degree than that observed for In(Bn-nonapa) (**11**). Metal–ligand complexes that form highly symmetric complexes with no fluxional isomerization typically show much more simple NMR spectra than those observed here, as in the spectra obtained previously for $[Ga(dedpa)]^+$ and $[In(octapa)]^-$.[22,23] Although it is interesting to obtain a general idea of the behavior and isomerization of these complexes in solution, to properly assess their potential in vivo as radiopharmaceuticals they must be studied using radiolabeling and serum stability experiments.

The new acyclic ligands H_3nonapa (**4**), Bn-H_3nonapa (**3**), and Bn-H_3trenpa (**7**) were radiolabeled with ^{111}In under conditions similar to those in our previous work with the acyclic chelating ligand H_4octapa (NaOAc buffer, pH 4.5, room temperature (RT)).[22] It was found that the potentially nonadentate ligand H_3nonapa efficiently radiolabeled ^{111}In, providing a radiochemical yield of ~97% after 10 min at ambient temperature (Table 1, Fig. 4). The benzylated derivative of H_3nonapa (Bn-H_3nonapa) achieved less optimal radiolabeling kinetics, with a still respectable yield of ~93% after 45 min at ambient temperature. The potentially decadentate, tren-based ligand, Bn-H_3trenpa, displayed poor radiolabeling performance, with a radiochemical yield of only ~15% after 45 min at room temperature. Elevated temperatures may have increased the radiochemical yield of Bn-H_3trenpa beyond ~15%; however, the purpose of these acyclic ligands is to provide facile room temperature radiolabeling kinetics and so this eventuality was not explored.

Once radiolabeled with ^{111}In, the radiometal complexes were incubated with mouse blood serum as a transchelation challenge (e.g., to transferrin in serum) to estimate their in vivo inertness.

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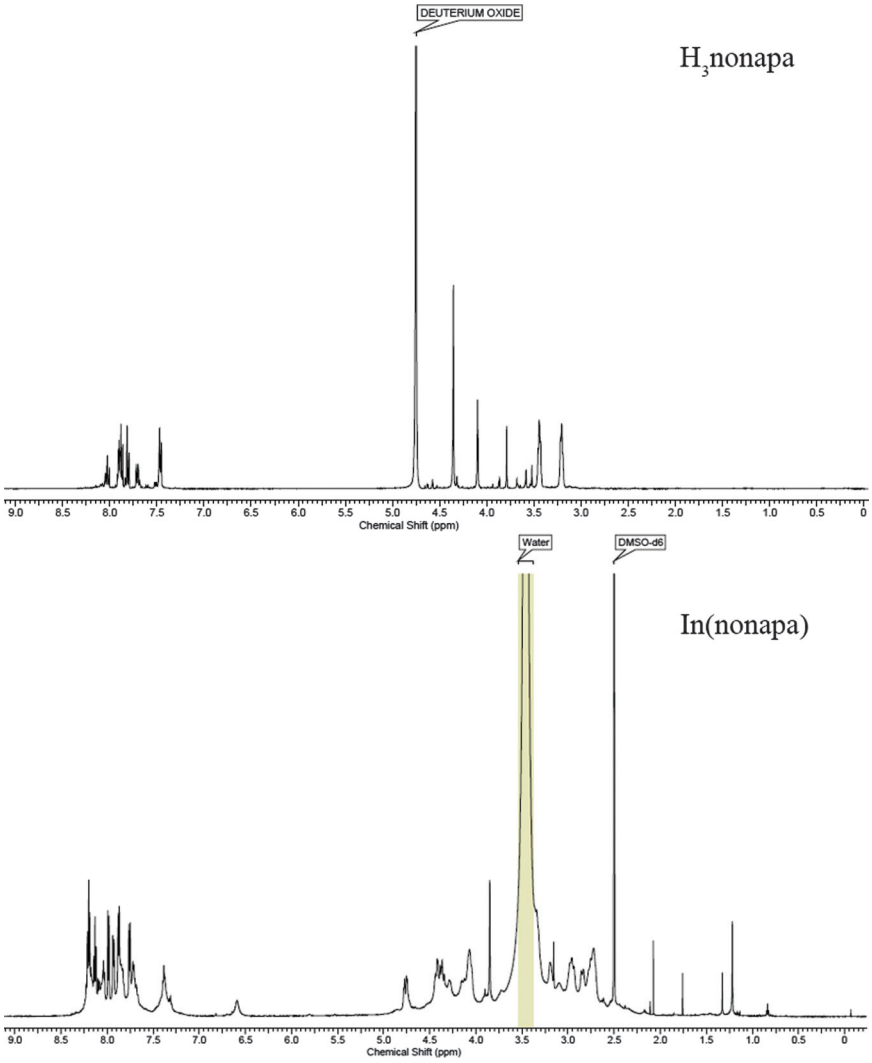
Fig. 1. ^{1}H NMR spectra of H_3nonapa (**4**; 400 MHz, 25 °C, D_2O, top) and the In^{3+} complex In(nonapa) (**12**; 600 MHz, 25 °C, DMSO-d_6, bottom).



^{111}In(nonapa) demonstrated excellent stability in mouse serum after 1 h of 93.5% ± 0.4%, but after 24 h had undergone significant transchelation with only 44.5% ± 25.9% remaining intact (Fig. 5). Of the three new ligands investigated, H_3nonapa was the most promising, with ^{111}In(Bn-nonapa) remaining only 16.6% ± 0.4% intact after 24 h in mouse serum, and ^{111}In(Bn-trenpa) providing insufficient radiolabeling yields (~15%) to warrant evaluation by a serum stability challenge. It was observed that the serum stability and radiolabeling efficiency were decreased for ^{111}In complexes of Bn-H_3nonapa compared to that of H_3nonapa (Table 1, Fig. 5), suggesting that benzylating the secondary amines of H_3nonapa to tertiary amines with Bn-H_3nonapa may have resulted in weaker coordination to indium, which was therefore more easily transchelated by serum proteins. This is a trend that was observed for the gallium complex of H_2dedpa, where alkylating the secondary amines to tertiary amines generally resulted in decreased stability and inertness to transchelation in apo-transferrin and serum stability challenges.[23,47,48] It is important to note that macroscale synthesis of nonradioactive In(Bn-nonapa)] produced a water insoluble complex, and although poor solubility is expected to be less of a problem at the extremely dilute conditions used for radiolabeling with ^{111}In, it may have contributed to the inferior stability with ^{111}In by means of precipitation or nonspecific association with serum proteins. In consideration of the superior radiolabeling and serum stability properties of the previously studied chelators H_4octapa, H_5decapa, DOTA, and DTPA, the three new ligands, H_3nonapa (**4**), Bn-H_3nonapa (**3**), and Bn-H_3trenpa (**7**), were not investigated for in vivo applications with ^{111}In.[20,22] These new ligands may prove useful in the future for larger radiometals that could benefit from their high denticities, such as ^{177}Lu, $^{86/90}$Y, and ^{225}Ac.

Conclusions

The four new acyclic ligands Bn-H_3nonapa (**3**), H_3nonapa (**4**), *p*-NO_2-Bn-H_3nonapa (**10**), and Bn-H_3trenpa (**7**) were synthesized and studied with nonradioactive In^{3+}, and with radioactive $^{111}In^{3+}$. The coordination of these ligands to In^{3+} was confirmed by high-resolution mass spectrometry and nuclear magnetic resonance spectrometry. The ^{1}H NMR spectra of the complexes In(nonapa) (**12**), In(Bn-nonapa) (**11**), and In(Bn-trenpa) (**13**) revealed broad and complicated splitting patterns, suggesting low symmetry and (or) fluxional isomerization, or the presence of multiple isomers in solution. The poor solubility of the In(Bn-nonapa) and In(Bn-trenpa) complexes in aqueous conditions was not promising for potential in vivo applications. Radiolabeling experiments were performed with $^{111}In^{3+}$, which demonstrated H_3nonapa (**4**) to be the most proficient ligand studied herein, achieving radiochemical yields of ~97% in 10 min at ambient temperature, and possessing stability to transchelation by mouse serum of 44.5% ± 25.9% after 24 h. Although the radiolabeling kinetics of H_3nonapa

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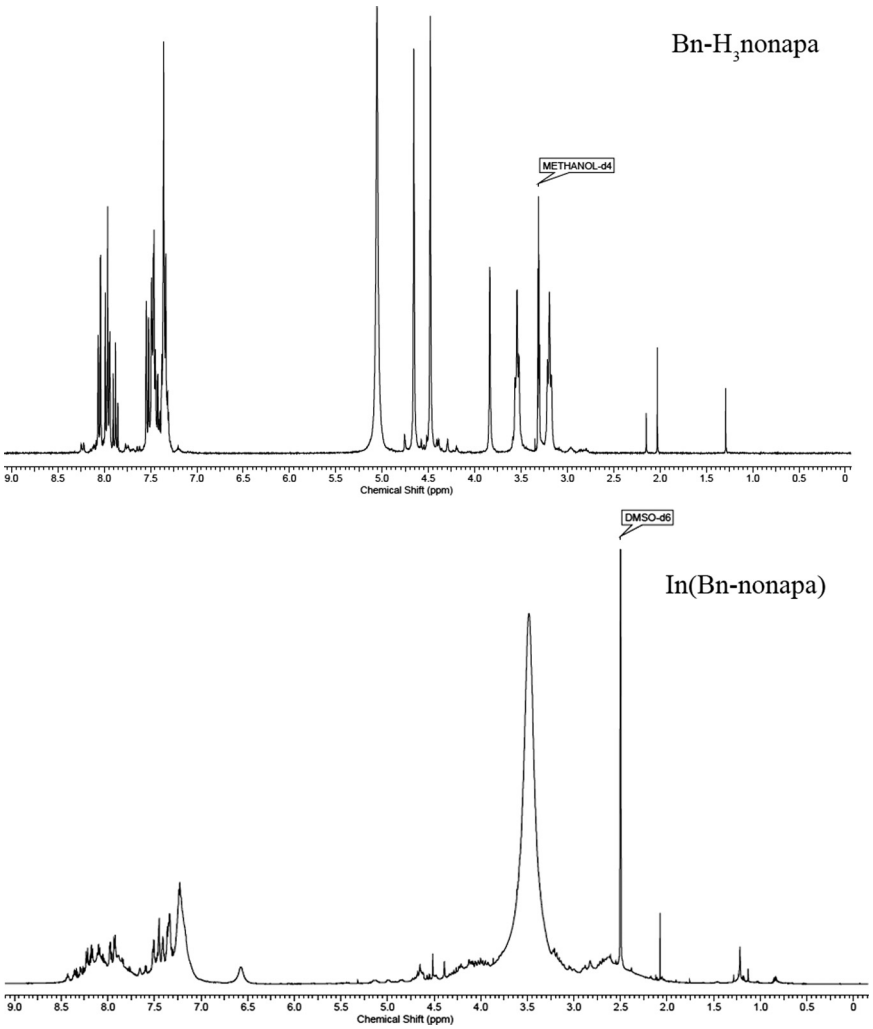
Fig. 2. ^{1}H NMR spectra of Bn-H_3nonapa (**3**; 300 MHz, 25 °C, MeOD, top) and the In^{3+} complex In(Bn-nonapa) (**11**; 600 MHz, 25 °C, DMSO-d_6, bottom).



(**4**) were excellent, serum stability results were inferior to the previously studied ligands DOTA, DTPA, and H_4octapa, suggesting that these ligands are not robust enough for ^{111}In radiopharmaceutical applications. The ligand Bn-H_3nonapa (**3**) possessed poor radiolabeling properties with ^{111}In, requiring 45 min at ambient temperature to achieve radiochemical yields of ~93%, and demonstrating poor serum stability (16.6% ± 0.4% after 24 h). The ligand Bn-H_3trenpa (**7**) was only able to achieve radiochemical yields of ~15% after 45 min at ambient temperature, which was deemed unacceptable for further study. Owing to the high denticity of these ligands (potentially 9–10 coordinate), they may have value for study with large isotopes like ^{177}Lu, $^{86/90}$Y, and ^{225}Ac.

Experimental

General materials and methods

All solvents and reagents were purchased from commercial suppliers (Sigma-Aldrich, St. Louis, MO; TCI America, Portland, OR; Fisher Scientific, Waltham, MA) and were used as received unless otherwise indicated. Methyl-6-bromomethylpicolinate was synthesized according to a literature protocol.[22] Water used was ultrapure (18.2 MΩ cm^{-1} at 25 °C, Milli-Q, Millipore, Billerica, MA). The analytical thin-layer chromatography (TLC) plates were aluminum-backed ultrapure silica gel (Siliaplate, 60 Å pore size, 250 μm plate thickness, Silicycle, Quebec, QC). Flash column silica gel was provided by Silicycle (Siliaflash Irregular Silica Gels F60, 60 Å pore size, 40–63 mm particle size, Silicycle, Quebec, QC). Automated column chromatography was performed using a Teledyne Isco (Lincoln, NE) CombiFlash R_f automated system with solid load cartridges packed with flash column silica gel and RediSep R_f Gold reusable normal-phase silica columns and neutral alumina columns (Teledyne Isco, Lincoln, NE). ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV300, AV400, or AV600 instruments; all spectra were internally referenced to residual solvent peaks except for ^{13}C NMR spectra in D_2O, which were externally referenced to a sample of CH_3OH/D_2O. Low-resolution mass spectrometry was performed using a Waters liquid chromatography-mass spectrometer (LC–MS) consisting of a Waters ZQ quadrupole spectrometer equipped with an electrospray/chemical ionization (ESCI) ion source and a Waters 2695 high-performance liquid chromatography (HPLC) system (Waters, Milford, MA). High-resolution electrospray-ionization mass spectrometry (HR-EI-MS) was performed on a Waters Micromass LCT time of flight instrument. Microanalyses for C, H, and N were performed on a Carlo Erba EA 1108 elemental analyzer. The HPLC system used for purification of nonradioactive compounds consisted of a semipreparative reverse phase C18 Phenomenex synergi hydro-RP (80 Å pore size, 250 mm × 21.2 mm, Phenomenex, Torrance, CA) column connected to a Waters 600 controller, a Waters 2487 dual wavelength absorbance detector, and a Waters delta 600 pump. ^{111}In(chelate) mouse serum stability experiments were analyzed

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Fig. 3. ^{1}H NMR spectra of Bn-H_3trenpa (**7**; 300 MHz, 25 °C, MeOD, top) and the In^{3+} complex In(Bn-trenpa) (**13**; 400 MHz, 25 °C, DMSO-d_6, bottom).

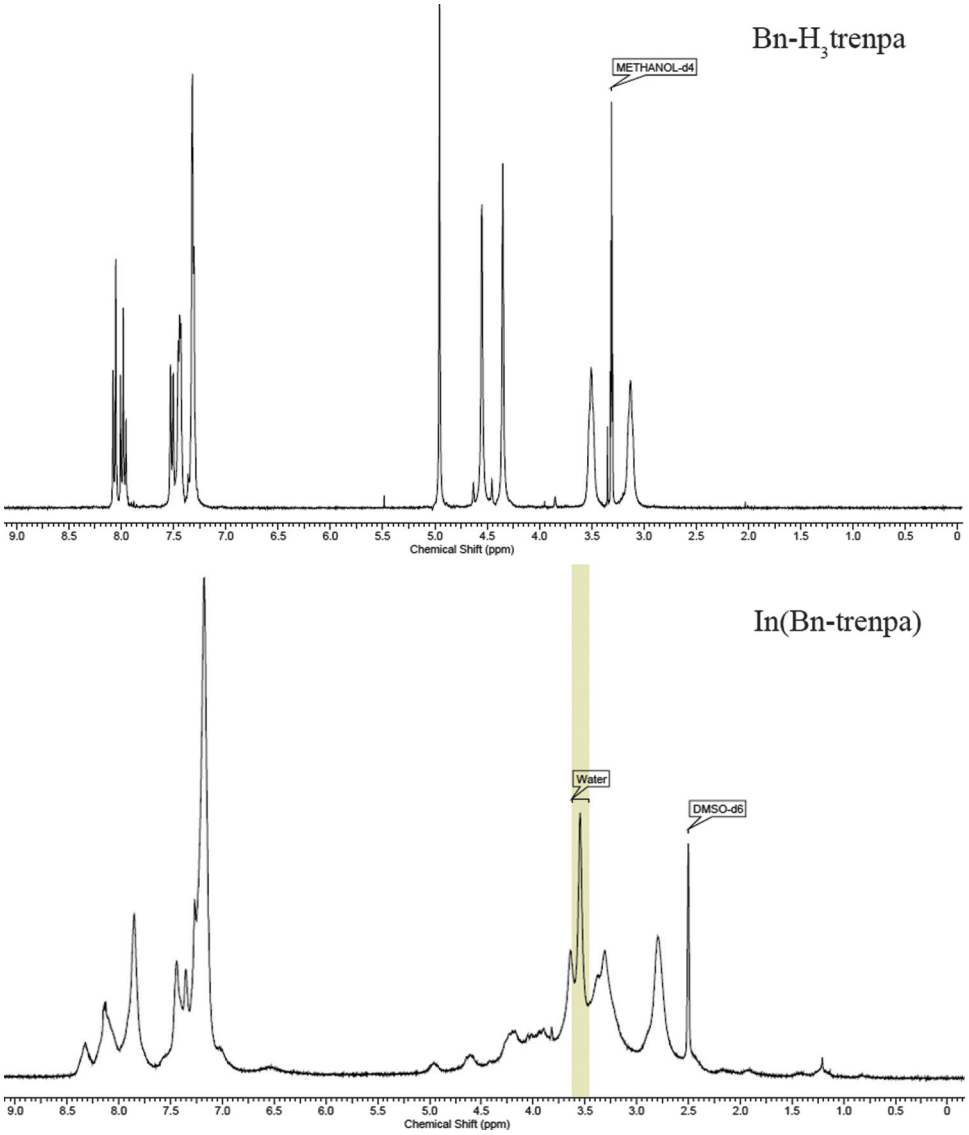

Table 1. Radiolabeling experiments with ^{111}In and the novel acyclic ligands Bn-H_3nonapa (**3**), H_3nonapa (**4**), and Bn-H_3trenpa (**7**), and the previously published ligands H_2dedpa, H_4octapa, H_5decapa, DOTA, and DTPA.[22]

Complex	Radiolabeling conditions	RCY (%)[a]	HPLC t_R (min)[b]	Serum stability 1 h (%)[c]	Serum stability 24 h (%)[c]
[^{111}In(nonapa)]	pH 4.5, 10 min, RT	97	5.0	93.5±0.4	44.5±25.9
[^{111}In(Bn-nonapa)]	pH 4.5, 45 min, RT	93	8.9	27.0±0.9	16.6±0.4
[^{111}In(Bn-trenpa)]	pH 4.5, 45 min, RT	15	10.5	NA[d]	NA[d]
[^{111}In(dedpa)]$^{+}$	pH 4.5, 10 min, RT	99	5.9	96.1±0.1	19.7±1.5
[^{111}In(octapa)]$^{-}$	pH 4.5, 10 min, RT	99	4.7	93.8±3.6	92.3±0.1
[^{111}In(decapa)]$^{2-}$	pH 4.5, 10 min, RT	95	5.4	89.7±1.6	89.1±1.7
[^{111}In(DOTA)]$^{-}$	pH 4.5, 30 min, 90 °C	97	3.5	89.6±2.1	89.4±2.2
[^{111}In(DTPA)]$^{2-}$	pH 4.5, 10 min, RT	99	6.5	86.5±2.2	88.3±2.2

[a]RCY = radiochemical yield.
[b]t_R = retention time.
[c]Blood serum stability results evaluated by PD-10 size-exclusion column elution.
[d]NA = not applicable.

using GE Healthcare Life Sciences PD-10 desalting columns (size exclusion for molecular weight (MW) < 5000 Da; 1 Da = 1 g/mol) and counted with a Capintec CRC 15R well counter. Radiolabeling of DOTA with ^{111}In was performed using a Biotage Initiator microwave reactor (μW). Analyses of radiolabeled complexes were carried out using a Waters xbridge BEH130 C18 reverse phase (150 mm × 6 mm) analytical column on a Waters Alliance HT 2795 separation module equipped with a Raytest Gabi Star NaI (Tl)

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Fig. 4. HPLC radiotrace of ^{111}In(nonapa) (t_R = 5.0 min, 97% RCY) showing small impurities.

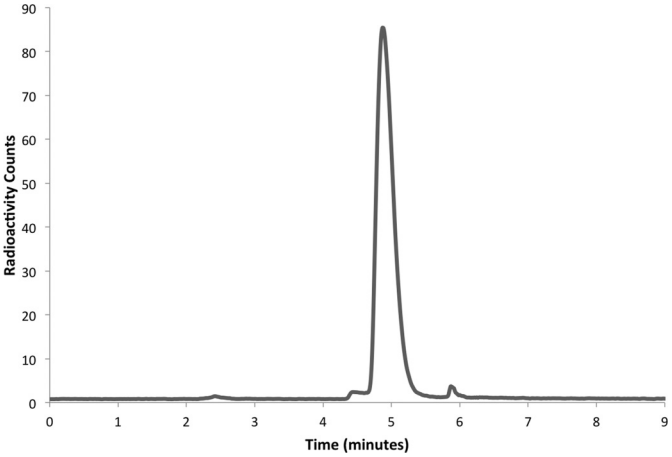
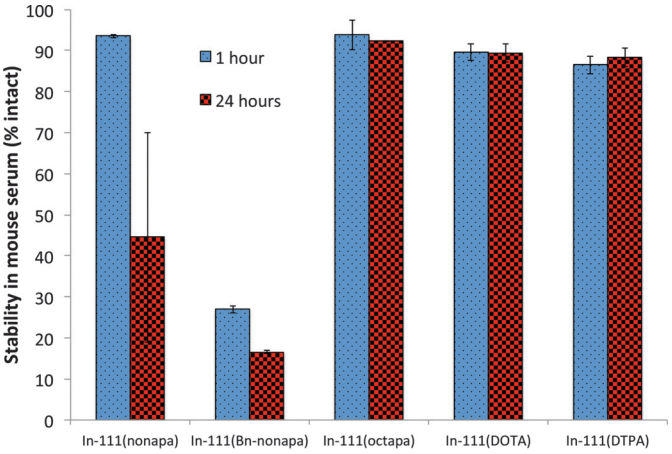


Fig. 5. Mouse serum stability assay of $^{111}In^{3+}$ complexes of ligands from Table 1, determined after 1 and 24 h by PD-10 size-exclusion column elution.



detector and a Waters 996 photodiode array (PDA) detector. ^{111}In was cyclotron produced (Advanced Cyclotron Systems, Model TR30) by proton bombardment through the reaction ^{111}Cd(*p*,*n*)^{111}In and was provided by Nordion as $^{111}InCl_3$ in 0.05 mol/L HCl.

N,*N*″-[Benzyl]diethylenetriamine (1)

Diethylenetriamine (5.0 mL, 46.3 mmol) was added to dry methanol (distilled over CaH_2, 100 mL), followed by benzaldehyde (9.43 mL, 96.6 mmol), and the mixture was refluxed for 2 h under Ar. The reaction mixture was cooled (0 °C) in an ice bath and $NaBH_4$ (12.2 g, 323 mmol) was added slowly and in small portions to prevent boiling. The reaction mixture was stirred overnight for ~16 h at ambient temperature. The reaction mixture was concentrated in vacuo and then a saturated aqueous solution of $NaHCO_3$ (~100 mL) and chloroform (200 mL) was added. The aqueous layer was extracted twice more with dichloromethane (100 mL); the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a yellow oil. The crude product was purified by silica chromatography (CombiFlash R_f automated column system; 120 g HP silica; A: dichloromethane, B: methanol, A: gradient 100% to 40%) to afford **1** as a yellow oil (8.15 g, 28.8 mmol, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33–7.24 (m, 10H, Bn-*H*), 3.80 (s, 4H, Bn-C*H*$_2$-N), 2.75 (m, 8H, ethylene-*H*), 1.44 (s, 3H, -N*H*-). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 140.17, 129.71, 129.06, 128.64, 128.28, 127.32, 55.12, 53.72, 44.68. HR-ESI-MS calcd. for [$C_{18}H_{25}N_3$ + H]$^+$: 284.2127; found [M + H]$^+$: 284.2133, PPM (parts per million) = 2.1.

N,*N*″-[(Benzyl)-*N*,*N*′,*N*″-[(6-methoxycarbonyl)pyridine-2-yl]methyl]diethylenetriamine (2)

To a solution of **1** (260 mg, 0.917 mmol) and sodium carbonate (excess, ~500 mg) in dry acetonitrile (distilled over CaH_2, 10 mL) was added methyl-6-bromomethylpicolinate (synthesized according to a literature procedure;[22] 654 mg, 2.84 mmol). The resulting solution was stirred at 60 °C for 20 h under Ar. Sodium carbonate was removed by filtration and the solvent was removed in vacuo. The crude product was purified by silica chromatography (CombiFlash R_f automated column system; 40 g HP silica; A: dichloromethane, B: methanol, A: gradient 100% to 20%) to afford **2** as a yellow oil (541 mg, 0.741 mmol, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.90–7.88 (m, 3H), 7.67–7.65 (m, 4H), 7.20–7.13 (m, 10H, Bn), 3.91 (s, 9H), 3.75 (s, 4H, Pic-C*H*$_2$-N), 3.71 (s, 2H, (Pic-C*H*$_2$-N), 3.50 (s, 4H, Bn-C*H*$_2$-N), 2.54 (m, 8H, dien-*H*). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.47, 160.78, 146.72, 138.66, 136.99, 128.35, 127.94, 126.74, 126.50, 123.19, 60.77, 60.17, 58.89, 52.54, 51.81. HR-ESI-MS calcd. for [$C_{42}H_{46}N_6O_6$ + H]$^+$: 731.3557; found [M + H]$^+$: 731.3549, PPM = –1.1.

Bn-H_3nonapa, *N*,*N*″-[(benzyl)-*N*,*N*′,*N*″-[(6-carboxy)pyridine-2-yl]methyl]diethylenetriamine (3)

To a solution of **2** (375 mg, 0.513 mmol) in a mixture of tetrahydrofuran/deionized water (3:1, 5 mL) was added LiOH (300 mg). The reaction mixture was stirred at ambient temperature for 2 h. A portion of HCl was added (5 mL, 6 mol/L), and then the mixture was reduced to dryness in vacuo. The mixture was dissolved in deionized water (4 mL) and purified via semipreparative reverse-phase (RP) HPLC (10 mL/min, gradient: A: 0.1% TFA (trifluoroacetic acid) in deionized water, B: CH_3CN; 5% to 100% B linear gradient in 25 min; t_R = 16 min, broad). Product fractions were pooled, concentrated in vacuo, dissolved in HCl (5 mL, 6 mol/L), and then concentrated in vacuo again to remove trifluoroacetic acid. The HCl salt of **3**, Bn-H_3nonapa·4HCl·H_2O, was obtained as a light yellow solid (330.3 mg, 93%, using the molecular weight of the HCl salt as determined by elemental analysis). ^{1}H NMR (300 MHz, MeOD) δ: 8.05 (d, *J* = 7.8 Hz, 2H), 7.99–7.94 (m, 3H), 7.88 (t, *J* = 7.7 Hz, 1H), 7.54–7.42 (m, 3H), 7.38–7.30 (m, 10H), 4.66 (s, 4H), 4.48 (s, 4H), 3.84 (s, 2H), 3.54 (m, 4H, dien-*H*), 3.19 (m, 4H, dien-*H*). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.85, 164.65, 151.69, 150.67, 146.87, 146.17, 145.73, 141.94, 139.81, 131.72, 130.33, 129.23, 129.13, 128.86, 128.42, 127.67, 125.84, 125.20, 59.72, 56.78, 56.35, 49.59, 49.48. HR-ESI-MS calcd. for [$C_{39}H_{40}N_6O_6$ + H]$^+$: 689.3088; found [M + H]$^+$: 689.3077, PPM = –1.5. Elemental analysis calcd. for Bn-H_3nonapa·4HCl·H_2O ($C_{39}H_{40}N_6O_6$·4HCl·H_2O = 852.562) (%): C 54.94, H 5.44, N 9.86; found: C 55.21 (Δ = 0.27), H 5.65 (Δ = 0.21), N 9.54 (Δ = 0.32).

H_3nonapa, *N*,*N*′,*N*″-[(6-carboxy)pyridine-2-yl)methyl]-diethylenetriamine (4)

To a solution of **2** (60.0 mg, 0.0822 mmol) in glacial acetic acid (10 mL) was added Pd/C (6 mg, ~10 wt%). Hydrogen gas was purged and vented for 3 min through the reaction mixture, which was then stirred under a hydrogen atmosphere (balloon) for 16 h. The Pd/C was filtered out on a fine fritted glass filter and rinsed with HCl (3 mol/L) and acetonitrile; the filtrate was then evaporated to dryness in vacuo. The crude product was then deprotected without further purification by adding HCl (10 mL, 12 mol/L) and refluxing for 16 h. The reaction mixture was concentrated in vacuo, dissolved in deionized water (4 mL) and purified via semipreparative reverse-phase HPLC (10 mL/min, gradient: A: 0.1% TFA (trifluoroacetic acid) in deionized water, B: CH_3CN; 5% to 100% B linear gradient in 25 min; t_R = 8.1 min, broad). Product fractions were pooled, concentrated in vacuo, dissolved in HCl (5 mL, 6 mol/L), and then concentrated in vacuo again to remove trifluoroacetic acid. The HCl salt of H_3nonapa (**4**) was obtained as a light yellow solid (17.3 mg, 47%). ^{1}H NMR (400 MHz, D_2O) δ: 8.05–8.01 (m, 1H),

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7.91–7.86 (m, 3H), 7.84–7.80 (m, 2H), 7.72–7.70 (m, 1H), 7.47–7.45 (m, 2H), 4.36 (s, 4H), 4.10 (s, 2H), 3.44 (m, 4H), 3.21 (m, 4H). ^{13}C NMR (100 MHz, D_2O) δ: 168.00, 166.61, 163.27, 162.92, 157.85, 151.07, 147.73, 147.52, 146.89, 141.01, 139.61, 129.95, 129.50, 127.40, 126.69, 125.53, 125.12, 124.75, 57.09, 52.03, 50.59, 45.60. HR-ESI-MS calcd. for $[C_{25}H_{28}N_6O_6 + H]^+$: 509.2149; found $[M + H]^+$: 509.2144, PPM = −0.9.

***N*,*N*′,*N*″-Tris[benzyl]ethylamine (5)**

Tris(2-aminoethyl)amine (tren; 2.0 mL, 13.4 mmol) was added to dry methanol (distilled over CaH_2, 25 mL), followed by benzaldehyde (3.92 mL, 44.1 mmol), and the mixture was refluxed for 2 h under Ar. The reaction mixture was cooled (0 °C) in an ice bath and $NaBH_4$ (4.10 g, 107 mmol) was added slowly and in small portions to prevent boiling. The reaction mixture was stirred overnight for ~16 h at ambient temperature. The reaction mixture was concentrated in vacuo and then a saturated aqueous solution of $NaHCO_3$ (~100 mL) and chloroform (100 mL) was added. The aqueous layer was extracted twice more with dichloromethane (100 mL), the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a yellow solid. The crude product was purified by silica chromatography (CombiFlash R_f automated column system; 120 g HP silica; A: dichloromethane with 0.5% triethylamine, B: methanol, A: gradient 100% to 50%) to afford **5** as an orange oil (938 mg, 2.25 mmol, 17%). The product streaked very badly on the column, and significant yield was lost. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.25 (m, 15H, Bn-*H*), 3.71 (s, 6H, Bn-CH_2-N), 3.24 (s, 3H, -N*H*), 2.64–2.56 (m, 12H, dien-*H*). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 139.14, 127.99, 127.82, 126.62, 53.55, 53.14, 46.44. HR-ESI-MS calcd. for $[C_{27}H_{36}N_4 + H]^+$: 417.3018; found $[M + H]^+$: 417.3018, PPM = 0.

***N*,*N*′,*N*″-Tris[benzyl]-tris[(6-methoxycarbonyl)pyridine-2-yl)methyl]ethylamine (6)**

To a solution of **5** (299 mg, 0.717 mmol) and sodium carbonate (excess, ~1 g) in dry acetonitrile (distilled over CaH_2, 10 mL) was added methyl-6-bromomethylpicolinate (synthesized according to a literature procedure)[22] (495 mg, 2.15 mmol). The solution was stirred at 60 °C for 20 h under Ar. Sodium carbonate was removed by filtration and the solvent was removed in vacuo. The crude product was purified by silica chromatography (CombiFlash R_f automated column system; 80 g HP silica; A: hexanes, B: ethyl acetate, A: gradient 100% to 0%) to afford **6** as an orange oil (394 mg, 0.456 mmol, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.96–7.93 (m, 3H), 7.72–7.70 (m, 6H), 7.28–7.18 (m, 15H), 3.97 (s, 9H, methyl ester), 3.78 (s, 6H), 3.51 (s, 6H), 2.46 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.68, 161.09, 146.92, 138.99, 137.13, 128.51, 128.11, 126.89, 125.61, 123.34, 60.39, 59.04, 52.91, 52.73, 51.96. HR-ESI-MS calcd. for $[C_{51}H_{57}N_7O_6 + H]^+$: 864.4449; found $[M + H]^+$: 864.4466, PPM = 2.0.

Bn-H_3trenpa, *N*,*N*′,*N*″-tris[benzyl]-tris[(6-carboxy)pyridine-2-yl)methyl]ethylamine (7)

To a solution of **6** (237 mg, 0.274 mmol) in a mixture of tetrahydrofuran/deionized water (3:1, 5 mL) was added LiOH (170 mg). The reaction mixture was stirred at ambient temperature for 2 h. A portion of HCl was added (5 mL, 6 mol/L), and then the mixture was reduced to dryness in vacuo. The solid was dissolved in deionized water (4 mL) and purified via semipreparative reverse-phase HPLC (10 mL/min, gradient: A: 0.1% TFA (trifluoroacetic acid) in deionized water, B: CH_3CN; 5% to 100% B linear gradient in 25 min; t_R = 17.7 min, broad). Product fractions were pooled, concentrated in vacuo, dissolved in HCl (5 mL, 6 mol/L), and then concentrated in vacuo again to remove trifluoroacetic acid. The HCl salt of **7**, Bn-H_3trenpa·6HCl, was obtained as a yellow solid (229 mg, 0.220 mmol, 80%, using the molecular weight of the HCl salt as determined by elemental analysis). ^{1}H NMR (300 MHz, MeOD) δ: 8.08–7.96 (m, 6H), 7.53–7.43 (m, 9H), 7.36–7.31 (m, 9H), 4.55 (s, 6H), 4.35 (s, 6H), 3.51 (m, 6H, ethylene-*H*), 3.13 (m, 6H, ethylene-*H*). ^{13}C NMR (75 MHz, MeOD) δ: 167.09, 151.99, 151.86, 148.35, 148.17, 140.95, 140.83, 132.83, 132.74, 131.43, 131.40, 130.54, 130.41, 130.32, 128.75, 126.65, 126.42, 60.79, 60.36, 57.10, 56.90, 51.71. HR-ESI-MS calcd. for $[C_{48}H_{51}N_7O_6 + H]^+$: 822.3979; found $[M + H]^+$: 822.3977, PPM = −0.3. Elemental analysis calcd. for Bn-H_3trenpa·6HCl ($C_{16}H_{18}N_4O_4$·6HCl = 1040.097) (%): C 55.4, H 5.52, N 9.42; found: C 55.50 (Δ = 0.10), H 5.83 (Δ = 0.31), N 9.64 (Δ = 0.22).

***N*,*N*″-[*p*-Nitrobenzyl]diethylenetriamine (8)**

Diethylenetriamine (0.775 mL, 7.18 mmol) was added to dry methanol (distilled over CaH_2, 15 mL), followed by *p*-4-nitrobenzaldehyde (2.17 mg, 14.4 mmol), and then refluxed for 2 h. The reaction mixture was cooled (0 °C) in an ice bath and $NaBH_4$ (2.72 g, 71.8 mmol) was added slowly and in small portions to prevent boiling. The reaction mixture was stirred overnight for ~16 h at ambient temperature. The reaction mixture was concentrated in vacuo and then a saturated aqueous solution of $NaHCO_3$ (~50 mL) and chloroform (50 mL) was added. The aqueous layer was extracted twice more with dichloromethane (50 mL), the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford an orange oil. The crude product was purified by silica chromatography (CombiFlash R_f automated column system; 80 g HP silica; A: dichloromethane, B: methanol, A: gradient 100% to 40%) to afford **8** as a yellow oil (1.07 g, 2.87 mmol, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.14–8.11 (m, 4H, NO_2-Bn-*H*), 7.49–7.47 (m, 4H, NO_2-Bn-*H*), 3.88 (s, 4H), 2.73 (s, 8H), 1.71 (s, 3H, -N*H*-). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 148.30, 146.83, 128.46, 123.42, 53.03, 49.15, 48.86. HR-ESI-MS calcd. for $[C_{18}H_{23}N_5O_4 + H]^+$: 374.1828; found $[M + H]^+$: 374.1829, PPM = 0.2.

***N*,*N*″-[(*p*-Nitrobenzyl)-*N*,*N*′,*N*″-[(6-methoxycarbonyl)pyridine-2-yl]methyl]diethylenetriamine (9)**

To a solution of **8** (92.1 mg, 0.246 mmol) and sodium carbonate (excess, ~300 mg) in dry acetonitrile (distilled over CaH_2, 10 mL) was added methyl-6-bromomethyl picolinate (synthesized according to a literature procedure;[22] 170.2 mg, 0.739 mmol). The solution was stirred at 60 °C for 20 h under Ar. Sodium carbonate was removed by filtration and the solvent was removed in vacuo. The crude product was purified by silica chromatography (CombiFlash R_f automated column system; 40 g HP silica; A: dichloromethane, B: methanol, A: gradient 100% to 20%) to afford **9** as an orange oil (130 mg, 0.158 mmol, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.08–8.04 (m, 4H), 7.95–7.91 (m, 3H), 7.76–7.71 (m, 2H), 7.65–7.57 (m, 3H), 7.44–7.40 (m, 5H), 3.95 (s, 6H, methyl ester), 3.94 (s, 3H, methyl ester), 3.78 (s, 4H), 3.75 (s, 2H), 3.63 (s, 4H), 2.62–2.58 (m, 8H, dien-*H*). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.50, 160.43, 159.89, 147.21, 146.96, 146.89, 137.37, 137.09, 129.02, 125.61, 123.65, 123.60, 123.50, 123.39, 123.31, 60.78, 60.34, 58.31, 52.80, 52.27. HR-ESI-MS calcd. for $[C_{42}H_{44}N_8O_{10} + H]^+$: 821.3259; found $[M + H]^+$: 821.3268, PPM = 1.1.

***p*-NO_2-Bn-H_3nonapa, *N*,*N*″-[(*p*-nitrobenzyl)-*N*,*N*′,*N*″-[(6-carboxy)pyridine-2-yl]methyl]diethylenetriamine (10)**

To a solution of **9** (130 mg, 0.158 mmol) in a mixture of tetrahydrofuran/deionized water (3:1, 5 mL) was added LiOH (150 mg). The reaction mixture was stirred at ambient temperature for 2 h. A portion of HCl was added (5 mL, 6 mol/L), and then the mixture was reduced to dryness in vacuo. The mixture was dissolved in deionized water (4 mL) and purified via semipreparative reverse-phase HPLC (10 mL/min, gradient: A: 0.1% TFA (trifluoroacetic acid) in deionized water, B: CH_3CN; 5% to 100% B linear gradient in 25 min; t_R = 18 min, broad). Product fractions were pooled, concentrated in vacuo, dissolved in HCl (5 mL, 6 mol/L), and then concentrated in vacuo again to remove trifluoroacetic acid. The product *p*-NO_2-Bn-H_3nonapa (**10**) was obtained as a light-orange solid (20 mg, 16%). Due to instability and decomposition, elemental analysis and ^{13}C NMR were not obtained. Further work with this scaffold has not been attempted due to instability. ^{1}H NMR (300 MHz, D_2O) δ: 8.03–7.79 (m, 10H), 7.55–7.45 (m, 7H), 4.56 (s, 4H),

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4.41 (s, 4H), 3.81 (s, 2H), 3.48–3.44 (m, 4H, dien-*H*), 3.15–3.10 (m, 4H, dien-*H*).

[In(Bn-nonapa)] (11)

Bn-H_3nonapa·4HCl·H_2O (HCl salt of **3**; 10.0 mg, 0.0117 mmol) and $In(ClO_4)_3·6H_2O$ (8.00 mg, 0.0153 mmol) were dissolved in HCl (aq; 1 mL, 0.1 mol/L) in a 20 mL screw cap vial. The pH was adjusted to ~4.5 with NaOH (aq; 0.1 mol/L) with stirring. The reaction mixture was stirred at 60 °C for 1 h, then evaporated to dryness to afford [In(Bn-nonapa)] (**11**) as a white solid in a quantitative yield. The reaction mixture turned cloudy white during the reaction, as the insoluble and neutrally charged metal complex formed. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.45–8.7.6 (broad m, 9H, pyr-*H*), 7.52–7.23 (broad m, 10H, benzyl-*H*), 4.65–3.70 (broad m, 8H), 3.30–2.55 (broad m, 8H), 2.45–2.10 (broad m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 166.26, 164.19, 153.79, 153.26, 153.09, 148.05, 147.80, 147.73, 147.64, 147.54, 142.16, 139.26, 138.39, 138.06, 133.51, 132.19, 131.90, 131.70, 128.86, 128.68, 128.62, 128.42, 128.33, 128.25, 127.86, 127.10, 126.32, 126.03, 123.43, 123.04, 122.93, 122.70, 118.20, 64.11, 59.73, 57.98, 53.33, 52.26, 49.60, 49.47, 49.10, 48.89, 44.15. HR-ESI-MS calcd. for $[C_{39}H_{37}{}^{115}InN_6O_6 + H]^+$: 801.1892; found $[M + H]^+$: 801.1888, PPM = −0.5.

[In(nonapa)] (12)

H_3nonapa (**4**; 14.9 mg, 0.0222 mmol) and $In(ClO_4)_3·6H_2O$ (15.0 mg, 0.0288 mmol) were dissolved in HCl (aq; 1 mL, 0.1 mol/L) in a 20 mL screw cap vial. The pH was adjusted to ~4.5 with NaOH (aq; 0.1 mol/L) with stirring. The reaction mixture was stirred at 60 °C for 1 h, then evaporated to dryness to afford [In(nonapa)] (**12**) as a white solid in a quantitative yield. No product was observed to precipitate from the yellow reaction mixture. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.21–7.75 (m, 8H, pyr-*H*), 7.38–7.31 (m, 1H, pyr-*H*), 4.77–4.75 (m, 1H), 4.44–3.70 (m, 7H), 3.34–2.70 (m, 8H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 164.56, 163.57, 153.20, 152.44, 152.02, 146.56, 146.27, 141.69, 141.59, 141.15, 137.85, 129.06, 128.68, 127.54, 126.47, 125.26, 125.12, 123.90, 122.02, 121.94, 52.60, 51.84, 51.78, 51.00, 50.15, 49.99, 49.12, 44.27, 43.10, 29.89, 29.10. HR-ESI-MS calcd. for $[C_{25}H_{25}{}^{115}InN_6O_6 + H]^+$: 621.0953; found $[M + H]^+$: 621.0958, PPM = 0.8.

[In(Bn-trenpa)] (13)

Bn-H_3trenpa·6HCl (HCl salt of **7**; 41.5 mg, 0.0399 mmol) and $In(ClO_4)_3·6H_2O$ (27.0 mg, 0.0519 mmol) were dissolved in HCl (aq; 1 mL, 0.1 mol/L) in a 20 mL screw cap vial. The pH was adjusted to ~4.5 with NaOH (aq; 0.1 mol/L) with stirring. The reaction mixture was stirred at 60 °C for 1 h, then evaporated to dryness to afford [In(Bn-trenpa)] (**13**) as a white solid in a quantitative yield. The reaction mixture turned cloudy white during the reaction, as the insoluble and neutrally charged metal complex formed. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.33–7.85 (m, 8H), 7.44–7.18 (m, 16H), 4.93–4.64 (broad m, 2H), 4.18–3.64 (broad m, 8H), 3.40–3.00 (broad m, 8H), 3.00–2.52 (broad m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.16, 164.57, 163.82, 163.08, 158.99, 158.17, 153.86, 153.16, 147.81, 147.73, 147.56, 144.88, 144.46, 138.18, 137.64, 137.57, 131.67, 131.08, 129.10, 128.81, 128.75, 128.47, 128.29, 127.86, 127.33, 127.16, 127.10, 126.75, 126.20, 125.96, 125.85, 123.58, 123.43, 123.09, 122.65, 59.90, 58.65, 58.48, 58.33, 58.25, 57.98, 57.45, 57.38, 57.19, 50.45, 50.34, 48.01, 47.75. HR-ESI-MS calcd. for $[C_{48}H_{48}{}^{115}InN_7O_6 + H]^+$: 934.2783; found $[M + H]^+$: 934.2775, PPM = −0.9.

^{111}In radiolabeling studies

The chelating ligands Bn-H_3nonapa (**3**), H_3nonapa (**4**), and Bn-H_3trenpa (**7**) were made up as stock solutions (1 mg/mL, ~10^{-3} mol/L) in deionized water. An aliquot of each ligand stock solution was transferred to screw cap mass spectrometry vials and made up to 1 mL with pH 5.0 NaOAc (10 mmol/L) buffer to a final concentration of ~365 μmol/L for each sample. An ~10 μL aliquot of the $^{111}InCl_3$ stock solution (~1 mCi for labeling studies and ~3–5 mCi for mouse serum competitions) was transferred into each vial, allowed to react at ambient temperature for 10 min, and then analyzed by RP-HPLC to confirm radiolabeling and calculate yields. If the reaction was not complete, it was left for a 45 min total reaction time and then checked again by RP-HPLC. Previously obtained radiolabeling yields and serum stability results for the chelators DTPA, DOTA, H_2dedpa, H_4octapa, and H_5decapa are also presented and were performed under identical experimental protocols at the same facilities (TRIUMF/Nordion).[22] Areas under the peaks observed in the radioactive HPLC trace were integrated to determine radiolabeling yields. Elution conditions used for RP-HPLC analysis were gradient: A: 10 mmol/L NaOAc buffer pH 4.5, B: CH_3CN; 0% to 100% B linear gradient 20 min. The radiometal complex $[^{111}In(dedpa)]^+$ used a modified HPLC gradient of A: 10 mmol/L NaOAc buffer pH 4.5, B: CH_3CN; 0% to 5% B linear gradient 20 min. $[^{111}In(Bn\text{-}nonapa)]$ t_R = 8.9 min, $[^{111}In(nonapa)]$ t_R = 5.0 min, and $[^{111}In(Bn\text{-}trenpa)]$ t_R = 10.5 min.

Mouse serum stability challenge

The compounds $[^{111}In(Bn\text{-}nonapa)]$, $[^{111}In(nonapa)]$, and $[^{111}In(Bn\text{-}trenpa)]$ were prepared with the radiolabeling protocol as described above. Mouse serum was removed from the freezer and allowed to thaw at ambient temperature for 30 min. In triplicate for each ^{111}In complex listed above, solutions were made in sterile vials with 750 μL mouse serum, 500 μL of ^{111}In complex (10 mmol/L NaOAc buffer, pH 5.5), 250 μL phosphate buffered saline (PBS), and were left to sit at ambient temperature. After 1 h, half of the mouse serum competition mixture (750 μL) was removed from each vial, diluted to a total volume of 2.5 mL with phosphate buffered saline, and then counted in a Capintec CRC 15R well counter to obtain a value for the total activity to be loaded on the PD-10 column. The 2.5 mL of diluted mouse serum competition mixture was then loaded onto a PD-10 column that had previously been conditioned via elution with 20 mL of PBS. The 2.5 mL of loading volume was allowed to elute into an ^{111}In waste container, and then the PD-10 column was eluted with 3.5 mL PBS and collected into another sterile vial. This 3.5 mL of collected eluent, which contained ^{111}In bound/associated with serum proteins (size-exclusion for MW < 5000 Da), was counted in a well counter, and then compared to the total amount of activity that was loaded on the PD-10 column to obtain the percentage of ^{111}In that was bound to serum proteins and therefore no longer chelate bound. The percent stability values shown in Table 1 represent the percentage of ^{111}In that was retained on the PD-10 column and therefore still chelate bound.

Supplementary material

^{1}H/^{13}C NMR spectra of final synthesized compounds are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjc-2013-0542.

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References

(1) Wadas, T. J.; Wong, E. H.; Weisman, G. R.; Anderson, C. J. *Chem. Rev.* **2010**, *110*, 2858. doi:10.1021/cr900325h.

(2) Ramogida, C. F.; Orvig, C. *Chem. Commun.* **2013**, *49*, 4720. doi:10.1039/c3cc41554f.

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(3) Price, E. W.; Orvig, C. *Chem. Soc. Rev.* **2014**, *43*, 260. doi:10.1039/c3cs60304k.
(4) Zeglis, B. M.; Lewis, J. S. *Dalton Trans.* **2011**, *40*, 6168. doi:10.1039/c0dt01595d.
(5) Anderson, C. J.; Welch, M. J. *Chem. Rev.* **1999**, *99*, 2219. doi:10.1021/cr980451q.
(6) Bartholomä, M. D. *Inorg. Chim. Acta* **2012**, *389*, 36. doi:10.1016/j.ica.2012.01.061.
(7) Clarke, E. T.; Martell, A. E. *Inorg. Chim. Acta* **1991**, *190*, 37. doi:10.1016/S0020-1693(00)80229-7.
(8) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum Press, New York, 1974–1989, Vol. 1–6.
(9) Liu, S.; Pietryka, J.; Ellars, C. E.; Edwards, D. S. *Bioconjugate Chem.* **2002**, *13*, 902. doi:10.1021/bc010134h.
(10) Liu, S.; He, Z.; Hsieh, W.-Y.; Fanwick, P. E. *Inorg. Chem.* **2003**, *42*, 8831. doi:10.1021/ic0349914.
(11) Li, L.; Bading, J.; Yazaki, P. J.; Ahuja, A. H.; Crow, D.; Colcher, D.; Williams, L. E.; Wong, J. Y. C.; Raubitschek, A.; Shively, J. E. *Bioconjugate Chem.* **2007**, *19*, 89. doi:10.1021/bc700161p.
(12) Decristoforo, C.; Hernandez Gonzalez, I.; Carlsen, J.; Rupprich, M.; Huisman, M.; Virgolini, I.; Wester, H.-J.; Haubner, R. *Eur. J. Nucl. Med. Mol. Imaging* **2008**, *35*, 1507. doi:10.1007/s00259-008-0757-6.
(13) Chakraborty, S.; Shi, J.; Kim, Y.-S.; Zhou, Y.; Jia, B.; Wang, F.; Liu, S. *Bioconjugate Chem.* **2010**, *21*, 969. doi:10.1021/bc900555q.
(14) Tatsi, A.; Maina, T.; Cescato, R.; Waser, B.; Krenning, E.; de Jong, M.; Cordopatis, P.; Reubi, J.; Nock, B. *EJNMMI Res* **2012**, *2*, 1. doi:10.1186/2191-219X-2-1.
(15) Breeman, W. A. P.; de Jong, M.; Visser, T. J.; Erion, J. L.; Krenning, E. P. *Eur. J. Nucl. Med. Mol. Imaging* **2003**, *30*, 917. doi:10.1007/s00259-003-1142-0.
(16) Sun, Y.; Anderson, C. J.; Pajeau, T. S.; Reichert, D. E.; Hancock, R. D.; Motekaitis, R. J.; Martell, A. E.; Welch, M. J. *J. Med. Chem.* **1996**, *39*, 458. doi:10.1021/jm9505977.
(17) Bakker, W. H.; Albert, R.; Bruns, C.; Breeman, W. A. P.; Hofland, L. J.; Marbach, P.; Pless, J.; Pralet, D.; Stolz, B.; Koper, J. W.; Lamberts, S. W. J.; Visser, T. J.; Krenning, E. P. *Life Sci.* **1991**, *49*, 1583. doi:10.1016/0024-3205(91)90052-D.
(18) Lub-de Hooge, M. N.; Kosterink, J. G. W.; Perik, P. J.; Nijnuis, H.; Tran, L.; Bart, J.; Suurmeijer, A. J. H.; de Jong, S.; Jager, P. L.; de Vries, E. G. E. *Br. J. Pharmacol.* **2004**, *143*, 99. doi:10.1038/sj.bjp.0705915.
(19) Liu, S.; Edwards, D. S. *Bioconjugate Chem.* **2001**, *12*, 630. doi:10.1021/bc010013h.
(20) Price, E. W.; Zeglis, B. M.; Cawthray, J. F.; Ramogida, C. F.; Ramos, N.; Lewis, J. S.; Adam, M. J.; Orvig, C. *J. Am. Chem. Soc.* **2013**, *135*, 12707. doi:10.1021/ja4049493.
(21) Price, E. W.; Zeglis, B. M.; Lewis, J. S.; Adam, M. J.; Orvig, C. *Dalton Trans.* **2014**, *43*, 119. doi:10.1039/c3dt51940f.
(22) Price, E. W.; Cawthray, J. F.; Bailey, G. A.; Ferreira, C. L.; Boros, E.; Adam, M. J.; Orvig, C. *J. Am. Chem. Soc.* **2012**, *134*, 8670. doi:10.1021/ja3024725.
(23) Boros, E.; Ferreira, C. L.; Cawthray, J. F.; Price, E. W.; Patrick, B. O.; Wester, D. W.; Adam, M. J.; Orvig, C. *J. Am. Chem. Soc.* **2010**, *132*, 15726. doi:10.1021/ja106399h.
(24) Bailey, G. A.; Price, E. W.; Zeglis, B. M.; Ferreira, C. L.; Boros, E.; Lacasse, M. J.; Patrick, B. O.; Lewis, J. S.; Adam, M. J.; Orvig, C. *Inorg. Chem.* **2012**, *51*, 12575. doi:10.1021/ic302225z.
(25) Brillouet, S.; Dorbes, S.; Courbon, F.; Picard, C.; Delord, J. P.; Benoist, E.; Poirot, M.; Mestre-Voegtlé, B.; Silvente-Poirot, S. *Bioorg. Med. Chem.* **2010**, *18*, 5400. doi:10.1016/j.bmc.2010.05.031.
(26) Havas, F.; Danel, M.; Galaup, C.; Tisnès, P.; Picard, C. *Tetrahedron Lett.* **2007**, *48*, 999. doi:10.1016/j.tetlet.2006.12.012.
(27) Bartholomä, M. D.; Louie, A. S.; Valliant, J. F.; Zubieta, J. *Chem. Rev.* **2010**, *110*, 2903. doi:10.1021/cr1000755.
(28) Stimmel, J. B.; Kull Jr, F. C. *Nucl. Med. Biol.* **1998**, *25*, 117. doi:10.1016/S0969-8051(97)00151-0.
(29) Stimmel, J. B.; Stockstill, M. E.; Kull, F. C. *Bioconjugate Chem.* **1995**, *6*, 219. doi:10.1021/bc00032a010.
(30) Camera, L.; Kinuya, S.; Garmestani, K.; Wu, C.; Brechbiel, M. W.; Pai, L. H.; McMurry, T. J.; Gansow, O. A.; Pastan, I.; Paik, C. H.; Carrasquillo, J. A. *J. Nucl. Med.* **1994**, *35*, 882.
(31) Harrison, A.; Walker, C. A.; Parker, D.; Jankowski, K. J.; Cox, J. P. L.; Craig, A. S.; Sansom, J. M.; Beeley, N. R. A.; Boyce, R. A.; Chaplin, L.; Eaton, M. A. W.; Farnsworth, A. P. H.; Millar, K.; Millican, A. T.; Randall, A. M.; Rhind, S. K.; Secher, D. S.; Turner, A. *Int. J. Rad. Appl. Instrum. B* **1991**, *18*, 469. doi:10.1016/0883-2897(91)90107-V.
(32) Cooper, M. S.; Ma, M. T.; Sunassee, K.; Shaw, K. P.; Williams, J. D.; Paul, R. L.; Donnelly, P. S.; Blower, P. J. *Bioconjugate Chem.* **2012**, *23*, 1029. doi:10.1021/bc300037w.
(33) Hancock, R. D. *J. Chem. Educ.* **1992**, *69*, 615. doi:10.1021/ed069p615.
(34) Liu, S.; Edwards, D. S. *Bioconjugate Chem.* **2001**, *12*, 7. doi:10.1021/bc000070v.
(35) Kukis, D. L.; DeNardo, S. J.; DeNardo, G. L.; O'Donnell, R. T.; Meares, C. F. *J. Nucl. Med.* **1998**, *39*, 2105.
(36) Jang, Y. H.; Blanco, M.; Dasgupta, S.; Keire, D. A.; Shively, J. E.; Goddard, W. A. *J. Am. Chem. Soc.* **1999**, *121*, 6142. doi:10.1021/ja983706q.
(37) Milenic, D. E.; Garmestani, K.; Chappell, L. L.; Dadachova, E.; Yordanov, A.; Ma, D.; Schlom, J.; Brechbiel, M. W. *Nucl. Med. Biol.* **2002**, *29*, 431. doi:10.1016/S0969-8051(02)00294-9.
(38) Kang, C. S.; Sun, X.; Jia, F.; Song, H. A.; Chen, Y.; Lewis, M.; Chong, H.-S. *Bioconjugate Chem.* **2012**, *23*, 1775. doi:10.1021/bc200696b.
(39) Chappell, L. L.; Ma, D.; Milenic, D. E.; Garmestani, K.; Venditto, V.; Beitzel, M. P.; Brechbiel, M. W. *Nucl. Med. Biol.* **2003**, *30*, 581. doi:10.1016/S0969-8051(03)00033-7.
(40) Rasaneh, S.; Rajabi, H.; Babaei, M. H.; Johari Daha, F. *J. Labelled Compd. Radiopharm.* **2010**, *53*, 575. doi:10.1002/jlcr.1780.
(41) Förster, G. J.; Engelbach, M. J.; Brockmann, J. J.; Reber, H. J.; Buchholz, H. G.; Mäcke, H. R.; Rösch, F. R.; Herzog, H. R.; Bartenstein, P. R. *Eur. J. Nucl. Med.* **2001**, *28*, 1743. doi:10.1007/s002590100628.
(42) Wu, C.; Kobayashi, H.; Sun, B.; Yoo, T. M.; Paik, C. H.; Gansow, O. A.; Carrasquillo, J. A.; Pastan, I.; Brechbiel, M. W. *Bioorg. Med. Chem.* **1997**, *5*, 1925. doi:10.1016/S0968-0896(97)00130-2.
(43) Brechbiel, M. W.; Gansow, O. A.; Atcher, R. W.; Schlom, J.; Esteban, J.; Simpson, D.; Colcher, D. *Inorg. Chem.* **1986**, *25*, 2772. doi:10.1021/ic00236a024.
(44) Balogh, E.; Mato-Iglesias, M.; Platas-Iglesias, C.; Toth, E.; Djanashvili, K.; Peters, J. A.; de Blas, A.; Rodriguez-Blas, T. *Inorg. Chem.* **2006**, *45*, 8719. doi:10.1021/ic0604157.
(45) Mato-Iglesias, M.; Balogh, E.; Platas-Iglesias, C.; Toth, E.; Blas, A. d.; Rodriguez-Blas, T. *Dalton Trans.* **2006**, *45*, 5404. doi:10.1039/b611544f.
(46) Platas-Iglesias, C.; Mato-Iglesias, M.; Djanashvili, K.; Muller, R. N.; Elst, L. V.; Peters, J. A.; de Blas, A.; Rodríguez-Blas, T. *Chem. Eur. J.* **2004**, *10*, 3579. doi:10.1002/chem.200306031.
(47) Boros, E.; Ferreira, C. L.; Yapp, D. T. T.; Gill, R. K.; Price, E. W.; Adam, M. J.; Orvig, C. *Nucl. Med. Biol.* **2012**, *39*, 785. doi:10.1016/j.nucmedbio.2012.01.003.
(48) Boros, E.; Ferreira, C. L.; Patrick, B. O.; Adam, M. J.; Orvig, C. *Nucl. Med. Biol.* **2011**, *38*, 1165. doi:10.1016/j.nucmedbio.2011.05.004.