Relevance of Palladium to Radiopharmaceutical Development Considering Enhanced Coordination Properties of TE1PA

Julie Pineau, [a] Luís M. P. Lima, [b] Carlos Platas-Iglesias, [c] Jan Rijn Zeevaart, [d] Cathryn H. S. Driver,[d] Nathalie Le Bris,[a] and Raphaël Tripier,[a]

Abstract: The limited use of palladium-103 and -109 radioisotopes for molecular radiotherapy is surely due to the lack of appropriate ligands capable of fulfilling all criteria required for application in nuclear medicine. Furthermore, the thermodynamic properties of these complexes in solution remain difficult to establish. The challenge is compounded when considering that radiolabeling of compounds for translation to clinical trials requires fast complexation. Thus, the coordination of Pd(II) and 103/109Pd-nuclei is a huge challenge in terms of molecular design and physicochemical characterization. Herein, we report a comprehensive study highlighting TE1PA, a monopicolinate cyclam – already established in nuclear imaging with 64Cu-PET (positron emission tomography) imaging tracers – as a highly relevant chelator for natural Pd and subsequently 109Pd-nuclide. The structural, thermodynamic, kinetic and radiolabeling studies of Pd(II) with TE1PA, as well as the comparison of this complex with three structurally related derivatives, support palladium-TE1PA radiopharmaceuticals as leading candidates for targeted nuclear medicine.

Introduction

Palladium(II) is recognized as a cation of interest in medicine since the 1960s when its antimicrobial properties, similar to those of platinum(II), were explored.[1] In 1979, antiviral, antifungal and antimicrobial properties of Pd(II) compounds were reported by Graham and Williams.[2] Nowadays, several Pd(II)-based drugs or therapeutic agents are used in more sophisticated techniques, such as targeted photodynamic therapy for localized cancer treatment.[3]

In nuclear medicine, palladium radioisotopes were first used in the early 1970s. 103Pd-based molecules were described for the control of homograft rejection,[4] and 109Pd-metal was introduced in 1987 as implanted seeds for brachytherapy.[5] Despite these early applications, further developments have remained very limited. However, these radiometers deserve special attention in therapy on account of their radiophysical characteristics, given the current needs for relevant radiopharmaceuticals. 103Pd- and 109Pd-radioisotopes have half-lives (t1/2) of 17 days and 13.7 h, respectively. Palladium-103 decays by electron capture (EC) and Auger electron (AE) emissions to the 103mRh-daughter (t1/2 = 56 min), which is another AE emitter that decays to stable Rh.[6] It is commercially available via cyclotron production as a high specific activity, no-carrier added product.[7] 109Pd-nuclide decays by β− particle emission (Eβ−(max) = 1.12 MeV, 100% yield) to the daughter nuclide, silver-109 m (t1/2 = 7.2 h), which in turn emits a photon (88 keV, 3.6% yield) accompanied by conversion electron emissions and AE emissions leading to the stable 109Ag-nuclide.[8] Palladium-109 is easily produced with moderate specific activity from an isotopically enriched 109Pd-target in a neutron reactor.[9]

The optimal use of therapeutic radiopharmaceuticals requires combination with a radiodiagnostic agent that allows for patient disease staging and treatment monitoring. Diagnostic radiotracers employ radionuclides, commonly metallic elements, with emissions that can be detected by specialized cameras and allow for disease imaging. These emissions include positrons (β+) and photons (γ) for PET (positron emission...
tomography) and SPECT (single-photon emission computed tomography), respectively.\textsuperscript{[3]} For the development of radiopharmaceuticals utilizing metallic radionuclides, the radiometals produced in their cationic form must be complexed with a chelator that fulfills all the necessary criteria for in vivo applications. The complex formed with the radiometal should be very stable, both in terms of thermodynamic stability and kinetic inertness.\textsuperscript{[10]} Moreover, the chelator must be bifunctional, in other words, besides its coordination properties, it must have an additional functional group suitable for conjugation to targeting biomolecules without compromising complex stability.\textsuperscript{[11]} The perfect match between the metallic cation and the design of the chelator is therefore the crucial factor for achieving the desired physico-chemical properties of the complex.

Given the wide range of radionuclides of interest for medical applications, a large panel of chelating agents have been reported in the literature.\textsuperscript{[12]} The current quest is to find versatile chelating agents capable of complexing a theranostic pair, meaning two radionuclides suitable for both diagnosis and therapy.\textsuperscript{[13]}

Radionuclides for therapy, such as lutetium-177 or yttrium-90 (\(\beta^+\)), have been successfully paired with gallium-68 (\(\beta^+\)) for PET imaging.\textsuperscript{[14]} However this pairing is not ideal as the corresponding half-lives of the radionuclides are not well matched (6.7 days for \(\text{\textsuperscript{177}}\text{Lu}\), 2.7 days for \(\text{\textsuperscript{86}}\text{Y}\) vs. 68 min for \(\text{\textsuperscript{68}}\text{Ga}\)-nucleide) and their coordination properties differ considerably, with typical coordination numbers of 8–10 for \(\text{\textsuperscript{177}}\text{Lu}\) and \(\text{\textsuperscript{86}}\text{Y}\) vs. 6 for \(\text{\textsuperscript{68}}\text{Ga}\). In addition to these heterotopic pairs, a few pairs of homotopic elements, such as \(\text{\textsuperscript{64}}\text{Cu}/\text{\textsuperscript{63}}\text{Cu}\)-isotopes, are of an increasing interest.\textsuperscript{[15]} The \(\beta^+\) emitter, copper-64 (\(t_{1/2} = 12.7\) h) is produced worldwide for PET imaging in preclinical and clinical trials. The therapeutic partner, copper-67 (\(t_{1/2} = 61.8\) h), is a \(\beta^+\) emitter but its rather low emission energy \((E_{\beta^+} = 0.577\) MeV\) might limit its treatment potential; therefore, finding alternative radionuclides with suitable therapeutic emissions to be paired with copper-64 would be highly beneficial.

While presenting interesting properties, palladium radioisotopes have been sparsely used in the context of radiopharmaceutical development, and only a few ligands such as DTPA\textsuperscript{[16]} and porphyrins were explored.\textsuperscript{[17]} The most reasonable explanation for this is that the palladium coordination chemistry does not match the chelators considered as gold standards in nuclear medicine, namely DOTA (1,4,7,10-tetraaza-cyclododecan-1,4,7,10-tetraacetic acid) and NOTA (1,4,7-triaza-cyclononan-1,4,7-triacetic acid) derivatives. Palladium, a second-row transition metal with the electronic configuration \((\text{1s}^2\text{2s}^2\text{2p}^6\text{3s}^2\text{3p}^6\text{3d}^{10})\) has very different coordination properties from the metallic cations such as \(\text{\textsuperscript{177}}\text{Lu}\) or \(\text{\textsuperscript{68}}\text{Ga}\). The coordination of both \(\text{Cu}(II)\) and \(\text{Pd}(II)\), and potentially used for the development of new radiopharmaceuticals based on the \(\text{\textsuperscript{64}}\text{Cu}/\text{\textsuperscript{109}}\text{Pd}\) or \(\text{\textsuperscript{64}}\text{Cu}/\text{\textsuperscript{109}}\text{Pd}\)-theranostic pairs. For a better understanding of the different factors that drive complexation, three other ligands (Figure 1) were studied in addition to TE1PA: cyclam, TE1Bn (benzyl cyclam) and TE1Py (pyridylmethyl cyclam).

### Results and Discussion

#### Synthesis and solid-state studies

TE1Py was obtained following a new synthetic route starting from the easily synthesized triBoc-cyclam (see procedure in Supporting Information, Scheme S1 and Figures S1-S4). All other chelators were obtained using established syntheses methods (see Supporting Information). The synthesis of the Pd(II) complexes was performed in refluxing aqueous solution using \(\text{Na}_2\text{PdCl}_4\) over 16 h at pH 4 (to avoid metal hydroxide precipitation). All complexes were obtained as yellowish powders in yields of around 60% following purification (see procedures in Supporting Information and Figure S5 to S14).

The structures of \([\text{Pd(TE1Bn)}]^+\) and \([\text{Pd(TE1PA)}]^+\) obtained by X-ray diffraction are shown in Figures 2 and 3, while those of \([\text{Pd(cyclam)}]^+\) and \([\text{Pd(TE1Py)}]^+\) are presented in the Supporting Information (Figures S15-16). Selected bond lengths and angles for all complexes are given in Table 1 (additional data in Tables S1 to S5). Crystals of \([\text{Pd(cyclam)}]\text{Cl}_2\text{H}_2\text{O}\) suitable for X-ray diffraction studies were obtained by slow evaporation from aqueous solution at pH 7. The structure is very similar to those of \([\text{Pd(cyclam)}]\text{Cl}_2\text{H}_2\text{O}\) and \([\text{Pd(cyclam)}]\text{Cl}_2\) reported previously,\textsuperscript{[26,27]} though with slightly different bond distances and angles. The structure evidences square-planar coordination with the Pd(II) ion located in the plane defined by the four

\[
\begin{align*}
\text{cyclam} & \quad \text{TE1Bn} & \quad \text{TE1Py} & \quad \text{TE1PA}
\end{align*}
\]

Figure 1. Ligands discussed in this study.

\textsuperscript{[1]} for Pd(II) coordination.\textsuperscript{[21]} Cyclam is a well-known scaffold forming highly stable and inert complexes with small transition metal ions such as \(\text{Ni}(II)\), \(\text{Co}(II)\) or \(\text{Cu}(II)\).\textsuperscript{[22]} Furthermore, cyclam forms a square-planar complex with Pd(II) with an extremely high stability constant \((\log \beta = 56.9)^{[23]}\)

Our group developed the monopicolinate cyclam derivative TE1PA, which exhibits improved properties for \(\text{\textsuperscript{64}}\text{Cu}-\text{immuno-PET}\) imaging in terms of radionabeling yield, conjugation to antibody, biodistribution, and pharmacokinetics as compared to those of DOTA and NOTA derivatives.\textsuperscript{[24,25]} Thus, we sought to investigate the coordination of TE1PA with natural Pd and palladium-109 to assess whether this chelator could be used for the coordination of both Cu(II) and Pd(II), and potentially used for the development of new radiopharmaceuticals based on the \(\text{\textsuperscript{64}}\text{Cu}/\text{\textsuperscript{109}}\text{Pd}\) or \(\text{\textsuperscript{64}}\text{Cu}/\text{\textsuperscript{109}}\text{Pd}\)-theranostic pairs. For a better understanding of the different factors that drive complexation, three other ligands (Figure 1) were studied in addition to TE1PA: cyclam, TE1Bn (benzyl cyclam) and TE1Py (pyridylmethyl cyclam).
nitrogen atoms. The hydrogen atoms of the two amino functions separated by a propylene bridge point to the same side of the N₄ plane, and opposite to the other two hydrogen atoms, resulting in a trans-III configuration according to Bosnich’s nomenclature.\[28\] The two six-membered chelate rings adopt chair conformations.

Crystals of [Pd(TE1Bn)(PdCl₄)] were obtained by slow evaporation under heating of a solution of the complex in DMSO. The structure resembles that of the parent unsubstituted complex, [Pd(cyclam)]⁺, in that a trans-III configuration is obtained with minimal distortion from a square-planar geometry (Figure 2). The presence of the benzyl group on N(1I) does not significantly affect the Pd(1)-N(1I) bond length value of 2.012(19) Å, which is similar to the Pd-NH bonds (Table 1). In the five-membered chelate rings, N(1I)-Pd(1)-N(2I) and N(4I)-Pd(1)-N(3I) angles are respectively equal to 86.0(7)° and 89.4(7)°. These N-Pd-N angles show similar tendencies to the two differently regiosubstituted N,N’-dibenzyl-cyclam palladium complexes described by Turner.\[29\]

Crystals of [Pd(TE1Py)(PdCl₄)] were obtained by slow evaporation of an aqueous solution of the complex at room temperature. While the crystal structure had a poor quality (Figure S16), it revealed a trans-III configuration similar to that obtained for the Pd-TE1Bn complex; the bond lengths and angles have to be considered cautiously because of the low resolution. The substitution of the benzyl group of TE1Bn by a

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**Table 1.** Selected bond lengths (Å) and angles (°) for all complexes.

| Configuration   | [Pd(cyclam)]⁺ | [Pd(TE1Bn)]²⁺ | [Pd(TE1Py)]²⁺ | [Pd(HTe1PA)]⁺ | [Pd(HTe1PA)]⁺ | [Pd(HTe1PA)]⁺ |
|-----------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                 | trans-III     | trans-III     | trans-III     | trans-III     | trans-I       | trans-III     |
| Pd(1)-N(1)      | 2.039(6)      | 2.012(19)     | 2.074(6)      | 2.059(5)      | 2.080(5)      | 2.009(10)     |
| Pd(1)-N(2)      | 2.031(5)      | 1.978(15)     | 2.024(6)      | 2.044(5)      | 2.035(5)      | 1.998(12)     |
| Pd(1)-N(3)      | –             | 2.036(16)     | 2.042(6)      | 2.057(5)      | 2.038(5)      | 2.007(10)     |
| Pd(1)-N(4)      | –             | 1.997(16)     | 2.040(6)      | 2.046(6)      | 2.045(5)      | 2.050(12)     |
| Pd(1)-N(5)      | –             | –             | –             | 5.015         | 3.066         | 5.002         |
| N(2)-Pd(1)-N(2) | –             | –             | –             | –             | 2.18(2)       | –             |
| N(1)-Pd(1)-N(2) | 85.3(3)       | 86.0(7)       | 84.3(3)       | 85.49(18)     | 84.98(19)     | 84.2(5)       |
| N(2)-Pd(1)-N(3) | 94.7(3)       | 86.8(6)       | 94.54(6)      | 95.0(2)       | 96.8(2)       | 95.8(4)       |
| N(3)-Pd(1)-N(4) | –             | 89.7(7)       | 84.31(6)      | 85.2(2)       | 84.8(2)       | 84.5(5)       |
| N(4)-Pd(1)-N(1) | –             | 98.0(7)       | 96.87(6)      | 94.4(2)       | 93.2(2)       | 95.6(4)       |

[a] structure of poor quality and low resolution
pyridylmethyl group in TE1Py does not significantly affect the bond lengths of the Pd(II) coordination sphere, but has a greater impact on the various bond angles. The values of the N(1)–Pd(1)–N(2) and N(3)–Pd(1)–N(4) angles, corresponding to the five-membered chelate rings, are below 85°, while the N(2)–Pd(1)–N(3) and N(4)–Pd(1)–N(1) angles are above 94°.

Three different crystal structures were obtained for the TE1PA complex by evaporation of aqueous solutions at different pH values (pH < 0, 3.5 and 7) (Figure 3). In each case, the Pd(II) ion is four-coordinated by the nitrogen atoms of the cyclam core. The nitrogen atom of the picolinate unit is not bound to palladium, regardless of whether the carboxylate function is protonated or not. The structure of [Pd(HTE1PA)][PdCl4] obtained from a 5 M HCl aqueous solution shows a trans-III configuration, while interestingly those obtained from an aqueous solution at pH 3.5 reveal a trans-I configuration. Crystals of [Pd(TE1PA)][ClO4]2H2O obtained at pH 7 (by adjusting the pH with NaOH) after addition of NaClO4 again indicate a structure with the trans-III configuration. The Pd–N bond lengths of the trans-III complex obtained in 5 M HCl are between 2.044(5)–2.059(5) Å, while those of the trans-III complex isolated at pH 7 are slightly shorter, ranging from 1.998(12) to 2.050(12) Å. The Pd(1)–N(1) bond, which involves the picolinate substituent, shows very different distances of 2.059(5) (5 M HCl) against 2.009(10) Å (complex at pH 7). A similar situation is observed for the Pd(1)–N(2) and Pd(1)–N(3) bonds, while the Pd(1)–N(4) distances are virtually identical. For the trans-I complex, the Pd(1)–N(1) distance of 2.080(5) Å is much longer than the Pd-NH distances of Pd(1)–N(2) at 2.035(5) Å, Pd(1)–N(3) at 2.038(5) Å, and Pd(1)–N(4) at 2.045(5) Å (Table 1), but also much longer than the Pd(1)–N(1) distance in the trans-III complex. For both the trans-III and trans-I configurations, the angles characterizing the five-membered chelate rings (N(1)–Pd(1)–N(2) and N(3)–Pd(1)–N(4), < 86°) are lower than those involving six-membered chelate rings (N(2)–Pd(1)–N(3) and N(4)–Pd(1)–N(1), > 93°). In both trans-III complexes, the distance between the metal center and the nitrogen atom of the pyridyl unit N(S) is > 5 Å, since the picolinate arm is orientated away from the central plane of the cyclam unit. On the contrary, in the trans-I configuration the protonated picolinate arm points towards the cyclam core, generating an intramolecular hydrogen bonding interaction between one NH group of the macrocycle and one oxygen atom of the carboxylic acid group (N(2)–H(2)–O(1), 2.18(2) Å).

Structural studies in solution

The 1H and 13C NMR spectra of [Pd(cyclam)]2+ were performed in D2O or in methanol-d4 (Figures S5–S6). The 13C NMR spectrum surprisingly indicated two series of peaks not visible in the 1H NMR spectrum. The proportion of the minor species was dependent on the solvent, ranging from 10% in methanol-d4 to about 30% in D2O irrespective of the pD (4 and 7). Sadler and coll. reported in 200427 that only one species with the trans-III configuration existed in solution, suggesting that the presence of a second species, such as the trans-I configuration, is possible depending on experimental conditions. DFT calculations indeed predict that the trans-I and trans-III configurations are significantly more stable than trans-II and cis-V (Table S6). The relative free energies calculated showed that the trans-III isomer is favored by only 2.5 kcal mol⁻¹ over trans-I. Calculations of the 13C NMR shifts using relativistic DFT calculations allowed for assignment of the major species present in solution to the trans-III isomer, in agreement with the relative energies and the X-ray structures (Table S7).

For the [Pd(TE1Bn)]2+ complex, the NMR study performed in DMSO-d6 for solubility reasons, exhibited one main series of peaks in both 1H and 13C NMR spectra (Figure S8). However, one other series of peaks corresponding to the minor isomer was also observed. This complex has already been described by Lindoy and co-workers,29 but without any details concerning the species present in solution. However, the NMR spectra clearly indicated more peaks than expected for one single isomer. The relative energies calculated for [Pd(TE1Bn)]2+ by DFT are very sensitive to the inclusion of explicit second-sphere water molecules. Calculations performed on the [Pd(TE1Bn)][PdCl4]2H2O system suggest that the trans-III isomer is the most stable configuration, roughly by 2 kcal mol⁻¹ with respect to the trans-I configuration. Thus, most likely both trans-III and trans-I isomers are present in solution, though an unambiguous assignment of the major species present in solution cannot be performed on the grounds of our NMR data. The NMR spectra of [Pd(TE1Py)]2+ in D2O (Figure S10) are very similar to those of the [Pd(TE1Bn)]2+ complex, with one main species most likely corresponding to the trans-III configuration.

For [Pd(TE1PA)]2+ spectra recorded in D2O at pD 7 (Figure S12) displayed two sets of signals with similar populations, assigned to the trans-I and trans-III configurations. Adjustment of the pD to 4 in the same sample did not change the proportions of the two populations (Figure S13). The DFT calculations revealed rather similar energies for these two isomers, while the trans-II and cis-V isomers are characterized by higher energies (Table S6). A summary of the different configurations obtained in solution for the studied Pd(II) complexes is given in Table S8.

Thermodynamic studies

Cyclam is known to form a Pd(II) complex displaying outstandingly high thermodynamic stability,30 but to our knowledge this property has only been investigated in one other cyclam derivative, tetra(2-hydroxyethyl) cyclam.30 The reason for this is the notorious difficulties in studying Pd(II) complexation in aqueous media, namely: the marked tendency of this metal cation to form insoluble hydrolysis species in all but the most acidic pH ranges, the frequently high stability of its polyamine complexes, and a predisposition to display slow complexation kinetics with structurally rigid ligands. In order to enable a detailed study of the complexation properties of Pd(II) with monosubstituted cyclam derivatives, a combination of UV spectrophotometry and potentiometry performed in aqueous medium at 25 °C was used. To overcome the expected high

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values of the formation constants for the complexes, the UV
titrations were run in a very acidic solution (pH = 0–1.5) at a
1.0 M concentration of (K,H)Cl electrolyte. In such low pH
conditions, Pd(II) complexation could be observed for both
TE1PA and TE1Py. Fitting of the UV spectroscopy data then
allowed for determination of the formation constant for the
protonated species [Pd(H,TE1PA)]^{2+} or [Pd(HTE1Py)]^{2+} with
subsequent establishment of the remaining equilibrium con-
stants for the existing species (constants were fitted to the
potentiometric titration of the corresponding system). No
formation constant for any complex species of TE1Bn could be
determined as the complex appears to be fully formed under
the used experimental conditions. However, from the UV data it
can be speculated that its log K value should be higher than ca.
43. The ligand protonation equilibria were studied by poten-
tiometry in 1.0 M KCI aqueous medium, as were the protonation
equilibria of the Pd(II) complexes since these are fully formed at
pH ≥ 2.0. The protonation and complexation constants are
presented in Table 2 (and Table S9), while species distribution
diagrams are presented in Figures S17 and S18.

The protonation constants of the ligands display the usual
pattern found for cyclam derivatives of two basic protonation
centers while the remaining ones are more acidic. A previously
observed feature, consistent with cyclam, is that both TE1Py
and TE1Bn show the uncommon inversion of values for two
acidic protonation centers where the third constant is lower
than the fourth. This feature has been attributed to the
increased strain exhibited upon addition of the third proton to
the cyclam skeleton.[31] For the Pd(II) complexes, using the
previously UV-determined formation constants to fit the
potentiometric data, it was found that TE1PA shows a very high
stability constant (log K_{ML} = 38.4), while TE1Py displays an
even higher constant (log K_{ML} = 42.6). Both values are much higher
than for the tetra(2-hydroxyethyl) cyclam derivative (log K_{ML} =
18.32),[32] despite still being significantly lower than the value
estimated for cyclam itself (log K_{ML} = 56.9).[23]

Inertness

The slow dissociation of the complexes is probably the most
important feature to be evaluated for the selection of medically
relevant compounds. The acid-assisted dissociations of the
cyclam and TE1PA complexes were studied in acidic aqueous
solutions. The dissociation was monitored using 1H NMR by
following the changes of the complexes at 90 °C in 5 M DCl. The
unchanged spectra (Figure S19–S20) unambiguously showed
absolutely no evolution of the structures even after five months
incubation with constant stirring. This confirms the perfect
matching of the cyclam core with Pd(II), and that the presence
of the picolinate pendant in TE1PA, even if not involved in
the metal coordination scheme, does not compromise kinetic
inertness.

109Pd Radiolabeling

Considering the very high stability constants of the Pd(II)-
cyclam derivative complexes and their inertness in acidic
medium, all cyclam derivatives were studied for complexation of
the 109Pd-radioisotope (see procedure in Supporting Informa-
tion). The aim was to compare the macrocycles, and specifically
TE1PA and cyclam, in terms of their 109Pd-labeling efficiency
(the kinetic process of complexation) and inertness under
radiolabeling conditions for potential application in developing
new palladium-based radiopharmaceuticals.

The 109Pd-radiouncleotide was produced with a specific
activity of 2.9 GBq/mg (78 mCi/mg) from a 94% enriched
109Pd-target following high-flux neutron beam irradiation.
After the precipitation and separation of Ag-radiouncleotide
impurities from the processed [109Pd][PdCl_{4}^{2-}] solution, gamma ray spectroscopy (88 keV (gamma from 109mAg-
daughter nuclide), 311, 413, 602, 636, 647, 781 keV) indicated
a radionuclidic purity > 99.9% with a radiochemical yield of
90%. While palladium-109 is easily accessible, the disadvant-
age is that the molar activity of the obtained radionuclide is
only moderate. In the long run, this might limit biological
application, however it is more than sufficient for evaluation
of chelator complexation properties.

In order to compare the efficiency of the different
chelators for 109Pd-complexation, the radiolabeling reactions
were performed in 0.1 M NH_{4}OAc at different pH values
(pH 3.5, 7 and 8.5), two temperatures (25 and 90 °C), and
different reaction times (10 and 30 min). An approximate
two-fold excess molar ratio of chelator:metal (2–2.5 : 1)(based
on starting mass of irradiated target and subsequent
approximate concentration of 109Pd-solution) was used to
ensure maximum metal complexation. The reactions were
then analyzed by radio-HPLC (Figure S21) to determine the
radiolabeling efficiency (% LE).

The radiometal complexation for all chelators was very
good (>90% LE) at pH 3.5 and 90 °C over 30 min. The relatively
long reaction time at this temperature provided enough energy
to activate metal-chelator bond formation and to overcome the
kinetic process of complexation to all four ligands. For
comparison purposes, the radiolabeling efficiencies were eval-
uated at different temperatures over the shorter reaction time
of 10 min. These results are presented in Figure 4, Figure S22
and Table S10.
With 99% LE at 90°C and 96% at room temperature within 10 min, TE1PA clearly presented the best efficiency for complexation of 109Pd-nuclide among the four chelators. While heating generally improved the radiolabeling efficiency, the chart in Figure 4 clearly demonstrates that the functionalization of the cyclam backbone accelerates the radiolabeling, especially at low temperature. Indeed, the poor LE obtained for cyclam is evidence of slow complexation kinetics, particularly at short radiolabeling times, and indicates that longer times (30 min) and high temperatures are required to improve complexation (90% LE). The differences between LEs of TE1Bn, TE1Py, and TE1PA at 90°C are not very significant. However, these chelators show rather different performances at room temperature (96% for TE1PA vs. 78% and 70% for TE1Py and TE1Bn, respectively). Most likely, the picolinate moiety at pH 3.5 is rather efficient in chelating Pd(II) close to the macrocyclic cavity, perhaps assisted by the intramolecular hydrogen bond observed in the X-ray structure (N(2)–H(2)···O(1)). This intermediate can then evolve to the final complex upon coordination of Pd(II) to the N atoms of the macrocycle. The results for labelling experiments performed at other pH values (7.0 and 8.5, Figures S23–S25) evidence a noticeable drop in radiolabeling efficiency for all chelators with respect to pH 3.5, except for TE1PA. Under these radiolabeling conditions, the [109Pd][Pd(TE1PA)]+ complex is formed with a very high radiolabeling yield as compared to the other chelators, (LE > 85% for TE1PA, and < 40% for cyclam, TE1Bn and TE1Py; 90°C, 30 min). Even at a pH of 8.5 at room temperature with a short reaction time, the LE of TE1PA stay moderate (35%) as compared to the LE of the other chelators that falls to values < 5%. Interestingly, the 109Pd-labelling reactions completed in a 0.01 M PBS buffer (pH 6.5–7.0) for 10 min at 25 °C or 90 °C resulted in very high LEs (≥ 95%) for all chelators, except for cyclam at room temperature (38%) (Figure S25). The higher labelling efficiencies are most likely as a result of the PBS buffer preventing extensive precipitation of palladium hydroxide, while the conformational rearrangement necessary for cyclam to accommodate the metal is not kinetically favored and the LE is therefore limited at lower temperatures.

To further investigate and compare the radiolabeled complex inertness with regard to transchelation, an EDTA challenge was performed under physiological pH conditions in PBS buffer (0.01 M, pH 7). TE1PA and cyclam were radiolabeled with palladium-109 in PBS buffer at 90°C to obtain maximum radionuclide complexation (> 98%) and then challenged by an EDTA solution (1000 eq) at room temperature. Transchelation to EDTA was then monitored by radio-HPLC over 24 h (Figure 5).

Not much transchelation was observed at the early time points with the behavior of both radio-complexes being quite similar (only 2–3% loss of the radiometal). However, a significant improvement in inertness of [109Pd][Pd(TE1PA)]+ over [109Pd][Pd(cyclam)]2+ is observed after 24 h at room temperature, ultimately demonstrating the enhanced properties of the picolinate derivative.

**Conclusion**

Considering their radiophysical properties, both 109Pd- and 103Pd-isotopes are very attractive radionuclides for therapeutic purposes in nuclear medicine. Moreover, these radionuclides could be associated with β−-emitters to form attractive theranostic pairs as effective solutions for the monitoring and treatment of patients with cancers. However, the progress towards this end has been hindered by the lack of efficient chelators able to comply with the required physicochemical specifications in terms of kinetics and thermodynamics. After a thorough review of the literature, macrocyclic polyamines, especially cyclam and its derivatives, appeared to be good candidates for Pd(II) complexation. Thus, cyclam-based chelators efficient for 64Cu-radiolabelling, such as TE1PA, should also be efficient for Pd(II) coordination. The comparative study of the Pd(II) complexes obtained with cyclam, TE1Bn, TE1Py, and TE1PA clearly showed that all complexes displayed mainly the trans-III configuration in the solid state, but both trans-I and trans-III configurations...
were present in solution. We succeeded in implementing, to our knowledge, the first thermodynamic study combining potentiometry and UV spectroscopy that allowed measurements of the complexation constants obtained with the mono-functionalized chelators. As expected, ligand function-

decreased the thermodynamic stability of the complexes, which nonetheless still presented extremely high stability constants (log $K_{eq} = 38.4$ for [Pd(TE1PA)$]^{3+}$). Radiolabeling studies unambiguously showed that the presence of the picolinate pendant on the cyclam backbone improved both the $^{110m}$Pd-radiolabelling efficiency in mild conditions and the inerterness with respect to transchelation. This paves the way for the appealing use of bifunctional TE1PA derivatives$^{[25]}$ for therapeutic purposes with Pd radioisotopes, as well as for a theranostic application when combined with copper-64 for PET imaging.

**X-ray structures:** Deposition Numbers 2159599 (for [Pd(cyclam)$_2$])$_2$, 2159601 (for [Pd(TB1Im)$_2$])$_2$, 2156052 (for [Pd(cyclam)$_2$]$_2$) obtained from a 5 M HCl solution, 2159598 (for [Pd(HTE1PA)$_2$] at pH 3.5), 2159600 (for [Pd(TE1PA)$_2$]$_2$ at pH 7) contain the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinforma-
tionszentrum Karlsruhe Access Structures service.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Data Availability Statement**

The data that support the findings of this study are available in the supplementary material of this article.

**Keywords:** cyclam - cyclam monopicolinate - complexation - palladium(II) - palladium-109 - radiolabelling

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