

Radiolabelled Cyclic Bisarylmercury: High Chemical and *in vivo* Stability for Theranostics

Ian Moore F. Gilpin,^{*,[a, b]} Martin Ullrich,^[a] Thomas Wünsche,^[a] Kristof Zarschler,^[a] Ondřej Lebeda,^[c] Jens Pietzsch,^[a, b] Hans-Jürgen Pietzsch,^[a] and Martin Walther^{*,[a]}

We show the synthesis of an *in vivo* stable mercury compound with functionality suitable for radiopharmaceuticals. The designed cyclic bisarylmercury was based on the water tolerance of organomercurials, higher bond dissociation energy of Hg–Ph to Hg–S, and the experimental evidence that acyclic structures suffer significant cleavage of one of the Hg–R bonds. The bispidine motif was chosen for its *in vivo* stability, chemical accessibility, and functionalization properties. Radionuclide production results in ^{197(m)}HgCl₂(aq), so the desired mercury compound was formed via a water-tolerant organotin transmetallation. The Hg-bispidine compound showed high chemical stability in tests with an excess of sulfur-containing competitors and high *in vivo* stability, without any observable protein interaction by human serum assay, and good organ clearance demonstrated by biodistribution and SPECT studies in rats. In particular, no retention in the kidneys was observed, typical of unstable mercury compounds. The ^{197(m)}Hg analogue allowed full characterization by NMR and HRMS.

Conventional radiopharmaceutical drugs used in oncology fulfil one of two roles: diagnostic (tumour imaging) or therapeutic (cancer treatment). Due to the biochemical heterogeneity between the diagnostic drug and the therapeutic drug, the *in vivo* interactions observed for the diagnostic drug are not directly interchangeable with the actual behaviour of the therapeutic drug. Theranostics, the designed combination of therapeutic and diagnostic radiopharmaceuticals with biochem-

ical homogeneity, aims to bridge this information gap, thus allowing continual treatment evaluation.^[1] A current example of a theranostic matched pair is ⁶⁸Ga and ¹⁷⁷Lu labelled DOTA-TATE for the treatment of neuroendocrine tumours.^[2] Although no single radionuclide is suitable for every type of treatment (due to tumour size, receptor expression, etc.), ideally the radiopharmaceutical would use the same element for both imaging and therapy as the *in vivo* interactions would then be identical.^[1]

Therefore, our interest in radiomercury was motivated by the theranostically suitable decay emissions of ¹⁹⁷Hg and its metastable nuclear isomer ^{197m}Hg (collectively referred to as ^{197(m)}Hg). A broader selection of radionuclides in developed pharmaceuticals would allow for more personalized care,^[3] and compared with currently used radionuclides the mercury isotope possesses many apt qualities. The emitted conversion electrons have energies closely corresponding with the beta emissions of ¹⁷⁷Lu used for therapy,^[4] whilst a gamma emission is suitably near the energy of the ^{99m}Tc gamma emission diagnostically used in single-photon emission computed tomography (SPECT) medical imaging.^[5] The high number of Auger electron (AE) emissions per decay (^{197m}Hg: 19, ¹⁹⁷Hg: 23) also have great therapeutic potential, with similar energies to ¹²⁵I, which has already shown that even extracellular AE radiotherapy can effectively cause cell death.^[6–8] Combined with the practical half-lives of 23.82 h and 64.81 h (for the metastable and ground state nuclear isomers respectively)^[9] and the stable ¹⁹⁷Au decay product, the innately theranostic nuclide ^{197(m)}Hg is a promising candidate for radiopharmacy.^[10,11]


There is historical precedence for the medicinal use of radiomercury, as during the 1960s and early 1970s reactor produced ¹⁹⁷Hg with low specific activity did see clinical use in diagnostics, for kidney and brain imaging, in the radiolabelled chlormerodrin,^[12] a mercurial diuretic commercially traded from 1952^[13] till 1974.^[14] ¹⁹⁷Hg-chlormerodrin, whilst once popular amongst some physicians,^[15] was discontinued by the FDA in 1989.^[16] The lack of viable mercury compounds, coupled with the rapid progress of the ^{99m}Tc alternative, led to radiomercury's discontinuation and current obscure status in the long list of radionuclides in modern radiopharmaceutical research.^[17–20] Modern cyclotron production, using ^{nat}Au as the target material, yields ^{197(m)}Hg with such high specific activity that the medically relevant amount falls well below the lower limits of concern for mercury's chemical toxicity.^[10]


In contrast to historical popularity, mercury has attracted far less interest in modern chemistry, mainly due to concerns over toxicity.^[21] Today, a lot of mercury research is focused on its

[a] I. M. F. Gilpin, Dr. M. Ullrich, T. Wünsche, Dr. K. Zarschler, Prof. J. Pietzsch, Dr. H.-J. Pietzsch, Dr. M. Walther
Institute of Radiopharmaceutical Cancer Research
Helmholtz-Zentrum Dresden-Rossendorf
Bautzner Landstrasse 400, 01328 Dresden (Germany)
E-mail: m.walther@hzdr.de
iammooregilpin@gmail.com

[b] I. M. F. Gilpin, Prof. J. Pietzsch
Faculty of Chemistry and Food Chemistry
School of Science, Dresden University of Technology
Mommsenstrasse 9, 01062 Dresden (Germany)

[c] Dr. O. Lebeda
Department of Radiopharmaceuticals, Nuclear Physics Institute of the CAS
Řež 130, 250 68 Husinec-Řež (Czech Republic)

 Supporting information for this article is available on the WWW under <https://doi.org/10.1002/cmdc.202100131>

 © 2021 The Authors. ChemMedChem published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

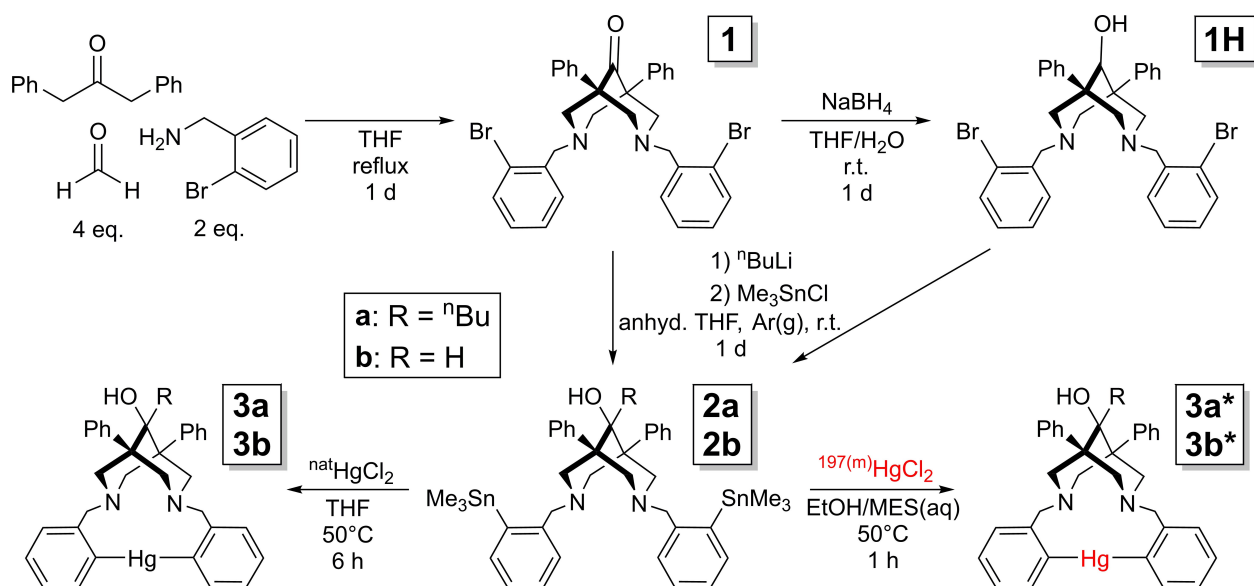
detection and removal from the environment and, although being phased out, organomercury's present-day main use is still in disinfectants (e.g. thiomersal). None of these uses require long term *in vivo* stability.^[22,23] Chelation therapy for mercury poisoning often uses thiols, such as dimercaptosuccinic acid (DMSA), due to low side-effects and cost, but as the priority is fast clearance, long term stability of the mercury compound formed is not essential.^[24] Beyond radiopharmaceuticals, the formation of a mercury compound that is highly stable in nanomolar concentrations could also transfer to trace mercury analysis in environmental science and toxicology.

As with other radiometals, the bifunctional chelator (BFC) model was deemed the most appropriate route for securing the radiomercury and providing a link to a tumour-targeting vector.^[19] Organomercurials inherently have the necessary high water tolerance and the Hg–Ph bond is preferred due to having the highest bond dissociation energy for any Hg bond.^[25] Research has shown that acyclic organomercury compounds suffer significant cleavage^[26,27] therefore a cyclic structure was chosen for increased stability. Functionalization of the bridge would allow for improved solubility and attachment of a tumour specific carrier. Therefore a bispidine backbone was chosen as a suitable candidate due to its known *in vivo* stability, ease of synthesis, and functionalization capabilities, along with its already thoroughly investigated potential for radiopharmaceuticals.^[28–32] Protonation of the tertiary amine moieties under physiological conditions would also improve the compound's water solubility due to the known bispidine 'proton sponge' effect.^[33] In order to form the desired highly stable bispidine carbon-metal bond, a different route from previous studies, which focussed on catalysis chemistry, was necessary.^[34–39] Trimethylstannyl was chosen as a viable leaving group for the transmetallation with mercury due to its tolerance for the unavoidable aqueous conditions used for the radio-metal's production and handling.^[40]

The objective of this work was the synthesis of a mercury compound, stable *in vivo*, which has solubility and functionality such that it could be attached to a cancer-targeting carrier for drug development in radiopharmacy.

The ^{nat}Hg-bispidine reference compound **3a** was synthesized under nonaqueous conditions in three main steps (Scheme 1): a 4-fold Mannich reaction, a lithiation followed by stannylation and finally a mercury transmetallation. Formation of **1** in high purity was possible with simple recrystallisation in a yield of 75% but the formation of **2a** proved more troublesome due to many difficult to separate side-products, affording the stannylated compound with a ~90% purity and 9% yield. However, the mercuration step proved high yielding with simple purification.

The reduced bispidine analogue was also prepared as the bispidol **1H** in high yield of 96%. Because its less sterically hindered or more reactive OH group it is better suited as the starting material for further conjugation and then afterward stannylation reactions. But the stannylated compound **2b** itself, before functionalization was carried out on the hydroxyl group, was lower yielding, with lower purity and much more prone to degradation than the butylated analogue **2a**. In apolar solvents the OH...N hydrogen bond causes the chair-boat conformer to be visible in NMR, overlapping with the chair-chair conformer useful in the mercuration reaction.^[41] The shorter reaction path along with the butyl moiety serving as a pseudo-conjugate, with regards to sterics, meant the focus of this study centred on **3a** for the proof of principle. The product **3a** was characterized by HPLC, MS (Figures 1 and 2), and NMR (see SI). Radiolabelling experiments of the stannylated precursor **2a** with ^{197(m)}HgCl₂(aq) were carried out in 1:1 (v/v) water/EtOH with a buffer to ensure a constant pH 6. After initial radio-TLC analyses of the reaction mixture showed a single peak not corresponding to Hg salts, the reaction was checked against a test developed by our lab in which the TLC plate is presoaked with a trithiol – tris(2-



Scheme 1. Cyclic bisarylmercury bispidine synthesis.

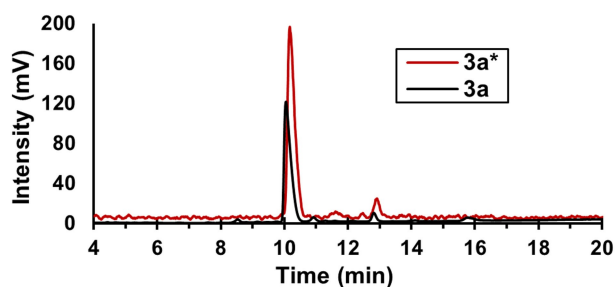


Figure 1. Overlay of radiochromatogram (γ , red) and UV absorption chromatogram (220 nm, black), for co-injection of $3a^*$ (retention time 10.2 min) and $3a$ (retention time 10.1 min).

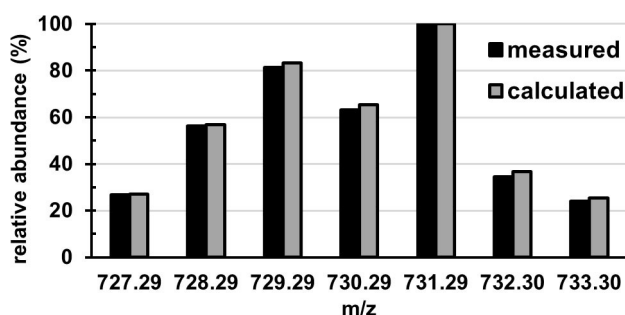


Figure 2. Relative abundance of the calculated and measured $[M+H]^+$ isotopic distribution pattern of substance $3a$ as analysed by mass spectrometry.

mercaptoethyl)ammonium oxalate^[42] – which readily binds to reactive Hg species (*i.e.* Hg^{2+} or RHg^+), fixing them to the baseline.

The results (Figure 3) showed the reaction product to be stable to this test, indicating complete binding of the Hg. The reaction solution was then measured by HPLC, with the gamma ray detector showing a single main peak. Confirmation for the formation of the $^{197(m)}Hg$ -radiolabelled bispidine $3a^*$ was done by co-injection with the non-radioactive analogue $3a$, which showed the same retention time for the UV trace as the gamma

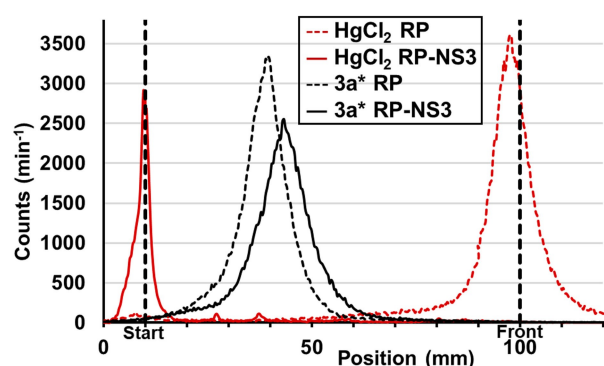


Figure 3. Species analysis of $^{197(m)}Hg$. Overlay of four radio-TLCs: RP–C18 (dotted lines) and trithiol impregnated RP–C18 (solid lines) of $^{197(m)}HgCl_2$ reference and $3a^*$. NS3 = tris(2-mercaptoethyl)ammonium oxalate.

trace of the HPLC (Figure 1). Chemical stability tests were performed on three aliquots of $3a^*$, each left for two days in a vial with an excess of sulfur-containing competitor and then analysed by radio-TLC, as shown in Figure 4.

The results show high chemical stability, as only the sulfide caused any measurable degradation ($\sim 4\%$), as seen at the baseline. The lipophilicity of $3a^*$ was determined, indicating how the compound would interact *in vivo*,^[43] by measuring the distribution coefficient under the physiological pH of blood (pH 7.4) using the “shake flask” method.^[44] The value $\log D_{7.4} = 2.27$ shows an acceptable level of lipophilicity for *in vivo* testing, being below the recommended upper limit of 3, over which the poor solubility can cause adverse effects, but above the lower limit of 1, below which would cause too fast a renal clearance.^[45] For the low amounts of $3a^*$ needed for *in vivo* testing, the water solubility is high enough for the aqueous conditions.

As a preliminary assessment for *in vivo* stability, $3a^*$ was incubated with human serum as per the procedure of Zarschler *et al.*^[46] The results (Figure 5) show that, unlike with $^{197(m)}Hg$ -radiolabelled EDTA used as reference, neither $^{197(m)}Hg$ release (demetallation) nor binding to serum proteins is detectable for $3a^*$. The only radioactive species detected matches with the mass of $3a^*$ highlighting its remarkable stability under these conditions. In contrast, a range of protein bands of different sizes are visible when $^{197(m)}Hg$ -radiolabelled EDTA was incubated with human serum due to substantial decomplexation and transchelation. For example, a prominent protein band in the size range of approximately 65 kDa is detectable in the autoradiographic scans, which corresponds most likely to radio-labelled serum albumin.

To test the actual *in vivo* stability of $3a^*$, a biodistribution was performed in healthy rats. The results (Figure 6) show rapid renal clearance by normal micturition, during a 24 h period, and no indication of demetallation\retention in the kidneys as observed with mercury salts.

These results were confirmed by the SPECT/CT study (Figure 7). No substantial retention or accumulation of activity in the kidneys was observed 24 hours after injection. In contrast to

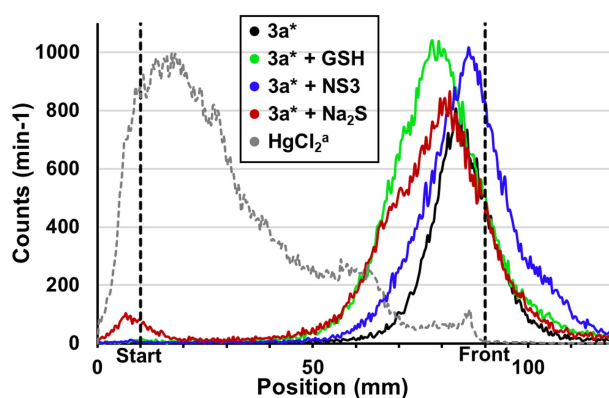


Figure 4. Degradation analysis of $^{197(m)}Hg$ -species. Overlay of five radio-TLCs (iTLC-SG) of $^{197(m)}HgCl_2$ reference, $3a^*$ reference, and $3a^*$ with competitor trials after 2 d at room temperature. ^aIntensity normalized with respect to $3a^*$. GSH = L-glutathione. NS3 = tris(2-mercaptoethyl)ammonium oxalate.

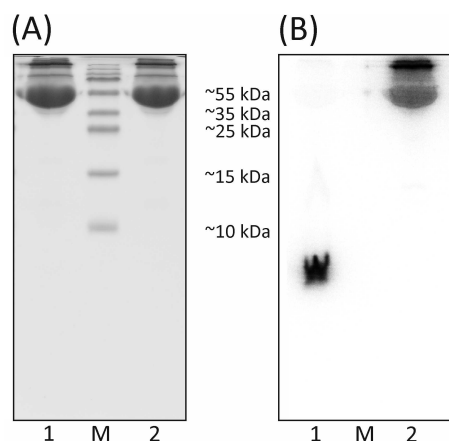


Figure 5. Analysis of $^{197\text{(m)}}$ Hg-incorporation into human serum proteins by SDS-PAGE. 20% SDS-polyacrylamide gel Coomassie blue staining (A), showing bands of human serum proteins, and autoradiograph (B), showing $^{197\text{(m)}}$ Hg-labelled bands. Lane 1: $^{197\text{(m)}}$ Hg-radiolabelled bispidine **3a***. Lane 2: $^{197\text{(m)}}$ Hg-radiolabelled EDTA. M: molecular-weight size marker.

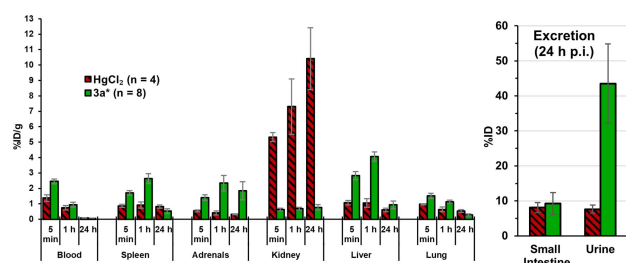


Figure 6. Biodistribution of **3a*** in healthy male Wistar rats ($^{197\text{(m)}}$ HgCl₂: n = 4, **3a***: n = 8). ~300 kBq per animal. All other organs measured well below 1% ID/g.

unbound mercury, however, there is fractional uptake by the liver with subsequent hepatobiliary clearance. **3a** also showed high bench stability, being left in solution for over eight months without degradation (measured by HPLC).

Limitations: Stannylation of the bispidine precursors **1** in order to form precursor **2a**, under milder conditions with Pd-based catalysts were unsuccessful after many attempts. Instead, the harsher conditions for the lithiation/stannylation low-yield route were required. The stannylated compounds **2a/b** are unstable under acidic conditions so purification with silica gel was not possible (even with wetting of a basic solvent system), therefore basic alumina was required. They are also prone to hydrolysis and as such have a poor shelf-life.

The radiolabelling conditions are pH-sensitive as a very low pH would risk hydrolysing the stannyl moieties, whilst too high a pH would form a mixture of different Hg species affecting the uniformity of the reaction conditions.

Future work: The next steps for this work would be testing of carrier attachment at the bispidine backbone, using methods already well established, e.g. amide, carbamate, thiourea, thiol and 'click' coupling.^[47–49] Followed by optimization of reaction conditions (with continued searching for suitable alternative leaving groups to remove the current bottle-neck in the

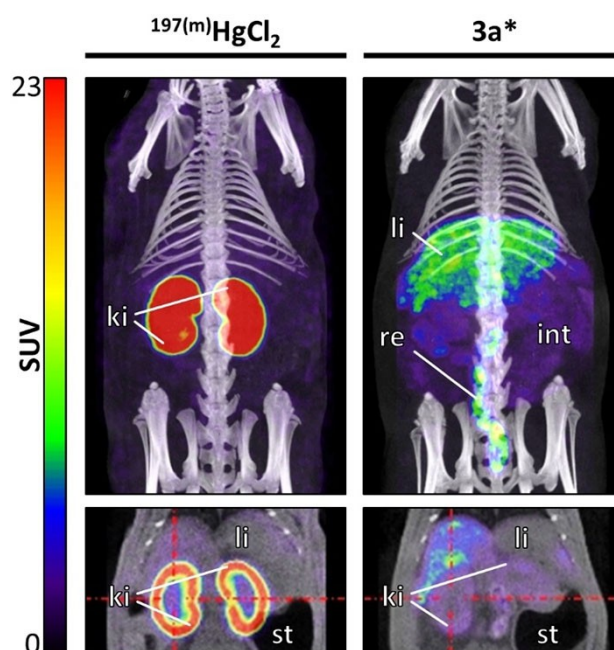


Figure 7. Standardized Uptake Value coloured SPECT/CT images of rats 24 h after injection of $^{197\text{(m)}}$ HgCl₂ (left, retention in kidneys) and **3a*** (right, rapid renal excretion with minor liver uptake followed by hepatobiliary clearance); maximum intensity projections (top) and coronal slice images (bottom); intestine (int), kidneys (ki), liver (li), rectum (re), stomach (st).

synthesis), testing of structural derivatives in search of improved characteristics (e.g. solubility) and investigations into the potential for mercury analytics.

Overall, these results show good promise for the development of a new class of thermodynamically stable organomercury compounds suitable for theranostic applications in radiopharmacy.

Acknowledgements

The authors gratefully acknowledge the excellent technical assistance of Ulrike Gesche for radiolabelling, and that of Regina Herrlich for the animal studies. The authors also thank Karin Landrock for the elemental analyses and the lipophilicity determination, Markus Laube for the HRMS assistance, and Constantin Mamat for the NMR assistance. The irradiations for $^{197\text{(m)}}$ Hg production were carried out at the CANAM infrastructure of the NPI CAS Řež supported through MEYS project no. LM2011019. Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Bispidine · Mercury · Organomercury · Radiopharmaceuticals · Theranostics

- [1] S. C. Srivastava, *Semin. Nucl. Med.* **2012**, *42*, 151–163.
- [2] U. Hennrich, K. Kopka, *Pharmaceuticals* **2019**, *12*, 114.
- [3] S. C. Srivastava, *J. Postgrad. Med.* **2013**, *47*, 31–46.
- [4] A. M. R. Pillai, F. F. (R.) Knapp, *Curr. Radiopharm.* **2015**, *8*, 78–85.
- [5] E. Boros, B. V. Marquez, O. F. Ikotun, S. E. Lapi, C. L. Ferreira, in *Ligand Des. Med. Inorg. Chem.* (Ed.: T. Storr), John Wiley & Sons, Ltd., **2014**, pp. 47–79.
- [6] A. Ku, V. J. Facca, Z. Cai, R. M. Reilly, *EJNMMI Radiopharm. Chem.* **2019**, *4*, 27.
- [7] P. Randhawa, A. P. Olson, S. Chen, K. L. Gower-Fry, C. Hoehr, J. W. Engle, C. F. Ramogida, V. Radchenko, *Curr. Radiopharm.* **2021**, *14*, 1–26.
- [8] Z. Cai, N. Al-saden, C. J. Georgiou, R. M. Reilly, *Int. J. Radiat. Biol.* **2020**, *96*, 1–11.
- [9] O. Lebeda, F. G. Kondev, J. Červenák, *Nucl. Inst. Methods Phys. Res. A* **2020**, *959*, 163481.
- [10] M. Walther, S. Preusche, S. Bartel, G. Wunderlich, R. Freudenberg, J. Steinbach, H.-J. Pietzsch, *Appl. Radiat. Isot.* **2015**, *97*, 177–181.
- [11] R. Freudenberg, R. Apolle, M. Walther, H. Hartmann, J. Kotzerke, *EJNMMI Phys.* **2018**, *5*, 15.
- [12] D. B. Sodee, B. Di Stefano, *Ohio State Med. J.* **1965**, *61*, 819–822.
- [13] M. Sittig, *Pharmaceutical Manufacturing Encyclopedia*, Noyes Publications, Westwood, **1988**.
- [14] J. R. Crout, *Fed. Regist.* **1974**, *39*, 42018–42019.
- [15] D. B. Sodee, *J. Nucl. Med.* **1968**, *9*, 645.
- [16] FDA, *Approved Drug Products with Therapeutic Equivalence Evaluations*, US Department Of Health And Human Service, **1989**.
- [17] M. J. Daly, W. Jones, T. G. Rudd, J. Tremann, *J. Nucl. Med.* **1979**, *20*, 63–66.
- [18] M. M. Staum, *J. Nucl. Med. Technol.* **1992**, *20*, 209–213.
- [19] T. I. Kostelnik, C. Orvig, *Chem. Rev.* **2019**, *119*, 902–956.
- [20] E. J. Anthony, E. M. Bolitho, H. E. Bridgewater, O. W. L. Carter, J. M. Donnelly, C. Imberti, E. C. Lant, F. Lermyte, R. J. Needham, M. Palau, P. J. Sadler, H. Shi, F.-X. Wang, W.-Y. Zhang, Z. Zhang, *Chem. Sci.* **2020**, DOI 10.1039/d0sc04082g.
- [21] S. C. Gad, T. Pham, in *Encycl. Toxicol.* (Ed.: P. Wexler), Academic Press, **2014**, pp. 207–210.
- [22] M. Horvat, in *Encycl. Anal. Sci.* (Eds.: A. Townshend, C. F. Poole, P. J. Worsfold), Academic Press, **2005**, pp. 545–557.
- [23] L. Wang, D. Hou, Y. Cao, Y. S. Ok, F. M. G. Tack, J. Rinklebe, D. O'Connor, *Environ. Int.* **2020**, *134*, 105281.
- [24] P. M. Wax, *J. Med. Toxicol.* **2013**, *9*, 303–307.
- [25] J. A. Dean, *Lange's Handbook of Chemistry*, McGraw-Hill, Inc., **1999**.
- [26] M. Wilhelm, W. Saak, H. Strasdeit, *Zeitschrift fur Naturforsch. - Sect. B J. Chem. Sci.* **2000**, *55 b*, 35–38.
- [27] L. Bytautas, *Croat. Chem. Acta* **2013**, *86*, 453–462.
- [28] P. Comba, M. Kerscher, K. Rück, M. Starke, *Dalton Trans.* **2018**, *47*, 9202–9220.
- [29] P. Comba, C. Haaf, H. Wadepohl, *Inorg. Chem.* **2009**, *48*, 6604–6614.
- [30] H. Cui, R. Goddard, K.-R. Pörschke, A. Hamacher, M. U. Kassack, *Inorg. Chem.* **2014**, *53*, 3371–3384.
- [31] A. M. Nonat, A. Roux, M. Sy, L. J. Charbonnière, *Dalton Trans.* **2019**, *48*, 16476–16492.
- [32] F. Bruchertseifer, P. Comba, B. Martin, A. Morgenstern, J. Notni, M. Starke, H. Wadepohl, *ChemMedChem* **2020**, *15*, 1591–1600.
- [33] Y. Miyahara, K. Goto, T. Inazu, *Tetrahedron Lett.* **2001**, *42*, 3097–3099.
- [34] H. Cui, R. Goddard, K.-R. Pörschke, *Organometallics* **2011**, *30*, 6241–6252.
- [35] A. Gogoll, C. Johansson, A. Axén, H. Grennberg, *Chem. Eur. J.* **2001**, *7*, 396–403.
- [36] J. Spieler, O. Huttenloch, H. Waldmann, *Eur. J. Org. Chem.* **2000**, 391–399.
- [37] L. A. Bulygina, N. S. Khrushcheva, A. S. Peregudov, V. I. Sokolov, *Russ. Chem. Bull.* **2016**, *65*, 2479–2484.
- [38] L. A. Bulygina, N. D. Kagamanov, N. S. Khrushcheva, K. A. Lyssenko, A. S. Peregudov, V. I. Sokolov, *J. Organomet. Chem.* **2017**, *846*, 169–175.
- [39] L. A. Bulygina, N. S. Khrushcheva, K. A. Lyssenko, A. S. Peregudov, *J. Organomet. Chem.* **2019**, *887*, 64–70.
- [40] M. Walther, O. Lebeda, S. Preusche, H.-J. Pietzsch, J. Steinbach, in *AIP Conf. Proc.*, American Institute of Physics, **2017**, p. 020023.
- [41] P. Comba, M. Kerscher, W. Schiek, in *Prog. Inorg. Chem.*, (Ed.: K.D. Karlin), John Wiley & Sons, Inc., **2007**, *55*, 613–704.
- [42] H. Spies, M. Glaser, H.-J. Pietzsch, F. E. Hahn, T. Lügger, *Inorg. Chim. Acta* **1995**, *240*, 465–478.
- [43] T. W. Johnson, K. R. Dress, M. Edwards, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5560–5564.
- [44] A. J. Leo, *Methods Enzymol.* **1991**, *202*, 544–591.
- [45] C. W. Lindsley, in *Encycl. Psychopharmacol.* (Eds.: I. P. Stolerman, L. H. Price), Springer, Berlin, Heidelberg, **2014**.
- [46] K. Zarschler, M. Kubeil, H. Stephan, *RSC Adv.* **2014**, *4*, 10157–10164.
- [47] H. Stephan, M. Walther, S. Fähnemann, P. Ceroni, J. K. Molloy, G. Bergamini, F. Heisig, C. E. Müller, W. Kraus, P. Comba, *Chem. Eur. J.* **2014**, *20*, 17011–17018.
- [48] G. Singh, K. Zarschler, S. Hunoldt, I. I. Santana Martínez, C. L. Ruehl, M. Matterna, R. Bergmann, D. Máthé, N. Hegedüs, M. Bachmann, P. Comba, H. Stephan, *Chem. A Eur. J.* **2020**, *26*, 1989–2001.
- [49] E. W. Price, C. Orvig, *Chem. Soc. Rev.* **2014**, *43*, 260–290.

Manuscript received: February 22, 2021
Revised manuscript received: April 26, 2021
Accepted manuscript online: May 5, 2021
Version of record online: June 16, 2021