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Synthesis of the first radiolabeled ^{188}Re *N*-heterocyclic carbene complex and initial studies on its potential use in radiopharmaceutical applications

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A novel approach towards the synthesis of radiolabeled organometallic rhenium complexes is presented. We successfully synthesized and analyzed the first ^{188}Re-labeled *N*-heterocyclic biscarbene complex, *trans*-dioxobis(1,1′-methylene-*bis*(3,3′-diisopropylimidazolium-2-ylidene))^{188}rhenium(V) hexafluorophosphate (^{188}Re-4) via transmetalation using an air-stable and moisture-stable silver(I) biscarbene complex. In order to assess the viability of this complex as a potential lead structure for *in vivo* applications, the stability of the ^{188}Re-NHC complex was tested in physiologically relevant media. Ultimately, our studies illustrate that the complex we synthesized dissociates rapidly and is therefore unsuitable for use in radiopharmaceuticals. However, it is clear that the transmetalation approach we have developed is a rapid, robust, and mild method for the synthesis of new ^{188}Re-labeled carbene complexes.

Keywords: ^{188}Re carbene complex; radiopharmaceutical application; *N*-heterocyclic carbene; transmetalation

Introduction

For almost half a century, radioactive isotopes of technetium and rhenium have received increasing attention for use in both diagnostic and therapeutic radiopharmaceuticals.[1,2] Technetium has one medically useful isotope, ^{99m}Tc ($t_{1/2}$ = 6.0 h). Rhenium, in contrast, has two isotopes used in nuclear medicine, ^{186}Re ($t_{1/2}$ = 3.72 days), and ^{188}Re ($t_{1/2}$ = 17.0 h), that have potential in both diagnostic and therapeutic applications. The two metals are congeners in Group VIIB, and as such, there are a number of important similarities between their coordination chemistries. Indeed, this similarity in fundamental chemistry has led many to envision the use of the two radiometals as a 'matched pair' for imaging (^{99m}Tc) and therapy ($^{186/188}$Re) using vectors bearing the same ligand scaffold.

However, any list of radiopharmaceuticals used in the clinic quickly reveals that the two radiometals are not utilized with similar frequency: ^{99m}Tc has been widely used in a number of diagnostic single photon emission computed tomography applications, whereas ^{186}Re and ^{188}Re have not been employed with equal success.[1,3] This disparity is in part due to a small but fundamental difference between the chemistry of the two metals. Upon coordination, rhenium is both harder to reduce and easier to oxidize under biologically relevant conditions than technetium. This susceptibility to redox chemistry reduces the stability of rhenium chelate complexes, which—in concert with the low specific activity of ^{186}Re and the successful clinical use of ^{90}Y and ^{177}Lu isotopes[4]—has consequently hampered the development of $^{186/188}$Re-based radiopharmaceuticals.[5]

Despite these difficulties, $^{186/188}$Re remain promising isotopes for nuclear medicine applications. Due to their emission of high-energy β^- particles that can penetrate several millimeters into solid tumors (^{186}Re: 1.069 MeV, 92.5%; ^{188}Re: 2.12 MeV, 100%), the two radiometals have significant potential as radiotherapeutics.[6–11] Moreover, based on the energies of the γ-photons emitted from each isotope—137 and 155 keV for ^{186}Re and ^{188}Re, respectively—both radioisotopes could be identified during radiotherapy by single photon emission computed tomography imaging.

Several ^{186}Re-based or ^{188}Re-based radiopharmaceuticals have been tested in preclinical studies, and some of them have been

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successfully translated to the clinic.[8–11] Specifically, $^{186/188}$Re-hydroxylethylene diphosphonate complexes are currently being used as palliative therapeutics for bone metastases,[11,12] and $^{186/188}$Re-complexes with thiolate-functionalities and amine/amide-functionalities can be used for the treatment of hepatocellular carcinoma (Figure 1(A–C)).[10,13–16] Tetradentate N_3S type complexes (Figure 1(D)) have been especially popular as chelate scaffolds for radiolabeled antibodies.[17,18] In addition, various organometallic rhenium(I)-tricarbonyl complexes have also proven suitable chelation architectures (Figure 1(E)).[19,20] While most targeted approaches with $^{186/188}$Re are based on chelate complexes, the isotopes have also been applied in a colloidal form,[21,22] similar to ^{99m}Tc sulfur colloids.[23–25]

While these successes no doubt represent steps in the right direction, more stable and easier-to-use rhenium chelators must be developed in order for the radiometal to fulfill its promise as a diagnostic and therapeutic radioisotope. Ultimately, the goal is simple: the creation of a $^{186/188}$Re chelator that allows rapid, mild, and cofactor-free attachment of the radiometal to complex biomolecular vectors.

With this aim in mind, we recently became interested in investigating new chelation architectures for ^{188}Re. We have decided to focus on the use of organometallic ^{188}Re-carbon bonds, specifically those seen in *N*-heterocyclic carbene (NHC) complexes of the metal.[26–29] Since the introduction of NHCs for the synthesis of transition metal complexes in the late 1960s,[30] this class of ligands has attracted a significant amount of attention in the field of organometallic catalysis.[31] Indeed, the study of organometallic NHC complexes has uncovered a number of exceptional catalysts,[32] a success which owes quite a bit to the discovery of several synthetic routes towards these ligand systems which increased their diversity and availability.[33–35]

To the best of our knowledge, NHC ligand scaffolds have not yet been applied to the coordination of $^{186/188}$Re, though they have recently been used for the complexation of ^{99}Tc.[36] Therefore, in the work at hand, we aim to present a synthetic approach towards the first NHC complex of generator-produced ^{188}Re and to assess its viability as a radiotracer for clinical use.

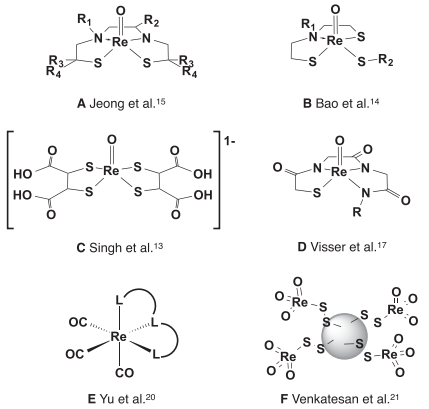

Figure 1. General design of $^{186/188}$Re tracers that already have been used in clinical applications: (A) N2S2-type amine/amidodithiolato ligand on an oxorhenium(V) core.[15] (B) NS3-type combination of amidodithiolate and thiolato ligands on an oxorhenium(V) core.[14] (C) DMSA or DMSA derivatives as stabilizing ligand for oxorhenium(V) cores.[13] (D) Oxorhenium(V) core with an N3S-type or MAG3 ligand,[17] used for coupling to biomacromolecules. (E) Rhenium(I)tricarbonyl core structure with three-coordinate bifunctional ligands, mostly used for coupling biomacromolecules.[20] (F) Schematic drawing of a rhenium/sulfur colloid used, for example, in radio-synovectomies.[21]

Experimental

Unless otherwise indicated, all solvents and reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) or VWR International GmbH (Darmstadt, Germany) and used without further purification. 18.2 MΩ/cm water was obtained from a PURELAB ultra device (ELGA LLC., Woodridge, Il, USA). *N*-Isopropylimidazole, 3,3′-diisopropyl-1,1′-methylenebisimidazolium *bis*(hexafluorophosphate), *bis*-[3,3′-diisopropyl-1,1′-methylene-*bis*-(imidazole-2-ylidene)silver(I)] *bis*(hexafluorophosphate) (**3**), trichloro-oxo-*bis*(triphenylphosphine)rhenium(V), and Dioxobis(1,1′-methylene-*bis*(3,3′-diisopropylimidazolium-2-ylidene))rhenium(V) hexafluorophosphate (**4**) were synthesized similar to the previously published procedures.[29,37–40] Samples of ^{188}Re were obtained from a 10-month old, 10.8 GBq ^{188}W/^{188}Re-generator (Isotope Technologies Garching GmbH, Garching, Germany) by elution using 10 mL of 0.9% sterile sodium chloride solution (Hospira Inc., Lake Forest, Il, USA) at a flow rate of 1 mL/min. The eluate is concentrated by passing over a Waters Sep-Pak Accell Plus QMA column (Milford, MA, USA) and loading of the activity to a 2.5 mL Dionex OnGuard II Ag (Thermo Scientific, Waltham, MA, USA) column. Subsequent elution with 2 mL of 0.9% sterile sodium chloride solution at a flow rate of 1 mL/min yielded the final $Na^{188}ReO_4$ solution (695 Ci/mg).

Electrospray ionization mass spectra were recorded with a Waters Acquity UPLC (Milford, MA, USA) with electrospray ionization SQ detector. High-resolution mass spectra (HRMS) were recorded with a Waters LCT Premier system (ESI). HPLC experiments were performed on a Shimadzu Prominence UFLC system equipped with (i) a flow cell Posi-Ram Model 4 radiodetector from LabLogic (Brandon, FL), Inca DGU-20A degasser, two LC-20AB pumps, a SPD-M20A photodiode array detector (190–600 nm bandwidth) and a SPD-M20A autosampler or (ii) a Flow-Ram Radio HPLC detector from LabLogic (Brandon, FL), Inca DGU-20A degasser, two LC-20AB pumps and a SPD-M20A photodiode array detector. In either case, a reversed phase Phenomenex® Luna 5 μm C18, 100 Å, 250 × 4.6 mm analytical column was used. Radioactivity measurements were performed with a Capintec CRC1243 Dose Calibrator (Capintec, Ramsay, NJ, USA).

N-isopropylimidazole (**1**)

N-isopropylimidazole was synthesized with slight modification according to procedures reported earlier.[37,38] Briefly, a solution of isopropylamine (14.8 g, 250 mmol, 1.00 eq) in methanol (30 mL) was added within 90 min to a cooled (0 °C) slurry of paraformaldehyde (7.89 g, 263 mmol, 1.05 eq) in methanol (30 mL). Subsequent addition of an aqueous glyoxal-solution (40%, 36.3 g, 250 mmol, 1.00 eq) containing ammonium carbonate (12.0 g, 125 mmol, 0.50 eq) and methanol (60 mL) led to an orange solution, which was warmed to ambient temperature and stirred for 16 h. The solution was concentrated to yield a reddish-brown, viscous liquid. The product was obtained by vacuum distillation (2.2×10^{-2} mbar, 55 °C) as a clear, almost colorless liquid (2.60 g, 23.6 mmol, 19%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.55 (s, 1H), 7.04 (t, $^2J_{HH}$ = 1.1 Hz, 1H), 6.95 (d, $^2J_{HH}$ = 1.3 Hz, 1H), 4.34 (hept, $^3J_{HH}$ = 6.7 Hz, 1H), 1.48 (d, $^3J_{HH}$ = 6.7 Hz, 6H). ^{13}C (101 MHz, $CDCl_3$): δ (ppm) = 135.1, 128.9, 116.6, 49.2, 23.7, 22.7. ESI-HRMS (*m/z*): calculated for $C_6H_{11}N_2$ ($[M+H]^+$): 111.0922, found: 111.0917.

1,1′-Methylene-*bis*(3,3′-diisopropylimidazolium) dihexafluorophosphate (**2**)

The title compound was synthesized analog to procedures reported earlier.[39] Briefly, *N*-isopropylimidazol (2.56 g, 23.3 mmol, 0.50 eq), dibromomethane (2.01 g, 11.6 mmol, 1.00 eq), and THF (10 mL) were placed in a pressure tube and heated to 130 °C with stirring for 72 h. After cooling to room temperature, the white solids were filtered off and washed with THF (3 × 30 mL) and diethyl ether (3 × 10 mL). The solids were redissolved in water, and an excess of ammonium hexafluorophosphate was added as a saturated, aqueous solution with stirring. The resulting white slurry was stirred for another 1 h at an ambient temperature before the off-white raw product was filtered off and washed with water (3 × 30 mL). Dissolution in a small amount of

acetonitrile and precipitation with diethyl ether yielded a white solid which was filtered, washed with diethyl ether (3 × 10 mL), and dried under reduced pressure to give 6.10 g (11.6 mmol, 85%) of **2** as white powder. ^{1}H NMR (400 MHz, DMSO-d6): δ (ppm) = 9.66 (br s, 2H), 8.10 (dd, $^{3}J_{HH}$ = 1.9 Hz, $^{4}J_{HH}$ = 1.9 Hz, 2H), 8.04 (dd, $^{3}J_{HH}$ = 1.9 Hz, $^{4}J_{HH}$ = 1.9 Hz, 2H), 6.67 (s, 2H), 4.71 (hept, $^{3}J_{HH}$ = 6.7 Hz, 2H), 1.51 (d, $^{3}J_{HH}$ = 6.7 Hz, 12H). ESI-HRMS (*m/z*): calculated for $C_{13}H_{22}F_6N_4P$ ($[M-PF_6]^+$): 379.1486, found: 379.1486. Elemental analysis: calculated for $C_{13}H_{22}F_{12}N_4P_2$: C 32.16%; H 4.16%; N 11.32%; found: C 31.71%; H 4.16%; N 11.24%.

Di(1,1′-methylene-*bis*(3,3′-diisopropylimidazolium-2-ylidene)) disilver(I) dihexafluorophosphate (3)

The title compound was synthesized similar to procedures described earlier.[29] Silver(I)oxide (6.72 g, 29.0 mmol, 2.50 eq) was added to a solution of bisimidazolium salt **2** (6.10 g, 11.6 mmol, 1.00 eq) in acetonitrile (50 mL). The reaction mixture was stirred for 4 h at an ambient temperature under exclusion of light. After removal of residual Ag_2O by filtration over a pad of Celite®, the clear solution was concentrated to 10 mL. Addition of diethyl ether led to the precipitation of an off-white solid, which was filtered off and washed with diethyl ether (3 × 30 mL). The raw product was redisolved in acetonitrile (10 mL) and again precipitated by addition of diethyl ether. The now white solid was collected by filtration, washed with diethyl ether (3 × 30 mL), and dried under reduced pressure to yield 4.12 g (4.24 mmol, 73%) of **3** as an off-white, flaky powder. ^{1}H NMR (400 MHz, DMSO-d6): δ (ppm) = 7.88 (br s, 4H), 7.73 (d, $^{3}J_{HH}$ = 1.9 Hz, 4H), 6.83 (br s, 2H), 6.40 (s, 2H), 4.68 (hept, $^{3}J_{HH}$ = 6.7 Hz, 4H), 1.43 (d, $^{3}J_{HH}$ = 6.7 Hz, 24H). ^{13}C NMR (101 MHz, DMSO-d6): δ (ppm) = 122.1, 119.8, 63.6, 54.2, 23.1. ESI-mass spectrometry (MS) (+, *m/z*): calculated for $C_{26}H_{40}Ag_2F_6N_8P$ ($[M-PF_6]^+$): 825.11, found: 825.2; ESI-MS (−, *m/z*): calculated for $C_{26}H_{40}Ag_2F_{18}N_8P_3$ ($[M+PF_6]^-$): 1115.04, found: 1115.0. ESI-HRMS (*m/z*): calculated for $C_{26}H_{40}Ag_2F_6N_8P$ ($[M\text{-}PF_6]^+$): 823.1120, found: 823.1142. Elemental analysis: calculated for $C_{26}H_{40}Ag_2F_{12}N_8P_2$: C 29.78%; H 4.23%; N 10.69%; found: C 29.61%H 4.39%; N 10.31%.

Dioxobis(1,1′-methylene-*bis*(3,3′-diisopropylimidazolium-2-ylidene))rhenium(V), $[Re(O)_2L_2]^+$ (4)

The title compound was synthesized with slight modification according to a previously published procedure[29]: $ReOCl_3(PPh_3)_2$ (21.4 mg, 25.7 μmol, 1.00 eq) and the silver carbene **3** (25.1 mg, 25.9 μmol, 1.00 eq.) were placed in a round bottomed flask. After addition of acetonitrile (5 mL), the reaction was stirred at room temperature for 2 h before $AgPF_6$ (6.5 mg, 25.8 μmol, 1.00 eq) was added and the brownish suspension was stirred for another 30 min at room temperature. The crude mixture was filtered to remove AgCl, diluted with toluene (5 mL), concentrated under reduced pressure to a total volume of approximately 4 mL, and again diluted with toluene (5 mL). Upon final concentration to a total volume of 4 mL, the title compound crashed out of the reaction mixture and was subsequently isolated by filtration. Washing with small portions of diethyl ether and drying under reduced pressure gave **4** as brownish solid (10.1 mg, 10.2 μmol, 40%). ^{1}H NMR (400 MHz, CD_3CN): δ (ppm) = 7.73 (d, $^{3}J_{HH}$ = 2.0 Hz, 4H), 7.50 (d, $^{3}J_{HH}$ = 2.0 Hz, 4H), 6.85 (d, $^{2}J_{HH}$ = 13.6 Hz, 2H), 6.38 (d, $^{2}J_{HH}$ = 13.6 Hz, 2H), 4.42 (br s, 4H), 1.54 (d, $^{3}J_{HH}$ = 6.6 Hz, 12H), 1.01 (d, $^{3}J_{HH}$ = 6.4 Hz, 12H). ^{13}C NMR (100 MHz, CD_3CN): δ (ppm) = 168.6, 125.0, 120.0, 65.7, 54.2, 25.2. ESI-HRMS (*m/z*): calculated for $C_{26}H_{41}F_{12}N_8O_2P_2Re$ ($[M+H]^+$): 684.2910, found: 684.2883.

Trichloro-oxo-*bis*(triphenylphosphine)rhenium(V), $^{188}ReOCl_3(PPh_3)_2$ (^{188}Re-5)

The compound was synthesized according to a previously published method.[40] An aliquot of 0.1 mL of the $^{188}ReO_4^-$ eluate (511 ± 27.5 μCi, $2.68 \times 10^{-6} \pm 1.44 \times 10^{-7}$ μmol, 695 Ci/mg, n = 10) was added to a mixture of PPh_3 (3.33 mg, 12.7 μmol) in HCl (0.3 mL, 37%) to yield a 9 M HCl solution. After addition of DCM (0.3 mL), the biphasic mixture was agitated for 5 min at an ambient temperature. Removal of the aqueous phase yielded the product $^{188}ReOCl_3(PPh_3)_2$ as a solution in dichloromethane (422 ± 28 μCi, $2.21 \times 10^{-6} \pm 1.48 \times 10^{-7}$ μmol, 82.7 ± 4.3%, 229 Ci/mg, *n* = 10).

Dioxobis(1,1′-methylene-*bis*(3,3′-diisopropylimidazolium-2-ylidene))rhenium(V), $[^{188}Re(O)_2L_2]^+$ (^{188}Re-4)

An aliquot of 0.1 mL of freshly prepared $^{188}ReOCl_3(PPh_3)_2$ (196 ± 14.3 μCi, $1.03 \times 10^{-6} \pm 7.50 \times 10^{-8}$ μmol, *n* = 6) was added to a freshly prepared suspension of $ReOCl_3(PPh_3)_2$ (1.25 mg, 1.50 μmol) and the silver carbene **3** (1.45 mg, 1.5 μmol) in acetonitrile (0.5 mL). The mixture was vigorously shaken for 2 h at ambient temperature, followed by filtration of precipitates. The crude mixture (146 ± 13.8 μCi, 75 ± 1.7%, RCP 92 ± 4.5%, specific activity 146 ± 14.0 μCi/mg, *n* = 6) was purified via HPLC, yielding the analytically pure title compound, containing approximately 40 μCi/mL $[^{188}Re(O)_2L_2]^+$.

Complex stability determinations

Stability testing of **^{188}Re-4** was carried out in water at different pH values (pH 5, pH 6, pH 7, and pH 8), in PBS at different pH values (pH 5, pH 6, and pH 7), and in fetal bovine serum (FBS) for incubation times of up to 4 h. Samples were prepared for each time point and each substrate by adding 50 μL of a freshly prepared solution of $[^{188}Re(O)_2L_2]^+$ (~40 μCi/mL) to 500 μL of the appropriate medium (H_2O, PBS, or FBS). Samples were incubated at 37 °C whilst agitated for 1 h, 2 h, 3 h, and 4 h. After incubation, 200 μL of each sample solution were subjected to analysis using an HPLC coupled with a Posi-Ram radiodetector for the determination of **^{188}Re-4** stability.

Variation of carrier concentration

The influence of cold carrier on the radiochemical yield of the transmetalation reaction was tested adding different amounts of cold $ReOCl_3(PPh_3)_2$ to the reaction solution. The reactions were performed following the synthetic protocol of $[^{188}Re(O)_2L_2]^+$ **^{188}Re-4**, with varying amounts of cold rhenium. Carrier concentrations of 0.0 μmol (0.00 mg), 0.5 μmol (0.42 mg), 1.0 μmol (0.83 mg), 1.5 μmol (1.25 mg), and 2.0 μmol (1.67 mg) of cold $ReOCl_3(PPh_3)_2$ were added. Simultaneously, the amounts of silver carbene **3** were adjusted to maintain molar ratios. In case of the carrier-free reaction, 0.5 μmol (0.48 mg) of **3** was used. Yields were determined by HPLC analysis of the reaction mixtures, collection of the fractions, and determination of their activities.

Results and discussion

A particularly promising family of structures in the pursuit of stable ^{188}Re-NHC bioconjugates is found in *trans*-dioxorhenium NHC complexes. Both their pseudo-octahedral coordination geometry—which provides steric shielding of the metal center—and the closed-shell electronic structure of the dioxorhenium(V) metal center account for the high stability of these complexes towards moisture, thermal stress, nucleophilic attack, and oxidation.[26–28,41] In addition, the accessibility of these complexes via transmetalation from stable silver(I) carbene complexes[29] abrogates the need for the *in situ* generation of free carbenes, which is often associated with the addition of cofactors and harsh reaction conditions.[33,34]

The synthesis of the *trans*-dioxorhenium biscarbene complex **4**—reported by Hor and coworkers in 2013—served as the starting point for the development of a route towards a ^{188}Re-labeled biscarbene complex.[29] Due to the relative inertness of *trans*-dioxorhenium carbene complexes towards air and moisture, the cold rhenium carbene complex **4** was synthesized under aerobic atmosphere.[26,27] It was generated from the commercially available complex $ReOCl_3(PPh_3)_2$ in the presence of the silver carbene **3** (Figure 2(A)). The complex is stable at room temperature in a mixture of acetonitrile and water, and no decomposition was observed up to 24 hours. Complex **4** was

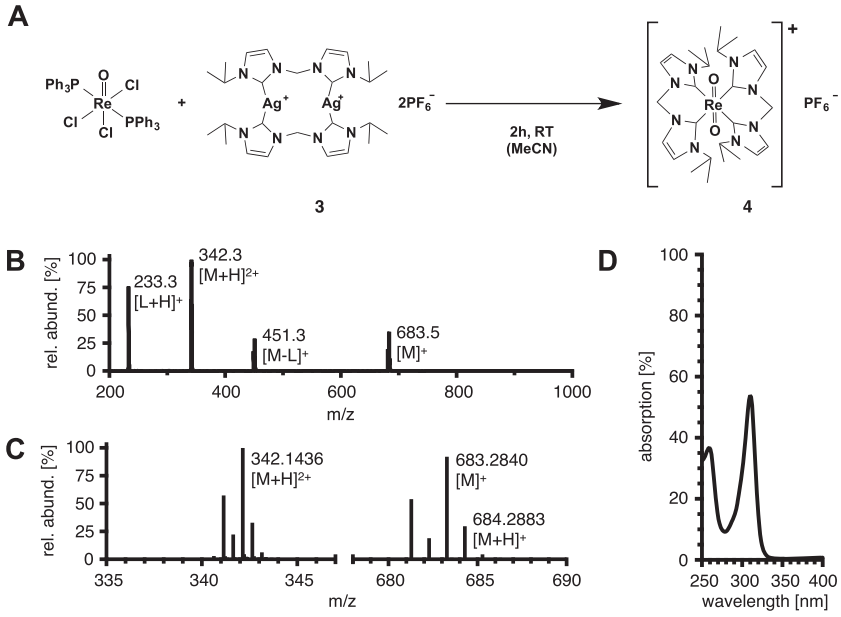

Figure 2. Synthesis and identification of cold dioxobis(1,1′-methylene-*bis*(3,3′-diisopropylimidazolium-2-ylidene))rhenium(V) hexafluorophosphate, **4**. (A) Reaction scheme for the transmetalation reaction to yield compound **4**. (B) Mass spectrometry of compound **4.** (C) High-resolution mass spectrometry, displaying the exact mass and the isotopic pattern of **4** and its protonated, dicationic form. (D) UV-spectrum of compound **4** with a strong absorption band at 310 nm.

identified via ^{1}H and ^{13}C-NMR as well as via MS and HRMS, confirming the elemental composition described earlier (Figure 2(B,C)).[29] The absorbance of the complex at 310 nm was attributed to a metal-to-ligand charge transfer band which has been previously identified for other octahedral *trans*-dioxorhenium compounds.[42]

Importantly, in stark contrast to the synthesis of the cold complex **4**, the ^{188}Re-labeled counterpart has to be synthesized starting from perrhenate ($^{188}ReO_4^-$), the ^{188}Re species produced by ^{188}W/^{188}Re-generators. In order to increase yields and decrease preparation time, we sought to synthesize the ^{188}Re carbene species in a procedure without intermediate work-up (Figure 3(A)). First, an aliquot of the ^{188}W/^{188}Re-generator eluent was obtained containing 511 ± 27.5 μCi ($2.68 \times 10^{-6} \pm 1.44 \times 10^{-7}$ μmol, 695 Ci/mg) of $^{188}ReO_4^-$ in sterile 0.9% saline solution. This eluent was mixed under ambient conditions with an excess of triphenylphosphine in concentrated hydrochloric acid and dichloromethane to give the intermediate product $^{188}ReOCl_3(PPh_3)_2$, **^{188}Re-5**, in 83 ± 4% yield with a reaction time of only 5 min.[40] In the second step, a solution of silver(I) carbene **3** in acetonitrile was added to the dichloromethane extract containing 378 ± 23.8 μCi **^{188}Re-5** ($1.99 \times 10^{-6} \pm 1.25 \times 10^{-7}$ μmol, 229 Ci/mg). The reaction mixture was then agitated for 2 h at room temperature.[29] Initial experiments showed that this procedure yields **^{188}Re-4** in high specific activity (278 Ci/mg). After HPLC purification of **^{188}Re-4** using acetonitrile and water as eluent, the title compound was obtained in 6.7 ± 7.8% radiochemical yield and >98% radiochemical purity.

Based on these somewhat meager radiochemical yields, it was reasoned that the tracer amounts of $^{188}ReOCl_3(PPh_3)_2$ in combination with impurities or side reactions could ultimately reduce the amount of $^{188}ReOCl_3(PPh_3)_2$ available for transmetalation and consequently reduce the final yields. This hypothesis was further supported by experiments in which

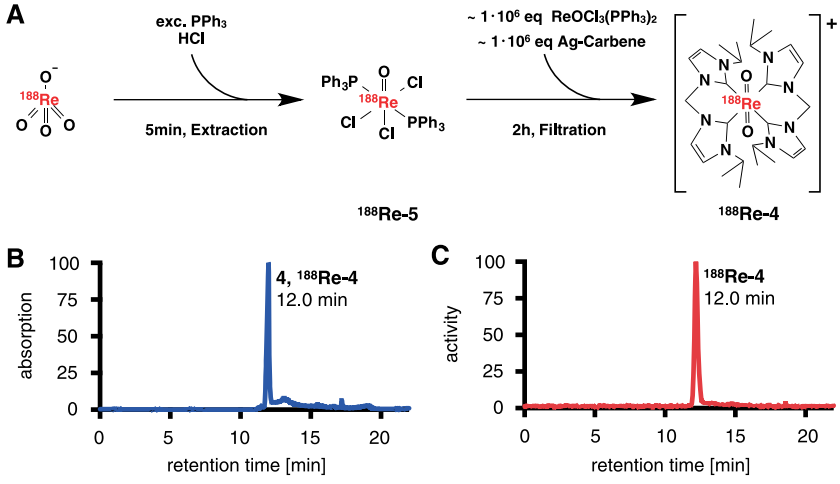

Figure 3. Synthesis of *trans*-dioxobis(1,1′-methylene-*bis*(3,3′-diisopropylimidazolium-2-ylidene))^{188}rhenium(V) hexafluorophosphate, **^{188}Re-4**. (A) Reaction scheme of the synthesis of compound **^{188}Re-4**. (B) UV and (C) radio HPLC signal of a purified sample of compound **^{188}Re-4**.

cold carrier $ReOCl_3(PPh_3)_2$ was added to the reaction mixture. In these cases, the yield of the transmetalation reaction forming **^{188}Re-4** was significantly increased, culminating in 62 ± 5% radiochemical yield with 1.0 μmol of added cold carrier (Figure 4(A)). After HPLC purification of the crude reaction mixtures, we obtained analytically pure solutions of **^{188}Re-4** with specific activities up to 386.4 ± 1.5 μCi/mg and a radiochemical purity >98% (Figure 3(B,C)). Interestingly, amounts of cold $ReOCl_3(PPh_3)_2$ carrier greater than 1.0 μmol did not lead to a further increase in yield but rather simply resulted in a reduction of specific activity (208.5 ± 2.5 μCi/mg and 191.5 ± 5.0 μCi/mg for 1.5 μmol and 2.0 μmol of cold carrier, respectively; Figure 4(B)).

Complex **4** is stable to air and moisture, one of the fundamental prerequisites for a successful chelation scaffold. However, in order to explore the utility of its radiolabeled counterpart, **^{188}Re-4** was incubated in water at different pH levels between 5.0 and 8.0 at 37 °C (Figure 4(C)). The HPLC analysis of **^{188}Re-4** in aqueous solutions over a period of 4 h revealed that approximately 25% of the rhenium complex decomposes within the first hour regardless of the pH value. After that point, decomposition is significantly slowed for the samples at pH 5.0, while for the samples at pH 6.0 and above, less than 50% of the initial amount of **^{188}Re-4** remained intact after four hours at 37 °C (Figure 4(C)). While decreasing amounts of **^{188}Re-4** were observed in these samples (t_R = 12.2 min, Figure 5(A)), the concomitant increase in any other radioactive peaks was not witnessed in the HPLC chromatogram. This strongly suggests that reoxidation to free $^{188}ReO_4^-$ is not the major decomposition pathway, as free $^{188}ReO_4^-$ would appear in the chromatogram at t_R = 7.5 min. Rather, it was observed that increasing amounts of activity were trapped on the HPLC column after decomposition of **^{188}Re-4**. However, when compared to the recently published, similar ^{99}Tc complex, the difference in stability against aqueous media is considerable.[36] Apart from the different metal center, the observed lower stability of **^{188}Re-4** may be attributed to the bulkiness of the isopropyl substituents and the terminal oxo-ligands, facilitating the de-coordination of the ligand.

In the next step, the stability of **^{188}Re-4** in solutions of phosphate buffered saline (PBS; 12 mM PO_4^{3-}, 139.7 mM Cl^-) was investigated.

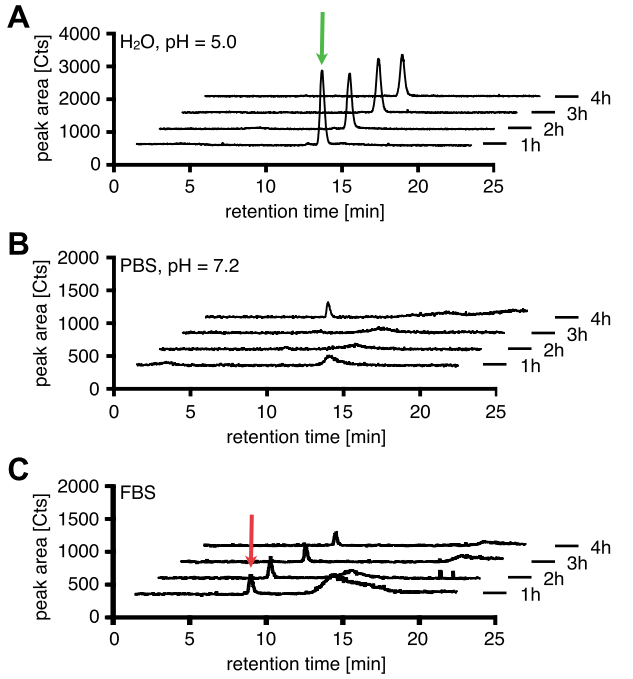

Figure 5. Stability of **^{188}Re-4**. Radio-HPLC of **^{188}Re-4** in H2O (A), PBS (B), and FBS (C) after incubation at 37 °C for 1, 2, 3, and 4 h, respectively. Green arrow: **^{188}Re-4;** Red arrow: $^{188}ReO4^-$.

In PBS solutions at three different pH values—pH 5.0, 6.0, and 7.0—the decomposition of **^{188}Re-4** was found to be rapid, with almost none of the radiolabeled complex remaining intact after 2 hours at 37 °C (Figure 5(B)). This finding may be attributed to the fact that the ionic strengths of the PBS solutions are significantly higher than those of non-buffered water of the same pH value (overall, 10^4–10^6-fold increase in ion concentration). It can be assumed that the anions, especially the chloride ions, are able to compete with and displace the carbenes at the metal center. In this case, the resultant free carbenes would immediately form imidazolium salts, which would be unable to bind to the rhenium once again.[26]

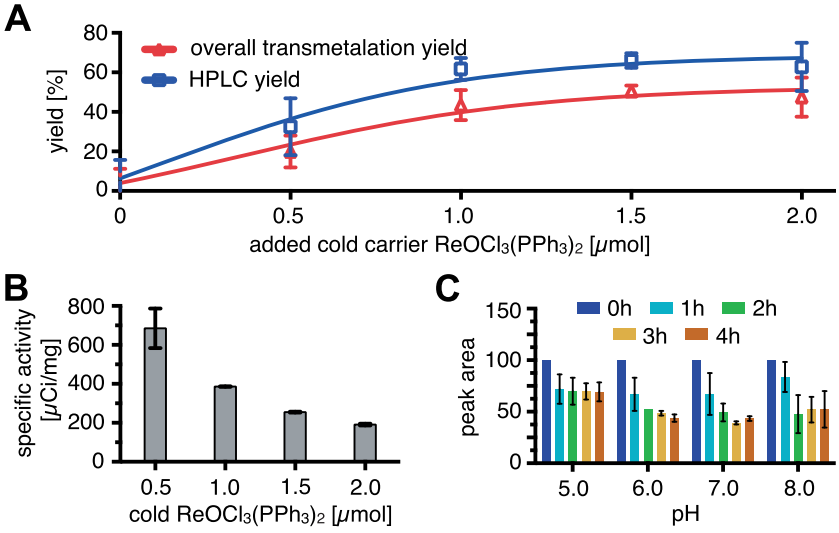

Figure 4. Yields, specific activities, and stability of **^{188}Re-4** in aqueous solution. (A) Effect of added cold carrier $ReOCl_3(PPh_3)_2$ on the yield of compound **^{188}Re-4**. (B) Specific activity of **^{188}Re-4** at different amounts of added cold carrier. (C) Stability of **^{188}Re-4** in H2O at different pH values over time. Peak areas are normalized to the initial amount of **^{188}Re-4**.

Finally, the stability of **^{188}Re-4** was investigated in fetale bovine serum, a medium that provides a reasonable facsimile of the *in vivo* environment. Perhaps not surprisingly, the stability of **^{188}Re-4** in FBS was similar to that in PBS. Here, almost complete decomposition of the rhenium carbene complex was observed within the first hour of incubation (Figure 5(C)). In addition to potentially coordinating anions, FBS contains a variety of biomacromolecules, which may undergo nucleophilic reactions with the metal center resulting in the decomposition of the complex. In contrast to PBS, broad radioactive peaks were detected when **^{188}Re-4** was dissolved in FBS. These might indicate the formation of adducts of ^{188}Re metal centers with biomolecules, ultimately resulting in the generation of ^{188}Re-perrhenate (as observed via HPLC; Figure 5(C)).

Conclusion

The principal aims of this study were to develop a methodology for the synthesis of radioactive, organometallic NHC complexes of rhenium (**^{188}Re-4**) and to assess the viability of NHCs as chelation architectures for $^{186/188}$Re-labeled radiopharmaceuticals. Unfortunately, as we have already discussed, the stability of **^{188}Re-4** under physiological conditions is too low for the complex to be of any meaningful use as a component of a radiopharmaceutical. Importantly, however, the synthetic method developed to make **^{188}Re-4** is a facile and straightforward approach for the synthesis of both carrier-free and carrier-added ^{188}Re-NHC complexes. Ultimately, carbenes can be envisioned to be novel and viable ligands for the selective and stable chelation of both ^{186}Re and ^{188}Re, and this methodological work appears to be an important first step toward this goal.

Naturally, more work has to be invested in the development of carbene ligands that confer greater stability under physiological environments. Particularly important will be the development of ligands that stabilize the dioxorhenium(V) core, preventing both reduction and oxidation under physiological conditions. One potential route towards these ligand systems could be the use of porphyrin-like carbene systems similar to those published by Alcalde *et al.*,[43] Hahn *et al.*,[44] and McKie *et al.*[45] These polydentate carbene ligands may exhibit greater kinetic and thermodynamic stability with the metal and, furthermore, limit the accessibility of the metal center to solvent molecules, thereby reducing the likelihood of displacement of the ligands and the oxidation of the metal center. Overall, it is our hope that the pursuit of novel ligands for rhenium will result in the development of stable and functionalizable $^{186/188}$Re chelators, which will in turn broaden the scope and applicability of these radioisotopes in the clinic.

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Conflict of Interest

The authors did not report any conflict of interest.

References

[1] R. K. Hom, J. A. Katzenellenbogen, *Nucl. Med. Biol.* **1997**, *24*, 485–498.
[2] S. Jürgens, W. A. Herrmann, F. E. Kühn, *J. Organomet. Chem.* **2014**, *751*, 83–89.
[3] a) J. R. Dilworth, S. J. Parrott, *Chem. Soc. Rev.* **1998**, *27*, 43–55; b) W. A. Volkert, T. J. Hoffman, *Chem. Rev.* **1999**, *99*, 2269–2292.
[4] a) K. A. David, M. I. Milowsky, L. Kostakoglu, S. Vallabhajosula, S. J. Goldsmith, D. M. Nanus, N. H. Bander, *Clin. Genitourin. Cancer* **2006**, *4*, 249–256; b) E. I. van Vliet, J. J. M. Teunissen, B. L. R. Kam, M. de Jong, E. P. Krenning, D. J. Kwekkeboom, *Neuroendocrinology* **2013**, *97*, 74–85.
[5] E. Deutsch, K. F. Libson, J.-L. Vanderheyden, A. R. Ketring, H. R. Maxon, *Nucl. Med. Biol.* **1986**, *13*, 465–477.
[6] W.-Y. Lin, C.-P. Lin, S.-J. Yeh, B.-T. Hsieh, Z.-T. Tsai, G. Ting, T.-C. Yen, S.-J. Wang, F. Knapp, M. Stabin, *Eur. J. Nucl. Med.* **1997**, *24*, 590–595.
[7] a) A. R. Ketring, *Nucl. Med. Biol.* **1987**, *14*, 223–232. b) P. G. Abrams, A. R. Fritzberg, Radioimmunotherapy of Cancer, Marcel Dekker, New York, **2000**.
[8] a) E. Torres-Garcia, G. Ferro-Flores, C. Arteaga de Murphy, L. Correá-Gonzales, P. A. Pichardo-Romero, *Arch. Med. Res.* **2008**, *39*, 100–109; b) L. Torres, M. Coca, J. Batista, A. Casaco, G. Lopez, I. Garcia, A. Perera, Y. Pena, A. Hernandez, Y. Sanchez, S. Romero, R. Leyva, A. Prats, R. Fernandez, *Nucl. Med. Commun.* **2008**, *29*, 66–75.
[9] K. C. Shin, J. C. Lee, H. J. Choi, J. M. Jeong, M. Son, Y. J. Lee, E. B. Lee, S. H. Hong, Y. W. Song, *Nucl. Med. Commun.* **2007**, *28*, 239–244.
[10] P. Bernal, J.-L. Raoul, G. Vidmar, E. Sereegotov, F. X. Sundram, A. Kumar, J. M. Jeong, P. Pusuwan, C. Divgi, P. Zanzonico, J. Stare, J. Buscombe, C. T. T. Minh, M. M. Saw, S. Chen, R. Ogbac, A. K. Padhy, *Int. J. Radiat. Oncol.* **2007**, *69*, 1448–1455.
[11] K. Liepe, R. Hliscs, J. Kropp, T. Grüning, R. Runge, R. Koch, F. F. Knapp, W.-G. Franke, *Canc. Biother. Rad.* **2000**, *15*, 261–265.
[12] a) M. Eisenhut, *Int. J. Appl. Radiat. Isot.* **1982**, *33*, 99–103; b) H. R. Maxon, E. A. Deutsch, S. R. Thomas, K. F. Libson, S. J. Lukes, C. C. Williams, S. Ali, *Radiology* **1988**, *166*, 501–507.
[13] J. Singh, K. Reghebi, C. R. Lazarus, S. E. M. Clarke, A. P. Callahan, F. F. Knapp, P. J. Blower, *Nucl. Med. Commun.* **1993**, *14*, 197–203.
[14] A. Bao, B. Goins, R. Klipper, G. Negrete, W. T. Phillips, *J. Nucl. Med.* **2003**, *44*, 1992–1999.
[15] J. M. Jeong, Y. J. Kim, Y. S. Lee, J. I. Ko, M. Son, D. S. Lee, J.-K. Chung, J. H. Park, M. C. Lee, *Nucl. Med. Biol.* **2001**, *28*, 197–204.
[16] S. Seifert, T. Heinrich, C. Jentschel, C. Smuda, R. Bergmann, H.-J. Pietzsch, *Bioconjugate Chem.* **2006**, *17*, 1601–1606.
[17] G. W. M. Visser, M. Gerretsen, J. D. M. Herscheid, G. B. Snow, G. van Dongen, *J. Nucl. Med.* **1993**, *34*, 1953–1963.
[18] a) S. Guhlke, A. Schaffland, P. O. Zamora, J. Sartor, D. Diekmann, H. Bender, F. F. Knapp, H. J. Biersack, *Nucl. Med. Biol.* **1998**, *25*, 621–631; b) K. Ogawa, T. Mukai, Y. Arano, M. Ono, H. Hanaoka, S. Ishino, K. Hashimoto, H. Nishimura, H. Saji, *Bioconjugate Chem.* **2005**, *16*, 751–757.
[19] a) K.-T. Chen, T.-W. Lee, J.-M. Lo, *Nucl. Med. Biol.* **2009**, *36*, 355–361; b) K. Ogawa, H. Kawashima, S. Kinuya, K. Shiba, M. Onoguchi, H. Kimura, K. Hashimoto, A. Odani, H. Saji, *Ann. Nucl. Med.* **2009**, *23*, 843–848; c) D. Satpati, A. Korde, K. Kothari, H. D. Sarma, M. Venkatesh, S. Banerjee, *Canc. Biother. Rad.* **2008**, *23*, 741–748; d) C. A. Kluba, T. L. Mindt, *Molecules* **2013**, *18*, 3206–3226.
[20] J. Yu, U. O. Häfeli, J. Xia, S. Li, M. Dong, D. Yin, X. Wang, *Nucl. Med. Commun.* **2005**, *26*, 453–458.
[21] P. P. Venkatesan, S. Shortkroff, M. R. Zalutsky, C. B. Sledge, *Nucl. Med. Biol.* **1990**, *17*, 357–362.
[22] a) S.-J. Wang, W.-Y. Lin, B.-T. Hsieh, L.-H. Shen, Z.-T. Tsai, G. Tinge, F. Knapp, *Eur. J. Nucl. Med.* **1995**, *22*, 505–507; b) J. M. Jeong, Y. J. Lee, Y. J. Kim, Y. S. Chang, D. S. Lee, J.-K. Chung, Y. W. Song, M. C. Lee, *Appl. Radiat. Isot.* **2000**, *52*, 851–855.
[23] A. Garcia-Burillo, I. Roca Bielsa, O. Gonzalez, C. Zafon, M. Sabate, J. Castellvi, X. Serres, C. Iglesias, R. Vilallonga, E. Caubet, J. Fort, J. Mesa, M. Armengol, J. Castell-Conesa, *Eur. J. Nucl. Med. Mol. Imaging* **2013**, *40*, 1645–1655.
[24] U. Veronesi, G. Paganelli, G. Viale, V. Galimberti, A. Luini, S. Zurrida, C. Robertson, V. Sacchini, P. Veronesi, E. Orvieto, C. De Concetta, M. Intra, G. Tosi, D. Scarpa, *J. Natl. Cancer Inst.* **1999**, *91*, 368–373.

[25] W. Hauser, H. L. Atkins, P. Richards, *Radiology* **1969**, *92*, 1369–1371.
[26] H. Braband, T. I. Zahn, U. Abram, *Inorg. Chem.* **2003**, *42*, 6160–6162.
[27] T. I. Kückmann, U. Abram, *Inorg. Chem.* **2004**, *43*, 7068–7074.
[28] S. J. Hock, L.-A. Schaper, W. A. Herrmann, F. E. Kühn, *Chem. Soc. Rev.* **2013**, *42*, 5073.
[29] R. Lum, H. Zhang, W. Zhang, S.-Q. Bai, J. Zhao, T. S. A. Hor, *Dalton Trans.* **2013**, *42*, 871.
[30] a) K. Öfele, *J. Organomet. Chem.* **1968**, *12*, P42–P43; b) H. W. Wanzlick, H. J. Schönherr, *Angew. Chem. Int. Ed.* **1968**, *7*, 141–142.
[31] a) W. A. Herrmann, *Angew. Chem. Int. Ed.* **2002**, *41*, 1290–1309; b) D. Bourissou, O. Guerret, F. P. Gabbaï, G. Bertrand, *Chem. Rev.* **2013**, *100*, 39–92.
[32] a) M. Scholl, S. Ding, C. W. Lee, R. H. Grubbs, *Org. Lett.* **1999**, *1*, 953–956; b) T. Weskamp, F. J. Kohl, W. Hieringer, D. Gleich, W. A. Herrmann, *Angew. Chem. Int. Ed.* **1999**, *38*, 2416–2419.
[33] A. J. Arduengo, R. L. Harlow, M. Kline, *J. Am. Chem. Soc.* **1991**, *113*, 361–363.
[34] T. Weskamp, V. P. W. Böhm, W. A. Herrmann, *J. Organomet. Chem.* **2000**, *600*, 12–22.
[35] H. M. J. Wang, I. J. B. Lin, *Organometallics* **1998**, *17*, 972–975.
[36] M. Benz, B. Spingler, R. Alberto, H. Braband, *J. Am. Chem. Soc.* **2013**, *135*, 17566–17572.
[37] E. Mas-Marzá, E. Peris, I. Castro-Rodríguez, K. Meyer, *Organometallics* **2005**, *24*, 3158–3162.
[38] T. Strassner, Y. Unger, A. Zeller, WO2008000726 A1, **2008**.
[39] T. Scherg, S. K. Schneider, G. D. Frey, J. Schwarz, E. Herdtweck, W. A. Herrmann, *Synlett* **2006**, 2894–2907.
[40] E. C. Lisic, S. Mirzadeh, F. F. Knapp, *J. Labelled Comp. Rad.* **1993**, *33*, 65–75.
[41] a) H. Braband, E. Oehlke, U. Abram, *Z. anorg. allg. Chem.* **2006**, *632*, 1051–1056; b) D. M. P. Mingos, *J. Organomet. Chem.* **1979**, *179*, C29–C33; c) I. Demachy, Y. Jean, *Inorg. Chem.* **1996**, *35*, 5027–5031.
[42] a) C. S. Johnson, C. Mottley, J. T. Hupp, *Inorg. Chem.* **1992**, *31*, 5143–5145; b) M. S. Ram, L. M. Skeens-Jones, C. S. Johnson, X. L. Zhang, C. Stern, D. I. Yoon, D. Selmarten, J. T. Hupp, *J. Am. Chem. Soc.* **1995**, *117*, 1411–1421; c) J. H. Beard, J. Casey, R. K. Murmann, *Inorg. Chem.* **1965**, *4*, 797–803; d) D. W. Pipes, T. J. Meyer, *Inorg. Chem.* **1986**, *25*, 3256–3262.
[43] E. Alcalde, N. Mesquida, L. Perez-Garcia, C. Alvarez-Rua, S. Garcia-Granda, E. Garcia-Rodriguez, *Chem. Commun.* **1999**, 295–296.
[44] F. E. Hahn, V. Langenhahn, T. Lügger, T. Pape, D. Le Van, *Angew. Chem. Int. Ed.* **2005**, *44*, 3759–3763.
[45] R. McKie, J. A. Murphy, S. R. Park, M. D. Spicer, S.-Z. Zhou, *Angew. Chem. Int. Ed.* **2007**, *46*, 6525–6528.

