Inorg. Chem. **2008**, *47*, 8213−8221

Expedient Multi-Step Synthesis of Organometallic Complexes of Tc and Re in High Effective Specific Activity. A New Platform for the Production of Molecular Imaging and Therapy Agents

Patrick W. Causey,[†] Travis R. Besanger,[‡] Paul Schaffer,[†] and John F. Valliant*[,‡,§]

McMaster Nuclear Reactor, Department of Chemistry, and McMaster Institute of Applied Radiation Sciences (McIARS), McMaster University, Hamilton, Canada, L8S 4M1

Received April 29, 2008

For over thirty years, instant labeling kits which involve no purification steps have been the only method used to prepare ^{99m}Tc radiopharmaceuticals for clinical studies. To address the limitations imposed by instant kits, which is hindering the development of molecularly targeted Tc- and Re-based imaging and therapy agents, a new strategy for the rapid multistep synthesis and purification of organometallic technetium-based molecular probes and corresponding rhenium-based therapeutic analogues was developed. Beginning with MO_4^- (M = ^{99m}Tc, $^{186/188}$Re), the carbonyl precursor $[M(CO)_3(H_2O)_3]^+$ was synthesized in 3 min in quantitative yield in a microwave reactor. A dipicolyl ligand was added and the chelate complex was formed in high yield in 2 min using microwave heating at 150 °C. This was followed by a new purification strategy to remove unlabeled ligand which entailed using a copper resin/C18 solid phase extraction protocol giving the desired product in greater than 78% decay corrected yield (dcy). Conversion to the corresponding succinimidyl active ester was achieved following a 5 min microwave irradiation at 120 °C and C18 solid phase extraction purification in 60% dcy. A series of amides were prepared subsequently by microwave heating at 120 °C for 5 min producing the desired targets in greater than 86% dcy. The reported method represents a move away from traditional instant kits toward more versatile platform synthesis and purification technologies that are better suited for producing modern molecular imaging and therapy agents.

Introduction

There have been significant advances made recently in the methods used for the production of positron emission tomography (PET) agents. Modern technologies such as automated synthesis modules[1] and microfluidic chips[2] are being used to incorporate PET radionuclides, including those with very short half-lives, into targeting vectors rapidly and in high yield with minimal handling required. In most cases, purification is done using inline high performance liquid chromatography which ensures the agents are isolated in high effective specific activity.

In contrast, the methods used to prepare technetium radiopharmaceuticals have not changed significantly in over 30 years,[3] despite the numerous advantages of technetium-based agents.[4] ^{99m}Tc is widely available at a low cost, the radionuclide has excellent nuclear properties for imaging applications in terms of half-life (6 h) and gamma ray energy (E_γ = 140 keV), and it imparts a lower dose burden to

* To whom correspondence should be addressed. E-mail: valliant@mcmaster.ca.
[†] McMaster Nuclear Reactor.
[‡] Department of Chemistry.
[§] McMaster Institute of Applied Radiation Sciences (McIARS).

(1) (a) Iwata, R.; Takashashi, M.; Shinohara, M.; Ido, T. *J. Labelled Comp. Radiopharm.* **1982**, *19*, 1350–1351. (b) Alexoff, D. L. In *Handbook of radiopharmaceuticals*; Welch, M. J., Redvanly, C. S., Eds.; John Wiley and Sons Ltd: England, 2003; pp 283−306.
(2) Lee, C.; Sui, G.; Elizarov, A.; Shu, C. J.; Shin, Y. S.; Dooley, A. N.; Huang, J.; Daridon, A.; Wyatt, P.; Stout, D.; Kolb, H. C.; Witte, O. N.; Satyamurthy, N.; Heath, J. R.; Phelps, M. E.; Quake, S. R.; Tseng, H. *Science* **2005**, *310*, 1793–1796.
(3) Eckelman, W. C.; Richards, P. *J. Nucl. Med.* **1970**, *11*, 761.
(4) (a) *Technetium, Rhenium and Other Metals in Chemistry and Nuclear Medicine, 7*; Mazzi, U., Ed.; Servizi Grafici Editoriali snc: Padova, 2006. (b) *Technetium and Rhenium in Chemistry and Nuclear Medicine*; Nicolini, M., Bandoli, G., Mazzi, U., Eds.; Raven Press: New York, 1990. (c) Jurisson, S. S.; Lyden, J. D. *Chem. Rev.* **1999**, *99*, 2205. (d) Liu, S.; Edwards, O. S. *Chem. Rev.* **1999**, *99*, 2235. (e) Dilworth, J. R.; Parrott, S. J. *Chem. Soc. Rev.* **1998**, *27*, 43. (f) Hom, R. K.; Katzenellenbogen, J. A. *Nucl. Med. Biol.* **1997**, *24*, 485. (g) Eckelman, W. C. *Eur. J. Nucl. Med.* **1995**, *22*, 249. (h) Schwochan, K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2258.

10.1021/ic800775w CCC: $40.75
Published on Web 08/19/2008

patients than most PET radionuclides.[5] At present, ^{99m}Tc labeled compounds are produced using single step instant kits in which the radionuclide as $^{99m}TcO_4^-$ is added to a preformulated vial containing a reducing agent, buffer, and the ligand to be labeled. After mixing the components, the solution containing the product is typically filtered and administered directly to patients without any further purification.

While instant kits are attractive because they provide a convenient and highly reproducible means of producing Tc radiopharmaceuticals, they are in fact hindering the development of targeted agents. For molecular imaging applications, the excess ligand that is present in the kit can compete with the small quantity of labeled product for the target of interest. Without employing chromatographic methods, this feature severely limits the range of biological targets for which Tc-based probes can be developed.[6] Furthermore, the perceived need to use instant kits that have no ancillary purification methods restricts the choice of targeting vectors to the limited number of compounds that can be radiolabeled in a single step in a manner that yields only one product.

To see the impact of such limitations, one need only consider that if ^{18}F-based PET agents beyond simply fluoride had to be produced using single step instant kits, there would be no ^{18}F-based PET tracers in clinical use today. The impact and widespread use of automated and multistep ^{18}F-synthesis platforms demonstrates the potential value in modernizing the way in which Tc radiopharmaceuticals are produced. To do this will require the development of new rapid multistep labeling and conjugation methodologies that begin with pertechnetate and perrhenate in saline and complementary purification techniques to remove unlabeled ligand that are all readily automatable. Here, an expedient microwave-based synthesis platform that can be used to prepare targeted organometallic Tc(I) and Re(I) bifunctional chelate derivatives in high effective specific activity was developed.

Experimental Section

General Information. Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere using commercial grade solvents. Reagents were purchased from Sigma-Aldrich and used without further purification. Solid-phase extraction (SPE) procedures were performed using Waters 1.6 mL C_{18} SepPak cartridges. Compound **2** was prepared as described in the literature.[12] $[Re(CO)_3(OH_2)_3]Br$ and copper-loaded Amberlite IRC-748 resin were prepared according to literature procedures.[13,7] ^{1}H and ^{13}C NMR spectra were recorded on either Bruker AV200, AV600, or DRX500 spectrometers. ^{1}H chemical shifts are reported in ppm relative to the residual proton signal of the deuterated solvents. Coupling constants (J) are reported in Hertz (Hz). ^{13}C chemical shifts are reported in ppm relative to the carbon signal of the solvent. Low resolution mass spectra were obtained on a Waters/Micromass Quattro Ultima spectrometer for electrospray ionization experiments. High resolution mass spectra were obtained on a Waters/Micromass Global Q-ToF spectrometer. Infrared spectra were obtained on a BioRad FTS-40 FTIR spectrometer. Purification of all nonradioactive products was achieved by flash chromatography using Ultrapure Silica Gel from Silicycle (70−230 mesh) or a Biotage SP1 normal or reversed phase automated purification system. Microwave reactions were performed using a Biotage Initiator Sixty instrument. Analytical HPLC was performed using a Varian Pro Star model 330 PDA detector with a model 230 solvent delivery system and a C-18 Nucleosil column (4.6 × 250 mm) at a flow rate of 1.0 mL min^{-1} and monitoring at 254 nm. Specific activity measurements were performed on an Agilent 1200 series HPLC with an XDB-C18 column (1.8 μm, 4.6 × 50 mm) at a flow rate of 2.0 mL min^{-1} and monitoring at 254 nm. The elution protocols used were as follows: Method A: Solvent A = methanol, Solvent B = 0.05 M TEAP (tetraethylammonium phosphate) buffer (pH 2.2): Gradient elution 0−3 min, 0% A; 3−6 min 25% A; 6−9 min 33% A; 9−20 min 100% A; 20−22 min 100% A; 22−25 min 0% A; 25−30 min 0% A.[15] Method B: Solvent A = acetonitrile containing 0.1% v/v TFA, Solvent B = H_2O containing 0.1% TFA v/v: Gradient elution starting at 5% A to 100% A over 25 min. Method C: Solvent A = H_2O containing 20 mM triethylamine, pH adjusted to 3 with trifluoroacetic acid, Solvent B = acetonitrile containing 20 mM triethylamine, pH adjusted to 3 with trifluoroacetic acid: Gradient elution starting at 100% A to 60% A over 5 min. Method D: Solvent A = H_2O containing 20 mM triethylamine, pH adjusted to 3 with trifluoroacetic acid, Solvent B = acetonitrile containing 20 mM triethylamine, pH adjusted to 3 with trifluoroacetic acid: Gradient elution starting at 70% A to 50% A over 5 min.

A calibration curve for compound **2** ranging in concentration from 0.02 to 1.0 mM was generated by UV-HPLC using method B with an internal standard of 2-pyridinecarboxaldehyde (actual concentrations: 0.017, 0.033, 0.066, 0.133, 0.266, 0.533, 1.066 mM). Subsequent determination of the limits of quantification (LOQ = 0.062 mM) and limits of detection (LOD = 0.020 mM) are based upon the linear regression analysis of the data points which were each measured in triplicate.

Specific activity for compounds **5b**, **6b**, and **7b** were determined by generating UV absorbance calibration curves for the corresponding nonradioactive rhenium standards **5a**, **6a**, and **7a** using HPLC method C for **5b** and method D for **6b** and **7b**. 1-(2-Methoxyphenyl)-piperazine was used as an internal standard, and the amount of rhenium standard compound used for linear regression analyses ranged from 25 ng to 20 000 ng. The signal-to-noise ratios for all points on the calibration curve were greater than 10. Samples of approximately 2−5 mCi/100 μL were injected and the corresponding UV absorbance was translated into mass of sample, and the collected activity was assayed to determine the final specific activities. Measurements were done in triplicate.

Crystallographic Details. X-ray crystallographic data for **4a** were collected from a sample mounted with epoxy on the end of a thin glass fiber at 173 K on a Bruker Apex2 Mo diffractometer (λ = 0.710 73 Å) with a 3-circle D8 goniometer, fine focus sealed tube source and graphite monochromator. Data processing was carried out by use of the program Apex2.[8] A numerical face correction was applied using the Apex2 software. The structure was solved by using the direct-methods procedure in the Bruker SHELXTL[9] program library and refined by full-matrix least-squares methods on F^2. All non-hydrogen atoms were refined using

(5) (a) Mahmood, A.; Jones, A. G. In *Handbook of radiopharmaceuticals*; Welch, M. J., Redvanly, C. S., Eds.; John Wiley and Sons Ltd: England, 2003; pp 323−362. (b) Funk, T.; Sun, M.; Hasegawa, B. H. *Med. Phys.* **2004**, *31*, 2680–2686.
(6) Eckelman, W. C. *Nucl. Med. Biol.* **2006**, *33*, 449–451.
(7) Lazarova, N.; James, S.; Babich, J.; Zubieta, Z. *Inorg. Chem. Commun.* **2004**, *7*, 1023–1026.
(8) *M86-Exx078 APEX2 User Manual*; Bruker AXS Inc.: Madison, WI, 2006.
(9) Sheldrick, G. M. *SHELXTL*, Release 6.14; Siemens Crystallographic Research Systems: Madison, WI, 2000.

anisotropic thermal parameters, and hydrogen atoms were added as fixed contributors at calculated positions, with isotropic thermal parameters based on the atom to which they are bonded.

Preparation of $[C_{17}H_{21}N_3O_2Re(CO)_3]Br$ (3a). Compound **2** (0.29 g, 0.97 mmol) and compound **1a** (0.65 g, 1.60 mmol) were combined in a 5 mL microwave vial. A water-acetonitrile mixture (90:10) was added (3.5 mL) and the reaction vial crimp sealed prior to heating in the microwave at 150 °C for 5 min with stirring. The product was purified by loading the reaction solution onto a C_{18} SepPak cartridge, washing with water (5 mL), and eluting the desired product with acetonitrile (4 mL). The acetonitrile was removed under reduced pressure where the resulting amber colored oil solidified upon standing (0.50 g, 82%). Recrystallization of the resulting solid from methanol gave colorless crystals of the desired product. Mp132 °C. ^{1}H NMR (δ (ppm), CD_3OD, 600 MHz): 8.87 (d, J = 5.4 Hz, 2H, PyH), 7.94 (dd, 2H, PyH), 7.56 (d, J = 7.8 H, 2H, PyH), 7.40 (dd, 2H, PyH), 4.86 (m, 4H, $PyCH_2$), 3.85 (m, 2, NCH_2), 2.47 (t, J = 7.2 Hz, 2H, CH_2CO), 2.01 (m, 2H, $NCH_2C\underline{H}_2$), 1.73 (m, 2H, $C\underline{H}_2CH_2CO$). ^{13}C NMR (δ (ppm), CD_3OD, 150 MHz): 197.19, 196.41, 176.91, 162.18, 153.15, 141.60, 126.87, 124.58, 71.55, 68.71, 34.31, 25.63, 23.09. FTIR (KBr, υ/cm^{-1}): 2028, 1913, 1739, 1728. HRMS (ES^+) calcd for $[C_{20}H_{21}N_3O_5Re]^+$, 570.1039 (M^+); found 570.1027.

Preparation of $[C_{21}H_{24}N_4O_4Re(CO)_3]Br$ (4a). Compound **3a** (0.18 g, 0.28 mmol) was combined with *N*-hydroxysuccinimide (0.16 g, 1.4 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (0.27 g, 1.4 mmol) in a 5 mL microwave vial, and anhydrous acetonitrile (4 mL) was added. The reaction vial was crimp sealed, and the mixture heated in the microwave at 120 °C for 5 min with stirring. The solvent was removed under reduced pressure, and the desired product isolated by silica gel chromatography (2% methanol/98% dichloromethane to 20% methanol/80% dichloromethane) as a viscous amber oil. The oil solidified upon standing under ambient conditions, and recrystallization from methanol gave colorless crystals suitable for X-ray structure determination (0.19 g, 92%). ^{1}H NMR (δ(ppm), $CDCl_3$, 500 MHz): 8.65 (d, J = 5.3 Hz, 2H, PyH), 8.01 (m, 2H, PyH), 7.82 (m, 2H, PyH), 7.20 (m, 2H, PyH), 6.22 (d, J = 16.2 Hz, 2H, $PyCH_2$), 4.41 (d, J = 16.2 Hz, 2H, $PyCH_2$), 3.79 (m, 2H, NCH_2), 2.84 (s, 4H, CH_2CH_2), 2.80, (t, J = 5.6 Hz, 2H, CH_2CO), 2.28 (m, 2H, $NCH_2C\underline{H}_2$), 1.93 (m, 2H, $C\underline{H}_2CH_2CO$). ^{13}C NMR (δ (ppm), $CDCl_3$, 150 MHz): 195.96, 169.23, 168.26, 161.36, 150.63, 140.34, 125.65, 125.29, 70.54, 67.05, 30.79, 25.79, 24.85, 22.32. FTIR (KBr, υ/cm^{-1}): 2029, 1924, 1735. HRMS (ES^+) calcd for $[C_{24}H_{24}N_4O_7Re]^+$, 667.1203 (M^+); found 667.1224.

Preparation of $[C_{23}H_{35}N_5ORe(CO)_3]Br$ (5a). Compound **4a** (32.3 mg, 0.042 mmol) was dissolved in acetonitrile (1.5 mL) and transferred to a 5 mL microwave vial. *N,N*-Diethylethylenediamine (0.04 mL, 0.28 mmol) was added, the vial crimp sealed, and the reaction mixture heated in the microwave at 120 °C for 5 min with stirring. The solvent was removed under reduced pressure, and the resulting dark oil purified by reverse phase silica gel chromatography (95% water/5% acetonitrile to 0% water/100% acetonitrile) to yield the desired product as a viscous amber oil (25 mg, 80%). Recrystallization from dichloromethane/diethyl ether gave colorless crystals of the desired product. ^{1}H NMR (δ (ppm), CD_3OD, 600 MHz): 8.86 (d, J = 5.4 Hz, 2H, PyH), 7.94 (m, 2H, PyH), 7.55 (d, J = 7.8 Hz, 2H, PyH), 7.37 (m, 2H, PyH), 3.82 (m, 2H, $NCH_2C\underline{H}_2N(Et)_2$), 3.59 (t, J = 6.6 Hz, 2H, $C(O)NHC\underline{H}_2$), 3.28 (m, 6H, $N(C\underline{H}_2CH_3)_2$ and $NC\underline{H}_2CH_2CH_2$), 2.42 (t, J = 7.8 Hz, 2H, $C\underline{H}_2C(O)$), 2.00 (m, 2H, $NCH_2C\underline{H}_2CH_2$), 1.74 (m, 2H, $NCH_2CH_2C\underline{H}_2$), 1.34 (t, J = 7.2 Hz, 6 Hz, $CH_2C\underline{H}_3$); the resonances for the $(PyC\underline{H}_2)_2NCH_2$ protons are obscured by the CD_3OD signal. ^{13}C NMR (δ (ppm), CD_3OD, 150 MHz): 197.18, 196.28, 177.10, 177.01, 162.51, 161.89, 153.08, 141.67, 126.38, 124.52, 71.27, 68.79, 52.47, 36.10, 35.66, 25.88, 23.57, 9.14. IR (KBr, υ/cm^{-1}): 2030, 1927, 1702, 1654. HRMS (ES^+) (m/z): $[M]^+$ calculated for $[C_{26}H_{35}N_5O_4Re]^+$ 668.2221, found 668.2247.

Preparation of $[C_{24}H_{28}N_4ORe(CO)_3]^+$(6a). The procedure was the same as that used to prepare **5a**. Yield: 27 mg (77%). ^{1}H NMR (δ (ppm), $CDCl_3$, 200 MHz): 8.64 (d, J = 4.8 Hz, 2H, PyH), 7.78 (m, 2H, PyH), 7.68 (br s, 1H, NH), 7.50 (m, 2H, PyH), 7.31 (m, 2H, PyH), 7.25 (m, 5H, PhH), 5.34 (d, J = 16.4 Hz, 2H, $PyCH_2$), 4.48 (m, 2H, $PhCH_2$), 4.31 (d, J = 16.4 Hz, 2H, $PyCH_2$), 3.66 (m, 2H, NCH_2), 2.37 (m, 2H, CH_2CO), 1.91 (m, 2H, $NCH_2C\underline{H}_2$), 1.70 (m, 2H, $C\underline{H}_2CH_2CO$). ^{13}C NMR (δ (ppm), $CDCl_3$, 50 MHz): 195.53, 173.12, 160.3, 151.16, 140.44, 138.60, 128.55, 127.85, 127.22, 125.67, 124.00, 70.61, 67.41, 43.45, 35.35, 24.33, 22.75. IR (KBr, υ/cm^{-1}): 2032, 1931, 1685. HRMS (ES^+) calcd for $[C_{27}H_{28}N_4O_4Re]^+$ 659.1668 (M^+), found 659.1642.

Preparation of $[C_{28}H_{35}N_5O_2Re(CO)_3]^+$ (7a). The procedure was the same as that used to prepare **5a**. Yield: 168 mg (82%). ^{1}H NMR (δ (ppm), CD_3OD, 600 MHz): 8.87 (d, J = 5.6 Hz, 2H, PyH), 7.94 (m, 2H, PyH), 7.55 (d, J = 7.9 Hz, 2H, PyH), 7.37 (m, 2H, PyH), 7.04 (m, 1H, ArH), 6.96 (m, 2H, ArH), 6.91 (m, 1H, ArH), 3.87 (s, 3H, OCH_3), 3.86 (m, 2H, $ReNC\underline{H}_2CH_2$), 3.77 (t, J = 5.0 Hz, 2H, $N_{amide}C\underline{H}_2CH_2N$), 3.74 (t, J = 4.9 Hz, 2H, $N_{amide}C\underline{H}_2CH_2N$), 3.06 (t, J = 4.9 Hz, $N_{amide}CH_2C\underline{H}_2N$), 3.00 (t, J = 5 Hz, 2H, $N_{amide}CH_2C\underline{H}_2N$), 2.61 (t, J = 7.1 Hz, 2H, $CH_2C(O)N$), 2.01(m, 2H, $NCH_2C\underline{H}_2$), 1.75 (m, 2H, $C\underline{H}_2CH_2C(O)N$); the resonances for the $(PyC\underline{H}_2)_2NCH_2$ protons are obscured by the CD_3OD signal. ^{13}C NMR (δ (ppm), CD_3OD, 150 MHz): 197.22, 196.40, 173.34, 162.20, 153.97, 153.18, 141.97, 141.64, 126.89, 126.34, 125.00, 124.55, 122.21, 119.75, 112.90, 71.54, 71.47, 68.76, 68.47, 56.01, 52.38, 51.89, 47.02, 44.16, 43.08, 33.31, 25.84, 23.36. IR (KBr, υ/cm^{-1}): 2030, 1918, 1686. HRMS (ES^+) (m/z): $[M]^+$ calculated for $[C_{31}H_{35}N_5O_5Re]^+$ 744.2196, found 744.2191.

Radiochemistry. Caution! *^{99m}Tc is a γ-emitter (E_γ = 140 keV, $t_{1/2}$ = 6 h) while ^{186}Re ($E_{max\beta}$ 1.07 MeV, $t_{1/2}$ = 3.8 d) and ^{188}Re ($E_{max\beta}$ 2.12 MeV, $t_{1/2}$ = 17 h) are both β^- and γ-emitters which should only be used in a licensed and appropriately shielded facility.* Perrhenate solutions ($^{186/188}ReO_4^-$; 74−185 MBq) were obtained from the McMaster Nuclear Reactor following neutron bombardment of naturally occurring rhenium. The average specific activity of the material was 111 MBq/μg.

Preparation of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ (1b). $^{99m}TcO_4^-$ from a commercial generator (1600 MBq, 1.5 mL) was added to a crimp sealed Emry microwave vial (5 mL) containing sodium tartrate (17 mg), sodium tetraborate (3.5 mg), sodium carbonate (3.2 mg), and potassium boranocarbonate (8.1 mg) under nitrogen. Note that the reaction vial containing the solid reagents was purged with nitrogen prior to use for 15 min. The reaction mixture was heated in the microwave at 130 °C for 3 min with stirring. γ-HPLC (Method A): t_R = 4 min; Yield: 1600 MBq (quantitative conversion).

Preparation of $[C_{17}H_{21}N_3O_2{}^{99m}Tc(CO)_3]^+$ (3b). Compound **2** in distilled water (1 mg/ mL) was added directly to the microwave vial containing $[^{99m}Tc(CO)_3(OH_2)_3]^+$ (1480 MBq), and the reaction mixture heated in the microwave at 150 °C for 2 min with stirring. The crude reaction mixture (1221 MBq) was then passed through a column packed with 400 mg of IRC-748-copper (II) resin (loading ~1.3 mmol/g) dropwise. Fractions containing the product were combined and loaded onto a C_{18} SepPak cartridge, washed with water (5 mL), and the desired product was eluted with acetonitrile (4 mL). γ-HPLC (Method A): t_R = 19.2 min; Yield: 943 MBq (78%, decay corrected).

Preparation of $[C_{21}H_{24}N_4O_4{}^{99m}Tc(CO)_3]^+$ (4b). The acetonitrile fraction containing compound **3b** (666 MBq) was dried using a Biotage V10 solvent evaporator (T = 35 °C, 5 min.), the residue dissolved in anhydrous acetonitrile (4 mL), and the solution transferred to a 5 mL vial containing *N*-hydroxysuccinimide (0.16 g, 1.4 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (0.27 g, 1.4 mmol). The reaction mixture was heated in the microwave at 120 °C for 5 min with stirring. The product was purified by loading the reaction mixture onto a C_{18} SepPak cartridge, washing with water (5 mL) and eluting the desired product with acetonitrile (4 mL). γ-HPLC (Method A): t_R = 20 min; Yield: 566 MBq (85%, decay corrected).

Preparation of $[C_{23}H_{35}N_5O^{99m}Tc(CO)_3]^+$ (5b). *N,N*-diethylethylenediamine (0.04 mL, 0.28 mmol) was added to compound **4b** (118 MBq) in acetonitrile (2 mL) in a 5 mL Emry vial, crimp sealed, and heated at 120 °C for 5 min with stirring. The product was verified by comparison of the γ-HPLC with the UV-HPLC of compound **5a**. γ-HPLC (Method A): t_R = 17.5 min., Yield: 107 MBq (91%, decay corrected). Specific activity: 4.13×10^8 MBq/mmol $\pm$ 0.02×10^8 MBq/mmol.

Preparation of $[C_{24}H_{28}N_4O^{99m}Tc(CO)_3]^+$ (6b). The procedure was the same as that used to prepare **5b**. The product was verified by comparison of the γ-HPLC with the UV-HPLC of compound **6a**. γ-HPLC (Method A): t_R = 21.2 min; Yield: 118 MBq (91%, decay corrected). Specific activity: 3.69×10^8 MBq/mmol $\pm$ 0.03 $\times 10^8$ MBq/mmol.

Preparation of $[C_{28}H_{35}N_5O_2{}^{99m}Tc(CO)_3]^+$ (7b). The procedure was the same as that used to prepare **5b**. The product was verified by comparison of the γ-HPLC with the UV-HPLC of the rhenium standard 7**a**. γ-HPLC (Method A): t_R = 22.5 min; Yield: 122 MBq (86%, decay corrected). Specific activity: 4.6×10^8 MBq/mmol $\pm$ 0.05×10^8 MBq/mmol.

Preparation of $[^{186/188}Re(CO)_3(OH_2)_3]^+$ (1c). *ortho*-Phosphoric acid (71 μL) was added to high purity water (medical grade, 5 mL), and the solution was degassed with N_2 for 15 min. An aqueous solution (0.5 mL) containing $^{186/188}ReO_4^-$ (42 MBq) was added, and an aliquot (1.5 mL) of that solution added to a sealed Emry vial containing sodium tartrate (16 mg), sodium tetraborate (4 mg), sodium carbonate (3.2 mg), sodium boranocarbonate (8.6 mg), and $BH_3 \cdot NH_3$ (17 mg) under nitrogen. Gas evolution was observed, and the solution turned amber in color. The reaction mixture was heated in a microwave at 150 °C for 3 min with stirring. The product was compared by γ-HPLC to the desired product synthesized according to the Mallinckrodt two-step kit formulation.[10] γ-HPLC (Method A): t_R = 3.5 min. Yield: 39.2 MBq (93%).

Preparation of $[C_{17}H_{21}N_3O_2{}^{186/188}Re(CO)_3]^+$ (3c). Compound **2** (0.29 g, 0.97 mmol) dissolved in degassed water for injection (WFI, 0.5 mL) was added directly to an aqueous solution of compound **1c** (77 MBq) in a crimp sealed Emry microwave vial, and the mixture heated in the microwave at 150 °C for 2 min with stirring. The product was purified by loading the reaction solution onto a C_{18} SepPak cartridge, washing with water (5 mL) and then acetonitrile (4 mL). γ-HPLC (Method A): t_R = 19.1 min; Yield: 68 MBq (90%).

Preparation of $[C_{21}H_{24}N_4O_4{}^{186/188}Re(CO)_3]^+$ (4c). The acetonitrile solution containing compound **3c** (9.9 MBq) was evaporated to dryness using a Biotage V10 solvent evaporator. Anhydrous acetonitrile (4 mL) was added and the mixture transferred to a 5 mL Emry vial containing *N*-hydroxysuccinimide (0.20 g, 1.8 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (0.36 g, 1.9 mmol). The reaction mixture was heated in the microwave at 120 °C for 5 min with stirring. The product was purified by loading the reaction solution onto a C_{18} SepPak cartridge, washing with water (5 mL), and eluting the desired product with acetonitrile (4 mL). γ-HPLC (Method A): t_R = 19.9 min.; Yield: 6.1 MBq (62%).

Preparation of $[C_{23}H_{35}N_5O^{186/188}Re(CO)_3]^+$ (5c). *N,N*-diethylethylenediamine (18.9 mg, 0.17 mmol) was added to compound **4c** (1.9 MBq) in acetonitrile (4 mL) in a 5 mL Emry vial, crimp sealed, and heated at 120 °C for 5 min with stirring. The product was verified by comparison of the γ-HPLC with the UV-HPLC of compound **5a**. γ-HPLC (Method A): t_R = 17.1 min.; Yield: 1.9 MBq (>99%).

Preparation of Compounds $[C_{24}H_{28}N_4O^{186/188}Re(CO)_3]^+$ (6c). The procedure was the same as that used to prepare **5c**. The product was verified by comparison of the γ-HPLC with the UV-HPLC of compound **6a**. γ-HPLC (Method A): t_R = 20.9 min; Yield: 1.7 MBq (>99%).

Preparation of $[C_{28}H_{35}N_5O_2{}^{186/188}Re(CO)_3]^+$ (7c). The procedure was the same as that used to prepare **5c**. The product was verified by comparison of the γ-HPLC with the UV-HPLC of compound **7a**. γ-HPLC (Method A): t_R = 21.9 min; Yield: 2.1 MBq (>99%).

Results and Discussion

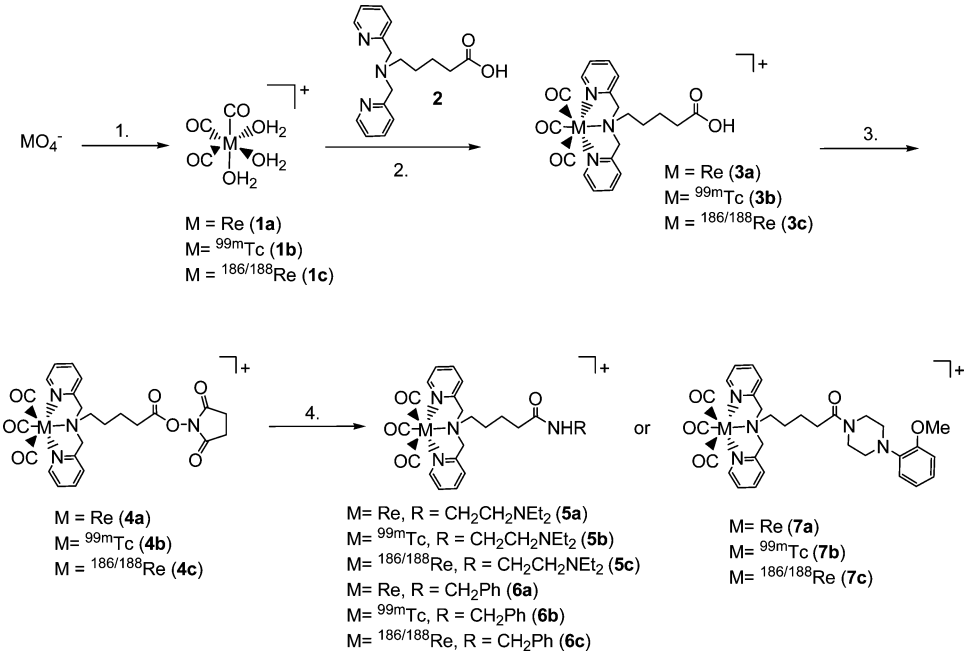
One of the challenges in creating a general purpose platform whereby Tc and Re can be conjugated to a variety of different targeting vectors is that the product transition metal complexes must be stable to a wide range of reaction conditions. Although there has been an elegant *in situ* synthesis and purification strategy developed for a bifunctional Tc(V) chelate system,[11] the difficulties of working in this oxidation state (isomer formation, thermal stability, etc.) may complicate the use of the technology as a general purpose platform. With the availability of the Tc(I)/Re(I) precursor *fac*-$[M(CO)_3(OH_2)_3]^+$, which can be prepared in water in a single step,[12] it is possible to prepare inert organometallic complexes with bifunctional tridentate chelating ligands.[13] Taking advantage of the robust nature of these complexes, a method for the rapid synthesis and purification of organometallic Re(I) and Tc(I) complexes, which includes a new method for the synthesis of the $[M(CO)_3]^+$ core, and a variety of functionalized derivatives was developed.

Microwave Synthesis of $[M(CO)_3(OH_2)_3]^+$ (M = ^{99m}Tc, $^{186/188}$Re) and Associated Chelate Complexes. To create a useful multistep labeling procedure it is essential that all steps be completed in high yield in as short a period of time as possible to minimize loss due to decay and to maximize the specific activity of the product. To achieve this goal, a series of experiments were performed to determine if microwave heating could be used to reduce the amount of time needed to prepare $[^{99m}Tc(CO)_3(OH_2)_3]^+$ (**1b**) and complexation by a bifunctional tridentate ligand (Scheme 1.) To this end, pertechnetate was added to a 5

(10) A two-component kit was kindly supplied by Mallinckrodt Medical, Inc. The kit was labeled with rhenium according to instructions supplied by the manufacturer. The radiochemical purity of the material was determined using g-HPLC to assess the percentage of free perrhenate.

(11) Misra, P.; Humblet, V.; Pannier, N.; Maison, W.; Frangioni, J. V. *J. Nucl. Med.* **2007**, *48*, 1381–1389.

Scheme 1. Expedient Synthesis of $M(CO)_3$-Bifunctional Chelate Complexes[a]



[a] 1. 130 °C, 5 min (M = Re), 130 °C, 3 min (M = ^{99m}Tc) or 150 °C, 3 min (M = $^{186/188}$Re). 2. 150 °C, 5 min (M = Re) or 150 °C, 2 min (M = ^{99m}Tc and $^{186/188}$Re). 3. 120 °C, 5 min, *N*-hydroxysuccinimide, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide. 4. 120 °C, 5 min, *N,N*-diethylethylenediamine, benzylamine, or 1-(2-methoxy)-phenylpiperazine.

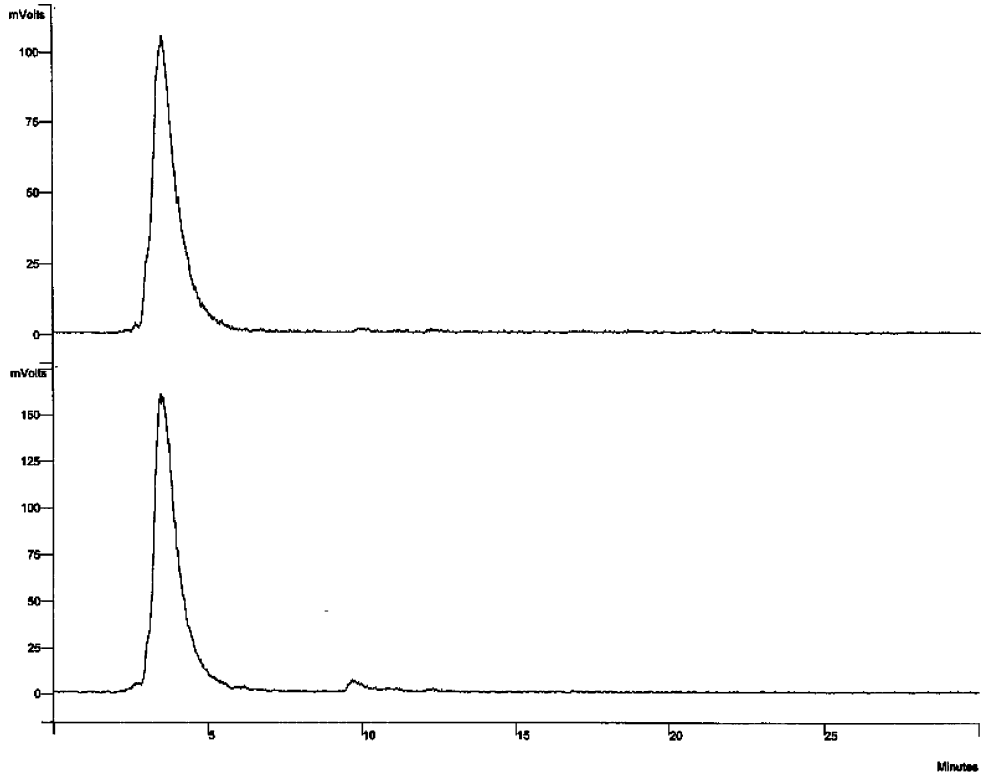


Figure 1. γ-HPLC chromatogram of **1b** produced by microwave (top) (T = 130 °C, time = 3 min) and conventional (bottom) heating (T = 95 °C, time = 30 min) (HPLC method A).

mL microwave vial containing the same components traditionally used to prepare **1b** (sodium-boranocarbonate, Na/K tartrate, sodium tetraborate decahydrate, and sodium carbonate), and the mixture heated in a microwave at a range of different temperatures for varying amounts of time. Radio-HPLC analyses of the resulting mixtures showed that the optimal conditions were 130 °C for 3 min and that the product was the same as the material produced using conventional heating (Figure 1.) At temperatures above 130 °C, degradation of the desired product to TcO_4^- occurred. By heating at 130 °C however, **1b** was prepared with exquisite reproducibility in nearly quantitative yield

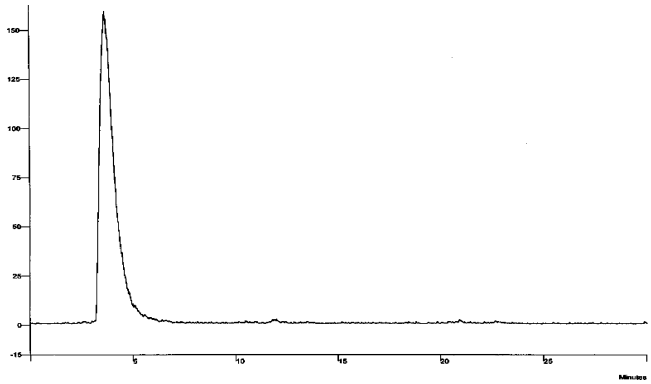


Figure 2. γ-HPLC chromatogram of **1c** formed by microwave heating (HPLC method A).

in mere minutes as opposed to greater than 20 min using conventional methods.[8]

The excellent yields and reduced synthesis times obtained for the production of **1b** lead us to investigate the synthesis of the radioactive rhenium analogues (i.e., $^{186/188}$Re) (Scheme 1). The current method used to prepare $[^{186/188}Re(CO)_3(OH_2)_3]^+$ **1c** involves the use of a two-step kit because of the greater reduction potential of ReO_4^- compared to TcO_4^-.[14,15] To produce the desired product following the conventional method, it is necessary to pretreat ReO_4^- with phosphoric acid and $BH_3 \cdot NH_3$ prior to performing the reductive carbonylation reaction using the Isolink kit. With microwave heating, the desired product can be produced in a single step by adding $BH_3 \cdot NH_3$ and phosphoric acid to the formulation used to prepare **1b**. Heating the reaction mixture at 150 °C for 3 min produced **1c** in close to quantitative yield (Figure 2.) The ability to produce $[^{186/188}Re(CO)_3(OH_2)_3]^+$ in a single pot is advantageous because it greatly decreases the overall reaction time and reduces the amount of sample handling required.

In addition to using microwave heating to prepare $[M(CO)_3(OH_2)_3]^+$ (**1b** and **1c**), we also explored its use in promoting metal complexation with a bifunctional chelating agent (Scheme 1). The ligand selected, which was developed by Zubieta and co-workers,[16] contains two pyridine groups and one tertiary amine which form a stable cationic complex with the tricarbonyl core. Labeling reactions were performed by direct addition of compound **2** in solution to the microwave vial containing **1b** or **1c**. Through a temperature course study, it was determined that quantitative conversion to the desired metal complex was achieved in less than two minutes at 150 °C. The identities of the metal complexes **3b** and **3c** were verified by comparison of the γ-HPLC traces of the products with UV-HPLC traces of fully characterized nonradioactive Re analogue **3a** (Figure 3.)

One of the limitations of using the tricarbonyl core for developing molecular imaging agents, particularly for targets that are expressed in low concentration, is that a substantial amount of ligand typically must be used during labeling reactions to achieve good radiochemical yields. This feature limits the maximum achievable specific activity unless ancillary purification methods like HPLC are employed. The impact of microwave heating on the radiochemical yield as a function of ligand concentration was tested to see if it was possible to reduce the amount of ligand required in a given reaction. At 10^{-3} M ligand concentrations, quantitative yields could be achieved and even at levels as low as 10^{-6} M some complex formation was observed (11%). This is in contrast to conventional heating at this level which produced primarily pertechnetate and unreacted starting material. Below a concentration of 10^{-6} M, the yield decreased dramatically, even with prolonged microwave heating.

Purification via Chemoselective Filtration. At 10^{-3} M ligand concentrations there was still an appreciable amount of unlabeled **2** remaining in solution. This material can be removed by semipreparative HPLC; however, such an approach is not desirable for producing agents that are destined to be used clinically or for an automated synthesis platform. To provide a means of producing in high effective specific activity, a convenient resin capture purification method was developed which takes advantage of the reactivity of the unlabeled chelate. The system is based on a simple copper functionalized solid-support which was used by Ley et al. to isolate bipyridyl derivatives of small molecules from reaction mixtures via coordination to the resin.[17]

Copper-loaded Amberlite IRC-748 resin (loading ~1.3 mmol/g) was prepared according to a literature procedure,[18] and a solution containing **2** and **3b** (1221 MBq) was passed through a syringe filled with approximately 400 mg of resin (Scheme 2.) To test the efficiency of the purification strategy (Figure 4,) a large excess of the ligand was employed during the labeling reaction (1.55 mM). After treatment with the copper resin, the amount of residual ligand was less than the detection limit of the HPLC (0.020 mM). For some batches of resin, formation of the Cu complex of **2** was detected by HPLC (verified through comparison to an authentic standard) which we believe is due to incomplete removal of free copper ion during the preparation of the resin. Notwithstanding, the copper complex if formed can be easily separated from the desired product using a C18 SPE cartridge, which is a procedure routinely used in automated radiochemistry systems. The average amount of radioactivity recovered was 78% across all experiments. This modest loss of activity is acceptable given the speed of the overall procedure, the exquisite purity of the product, and the low cost of ^{99m}Tc.

Rapid Evaporation and Activation. The next step was

(12) Alberto, R.; Ortner, K.; Wheatley, N.; Schibli, R.; Schubiger, A. P. *J. Am. Chem. Soc.* **2001**, *123*, 3135–3136.
(13) Banerjee, S. R.; Maresca, K. P.; Francesconi, L.; Valliant, J.; Babich, J. W.; Zubieta, J. *Nucl. Med. Biol.* **2005**, *32*, 1–20.
(14) Schibli, R.; Schwarzbach, R.; Alberto, R.; Ortner, K.; Schmalle, H.; Dumas, C.; Egli, A.; Schubiger, P. A. *Bioconjugate Chem* **2002**, *13*, 750–756.
(15) Park, S. H.; Seifert, S.; Pietzsch, H. J. *Bioconjugate Chem.* **2006**, *17*, 223–225.
(16) Banerjee, S. R.; Levadala, M. K.; Lazarova, N.; Wei, L.; Valliant, J. F.; Stephenson, K. A.; Babich, J. W.; Maresca, K. P.; Zubieta, J. *Inorg. Chem.* **2002**, *41*, 6417–6425.
(17) Ley, S. V.; Massi, A.; Rodriguez, F.; Horwell, D. C.; Lewthwaite, R. A.; Pritchard, M. C.; Reid, A. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1053–1055.
(18) Siu, J.; Baxendale, I. R.; Lewthwaite, R. A.; Ley, S. V. *Org. Biomol. Chem.* **2005**, *3*, 3140–3160.

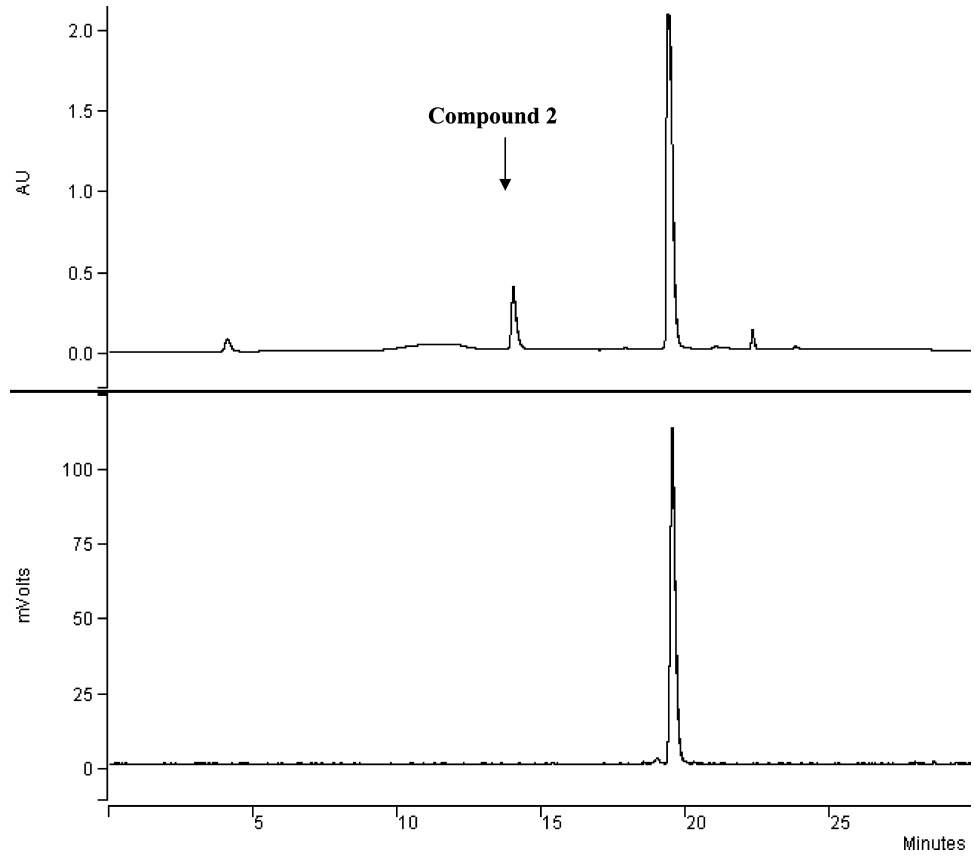
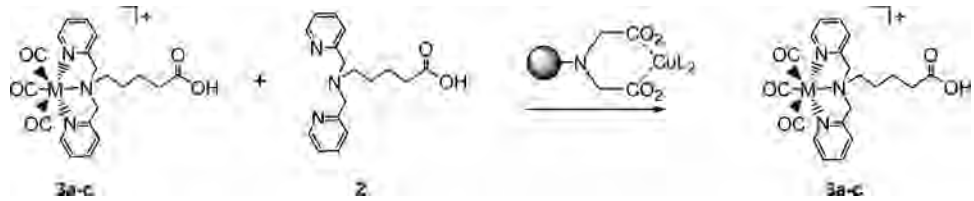


Figure 3. UV-HPLC chromatogram of crude reaction mixture for the synthesis of **3b** showing excess **2** along with co-injected **3a** (top) and the γ-HPLC chromatogram of the reaction mixture containing **3b** (bottom).

Scheme 2. Removal of Unreacted Ligand **2** by Chemoselective Filtration



to develop a convenient means to link the purified chelate complexes to targeting vectors. The approach taken involved conversion of the pendent acid group to an active ester so that the chelate complexes can be conjugated to amino groups. Generally, the preparation of active esters is best performed in organic solvents. This is not an option for traditional Tc instant kits as they are designed to work with the 0.9% saline solution used to elute $^{99m}TcO_4^-$ from the $^{99}Mo/^{99m}Tc$ generators. In contrast, for PET chemistry, organic solvents are used routinely to accelerate labeling reactions and to be able to utilize reactive compounds that would hydrolyze in aqueous solutions.

Following removal of unlabeled ligand using the copper resin, the aqueous solution containing **3b** was loaded onto a C_{18} SepPak cartridge which was subsequently washed with water (5 mL) to remove salts and then acetonitrile (4 mL) to elute the desired product. The acetonitrile fraction containing compound **3b** (666 MBq) was dried using a Biotage V10 solvent evaporator which is a new technology for rapid removal of solvents under mild conditions. The evaporation was complete in less than five minutes with heating at 35 °C. Analysis of the product following the evaporation procedure showed no signs of degradation which is consistent with the mild nature of the drying process and the known stability of the complex. If high effective specific activity is not a critical issue, the copper resin procedure can be omitted and the aqueous solution containing the metal complex concentrated directly following microwave heating.

Microwave heating was subsequently used to produce the active ester **4b**. Anhydrous acetonitrile was added to dried **3b** in a 5 mL microwave vial along with *N*-hydroxysuccinimide and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC). The reaction mixture was heated at 120 °C for 5 min, and the product isolated by loading the reaction solution onto a C_{18} SepPak cartridge, washing with water (5 mL), and eluting the desired product with acetonitrile (4 mL). The identity of **4b**, which was isolated in 85% yield, was verified by comparison of the γ-HPLC trace with the UV-HPLC trace of the rhenium standard **4a**. The nonradioactive Re analogue was also prepared in the microwave in the same manner used for the Tc complex and was isolated in high yield (92%). An X-ray crystal structure of the product was obtained

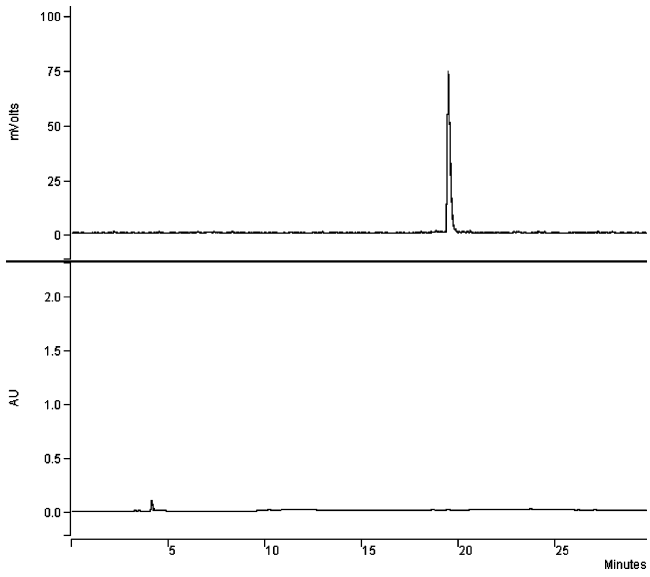


Figure 4. γ-HPLC (top) and UV-HPLC (bottom) chromatograms of **3b** after treatment with the copper resin.

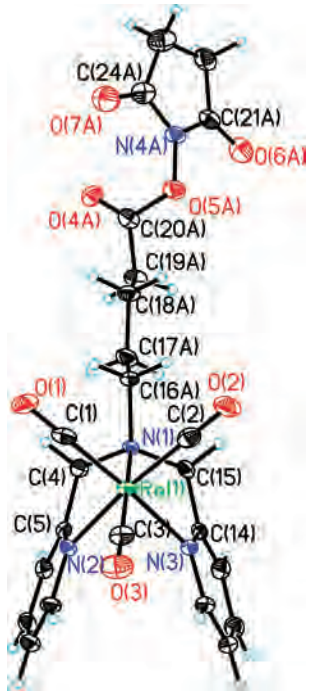


Figure 5. X-ray crystal structure of **4a** (30% thermal probability ellipsoids). The bromide anion and a water molecule were removed for clarity.

following recrystallization from methanol (Figure 5, Tables 1 and 2).

The reactivity of the active esters **4b** and **4c** were tested using a series of model amines including *N,N*-diethylethylenediamine, benzylamine, and 1-(2-methoxy)-phenylpiperazine (Scheme 1). Reactions were performed in the microwave at 120 °C for 5 min. Yields were typically greater than 90% and the products isolated by SPE. In the case of complex **4c**, reaction with a secondary amine produced the desired product **7b** in somewhat lower yield than for the technetium analogue but still acceptable at 86%. The reaction solvent was readily removed using the V10 evaporator so that the final products could be reconstituted in saline solutions suitable for direct administration to animal models. It is clear from these experiments that the active ester is sufficiently reactive even at high effective specific activity to efficiently couple with a variety of different amines. The typical specific activity of the ^{99m}Tc-labeled amides produced using this strategy was between 3.7×10^8 MBq/mmol and 4.6×10^8 MBq/mmol, in close agreement with a previously reported specific activity values for multistep labeling of small molecule radiotracers (4.1×10^8 MBq/mmol).[11]

Table 1. Crystal and Structure Refinement Data for **4a**

empirical formula	$C_{24}H_{26}BrN_4O_8Re$
formula weight	764.60
temperature	173(2) K
crystal system	monoclinic
space group	$P2(1)/c$
unit cell dimensions	a = 9.0064(6) Å
	b = 14.7027(11) Å, β = 91.7070(10)°
	c = 20.1519(15) Å
Z	4
goodness-of-fit on F^2	1.430
final R indices [I > 2σ(I)]	R1 = 0.0513, wR2 = 0.1929
R indices (all data)	R1 = 0.0593, wR2 = 0.2050

Table 2. Select Bond Lengths and Angles for Compound **4a**

Re(1)−C(2)	1.915(9)	C(2)−Re(1)−C(3)	87.8(4)
Re(1)−C(3)	1.916(8)	C(2)−Re(1)−C(1)	89.3(4)
Re(1)−C(1)	1.918(9)	C(3)−Re(1)−C(1)	85.3(4)
Re(1)−N(3)	2.181(7)	C(2)−Re(1)−N(3)	95.9(3)
Re(1)−N(2)	2.192(6)	C(3)−Re(1)−N(3)	97.9(3)
Re(1)−N(1)	2.240(6)	C(1)−Re(1)−N(3)	174.0(3)
N(4A)−O(5A)	1.379(13)	C(2)−Re(1)−N(2)	171.9(3)
O(4A)−C(20A)	1.210(14)	C(3)−Re(1)−N(2)	96.2(3)
O(5A)−C(20A)	1.372(14)	C(1)−Re(1)−N(2)	98.0(3)
N(4)−O(5)	1.383(14)	N(3)−Re(1)−N(2)	76.6(2)
N(4)−C(21)	1.404(18)	C(2)−Re(1)−N(1)	97.5(3)
N(4)−C(24)	1.488(18)	C(3)−Re(1)−N(1)	173.3(3)
O(4)−C(20)	1.211(16)	C(1)−Re(1)−N(1)	98.9(3)
O(5)−C(20)	1.380(14)	N(3)−Re(1)−N(1)	77.5(2)
O(6)−C(21)	1.184(16)	N(2)−Re(1)−N(1)	78.1(2)
		C(20A)−O(5A)−N(4A)	113.9(10)
		C(24A)−O(7A)−H(7AA)	109.5
		O(4A)−C(20A)−O(5A)	122.3(11)
		O(6A)−C(21A)−N(4A)	118.8(16)
		O(5)−N(4)−C(21)	122.5(12)
		O(5)−N(4)−C(24)	120.8(11)
		C(21)−N(4)−C(24)	110.1(12)
		C(20)−O(5)−N(4)	112.0(11)

Conclusions

An indirect labeling method for preparing organometallic Tc and Re chelate complexes was developed. The process which involved microwave heating, *in situ* purification, and rapid solvent evaporation was efficient, high yielding, and produced the desired products in high effective specific activity. The entire synthetic procedure, which can be easily converted into an automated synthesis platform, was completed in less than 30 min, and the products were generated in high effective specific activity. Performing the same series of reactions using conventional heating and evaporation methods required greater than 2 h, and the decay corrected yields were not as high (57%) as those obtained using the microwave-V10 evaporator system (86%). The approach described here represents a movement away from conventional instant kits toward automated multistep synthesis and purification platforms which should help accelerate the process of discovering novel ^{99m}Tc and Re molecular imaging

and therapy agents and increase the number of new targeted agents entering the clinic. The technique will also be applicable for use with other nonstandard radionuclides such as the PET isotope ^{94m}Tc which has a relatively short half-life ($t_{1/2}$ = 54 min).

Acknowledgment. This work was supported by funding from the Canadian Institutes for Health Research (CIHR), the Ontario Institute of Cancer Research, and the Cancer Imaging Network of Ontario (CINO). We thank the staff of the MAX Diffraction Facility at McMaster University for performing the X-ray analyses and preparing the structure for publication.

Supporting Information Available: Complete spectral data for all novel Re compounds and relevant HPLC chromatograms for the radiolabeled compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

IC800775W