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Aqueous Fluoride and the Preparation of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ and ^{99m}Tc–Carborane Complexes

Oyebola O. Sogbein, Andrew E. C. Green, and John F. Valliant*

Departments of Chemistry and Medical Physics & Applied Radiation Sciences, McMaster University, Hamilton, Ontario, Canada L8S 4M1

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A method for the preparation of η^5-metallocarborane complexes of technetium-99m in water was developed. The key to the procedure is the use of aqueous sodium or potassium fluoride, which prevents premature degradation of the Tc(I) starting material used to prepare the carborane complexes. Solid-phase extraction was used to purify Tc-metallocarboranes derived from both ortho and meta isomers, which were isolated in good to excellent yields in high radiochemical purities. In conjunction with these studies, a series of fluoride-based "kits" were developed to produce the key precursor $[^{99m}Tc(CO)_3(H_2O)_3]^+$ in the absence of any other stabilizing ligand. Using this approach, $[^{99m}Tc(CO)_3(H_2O)_3]^+$ could be prepared directly from $^{99m}TcO_4^-$ under a range of pH values, including neutral pH, which affords the opportunity to develop one-pot labeling procedures for base-sensitive targeting vectors.

Introduction

Considering the substantial body of work that has been reported on the organometallic chemistry of rhenium,[1] there are comparatively very few examples of organometallic ^{99m}Tc complexes that have been described.[2] This is a consequence of the radioactive nature of the element and the fact that reactions performed with ^{99m}Tc, the most widely used radionuclide in diagnostic medicine, are performed under highly dilute reaction conditions in aqueous solution.[3] This often negates the possibility of applying traditional synthetic procedures used in organometallic chemistry to prepare Tc compounds for nuclear medicine applications.

The most widely investigated class of η^5-organometallic ^{99m}Tc compounds are of the type $CpTc(CO)_3$ (Cp = cyclopentadienide).[4] The only successful direct labeling strategy for preparing these complexes involves incorporating an electron-withdrawing acetyl substitutent on the Cp ring as a means of preventing side reactions including dimerization of the ligand.[5] We recently reported that it is also possible to prepare organometallic complexes of ^{99m}Tc using nido-carboranes in place of Cp.[6] The dicarbollide dianion, which is isolobal to Cp, is an attractive alternative because it is compatible with aqueous solvents, it is easier to functionalize with a wider range of substituents than traditional organometallic ligands, and metal–carborane complexes are often more stable than their Cp analogues.

Carboranes have been investigated extensively as ligands for carrying a range of different radionuclides including isotopes of iodine, ^{211}At, and ^{57}Co.[7] The objective of this study was to determine if the fluoride-based method we reported for the preparation of Re–carborane complexes could be used to synthesize the ^{99m}Tc analogues. The main difference being that the reactions involving ^{99m}Tc are run

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at extremely low concentrations of the metal (between 10^{-7} and 10^{-9} M). Furthermore, reactions should ideally reach completion within one half-life of the isotope (6 h), so that the procedures can be adapted in the future to prepare radiotracers for clinical use.

Experimental Section

Instrumentation. NMR spectroscopy was performed on a Bruker Avance DRX500 spectrometer at ambient temperature. The chemical shifts (δ) for ^{1}H and ^{13}C were recorded relative to residual solvent peaks as internal standards or through reference to trimethylsilane (TMS). $BF_3{\cdot}Et_2O$ was used as the reference standard for ^{11}B NMR experiments. Electrospray ionization (ESI) mass spectrometry experiments were performed on a Micromass Quattro Ultima instrument where samples were dissolved in 1:1 CH_3OH/H_2O or 1:1 CH_3CN/H_2O mixtures.

HPLC experiments were performed on a Varian Prostar Model 230 instrument, fitted with a Varian Prostar model 330 PDA detector and an IN/US γ-RAM gamma detector. The wavelength for detection was set at $\lambda = 254$ nm, and the dwell time in the gamma detector was 0.5 s with a 10 μL loop. Two different analytical columns were used: (1) a Varian Dynamax (L $\times$ ID = 250 $\times$ 4.6 mm), MicroSorb-MV analytical column (300−5 μ, RP-C18) and (2) a Varian Nucleosil (L $\times$ ID = 250 $\times$ 4.6 mm), analytical column (300−5 μ, RP-C18). Elution protocols: (Method A) Solvent A = 0.1% CF_3CO_2H in H_2O, solvent B = 0.1% CF_3CO_2H in CH_3CN. Gradient elution, 0−3 min, 100% A to 95% A; 3−6 min, 75% A; 6−9 min, 66% A; 9−20 min, 0% A; 20−22 min, 0% A; 22−24 min, 95% A; 24−25 min, 100% A. (Method B) Solvent A = H_2O, solvent B = CH_3CN. Gradient elution, same as Method A. **(**Method C) Solvent A = tetraethylammonuium phosphate (TEAP; pH = 2−2.5), solvent B = CH_3OH. Gradient elution, 0−3 min, 100% A; 3−6 min, 100% A to 75% A; 6−9 min, 75% A to 67% A; 9−20 min, 67% A to 0% A; 20−22 min, 0% A; 22−25 min, 0% A to 100% A; 25−30 min, 100% A. The flow rate for all methods was set at 1 mL/min.

Caution: *^{99m}Tc is radioactive and decays primarily via gamma emission. The isotope should be handled using the appropriate shielding and in a licensed facility.*

Methods. Synthesis of *meta*-$[RC_2B_9H_{11}]^-$, R = $CH_2CH_2CO_2H$, 5. Compound **4** (0.10 g, 0.434 mmol) and powdered potassium hydroxide (0.20 g, 3.4 mmol) were combined in a 95:5 (v/v) mixture of absolute ethanol/ddH_2O (8 mL) at room temperature under N_2(g). The suspension was heated to 90 °C for 12 h, cooled to room temperature, and CO_2(g) bubbled through the homogeneous solution to precipitate the excess KOH as K_2CO_3. The heterogeneous mixture was passed through a fritted funnel packed with Celite, which was subsequently washed with absolute ethanol (3 $\times$ 10 mL). After concentration of the filtrate under reduced pressure, the mixture was purified by flash chromatography through silica gel (isocratic elution: 1:9 CH_3OH/CH_2Cl_2 + 0.1% AcOH). The oily residue was suspended in ddH_2O (10 mL) and lyophilized at −80 °C to yield a white solid (0.049 g, 50%). TLC R_f (85:15 CH_2Cl_2/CH_3OH + 0.1% AcOH) = 0.28; ^{1}H NMR (500.13 MHz, 5:1 D_2O/CD_3OD): δ −0.5−2.8 (bm, BH), 0.94 (m, CH_2), 1.08 (m, CH_2); ^{13}C{^{1}H} NMR (50.3 MHz, CD_3OD): δ 18.27, 23.47, 32.35, 58.28, 180.44; ^{11}B{^{1}H} NMR (160.5 MHz, CD_3OD): δ −5.14, −6.41, −22.20, −23.19, −35.11, −36.14; FTIR (KBr, cm^{-1}): ν 3220, 2533, 1429; ESMS (negative ion): 205.48 $[M - H]^-$.

General Procedure for the Synthesis of $[^{99m}Tc(CO)_3(OH_2)_3]^+$. A penicillin vial (10 mL) containing $K_2[BH_3{\cdot}CO_2]$ (8.5 mg, 62.6 μmol), $Na_2B_4O_7$ (1.9 mg, 11.0 μmol), NaF (10.5 mg, 250 μmol), and Na_2CO_3 (4.0 mg, 37.7 μmol) was sealed with a rubber stopper and flushed with N_2 for 10 min. ^{99m}Tc-generator eluent (10−50 mCi, 370−1850 MBq) in 500 μL of saline was added by syringe, and the solution heated to 70 °C for 30 min followed by cooling in an ice bath. Quality control was performed by gradient HPLC (method A, 4.9 min, yields ≥ 95%).

General Procedure for Radiolabeling Carboranes. The carborane ligand in 500 mM degassed aqueous solution of NaF (500 μL) was added to a penicillin vial, which was stoppered, flushed with N_2, and then heated at 85 °C for 30−60 min. An aliquot of the solution containing the ligand was added directly to the solution containing $[^{99m}Tc(CO)_3(OH_2)_3]^+$ by syringe, and the reaction heated to 85 °C for 1.5−4 h. The reaction was subsequently cooled in an ice bath and loaded onto a Sep-Pak (Waters, C18). The Sep-Pak cartridge was conditioned prior to use with absolute ethanol (10 mL), acetonitrile (10 mL), 1:1 acetonitrile/10 mM HCl (10 mL), and 10 mM HCl (10 mL). After conditioning and loading, 1 mL fractions were collected following elution with 10 mM HCl (7 $\times$ 1 mL), 1:4 acetonitrile/10 mM HCl (2 $\times$ 1 mL), 1:1 acetonitrile/10 mM HCl (2 $\times$ 1 mL), 4:1 acetonitrile/10 mM HCl (2 $\times$ 1 mL), and finally acetonitrile (10 $\times$ 1 mL).

Results and Discussions

We have reported that fluoride ion can be used to prepare Re−carborane complexes from $[NEt_4]_2[Re(CO)_3Br_3]$ and $[Re(CO)_3(OH_2)_3]Br$. The analogous Tc starting material $[^{99m}Tc(CO)_3(OH_2)_3]^+$ can be prepared from $^{99m}TcO_4^-$, which is the starting material for all technetium labeling reactions, using a commercially available kit. Unfortunately, $[Tc(CO)_3(OH_2)_3]^+$ prepared from the commercial kit does not react with carborane ligands to any appreciable extent with or without fluoride. After much consideration, our hypothesis was that tartrate, which is present in the kit as a stabilizer, was having a detrimental impact on the overall yield. The development of a kit *sans* tartrate, however, also did not yield the desired product because $[Tc(CO)_3(OH_2)_3]^+$ decomposed under the reaction conditions needed to label carboranes. On the basis of the results of the stabilizing effects of fluoride that were observed for the reactions involving $[Re(CO)_3(OH_2)_3]^+$, an investigation into a fluoride-based formulation for preparing $[Tc(CO)_3(OH_2)_3]^+$ was undertaken.

To determine if $[Tc(CO)_3(OH_2)_3]^+$ could be prepared in the presence of fluoride and to see if the product would react with a carborane ligand, a series of preliminary reactions were performed using ^{99}Tc ($E_{\beta_{max}} = 0.294$ MeV, $t_{1/2} = 2.11 \times 10^5$ years). This particular isotope of technetium allows reactions to be carried out on a macroscopic scale (i.e., millimole) so that the products can be fully characterized by conventional methods (NMR, mass spectrometry, etc.). To this end, $[^{99}Tc(CO)_3(OH_2)_3]^+$ was synthesized by reacting $^{99}TcO_4^-$ with a mixture of $NaBH_4$, Na_2CO_3, CO, and KF and heating the reaction to 100 °C for 30 min (Scheme 1). The final concentration of KF in the reaction vial was 0.1 M. In a separate vial, compound **2** was incubated with 0.1

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Scheme 1

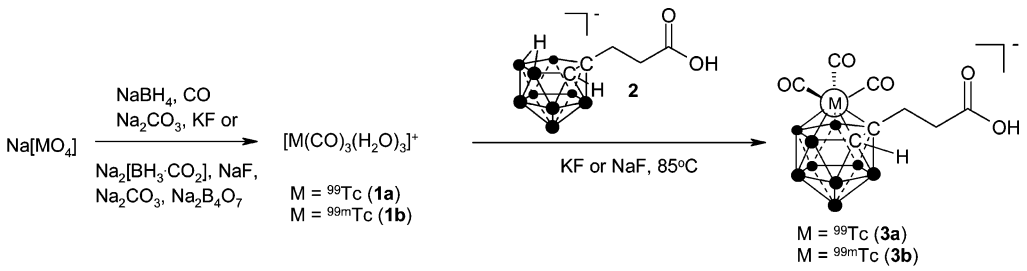

Table 1. Radiochemical Yield of **1b** Using Different Formulations (pH ≥ 12)[a]

	expt no.											
reagent	1	2	3	4	5	6	7	8	9	10	11	12
$K_2[BH_3{\cdot}CO_2]$ (mg)	4.0	4.0	4.0	5.5	7.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
$Na_2B_4O_7$ (mg)	3.5	4.5	3.0	3.0	3.0	3.0	2.5	2.2	1.9	1.9	1.9	1.9
NaF (mg)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	3.5	5.5	7.5
Na_2CO_3 (mg)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
yield (%)	20	15	25	35	55	55	75	80	80	80	85	85

[a] Further details are provided in the Experimental Section.

M KF at 85 °C for 1 h and subsequently added via syringe to the vial containing [^{99}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ and the reaction progress monitored by HPLC. An important observation was that pre-incubating the ligand with fluoride prior to complexation afforded better yields of the desired product for reasons that at present are not obvious.

The radiochromatogram (β^- detection) of the crude reaction mixture after 14 h showed a dominant peak at 19.1 min, which corresponded to that for the rhenium analogue. Despite the residual [^{99}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ present in the mixture, the product was readily isolated by HPLC in 25% yield. The negative-ion electrospray mass spectrum was consistent with the target mass, while the IR spectrum showed the expected features. This includes the carboxylic acid O−H stretch at 3451 cm^{-1}, the carborane B−H stretch at 2550 cm^{-1}, and the C≡O stretches at 2017 and 1928 cm^{-1}.

On the basis of the success in preparing [^{99}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ from $NaBH_4$ in the presence of fluoride, the reaction was repeated at the tracer level using ^{99m}TcO$_4^-$. HPLC (γ-detection) showed that after 30 min the desired product was the main reaction component, with unreacted ^{99m}TcO$_4^-$ and [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ being the only impurities. Interestingly, extended incubation of the reaction mixture at elevated temperatures for over 3 h resulted in only a small amount of decomposition of the technetium starting material. This observation is in contrast to [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ prepared in the absence of tartrate and fluoride which decomposed to a much greater extent under the same conditions, suggesting that fluoride ion has a unique stabilizing effect on the [Tc(CO)$_3$]$^+$ core.

The commercial kit that is normally used to prepare [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ consists of $Na_2[BH_3{\cdot}CO_2]$, Na/K-tartrate, $Na_2B_4O_7{\cdot}10H_2O$, and Na_2CO_3, and the ratio of each component had been established for optimal formation of the ^{99m}Tc-trisaquo species. The key ingredient is the boranocarbonate anion $(BH_3{\cdot}CO_2)^{2-}$, which acts as both the reducing agent (replacing $NaBH_4$), and the in situ source of CO.[8] As mentioned previously, when [^{99}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ prepared from the commercial kit was combined with a nido-carborane, the desired metallocarborane was not detected. Varying the reaction conditions, including the temperature and amount of ligand, did not facilitate the formation of the desired products. Because the boranocarbonate kit is so convenient for the routine preparation of [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ and avoids the need to use carbon monoxide, attempts were made to develop the equivalent fluoride-based kits.

Table 2. Radiochemical Yield of **1b** as a Function of Fluoride Concentration (pH ≥ 12)[a]

	expt no.				
reagent	13	14	15	16	17
$K_2[BH_3.CO_2]$ (mg)	8.5	8.5	8.5	8.5	8.5
$Na_2B_4O_7$ (mg)	1.9	1.9	1.9	1.9	1.9
NaF (mg)	8.5	9.5	10.5	11.5	25.5
Na_2CO_3 (mg)	4.0	4.0	4.0	4.0	4.0
yield (%)	90	95	95	95	95

[a] Further details are provided in the Experimental Section.

Tables 1−3 contain the results from the various experiments that were performed to optimize the radiochemical yield of [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ using fluoride in place of tartrate. Simply replacing tartrate in the commercial kit formulation with an equivalent amount of fluoride did not afford good yields of the desired product. Increasing the quantity of boranocarbonate, however, had a dramatic impact on the yield of [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ (Table 1, entries 4−6), which improved to 55%. After establishing the need to increase the amount of boranocarbonate, the next step was to adjust the quantity of borate. Borate acts to degrade excess boranocarbonate and prevent unwanted side-reactions with the Tc(I) cation once it has formed. The correct quantity of borate is crucial since an excess would act to degrade boranocarbonate too rapidly, whereas an insufficient quantity might lead to unwanted side-products. The commercial kit utilizes the decahydrate, $Na_2B_4O_7{\cdot}10H_2O$, and its molar ratio to $Na_2[BH_3{\cdot}CO_2]$ is approximately 1:6. Using this ratio as a

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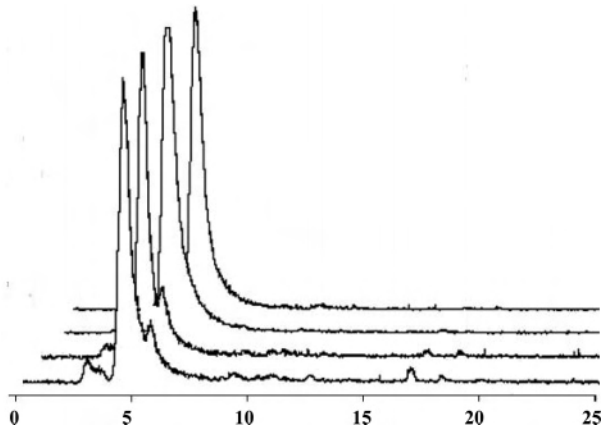

Figure 1. γ-HPLC radiochromatograms showing the conversion of $[^{99m}TcO_4]^-$ (t_R = 11 min) to $[^{99m}Tc(CO)_3(OH_2)_3]^+$ (t_R = 4.9 min) with increasing fluoride ion concentrations (Method C elution conditions): 260 mM (front), 404 mM (second from front), 500 nM (second from back), 1215 nM (back).

starting point, only poor yields of the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ cation were obtained. However, subsequent experiments established that higher yields of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ could be achieved when using reduced amounts of the anhydrous salt $Na_2B_4O_7$ instead of the decahydrated form (entries 7−9). In the end, the optimal ratio of anhydrous sodium borate to boranocarbonate was determined to be approximately 1:5, which prompted an investigation of the effect of fluoride ion concentration on the yield of $[^{99m}Tc(CO)_3(OH_2)_3]^+$.

We have demonstrated that, for certain ligands, increasing the concentration of fluoride from 0.1 to 1.0 M improved the yields of rhenacarboranes so long as the ligands remain soluble. Since the method for ^{99m}Tc-metallocarborane formation involved essentially the same procedure, it seemed logical that higher quantities of fluoride ion would also improve the average yield of $[^{99m}Tc(CO)_3(OH_2)_3]^+$. To test this hypothesis, a series of experiments were performed where the quantity of fluoride ion was increased incrementally (Table 1, entries 10−12; Table 2, entries 13−16), which, as predicted, gave corresponding improvements to the yield of $[^{99m}Tc(CO)_3(OH_2)_3]^+$. This is illustrated in Figure 1, which shows the γ-HPLC radiochromatograms from experiments involving different amounts of fluoride.

Entry 11 (260 mM NaF) in Table 1 showed high conversion of $[^{99m}TcO_4]^-$ to $[^{99m}Tc(CO)_3(OH_2)_3]^+$; however, some unidentifiable peaks appeared in the HPLC radiochromatogram (3.3, 5.5, and small peaks around 17 and 18 min). These peaks were not present for the reactions which employed 500 mM fluoride. Fluoride ion concentrations greater than this value produced the same results, hence further increasing the amount of fluoride was unnecessary.

With the existing commercial kit, to investigate the influence of pH on the efficiency of labeling it is necessary to adjust the pH of the solution after the synthesis of $[^{99m}Tc(CO)_3(OH_2)_3]^+$. A more convenient approach would be to design kits that would afford the ^{99m}Tc-trisaqua ion at specific pH values directly. Besides convenience, this approach would also create the opportunity for "one-pot" labeling procedures for biomolecules that are sensitive to high pH. To date however, $[^{99m}Tc(CO)_3(OH_2)_3]^+$ has only been prepared under highly basic reaction conditions. With the stabilizing influence of fluoride being apparent, attempts were made to prepare $[^{99m}Tc(CO)_3(OH_2)_3]^+$ directly at various pH values.

Table 3. Radiochemical Yield of **1b** as a Function of Different pH Values[a]

	expt no. (pH)			
reagent	18 (10−10.5)	19 (9−9.5)	20 (8−8.5)	21 (7−7.5)
$K_2[BH_3.CO_2]$ (mg)	8.5	8.5	8.5	8.5
$Na_2B_4O_7$ (mg)	1.9	1.9	1.9	1.9
NaF (mg)	10.5	10.5	10.5	10.5
$NaH_2PO_4{\cdot}H_20$ (mg)	0	0	7.8	8.0
Na_2HPO_4 (mg)	3.0	0	6.9	7.1
$NaHCO_3$ (mg)	5.0	5.2	0	0
Na_2CO_3 (mg)	0	4.0	0	0
yield (%)	95	95	95	95

[a] Further details are provided in the Experimental Section.

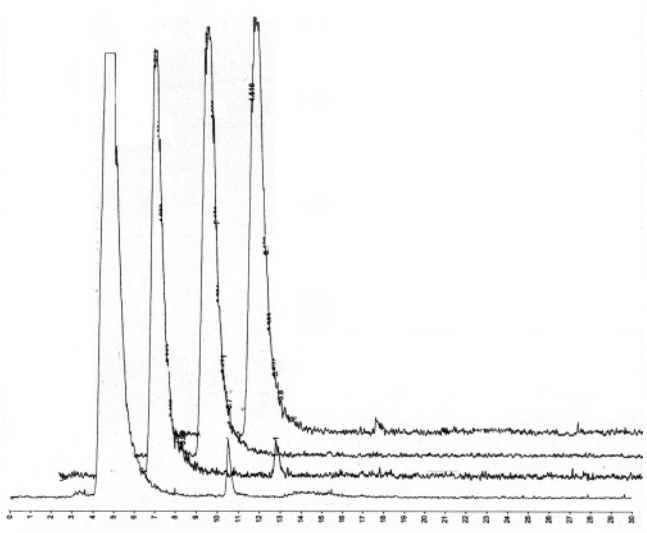

Figure 2. γ-HPLC radiochromatograms showing the conversion of $[^{99m}TcO_4]^-$ (t_R = 11 min) to $[^{99m}Tc(CO)_3(OH_2)_3]^+$ (t_R = 4.9 min) at different pH values (Method C elution conditions):10−10.5 (front), 9−9.5 (second from front), 8−8.5 (second from back), 7−7.5 (back).

A series of reactions were performed in different buffers where the pH of the mixtures were tested prior to and after formation of **1b**. This approach led to the successful development of several unique formulations for the preparation of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ at pH values ranging from 7 to 10.5. As illustrated in Table 3 (entries 18−21), the radiochemical yields of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ were unaffected by changes in pH. The γ-HPLC radiochromatograms from these entries are shown in Figure 2. This set of experiments represents the first report of the direct formation of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ from conditions that are not highly alkaline (pH ≥ 12) which makes developing one-pot kits for pH sensitive biomolecules a real possibility.

One aspect that was not discussed was the influence of temperature. The formation of the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ cation using the commercial kit is normally achieved at 100 °C. In the experiments described above, heating to reflux resulted in poor yields of the ^{99m}Tc-trisaqua ion. The optimal temperature for the fluoride mediated formation of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ was between 65 and 70 °C. Presum-

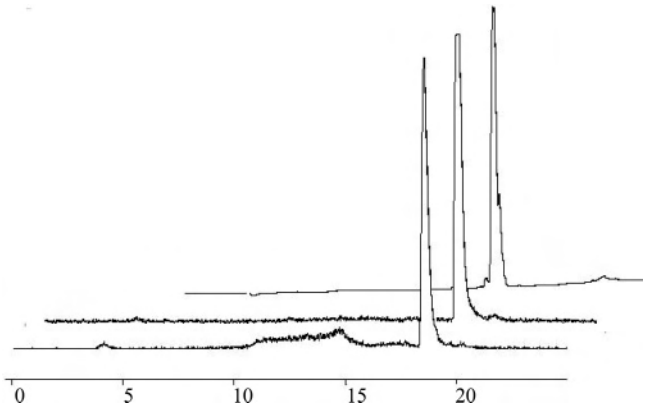

Figure 3. [Front] γ-HPLC radiochromatogram of the crude reaction mixture after 3 h at 85 °C; [Middle] γ-HPLC radiochromatogram of Sep-Pak purified **3b**; [Back] UV−HPLC chromatogram of the Re standard (method A elution conditions).

ably, the stabilizing influence of fluoride ion is diminished at temperatures above 70 °C leading to more rapid decomposition of [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$.

After establishing the necessary parameters needed to produce [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ in high yield, the synthesis of ^{99m}Tc-metallocarboranes was investigated. The initial experiments were performed with compound **2**[6] (Scheme 1) because the carborane is soluble in aqueous solutions and because the Re and ^{99}Tc-analogues which were prepared previously could be used as well-characterized reference standards. [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ was prepared from 185−740 MBq (5−20 mCi) of [^{99m}TcO$_4$]$^-$, using the optimal conditions described above (Table 2, entry 14). Compound **2**, which had been pre-incubated with fluoride ion, was subsequently added directly into the reaction vial. The temperature was raised to 85 °C from the initial 70 °C used to prepare [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$, and the reaction progress monitored by HPLC (Figure 3). After 3 h, the reaction was complete with the major product being the Tc-carborane with the minor impurities being [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ and [^{99m}TcO$_4$]$^-$.

The next step involved separating **3b** from residual [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$, Na[^{99m}TcO$_4$], and unreacted ligand. This could have been performed by semipreparative HPLC; however, this approach is time-consuming and impractical for routine clinical use. A convenient alternative involved solid-phase extraction using a commercially available C_{18} Sep-Pak cartridge. In the procedure described here, it was necessary to first condition the Sep-Pak with acetonitrile, ethanol, and 10 mM HCl. The entire crude reaction mixture was then loaded onto the column and eluted with 10 mM HCl. Residual [^{99m}TcO$_4$]$^-$ and [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ are both removed under these conditions, while the target complex remains on the Sep-Pak. After elution with acetonitrile, the product was obtained in greater than 99% purity (Figure 3).

One important feature of this purification protocol is that it results in the removal of excess ligand, giving the product in high effective specific activity. The importance of this result lies in the fact that removal of residual ligand from the purified product assists in the prevention of unwanted biological effects once the tracer is administered. Furthermore, if a ligand and its ^{99m}Tc complex have similar affinities for a particular receptor, then the excess unlabeled ligand can prevent binding of the ^{99m}Tc-labeled compound to the target.

Another important criterion that needed to be evaluated was stability. In vivo, there are many endogenous ligands that can degrade ^{99m}Tc complexes through transmetalation reactions.[3] To test the stability of the ^{99m}Tc-metallocarborane toward ligand exchange, compound **3b** was incubated with a 1000-fold excess of cysteine and histidine in separate experiments. After incubation at 37 °C in phosphate-buffered saline (PBS, pH = 7.2) for 24 h, the γ-radiochromatograms from both experiments indicated greater than 99% of the product remained intact. The stability of the complexes supports the potential use of ^{99m}Tc-metallocarboranes as synthons for preparing radiopharmaceuticals.

To study the factors that impact the yield of **3b**, pH, ligand concentration, and fluoride ion concentration were systematically varied. With the exception of the ligand concentration, these adjustments were made to the kit used to prepare [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$, taking advantage of the initial work on developing different fluoride kits, which allow for direct control of pH and fluoride ion concentration.

The mild reaction conditions afforded by the fluoride-based method for the preparation of the rhenacarboranes cannot necessarily be directly correlated to chemistry at the tracer level. The discrepancy lies in the fact that, at the tracer level, [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ is normally prepared under highly basic conditions whereas the corresponding rhenacarboranes are prepared effectively at neutral pH. Since the initial method used to prepare ^{99m}Tc-metallocarboranes involved direct incubation of the nido-carborane ligand with [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ without any adjustment of pH, the question remained as to whether fluoride mediates the metalation reaction or whether product formation was a consequence of the high pH.

To investigate the impact of basicity, labeling reactions were performed at various pH values using a fixed fluoride ion concentration of 0.1 M and reaction time of 3 h. As Figure 4 illustrates, the yield of **3b** increases with increasing pH and that a high pH is needed to afford good yields of the ^{99m}Tc-metallocarborane, which is not the case for rhenium at the macroscopic level. The results do demonstrate that it is possible, albeit in reduced yields, to prepare metallocarboranes at low pH, which will be important in cases where base-sensitive targeting vectors are attached to the cluster. These results also indicate that fluoride ion is clearly involved in mediating the formation of **3b** since a yield of 40% was observed at neutral pH at a ligand concentration of 10^{-2} M. In the absence of fluoride under the same conditions, no product was detected.

Figure 4 also summarizes the results of a series of experiments that were performed to evaluate the effect of changing the concentration of the ligand on the yield of the Tc-metallocarborane. The results clearly demonstrate that the concentration of the ligand does play a significant role since the percent conversion roughly doubles when the ligand concentration is increased to 10^{-2} from 10^{-4} M. The

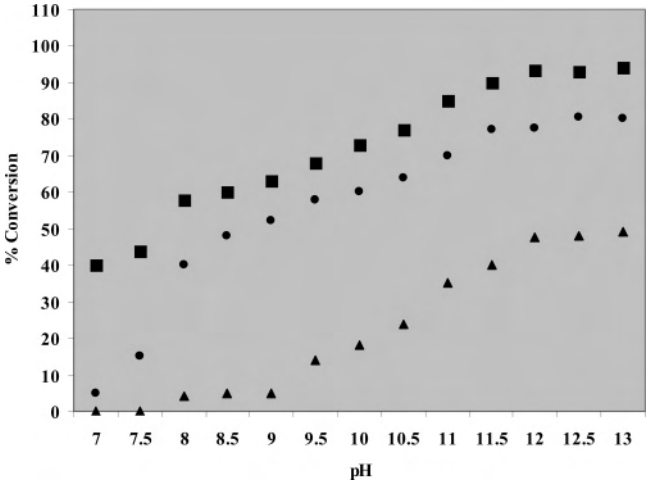

Figure 4. Formation of **3b** as a function of pH and ligand concentration for the reaction of **2** with $[^{99m}Tc(CO)_3(OH_2)_3]^+$ after 3 h at ligand concentrations of (▲) 10^{-4}, (●) 10^{-3}, and (■) 10^{-2} M.

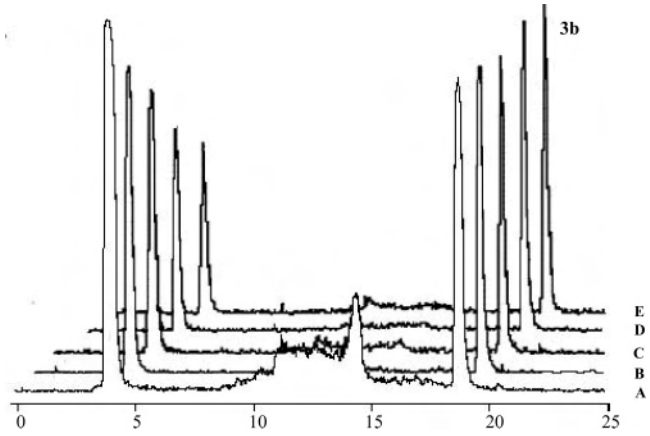

Figure 5. γ-HPLC radiochromatograms showing the formation of **3b** at [A] 10, [B] 50, [C] 100, [D] 500, and [E] 1000 mM NaF (pH ≥ 12; [L] = 10^{-3} M) (Method A elution conditions).

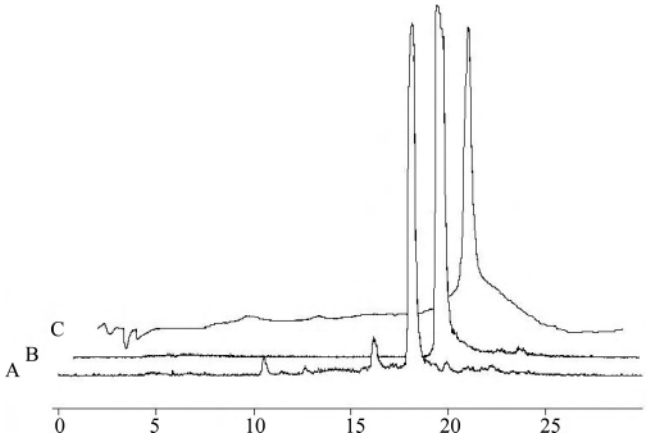

Figure 6. [A] γ-HPLC radiochromatogram of the crude reaction mixture after 90 min; [B] γ-HPLC radiochromatogram of Sep-Pak purified **6**; [C] UV chromatogram of the Re standard (Method A elution conditions).

importance of a low ligand concentration for preparations that do not involve further purification lies predominantly in minimizing side effects and/or receptor saturation once the radiotracer is administered in vivo. In this respect, both the Cp^- and the [*nido*-7,8-$C_2B_9H_{12}]^-$ anion are less efficient than the bi- and tridentate chelates for Tc(I) where Schibli and co-workers have demonstrated that mild radiolabeling conditions (30 min, 75 °C) can be used to prepare complexes of $[^{99m}Tc(CO)_3(OH_2)_3]^+$ with tridentate chelates in yields of ≥95% at ligand concentrations ranging from 10^{-4} to 10^{-6} M.[9] In the case of the AcCp system, the concentration of the ligand used was $10^{-3}-10^{-4}$ M. Although carborane concentrations of $10^{-3}-10^{-4}$ M do not produce as high a yield as the AcCp system, this limitation is overcome since excess ligand can readily be removed using the simple purification method described above. The general utility of this approach for purifying other carboranes requires further investigation.

Another important factor that was investigated was the impact of changing the fluoride ion concentration on the yield of ^{99m}Tc-metallocarborane formation. A series of experiments were performed where $[^{99m}Tc(CO)_3(OH_2)_3]^+$ was prepared in the presence of varying amounts of fluoride ion (10, 100, 500, and 1000 mM NaF) followed by the addition of compound **2**. The progress of the reaction was monitored by HPLC (Figure 5) which showed that increasing the fluoride ion concentration increases the yield of the product. This is consistent with the results observed for rhenium.

One of the attractive features of carboranes is that they can be derivatized at both the boron and the carbon vertexes with a range of different functionalities. Furthermore, dicarba-*closo*-dodecaboranes exist as three isomers in which the relative position of the cage carbon atoms change. This affords the opportunity to vary the relative orientation of substituents located off of the cage, which can in turn be used to optimize the binding of carboranes and metallocarboranes to the ligand-binding domain of target receptors.

To determine if Tc-labeled meta-carboranes could be prepared at the tracer level under similar conditions used to prepare ortho-Tc-metallocarboranes, the meta-carborane analogue of compound **2** was reacted with $[^{99m}Tc(CO)_3(OH_2)_3]^+$ (Scheme 2). To allow for a direct comparison of the reactivities of the two isomers toward the *fac*-$[^{99m}Tc(CO)_3]^+$ core, a series of reactions were performed using the nido-carborane **5**. This ligand was prepared by treating compound **4** with alcoholic KOH, which gave compound **5** in modest yield (50%).

At a ligand concentration of 10^{-3} M, compound **5** was combined with $[^{99m}Tc(CO)_3(OH_2)_3]^+$ (85 °C, pH 12) in 0.5 M fluoride. Under these conditions, nearly quantitative conversion of **5** to the desired product was observed in the HPLC radiochromatogram in approximately half of the reaction time (1.5 h) required to achieve the maximum yield of compound **3b** (Figure 6). Afterward, the Sep-Pak purification protocol described above was employed to isolate compound **6** in 70% yield free from residual ligand. Cysteine and histidine challenge experiments were performed under the same conditions used for **3b** where there was no sign of decomposition.

To determine if pendent donor groups would have any impact on the labeling reaction, a problem which has been

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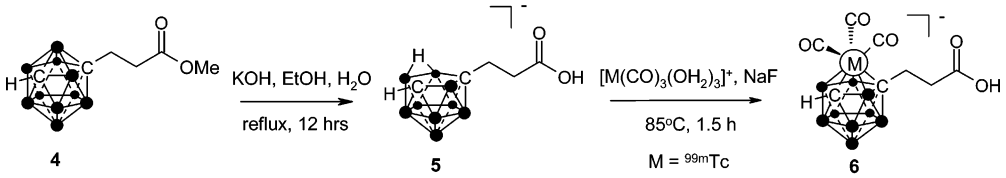
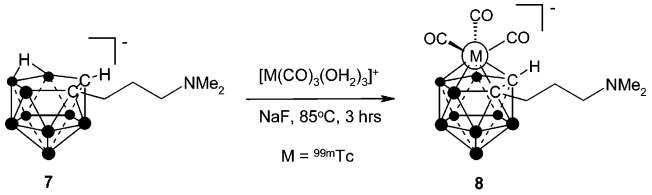
Scheme 2



Scheme 3



reported previously for more traditional organometallic ligands, attempts were made to label the dimethylamino carborane derivative **7**[6] (Scheme 3). The initial yield of **8** was not as good as that for compounds **3b** and **6** due primarily to the poor solubility of the ligand in aqueous fluoride. To improve the solubility, a small amount of base was added to liberate the amine (compound **7** is typically prepared as an internal salt) prior to complexation. With this modification, the isolated yield of the desired product improved to 70% after 3 h at a ligand concentration of 10^{-2} M.

Conclusions

In summary, aqueous fluoride can be used to facilitate the preparation of [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ and Tc-labeled carboranes. Simple formulations were developed that allow for the synthesis of [^{99m}Tc(CO)$_3$(OH$_2$)$_3$]$^+$ at specific pH values, including those that are much less basic than what is produced using the commercially available kit. In terms of the radiosynthesis of ^{99m}Tc-metallocarboranes, a small collection of ligands were successfully labeled and the products isolated free from any unreacted ligand. The products displayed resistance to ligand exchange by both cysteine and histidine. It was determined that a number of factors, most notably ligand and fluoride concentrations and pH influence the overall yield of the reaction. Having the ability to label carboranes with Tc creates the means to explore the possibility of using the substantial number of targeted carborane derivatives that have been developed for boron neutron capture therapy[10] as the basis for designing novel radiopharmaceuticals for imaging tumors. Furthermore, it may also be possible to use fluoride to generate "one-pot" kits for preparing Tc(I) radiopharmaceuticals and for improving the labeling yields of other ligand systems and bioconjugates, which did not show satisfactory conversion to the desired products when reactions were carried out using the existing commercial kit.

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