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Preparation and Properties of $^{99m}\text{Tc}(\text{CO})_3^+$ -Labeled *N,N*-Bis(2-pyridylmethyl)-4-aminobutyric Acid

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Abstract

Labeling biomolecules with $^{99m}\text{Tc}(\text{CO})_3^+$ (^{99m}Tc tricarbonyl) is attracting increasing attention. Although histidine is often considered an ideal bifunctional chelator for ^{99m}Tc (or ^{188}Re) tricarbonyl, the family of dipicolylamine carboxylate chelators may be a useful alternative because of the expected ease of synthesis and because the structure provides a pendent carboxylate for potential conjugation to biomolecules. The dipicolylamine chelator *N,N*-bis(2-pyridylmethyl)-4-aminobutyric acid (BPABA) was synthesized using 4-aminobutyric acid in place of glycine or aminopropionic acid in the literature, to avoid possible involvement of the carboxylate in the complex formation process by forming five- or six-membered chelation rings. Using a commercial tricarbonyl kit (Mallinckrodt), the complex formation properties of both BPABA and commercial histidine with ^{99m}Tc tricarbonyl were investigated, and the in vitro complex stabilities in saline and in serum were compared. Stability in vivo was also examined following i.v. administration to normal mice. BPABA was synthesized simply and quantitatively by reacting picolyl chloride with aminobutyric acid in one step. On RP HPLC, the product eluted essentially in one peak and the structure was confirmed by ESI-MS. After labeling, both BPABA and histidine were shown by RP HPLC to form tricarbonyl complexes. In both cases, after incubation at 100 °C for 20 min, only one predominant peak of $^{99m}\text{Tc}(\text{CO})_3^+$ -histidine or $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA was apparent, and both complexes were stable at room temperature in saline for at least 24 h. After incubation for 24 h in 37 °C serum, by SE HPLC, 20% of the $^{99m}\text{Tc}(\text{CO})_3^+$ -histidine was bound to serum protein compared to less than 10% for $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA. A 5000 molar excess of histidine at 100 °C for 6 h was unable to dissociate $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA. By contrast, BPABA easily dissociated $^{99m}\text{Tc}(\text{CO})_3^+$ -histidine under the same conditions. Both complexes were stable in vivo in mice, and $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA showed rapid and specific hepatobiliary clearance while $^{99m}\text{Tc}(\text{CO})_3^+$ -histidine was cleared through the kidneys. In conclusion, BPABA was easily synthesized and was shown to possess properties comparable to histidine for labeling of biomolecules with ^{99m}Tc tricarbonyl. However, it was found that the chelator concentration required for quantitative ^{99m}Tc tricarbonyl labeling with both BPABA and histidine were 2 orders higher than that required with more conventional labeling using MAG_3 . Finally, the complex $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA itself was found to clear exclusively via the hepatobiliary pathway and may have value as a potential hepatobiliary imaging agent.

INTRODUCTION

We have been investigating tumor imaging by pretargeting with antibodies conjugated with phosphodiamidate morpholino oligomers (MORFs) along with ^{99m}Tc -radio-labeled complementary MORFs as effector using MAG_3 as chelator (1–4). Our overall objective is to

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perform ^{188}Re radiotherapy using this MORF pretargeting approach. While MAG_3 performs well as a chelator for $^{99\text{m}}\text{Tc}$, it falls short of ideal for the chelation of ^{188}Re . Preliminary studies showed that, when using MAG_3 as a chelator for ^{188}Re in the labeling of MORF, oxidization to $^{188}\text{ReO}_4^-$ during preparation was excessive (5). Since more suitable chelators for ^{188}Re are therefore still needed, the ^{188}Re -tricarbonyl core was investigated as an alternative labeling strategy because of the predicted kinetic inertness of these complexes (6–8). Initially, the ^{188}Re tricarbonyl labeling was achieved with Hynic-MORF (9). Evaluation showed improved stability of the label to oxidation but with kidney levels high enough to be problematic with ^{188}Re (or $^{99\text{m}}\text{Tc}$). We suspected that the tricarbonyl complexes (10,11) was binding to robust groups such as imidazole in kidney tissue proteins possibly through interaction of the open position of the tricarbonyl complex of the bidentate Hynic chelator.

Numerous ligands have now been recommended as chelators for labeling biomolecules with technetium or rhenium tricarbonyl cores, and it now seems clear that bidentate chelators are not ideal because of the remaining open position (10,11). Of the tridentates considered, those containing the basic formula of [aromatic N-heterocycle]–[amine]–[carboxylate] have received the most attention (8,10–14), although the tridentates [aromatic N-heterocycle]–[amine]–[thiol] (15) and [thioether]–[thioether]–[carboxylate] (16) have also been tested. Examples of rhenium tridentate tricarbonyl complexes are shown in Figure 1. Recently, when *N,N*-dipicolyl-glycine or *N,N*-di(imidazolylmethyl)glycine was allowed to react with $\text{Re}(\text{CO})_3^+$, the complex with the two aromatic N-heterocyclic nitrogens and the amine in coordination was obtained leaving the carboxylate free (17). This suggested that tridentates with the formula of [aromatic N-heterocycle]–[amine]–[aromatic N-heterocycle] might be preferable for technetium or rhenium tricarbonyl labeling over [aromatic N-heterocycle]–[amine]–[carboxylate].

Lately we have become particularly interested in tridentates of *N,N*-dipicolyl- ω -amino acids. Besides providing the [aromatic N-heterocycle]–[amine]–[aromatic N-heterocycle] chelation site, these molecules have a pendent carboxylate group suitable for conjugation to the amines on biomolecules. They can be synthesized in one step simply by reacting picolyl chloride with the corresponding ω -amino acid (17).

We report herein on the labeling with $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ of a new dipicolylamino acid and the properties of the complex. While our ultimate goal is to develop a chelation strategy for $^{188}\text{Re}(\text{CO})_3^+$, in this investigation $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ was used instead because of the ready availability of $^{99\text{m}}\text{Tc}$ and its tricarbonyl kit with a recognition of the structural similarities between rhenium and technetium.

MATERIALS AND METHODS

Bis(2-pyridylmethyl)-4-aminobutyric acid (BPABA), also called dipicolylaminobutyric acid, was synthesized in house while histidine was purchased from Aldrich (St. Louis, MO). The MS analysis was performed by the UMMS Proteomics and Mass Spectrometry Facility. The Isolink kits for synthesizing $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ were gifts from Mallinckrodt Medical (St Louis, MO). Each lyophilized kit contained 8.5 mg of sodium tartrate, 2.85 mg of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, 7.15 mg of sodium carbonate, and 4.5 mg of sodium boranocarbonate. The $^{99\text{m}}\text{TcO}_4^-$ was eluted from a ^{99}Mo - $^{99\text{m}}\text{Tc}$ generator (Bristol-Myers Squibb Medical Imaging, Inc., North Billerica, MA). All other chemicals were reagent grade and were used without purification.

Synthesis of Bis(2-pyridylmethyl)-4-aminobutyric Acid (BPABA)

The reaction was achieved essentially according to a reported procedure (17) with a modification in separation method. The 2-chloromethylpyridine hydrochloride (33.7 g, 196 mmol) and 4-aminobutyric acid (8.3 g, 80 mmol) were dissolved in 100 mL of water, and the

pH was adjusted to 10 and maintained in the range of 8–10 for 4 d by adding 5 M NaOH at intervals (total ~18 g, 450 mmol). The black reaction mixture was washed with CHCl_3 (70 mL $\times$ 4), followed by pH adjustment to 8 and concentration of the aqueous phase via evaporation. Toward the end of the evaporation, concentrated HCl was added to facilitate removal of as much NaCl as possible. The product was characterized by ESI-MS and HPLC.

HPLC Analysis

Reverse phase HPLC (RP HPLC) with an YMC-Pack C-18 column (system 1) was used for separation and analysis during synthesis of BPABA, while a Vydac C-18 column (system 2) was used for analysis during the $^{99\text{m}}\text{Tc}$ tricarbonyl labeling. Size exclusion HPLC (SE HPLC) was also used for in vitro and in vivo stability investigations of the $^{99\text{m}}\text{Tc}$ tricarbonyl complexes (system 3).

System 1—Semipreparative YMC-Pack C-18 column (250 $\times$ 10 mm, 5 μm , 323 Å); 10 mM HCl as solvent A and acetonitrile as solvent B; flow 2.0 mL/min. Gradient table: 0–15 min, 90% A; 15–20 min, 90–70% A; 20–23 min, 70–90% A, and 23–35 min, 90% A.

System 2—The Vydac C-18 column (5 μm , 250 $\times$ 4.6 mm); 0.05 M TEAP (triethylamine phosphate, pH 2.0) as solvent A and methanol as solvent B; 1.0 mL/min. Gradient table: 1–3 min, 100% A; 3–6 min, 100%–75% A; 6–9 min, 75%–66% A; 9–20 min, 66%–0% A; 20–27 min, 0% A; 27–40 min, 0%–100% A; 40–50 min, 100% A.

System 3—The Superose-12 size-exclusion column; 0.10 M pH 7.2 phosphate buffer; isocratic flow 0.6 mL/min.

Formation of $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ Complexes

The $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ core was prepared fresh daily by introducing 1 mL of $^{99\text{m}}\text{TcO}_4^-$ saline solution into a kit and heating in boiling water. An incubation time of 20 min was routinely used. After neutralization with 120 μL of 1 N HCl, an equal volume of aliquot was added to a vial containing either 1 mM BPABA or 1 mM histidine of pH 7.0 and heated in a boiling water bath normally for 20 min. Both the labeled compounds of $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA or $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –histidine were characterized by RP HPLC (system 2) and SE HPLC.

In Vitro Stability of $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ -Labeled BPABA and Histidine

The stabilities of both complexes were tested by diluting the labeled solution 100 fold in saline or human serum, incubating at 37 °C, and analyzing at intervals using SE HPLC.

The affinity of $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ for BPABA relative to histidine was also measured by mixing $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA in 100 mM histidine at a molar ratio of BPABA to histidine of 1:5000 and incubating either at 37 °C for 24 h or in a boiling water bath 6 h. The identical procedure was also performed starting with $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –histidine in 100 mM BPABA.

In Vivo Stability and Biodistribution of $^{99\text{m}}\text{Tc}$ Tricarbonyl-Labeled BPABA

A group of four normal CD-1 mice (35–45 g) were each injected with 70 μCi of $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA or $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –histidine via a tail vein. After 35 min, the mice were sacrificed under halothane anesthesia. Selected organs were harvested and rinsed, and their radioactivity was measured in a Cobra II auto-gamma counter (Packard, Downers Grove, IL). To provide an estimate of in vivo stability, plasma, urine, and an extract from small intestine contents were analyzed by SE HPLC. Plasma was obtained by adding 0.5 mL of blood from each mouse into a tube containing 0.068 mL of 7.5% K_3EDTA followed by centrifugation at

650–700 g for 10 min. The small intestine extract was obtained by washing with 4 mL of water and centrifuging the wash at 2500g for 20 min.

RESULTS

Characterization of BPABA

Analysis by RP HPLC (System 1) reveals one major peak at 7.8 min with over 90% of peak area and a small peak at 8.7 min, presumably due to $(\text{PyCH}_2)_2\text{N}(\text{CH}_2)_3\text{COOH}$ and $(\text{PyCH}_2)_3\text{N}^+(\text{CH}_2)_3\text{COOH}$. The broad peak at 29 min is due to the solvent.

The ESI-MS analysis of BPABA shows a product peak attributed to $[(\text{PyCH}_2)_2\text{NH}^+(\text{CH}_2)_3\text{COOH} [\text{MH}^+ : 286.2 \text{ } m/z, \text{ abundance } 100\%; \text{ corresponding isotope peak at } 287.2 \text{ } m/z, \text{ abundance } 17\%]]$ and a byproduct peak due to the quaternary ammonium salt $(\text{PyCH}_2)_3\text{N}^+(\text{CH}_2)_3\text{COOH} [\text{M}^+ : 377.2 \text{ } m/z, \text{ abundance } 43\%; \text{ corresponding isotope peak at } 378.2 \text{ } m/z, \text{ abundance } 11\%]$. All other peaks are lower than 10% (data not presented). Confirmation of structure is apparent in that fragmentation of the molecular ion peak 286.2 m/z provide a series of fragment peaks: $(\text{PyCH}_2)_2\text{NH}^+(\text{CH}_2)_3\text{COOH}$, m/z 286.2, abundance 15.5%; $(\text{PyCH}_2)_2\text{N}(\text{CH}_2)_3\text{CO}^+$, m/z 268.2, abundance 25%; $(\text{PyCH}_2)_2\text{NH}_2^+$, m/z 200.2, abundance 13%; $\text{Py-CH=NH}^+(\text{CH}_2)_3\text{COOH}$ m/z 193.1, 25.0%; $\text{Py-CH=N}(\text{CH}_2)_3\text{CO}^+$ m/z 175.1, 16%.

Formation Kinetics of $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA vs $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –Histidine

On RP HPLC (System 2) the $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ core elutes at 4 min (Figure 2A) as described by the manufacturer and forms complexes with both BPABA and histidine (Figures 2B and 2C), resulting in single peaks and indicating a radiochemical purity of over 90%. Reaction times over 5–40 min in boiling water makes no difference in complex formation as shown by HPLC profiles identical to that of Figure 2B or 2C (data not presented). However, heating more than 60 min considerably increases the impurity peaks with both $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA and $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –histidine (data not presented). It was therefore judged safe in this study to employ a routine heating time of 20 min for complex formation.

In Vitro Stability of $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA vs $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –Histidine

Both $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA and $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –histidine are unaffected by 24 h incubation in 37 °C saline, but both show serum protein binding after 24 h incubation in 37 °C human serum (data not presented). The percentage of serum binding with $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –histidine is higher than that with $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA (20 vs 9.5% at 24 h), indicating that $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –histidine may be less stable against transchelation to proteins than $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA under these conditions.

Ligand exchange between BPABA and histidine was also investigated by SE HPLC (system 3). In this system, the $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ core itself does not elute probably due to interaction of the core with the cross-linked agarose matrix. The HPLC chromatograms of $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –histidine and $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA are shown in Figure 3A and 3D, respectively. Incubation of $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –histidine with a 5000 molar excess of BPABA pH 7 at 37 °C over 1 d shows minimal replacement of histidine by BPABA (Figure 3B); however, heating of the same mixture in a boiling water bath for 6 h induces almost total replacement of histidine by BPABA (Figure 3C). In contrast, incubation of $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ –BPABA with a 5000 molar excess of histidine shows no replacement of BPABA (Figure 3E) even at 100 °C for 6 h (Figure 3F). The capability of BPABA to replace histidine in its $^{99\text{m}}\text{Tc}(\text{CO})_3^+$ complex, but not vice versa, reflect a higher affinity of BPABA for $^{99\text{m}}\text{Tc}$ tricarbonyl.

Biodistribution and In Vivo Stability of $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$ vs $^{99m}\text{Tc}(\text{CO})_3^+-\text{Histidine}$

Table 1 presents the biodistribution in normal mice at 35 min postadministration of $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$ and $^{99m}\text{Tc}(\text{CO})_3^+-\text{histidine}$. The biodistribution of $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$ shows rapid hepatobiliary excretion with 57% in the small intestine, 5% in the large intestine, and 15% in liver including gall bladder at 35 min. Less than 8% was excreted into urine at this time. By SE HPLC analysis, only 30% (2.4%ID) of the radioactivity in urine was present as $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$. The remaining urine radioactivity was due to the approximately 5% radiochemical impurities in the $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$ injectate. While clearance of these impurities via the kidneys was rapid and quantitative, the excretion of $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$ via the kidney was largely negligible. By contrast, $^{99m}\text{Tc}(\text{CO})_3^+-\text{histidine}$ is predominantly excreted through kidney as reported previously (10, 11) with 50% of $^{99m}\text{Tc}(\text{CO})_3^+-\text{histidine}$ in urine in 35 min. Though rapid, the kidney excretion is not as rapid as typical kidney imaging agents such as $^{99m}\text{Tc}-\text{BPHA}$ (18), $^{99m}\text{Tc}-\text{DTPA}$ (19), and $^{99m}\text{Tc}-\text{MAG}_3$ (20).

The in vivo stabilities of $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$ and $^{99m}\text{Tc}(\text{CO})_3^+-\text{histidine}$ were evaluated by comparing the SE HPLC radiochromatograms of the injectates with that of urine, plasma, and extracts of small intestine obtained from animals administered the complexes 35 min earlier. As shown in Figure 4, $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$ is still intact in plasma as well as in the wash from the small intestines. Because of the negligible urinary excretion of $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$ (less than 8% ID) the HPLC profile of urine is not presented. Like $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$, $^{99m}\text{Tc}(\text{CO})_3^+-\text{histidine}$ is also intact in plasma and in urine. Because of the negligible hepatobiliary excretion of $^{99m}\text{Tc}(\text{CO})_3^+-\text{histidine}$, an HPLC profile of small intestine wash was not obtained in this case. These results show that both $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$ and $^{99m}\text{Tc}(\text{CO})_3^+-\text{histidine}$ are “stable” in vivo.

DISCUSSION

In this investigation, BPABA has been chosen as a model compound because of its ease of synthesis and the presence of a pendent carboxylate group for conjugation to biomolecules. In addition, the molecule was selected to avoid five- or six-membered chelation rings with the ^{99m}Tc tricarbonyl core. Thus free BPABA should exhibit similar labeling properties to conjugated BPABA and therefore should serve as a reliable model for the behavior of BPABA-conjugated biomolecules. The result of this investigation show that the properties of complex formation and stability of BPABA are comparable, if not superior to, that of histidine, often considered a model compound for ^{99m}Tc tricarbonyl labeling (10,11,14).

Our interest in tricarbonyl labeling is in connection with phosphoramidate morpholino (MORF) pretargeting in which it will eventually be necessary to radiolabel MORF with ^{188}Re and in a manner that avoids unacceptably large reoxidation to $^{188}\text{ReO}_4^-$. Because of the recognized kinetic inertness of these complexes (6–8), tricarbonyl labeling of biomolecules via BPABA is expected to avoid the oxidation problem at least to a greater degree than experienced in this laboratory with a more conventional labeling method for ^{188}Re using MAG_3 as a chelator (5). However, a drawback to the use of the tricarbonyl strategy resulting from kinetic inertness is “high” chelator concentration required for quantitative labeling (10,11), a phenomenon verified in this research. A chelator concentration of 10^{-4} or 10^{-5} M was required for a labeling efficiency of 90%, even with histidine. By contrast, the concentration of MAG_3 can be reduced to below 10^{-6} M when labeling MAG_3 -conjugated MORF or phosphorothioate DNA while still achieving a labeling efficiency of over 90% (21).

Parenthetically, it may be worth mentioning that the results presented here on the properties of $^{99m}\text{Tc}(\text{CO})_3^+-\text{BPABA}$ suggest that this complex may have utility for imaging the hepatobiliary system. The ^{99m}Tc -labeled hepatobiliary imaging agents include $^{99m}\text{Tc}-\text{IDAs}$ (22,23), ^{99m}Tc -pyridoxylidine glutamate (24), ^{99m}Tc -penicillamine (25), and $^{99m}\text{Tc}-\text{MAG}_3$ –

biocytin (26,27), with ^{99m}Tc -IDAs being the most extensively investigated. Both ^{99m}Tc -IDAs and ^{99m}Tc -MAG₃-biocytin are excreted exclusively via the hepatobiliary pathway in contrast to both ^{99m}Tc -pyridoxylidene glutamate and ^{99m}Tc -penicillamine. Similar to the ^{99m}Tc -IDAs (<97% ID) and ^{99m}Tc -MAG₃-biocytin (>90% ID), $^{99m}\text{Tc}(\text{CO})_3$ -BPABA is also cleared exclusively via the hepatobiliary pathway (97% ID).

The mechanism of hepatobiliary processing is reported to be different among these agents. As shown in Figure 5, the structures of the ^{99m}Tc -IDAs, ^{99m}Tc -MAG₃-biocytin and $^{99m}\text{Tc}(\text{CO})_3$ -BPABA are fundamentally different and, as a result, each is expected to clear by a different mechanisms of hepatobiliary excretion. For example, ^{99m}Tc -IDAs and ^{99m}Tc -MAG₃-biocytin must clear by different mechanisms since only the former can be blocked by endogenous bilirubin (26) with the result that hepatobiliary function in patients with high serum bilirubin levels can be evaluated only with ^{99m}Tc -MAG₃-biocytin. Since $^{99m}\text{Tc}(\text{CO})_3$ -BPABA possesses a different structure to those of ^{99m}Tc -IDAs and ^{99m}Tc -MAG₃-biocytin, the mechanism by which $^{99m}\text{Tc}(\text{CO})_3$ -BPABA clears through the liver may be different as well. Therefore, should $^{99m}\text{Tc}(\text{CO})_3$ -BPABA be investigated as a potential imaging agent for the hepatobiliary system, it may be shown to be cleared via a unique transporter, the function of which could then be evaluated.

CONCLUSION

The tridentate chelator *N,N*-bis(2-pyridylmethyl)-4-aminobutyric acid (BPABA) is easily synthesized and forms a “stable” complex with $^{99m}\text{Tc}(\text{CO})_3^+$. The property of stability might apply to technetium as well for use in radiotherapy studies. Further development efforts are needed to improve the labeling recipes for high specific activity. Finally, the $^{99m}\text{Tc}(\text{CO})_3$ -BPABA complex itself is hepatobiliary specific and might be useful in imaging malfunction of specific transporter in liver.

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Figure 1.
Examples of chemical structures between Re tri-carbonyl and tridentates of different classes.

Figure 2. Radiochromatograms by RP HPLC (System 2) of $^{99m}\text{Tc}(\text{CO})_3^+$ core (panel A) and $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA and $^{99m}\text{Tc}(\text{CO})_3^+$ -histidine following incubation at 37 °C for 40 min or less (panels B and C, respectively).

Figure 3.

Radiochromatograms by SE HPLC (System 3) of $^{99m}\text{Tc}(\text{CO})_3^+$ -histidine alone (panels A) and after incubation with a 5000-fold molar excess of BPABA for 24 h at 37 °C (panel B) and 6 h at 100 °C (Panel C). Also presented are radiochromatograms of $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA alone (panels D) and after incubation with a 5000-fold molar excess of histidine for 24 h at 37 °C (panel E) and 6 h at 100 °C (Panel F).

Figure 4.

Radiochromatograms by SE HPLC (System 3) of $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA injectate (top left panel) and $^{99m}\text{Tc}(\text{CO})_3^+$ -histidine injectate (top right panel) and analysis of plasma (middle row), extract from the small intestines (in the case of $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA) (left bottom panel), and urine (in the case of $^{99m}\text{Tc}(\text{CO})_3^+$ -hisidine) (right bottom panel) from an animal injected 35 min earlier.

Figure 5. Structures of reported hepatobiliary specific imaging agents. (A) ^{99m}Tc -IDAs, (B) ^{99m}Tc -MAG3-biocytin, and (C) $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA.

Table 1
Biodistribution in Normal Mice of $^{99m}\text{Tc}(\text{CO})_3^+$ -BPABA and $^{99m}\text{Tc}(\text{CO})_3^+$ -Histidine at 35 min^a

organ	BPABA		histidine	
	mean	SD	mean	SD
		(a) %ID/g		
liver	8.72	2.38	2.77	0.27
heart	0.29	0.08	0.69	0.07
kidneys	4.27	1.72	6.65	1.32
lungs	0.55	0.10	1.94	0.11
spleen	0.40	0.15	0.83	0.12
muscle	0.13	0.05	0.38	0.02
blood	0.73	0.16	1.54	0.16
		(b) %ID/organ		
liver	14.95	3.23	5.49	1.05
heart	0.05	0.01	0.14	0.03
kidneys	2.78	1.11	5.27	1.25
lungs	0.12	0.02	0.43	0.04
spleen	0.04	0.01	0.09	0.03
stomach	1.08	0.15	0.65	0.12
sm. intestines	57.35	8.78	3.35	0.28
lg. intestines	5.14	1.19	0.57	0.07
muscle	1.83	0.64	6.90	0.63
blood	1.87	0.29	4.85	0.74
whole body	92.5	1.7	50.6	4.0

^a Mean of %ID or %ID/g with standard deviation (SD), $n = 4$.