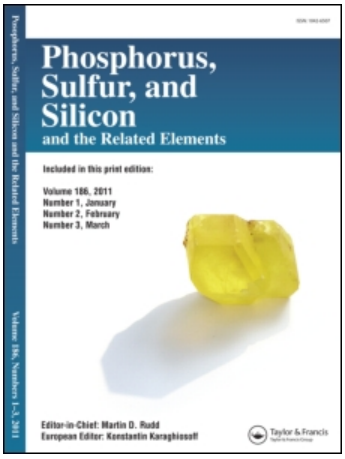

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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D. Scott Edwards; Shuang Liu

To cite this Article Edwards, D. Scott and Liu, Shuang(1999) 'Tc-99m Radiopharmaceuticals Based on a Ternary Ligand System Comprised of a Water Soluble Phosphine', Phosphorus, Sulfur, and Silicon and the Related Elements, 144: 1, 493 — 496
To link to this Article: DOI: 10.1080/10426509908546289
URL: http://dx.doi.org/10.1080/10426509908546289

Phosphorus, Sulfur and Silicon, 1999, Vol. 144-146, pp. 493-496
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Tc-99m Radiopharmaceuticals Based on a Ternary Ligand System Comprised of a Water Soluble Phosphine

D. SCOTT EDWARDS and SHUANG LIU

DuPont Pharmaceuticals Company, N. Billerica, MA 01862 USA

A series of Tc-99m-labeled, hydrazinonicotinamide (Hynic) functionalized biologically active molecules (BAMs) have been synthesized using different combinations of tricine and a water soluble phosphine (L) as ancillary ligands. One of these, DMP444, Tc-99m-(cyclo(D-Val-NMeArg-Gly-Asp-Mamb(5-(6-hydrazinonicotinylamido)hexanamide)))(tricine)(TPPTS) is currently in clinical trials as a thrombus imaging agent. The ternary ligand complex of a chemotactic peptide conjugate, Tc-99m-(fMLFKHynic)(tricine)(TPPTS) has been found to image infection in a rabbit model. The syntheses were performed in one step in high yield and high specific activity (~20,000 Ci/mmol) and the complexes were shown to be stable for > 6 h in the reaction mixture and upon dilution. The advantages of this ternary ligand system for Tc-99m radiopharmaceuticals include: the ability to form the complexes in high yield using low concentrations of the Hynic functionalized BAMs; stability both in vitro and in vivo; and the ability to tailor the lipophilicity and charge of the complexes by the choice of the water soluble phosphine.

Keywords: Tc-99m radiopharmaceuticals; peptides; water soluble phosphines

INTRODUCTION

Radiopharmaceuticals comprised of Tc-99m labeled biologically active molecules (BAMs) have been under extensive investigation for over a decade for imaging a variety of organs or disease states. The first BAMs to be used were generally monoclonal antibodies or other large proteins. These compounds suffered from slow extravazation and blood clearance due to their large size, resulting in low target to background ratios. The recent focus in this field has been on the use of small, highly active peptides or peptidomimetics as the BAMs. These small BAMs can be chemically synthesized and generally have fast localization and background clearance. Tc-99m labeled peptides are currently under clinical evaluation for imaging thrombosis, cancer, infection and inflammation. We describe a novel ternary ligand system comprised of a water soluble phosphine for the Tc-99m labeling of peptides and other small BAMs. The resulting Tc-99m complexes are formed in high yield and high specific activity and exhibit excellent in vitro and in vivo stability.

RESULTS AND DISCUSSION

The ternary ligand system is comprised of a hydrazinonicotinamide (Hynic) derivatize BAM, tricine (N-tris(hydroxymethyl)methylglycine), and a water soluble phosphine (L). (See Figure 1) The use of tricine to label Hynic derivatized proteins with Tc-99m has been previously described.[1] However, we found in applying this chemistry to a Hynic derivatized small peptide, the resulting binary ligand complex, ^{99m}Tc(Hynic)(tricine)$_2$, was not stable in solution and existed as a mixture of a numb of coordination isomers.[2] We reasoned that addition of a phosphine co-ligand to for a ternary ligand system would result in complexes with increased stability and fewer isomeric forms due to the steric and electronic influence of the phosphine ligand.[3]

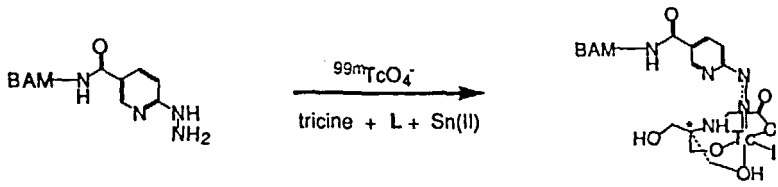

FIGURE 1 ^{99m}Tc(Hynic)(tricine)(phosphine) radiopharmaceuticals.

Using a platelet glycoprotein IIb/IIIa receptor antagonist cyclic peptide, cyclo(D-Val-NMeArg-Gly-Asp-Mamb(5-(6-hydrazinonicotinylamido)hexanamide)), derivatized with Hynic, we synthesized a series of ternary ligand complexes of the formula ^{99m}Tc(Hynic)(tricine)(L) using different sulfonated aryl, sulfonated aralkyl or carboxyalkyl phosphines. (See Figure 2) The syntheses were performed in one step in high yield. (See Table 1) The reaction of ~50 mCi of $^{99m}TcO_4^-$, 20 - 40 mg tricine, 1 - 10 mg phosphine, 20 - 100 µg stannous chloride, and 5 - 20 µg of the Hynic peptide in 1.0 mL of saline at 50 to 80 °C for 30 min resulted in >70% yield for all the phosphine tested, as determined by reverse-phase, radio-HPLC. The retention times for the complexes show the expected trend; the complexes with the more lipophilic phosphine having the longer retention times. The times reported are the average values for the two peaks that are seen for each complex, resulting from the resolution of two diastereomers formed due to the presence of a chiral substituent on the Hynic (i.e. the peptide) and the chiral technetium complex. The complexes with the sulfonated aryl phosphines, RP444, RP445, and RP446, were shown to be stable for >6 h in the reaction mixture and after dilution 100-fold with saline. These three complexes were evaluated as thrombus imaging agents in a canine deep vein thrombosis model.[4]

TABLE 1 Analytical and yield data for IIb/IIIa antagonist complexes

Phosphine	% Yield	Ret.Time(min)
TPPTS (RP444)	>90	10.4
TPPDS (RP445)	>90	12.8
TPPMS (RP446)	>90	15.9
TPEPTS	70	10.0
TPPPTS	83	12.7
TCEP	70	9.0

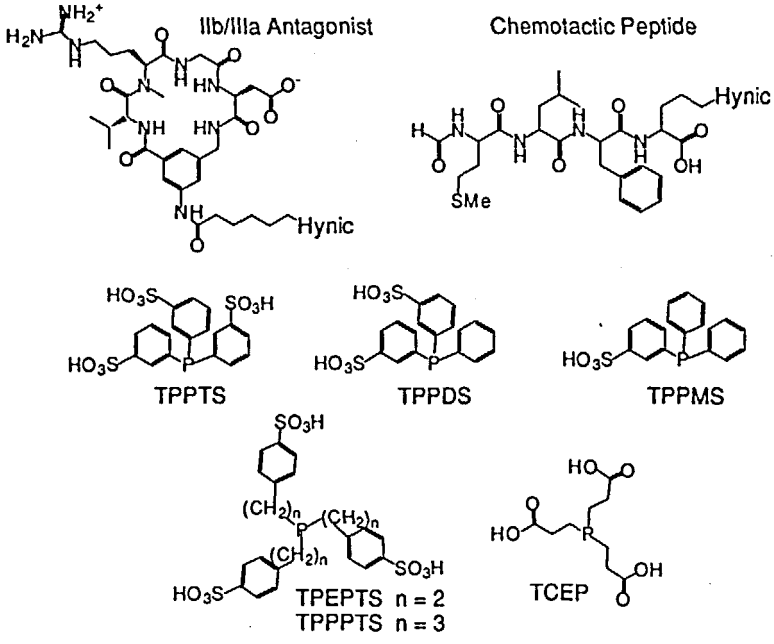

FIGURE 2 Structures of biologically active molecules and phosphines.

The complexes RP444, RP445 and RP446 localized in thrombi formed in the jugular veins of adult beagle dogs to a significant extent (2.9 ± 0.4 - 3.4 ± 0.9% i.d./g) and all cleared rapidly from the circulation ($t_{1/2}$ ~90 min). Clear images of the thrombi were obtained as soon as 15 min post-injection and by 2 h the thrombus-to-background

were obtained as soon as 15 min post-injection and by 2 h the thrombus-to-background ratios were ~10:1. RP444 is currently in clinical trials for imaging deep vein thrombosis (DVT), arterial thrombosis, and pulmonary embolism. For these studies, a patient typically receives 20 - 25 mCi of RP444 along with approximately 10 μg of the Hynic peptide conjugate, 20 mg of tricine, and 2-3 mg of TPPTS. Preliminary results indicate that RP444 is a promising radiopharmaceutical for imaging DVT.[5]

We have also synthesized the ternary ligand complex of the Hynic derivatized chemotactic peptide (CTP), formyl-Met-Leu-Phe-Lys-Hynic (see Figure 2), for use as an infection imaging agent.[6] The chemotactic peptide formyl-Met-Leu-Phe is a potent agonist (EC_{50} = ~3 nM) of the CTP receptor on white blood cells and plays a key role in the recruitment of white cells to sites of infection as part of the body's natural defense mechanism. The complex ^{99m}Tc(fMLFKHynic)(tricine)(TPPTS) was evaluated in a rabbit model of focal infection, in which an infection was instilled in a hind leg by injection of e. coli bacteria. The complex showed good uptake at the infection site (~0.6% i.d./g) and provided target-to-background ratios of ~12:1 at 4h post injection. However, since this complex is a very potent agonist and elicited a transient but substantial neutropenic response in the rabbits, it is not a candidate for clinical evaluation.

In summary, we have described a new ternary ligand system for labeling biologically active small molecules with Tc-99m for use as radiopharmaceuticals. We synthesized complexes using two very different peptides, one a cyclic IIb/IIIa receptor antagonist and the other a linear chemotactic peptide receptor agonist, and a series of water soluble phosphines. The complexes were formed in high yield and exhibited very good stability both in vitro and in vivo. The biological activity of the peptides was maintained as evidenced by the excellent images that were obtained in animal models of thrombosis and infection. This is quite remarkable considering the significant increase (>2x) in size and molecular weight in going from the peptides to the ternary complexes. The complexes are also formed using only 5 - 20 μg of the Hynic conjugates, which is critical if the peptide or other biologically active molecule is very potent, since radiopharmaceuticals must give diagnostically useful information without eliciting a clinically significant response or side-effect. We are currently exploring other radiopharmaceutical applications of this versatile ternary ligand system.

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