Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 111, No. 3, June 1999, pp. 425–436
© Indian Academy of Sciences

Formylation of functionalized P–H bonds – A novel approach to the design of synthons for use in biomedicine

KATTESH V KATTI
University of Missouri, Center for Radiological Research, 106 Allton Building Laboratories, DC117.0, Columbia, Missouri 65211, USA
e-mail: kattik@health.missouri.edu

Abstract. Formylation of phosphorus–hydrogen bonds has become a versatile strategy to producing compounds with phosphorus–carbon bonds. However, the utility of such reactions on functionalized phosphines is a recent development. Our laboratory has developed new approaches to the synthesis of phosphorus(III) hydrides functionalized with main group backbones (e.g. thioether: (P_2S_2), alkyl amino (P_2N_2)). The formylation of P_2S_2 and P_2N_2 functionalized phosphorus(III) hydrides, using formaldehyde, produced hydroxymethyl-substituted $(P(CH_2OH)_n)_n$ compounds. Recent results suggest that formylation of P-H bonds occur under mild conditions in aqueous media without the aid of transition metal catalysts. The hydroxymethyl (HMP) functionalized P_2S_2 and P_2N_2 phosphines are highly soluble in water and display oxidative-stability in aqueous media. The HMP functionalized phosphines react with the early (e.g. Re(V)) and late transition metals (e.g. Rh(I), Pd(II), Pt(II), Ag(I) and Au(I)), under biphasic (aqueous-organic) solvent media to produce a new generation of water-soluble transition metal compounds. The electronic characteristics of HMP groups dictate high kinetic inertness and thermal stability in aqueous media and therefore provide a novel access to stabilize specific oxidation states of transition metals in aqueous media. Our laboratory has also concentrated its efforts on the application of HMP-based ligands in the design of site-directed and tumor-specific radiopharmaceuticals. This interest stems from the fact that the development of *in vivo*-stable $^{186/188}$rhenium (and ^{99m}Tc) and ^{199}gold compounds and their conjugation to specific biomolecular vectors (e.g. peptides or proteins) will provide new avenues in the discovery of cancer diagnostic, and more importantly, therapeutic pharmaceuticals. Our studies have demonstrated that the HMP ligands upon complexation with technetium-99m and gold-198, produce complexes that are highly stable under *in vivo* conditions as evidenced through studies in experimental animal models. Details on ligand design and transition metal/organometallic chemistry as they relate to the development of aqueous-soluble transition metal/radiometal compounds with potential applications in nuclear medicine will be discussed.

Keywords. Organometallic chemistry; synthons; phosphorus-hydrogen bonds; formylation; transition metals; HMP functionalized phosphines; biomedicine.

1. Introduction

Chemical reactions that result in the formation of compounds that possess phosphorus-carbon bond(s) have been known for several decades[1]. Since that time, research in phosphorus chemistry has produced myriad organic and inorganic phosphorus compounds that contain phosphorus (III or V)–carbon bonds[1–3]. Phosphorus–carbon bonded compounds are versatile in terms of their usefulness as: (a) ligands for use in the development of catalytically useful transition metal compounds (e.g. tri(aryl) or tri(alkyl)

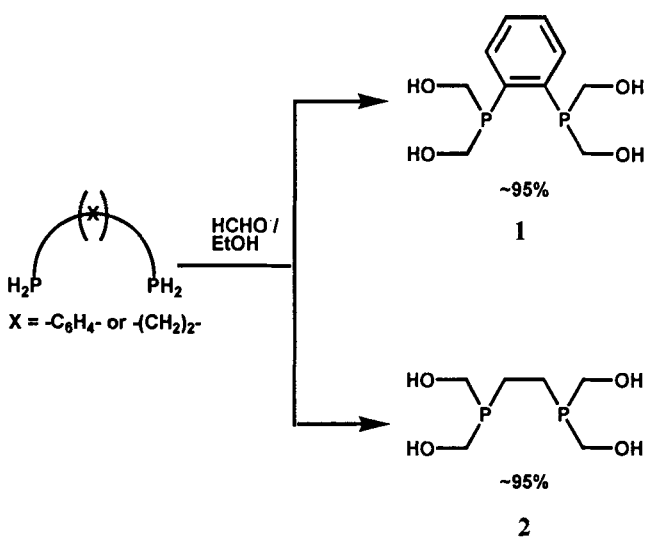
phosphines)[1,4]; (b) chemical precursors or synthons in the development of antimicrobial and antibactericidal agents[5]; (c) chemical intermediates in the development of broad spectrum herbicides (e.g. glyphosates and related compounds)[5] and; (d) chelating frameworks for use in complexation reactions with radiometals[6]. The technetium-bonded functionalized phosphines are being currently used as radiopharmaceuticals for the diagnosis of specific cancers and other diseases in humans (e.g. Technecard[7] and ^{99m}Tc-Tetrofosmin[8] are Tc-99m complexes derived from mono and bisphosphines respectively and are both being used as myocardial perfusion agents)[7,8].

The chemical, catalytic, biological and biomedical implications of phosphorus-carbon compounds are unlimited. This unique aspect of phosphorus chemistry continues to provide burgeoning interest in the discovery of chemical reactions that result in new compounds with phosphorus-carbon bonds. The following account will summarize contributions from our laboratory to the field of phosphorus chemistry. In particular, (a) the development of a new family of phosphorus-carbon-bonded water-soluble phosphines; (b) their utility in the design and development of transition metal/radiometal compounds; and (c) their potential applications in biomedicine will be described.

2. Experimental

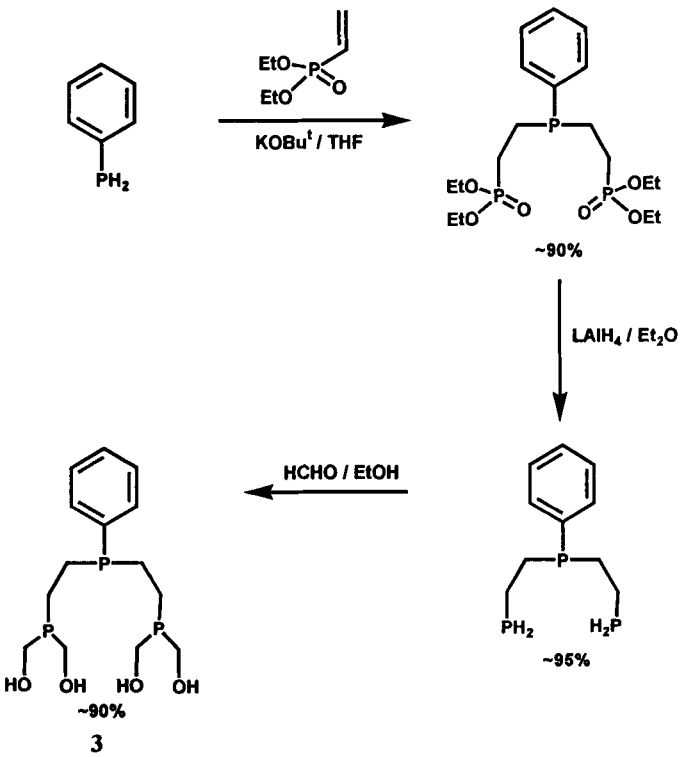
2.1 *Synthesis of hydroxymethyl functionalized phosphines*

The approach developed in our laboratory for the synthesis of hydroxymethyl functionalized phosphines involved hydroformylation of P-H bonds of appropriate phosphines as outlined in scheme 1. For example, the formylation of H_2PXPH_2 using formaldehyde produced R_2PXPR_2 (X = CH_2CH_2, R = CH_2OH, **1**, and X = C_6H_4; R = $-CH_2OH$ **2**) in good yields (scheme 1)[9,10].



Scheme 1.

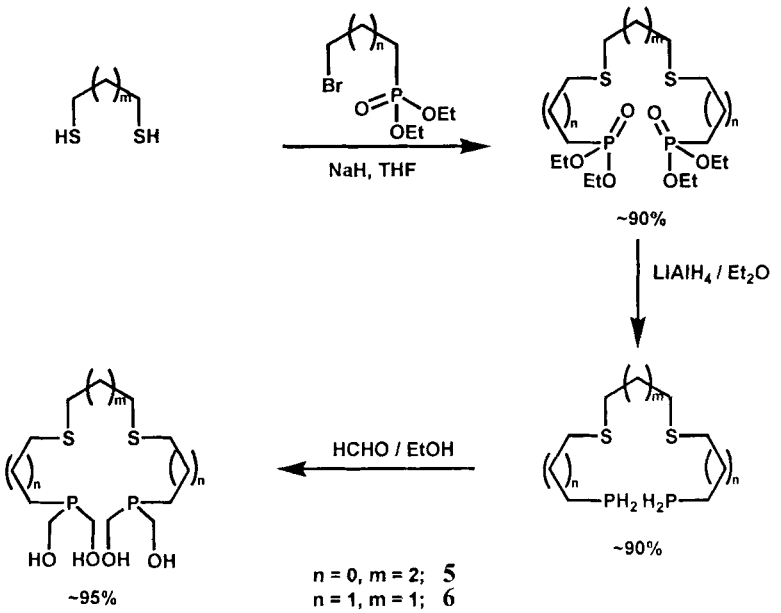
A tripodal phosphine **3** which combines the properties of aryl and alkyl functionalized phosphines was prepared via formylation reaction of a novel triphosphorus(III) hydride as outlined in scheme 2 [11].



Scheme 2.

Examples of bisphosphines functionalized with thioether frameworks are rare and such phosphines may show versatility in terms of their bonding with transition metals. A new approach to the design and development of thioether functionalized *bis* phosphines is outlined in scheme 3 [12]. This synthetic approach which makes use of alkane dithiols and bromoalkyl phosphonates is versatile because the chain length across the sulphur-sulphur and phosphorus-carbon skeletons can be readily varied to allow systematic tuning of chemical flexibility across the P^{III} centres [13]. This kind of chemical flexibility plays a vital role in the overall coordination chemistry with specific transition metals [13].

Our studies have demonstrated that the formylation of P-H bonds (schemes 1–3) occur under relatively mild conditions (e.g. at 25°C in ethanol) and in most instances the hydroxymethyl-functionalized phosphines are produced in > 90% yields in high purity. Further, the reactions outlined in schemes 1–3 are fairly general and have allowed the development of tetra and hexaphosphines as shown in figure 1 [14]. Complete characterization of all the new phosphines including the phosphorus hydride intermediates has been achieved using ^{31}P, ^{13}C and ^{1}H NMR spectroscopy. Fast atom bombardment mass spectroscopy of all new phosphines have provided parent ions corresponding to their molecular constitutions. In several instances, the structures of new phosphines have been



Scheme 3.

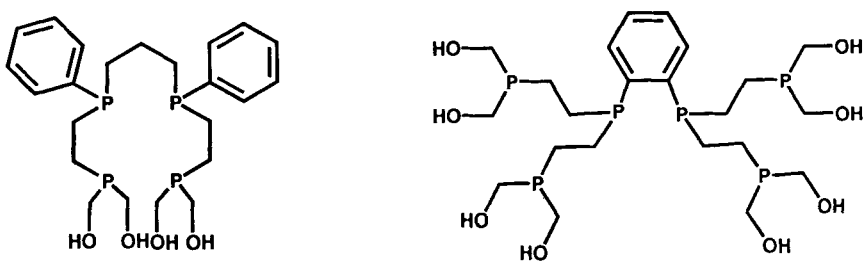


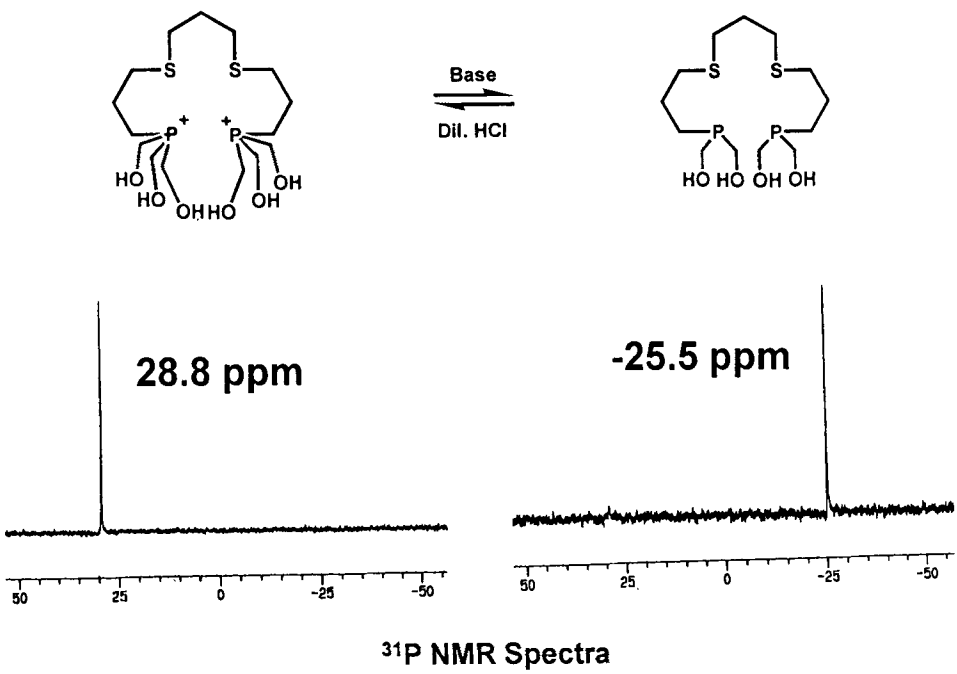
Figure 1. Examples of hydroxymethyl functionalized tetra- and hexaphosphines.

proved through X-ray diffraction analysis of single crystals of their transition metal complexes (vide infra).

2.2 *Oxidative-stability and aqua-behavior*

The two most important criteria for establishing applications in biomedicine are that the ligands under specific use must display aqueous-stability and reasonable solubility. It is important to note that the hydroxymethyl phosphines **1–6**, described in schemes 1–3, possess a high degree of oxidative-stability and are soluble in water or mixed water-ethanol media. The ethane bridged *bis*(dihydroxymethylphosphino)ethane **2** is comparable in terms of alkane chain length and necleophilicity of the P^{III} centres, to that of *bis*(dimethylphosphino) ethane (commonly referred to as DMPE). Whereas DMPE ignites

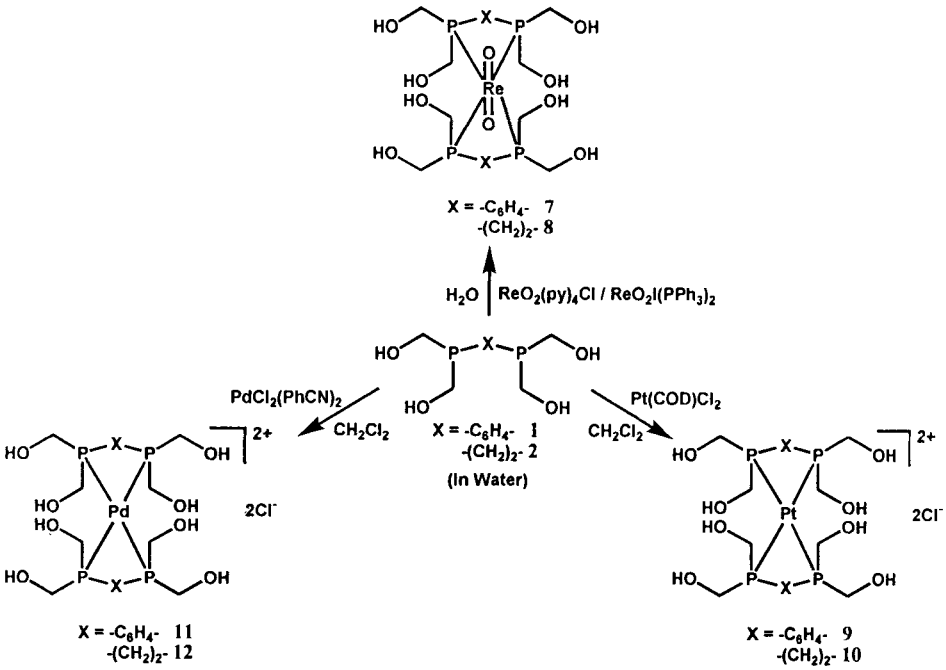
and catches fire upon contact with atmospheric moisture the corresponding hydroxymethyl phosphine analogue **2** is oxidatively stable in aqueous media. In fact our studies have demonstrated that the P_2S_2 phosphines **5** and **6** (shown in scheme 3) can be readily converted into their phosphonium chlorides upon interaction with hydrochloric acid according to scheme 4. The phosphonium chlorides revert back to the parent phosphines **5** or **6** upon treatment with triethyl amine or sodium bicarbonate buffer in aqueous media. It is important to recognize that the P_2S_2 phosphines **5** and **6** display remarkable oxidative-stabilities in aqueous media over the *p*H range 1–9 over extended periods of time.



Scheme 4.

2.3 *Coordination chemistry*

The hydroxymethyl phosphines **1** and **2** are excellent chelating agents for reactions with early and late transition metals as outlined in scheme 5. The reactions outlined in scheme 5 generally produced products in excess of 90% yields and occur under aqueous-organic biphasic media[9,10,15,16]. For example, the bisphosphines **1** and **2** dissolved in water react with $Pt(COD)\cdot Cl_2$ (COD = cyclooctadiene); dissolved in CH_2Cl_2 under biphasic conditions to produce the corresponding water-soluble Pt(II) complexes in near quantitative yields (scheme 9)[9,15]. The reactions outlined in scheme 5 to produce complexes **7–12** are "strictly" biphasic because upon simple vortexing of solutions of ligands (in aqueous phase) and metal precursors (dissolved in organic solvents), more than 95% of the metal precursors are transferred into the aqueous media. The metal



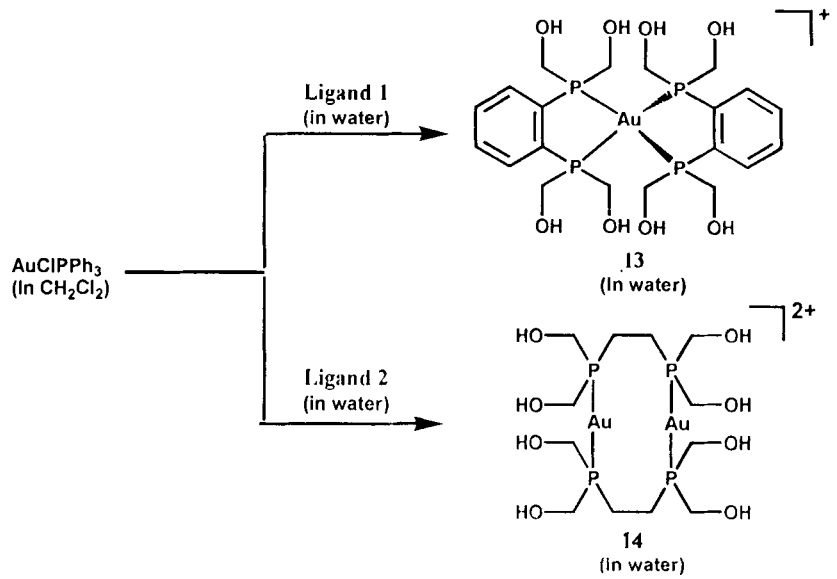
Scheme 5.

complexes are isolated in the aqueous phase upon simple separation from the organic phase[9–16].

The reactions of **1** (and **2**) with gold(**I**) precursors, as outlined in scheme 6, exemplify the rich transition metal chemistry of these ligands to producing products under biphasic conditions [17,18].

Detailed characterization of all the metal complexes, described in schemes 5 and 6, has been carried out using NMR spectroscopic (^{1}H, ^{13}C and ^{31}P NMR) and X-ray diffraction analysis [9,18]. For example, the dioxo Re(V) cores in **7** and **8** [10] and the square planar geometries of Pt(II) and Pd(II) in **9–12** [9,16] have been confirmed by X-ray diffraction studies of their single crystals. The distorted tetrahedral arrangement of phosphines around Au(**I**) in **13** and the near linear P-Au-P arrangement in **14** were also confirmed by X-ray crystallographic investigations [17,18]. The salient bond distance and other X-ray crystallographic parameters of complexes **9–14** are summarized in table 1.

While the reactions summarized in schemes 5 and 6 provide examples of transition metal complexes (e.g. **7–14**) that possess a ratio of 1:2 between the metal and the coordinated ligand, for specific biomedical (vide infra) and also catalytic applications, it is important to have a ratio of 1:1 between a transition metal and ligand [13]. In this context, the tripodal phosphine (described in scheme 2) and the thioether functionalized bisphosphines (outlined in scheme 3) provide ample opportunities for tuning the geometries and coordination number of transition metal centres. For example, the tridentate phosphine **3**, upon reactions with Pd(II), Pt(II) and Rh(I) precursors, produced the corresponding tripodally-coordinated water-soluble novel transition metal compounds



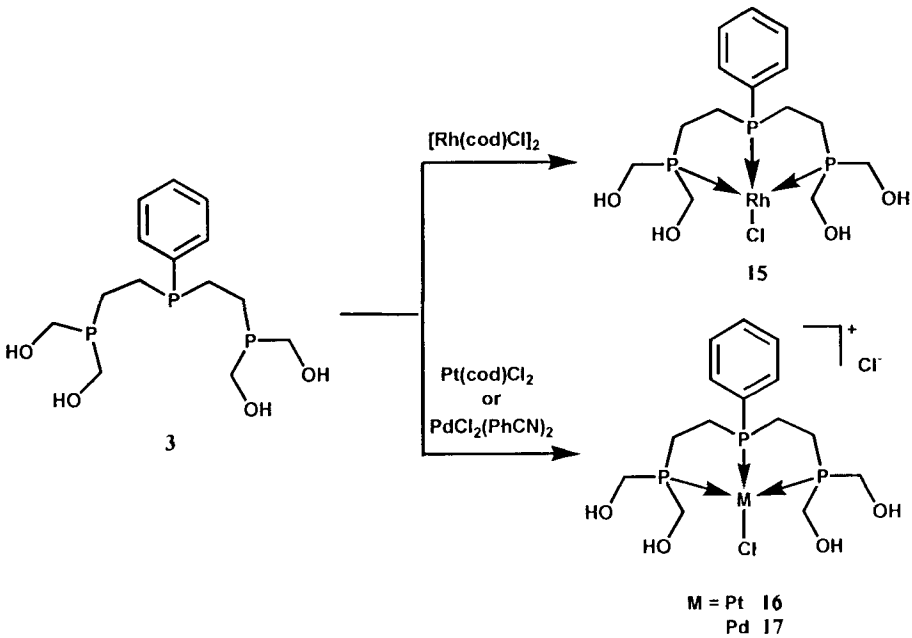
Scheme 6.

Table 1. X-ray crystallographic data of transition metal complexes derived from hydroxymethyl phosphines

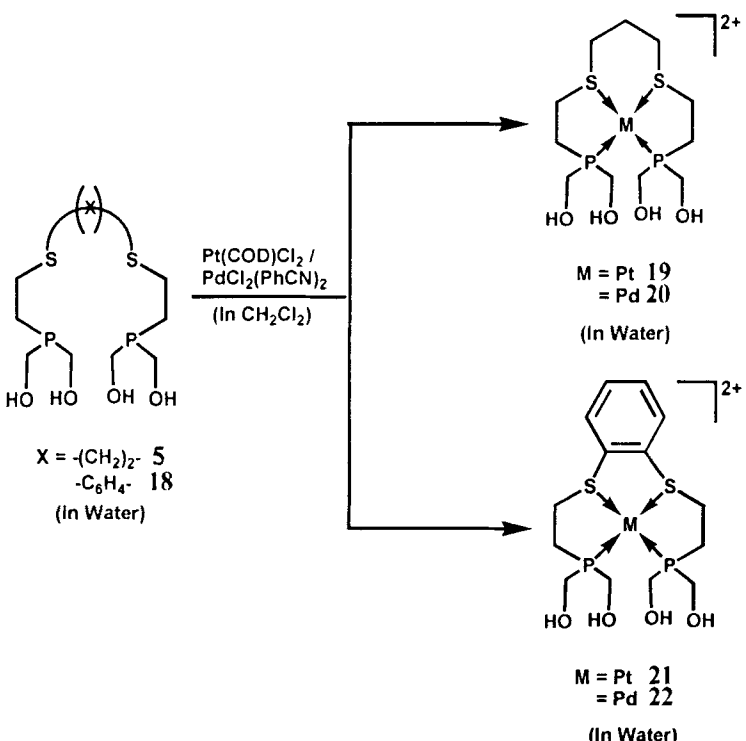
Coordination compounds of hydroxymethyl phosphines	P–M Distances (Å)	Geometry around metal centre	Reference
$[ReO_2\{HOH_2C)_2PC_6H_4P(CH_2OH)_2\}_2]I$ **7**	2·461 (1); 2·456 (1)	Octahedral	10
$[ReO_2\{(HOH_2C)_2PCH_2CH_2P(CH_2OH)_2\}_2]$ Cl **8**	2·480 (2); 2·474 (2)	Octahedral	10
$[Pt\{HOH_2C)_2PCH_2CH_2P(CH_2OH)_2\}_2]Cl_2$ **10**	2·304 (1); 2·315 (1)	Square planar	9
$[Pd\{(HOH_2C)_2PC_6H_4P(CH_2OH)_2\}_2]Cl_2$ **11**	2·312 (1); 2·322 (1)	Square planar	16
$[Au\{(HOH_2C)_2PC_6H_4P(CH_2OH)_2\}_2Cl$ **13**	2·368 (11); 2·368 (11); 2·354 (2); 2·378 (2)	Distorted tetrahedral	17, 18
$[Au_2\{HOH_2C)PCH_2CH_2P(CH_2OH)_2\}_2]Cl_2$ **14**	2·307 (3); 2·305 (4); 2·304 (4); 2·316 (3)	Linear	17, 18
$[Pd(HOH_2C)_2P(CH_2)_2S(CH_2)_3S(CH_2)_2P(CH_2OH)_2Cl_2$ **20**	2·266 (7); 2·285 (7)	Square planar	12
$[ReO_2(HOH_2C)_2P(CH_2)_2S(CH_2)_3S(CH_2)_2P(CH_2OH)_2]Cl$ **23**	2·425 (10); 2·418 (10)	Octahedral	13

as outlined in scheme 7. Phosphorus NMR spectroscopy was used as a diagnostic tool to confirm tripodal coordination in **15–17** (scheme 8)[11,19]. The ^{195}Pt NMR of **16** clearly established the tripodal linkage of the metal centre with the two disparate P^{III} centres[19].

The coordination chemistry of the water-soluble P_2S_2 ligands, as outlined in scheme 8, justifies the importance of heteroatomic thioether electron donors in achieving 1:1 metal-to-ligand ratio[12]. In sharp contrast, the simple hydroxymethyl bisphosphines **1** and **2** produced transition metal complexes with similar metals in 1:2 metal-to-ligand stoichiometry[9,10,15]. While reactions of the P_2S_2 ligand, illustrated in scheme 8, provide further examples of the ability of water-soluble hydroxymethyl phosphines to undergo

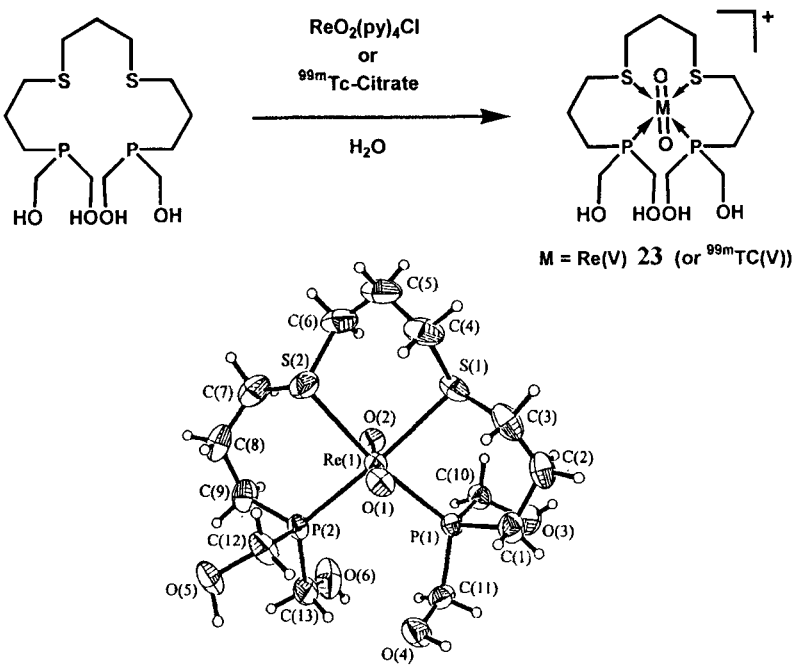


Scheme 7.



Scheme 8.

reactions with transition metal centres under biphasic conditions, scheme 9 describes coordination chemistry of this ligand system with a Re(V) precursor using water as a solvent. This reaction which results in a water-soluble Re(V) complex **23** (scheme 9) is of particular importance in biomedicine as it presents the prospects of using radiometals of diagnostic (e.g. ^{99m}Te–a ν emitter) and therapeutic (e.g. $^{186/188}$Re–a β emitter) importance in similar reactions (vide infra).



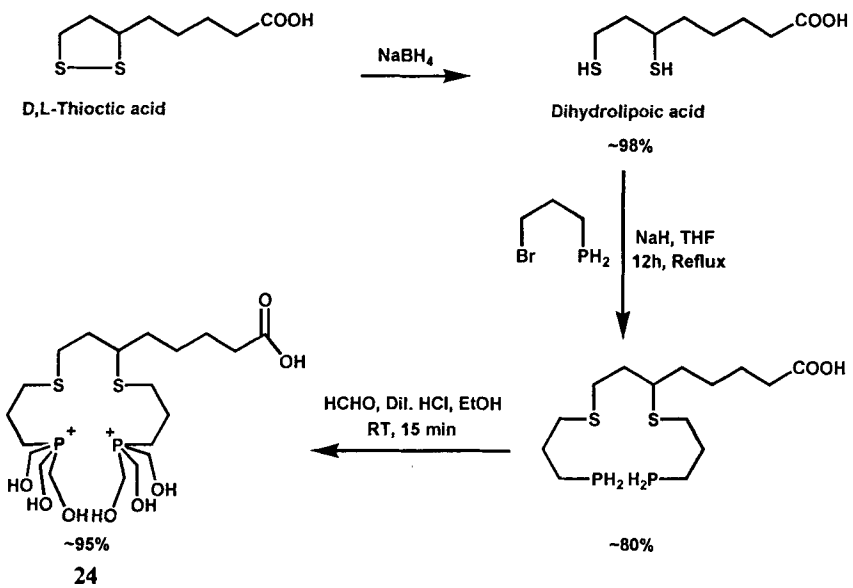
Scheme 9.

Extensive spectroscopic data and detailed X-ray crystallographic analysis of **19–23** have provided insights into the nature of coordination of different transition metals with the P_2S_2 ligand frameworks [12,13]. Important bonding parameters of **19–23** are summarized in table 1.

2.4 *Biomedical applications*

As part of our ongoing studies on the design and development of chelating systems for use in formulating high specific activity ^{99m}Tc- and ^{188}Re-labelled biomolecules, we recently reported results of studies with a new class of functionalized phosphines of the type: $P(CH_2OH)_3$ (THP) and $(HOH_2C)_2PXP(CH_2OH)_2$ (X = $-C_6H_4$ **1**, $-(CH_2)_2$ **2**) [20]. These ligands are oxidatively-stable in air and aqueous solution. They also produce water-soluble (and kinetically inert) complexes with a number of early (Re(V) and ^{99m}Te(V)) and late (Rh(I), Pd(II), Pt(II), Ag(I), and Au(I)) transition metals [9–19]. Radiochemical investigations of THP, **1** and **2**, complexes with ^{99m}Tc have shown that hydroxymethyl-functionalized phosphines (HMP) produce ^{99m}Tc-complexes having excellent *in vitro* stability. *In vivo* studies (in rats) of these complexes further demonstrated excellent

stability profiles as well as efficient clearance from non-target tissue with a high degree of excretion *via* the urinary pathway [20]. These attractive properties of HMP-based ligands prompted us to explore the design and development of bifunctional chelating agents, a prototype of which is outlined in scheme 10. The P_2S_2 ligands **6** (and its carboxylate compound **24**) undergo transmetallation reactions with technetium-99m precursors that contain easily exchangeable ligands (e.g. citrate or glucoheptonate groups). For example,



Scheme 10.

Table 2. Biodistribution of ^{99m}Tc-P_2S_2 in rats as a function of time after intravenous administration

Organ	15 min	1 h	2 h
Percent injected dose/organ[a]			
Brain	0·03 ± 0·01	0·01 ± 0·00	0·00 ± 0·00
Blood[b]	3·82 ± 0·88	1·92 ± 0·64	0·98 ± 0·26
Heart	0·09 ± 0·00	0·05 ± 0·02	0·03 ± 0·00
Lung	0·59 ± 0·35	0·66 ± 0·19	0·57 ± 0·32
Liver	11·50 ± 1·18	12·98 ± 0·67	12·06 ± 2·40
Spleen	0·25 ± 0·04	0·60 ± 0·28	0·79 ± 0·30
Stomach	0·36 ± 0·36	0·57 ± 0·39	0·97 ± 0·59
Large intestine	0·30 ± 0·05	0·16 ± 0·06	0·15 ± 0·00
Small intestine	46·82 ± 3·13	49·19 ± 3·86	50·81 ± 4·38
Kidneys	2·59 ± 0·40	1·79 ± 0·39	1·61 ± 0·16
Urine	16·99 ± 2·72	23·34 ± 3·99	25·64 ± 1·13

[a]Values represent the mean ± SD ($n = 5$) of the percent injected dose/organ (% ID/organ). Body weights of Sprague–Dawley rats ranged from 180–250 g.
[b]Total blood volume is estimated to be 6·5% of the body weight.

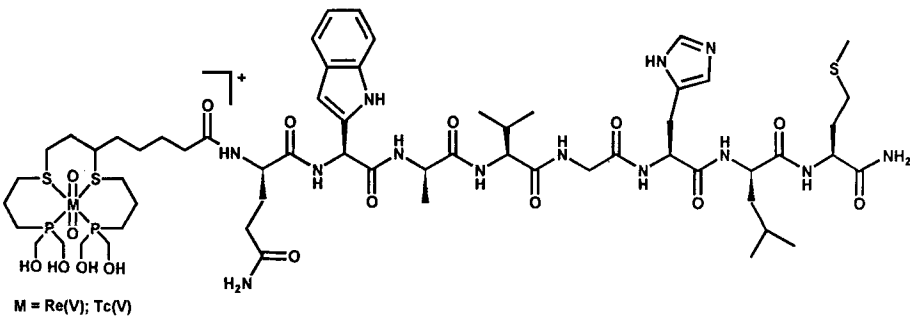


Figure 2. Conjugation of bombesin with rhenium (and technetium) complexes.

incubation of an aqueous solution of the P_2S_2 ligand **6** with ^{99m}Tc-citrate resulted in the formation of P_2S_2-Tc-99m complex (P_2S_2Tc) in > 98% as evidenced by high performance liquid chromatographic (HPLC) analysis[21]. The retention time of P_2S_2Tc complex is similar to that of its Re(V) analogue suggesting similar structural features for the P_2S_2Tc and Re(V)P_2S_2 complexes (scheme 9)[21,22].

In vivo studies of the ^{99m}Tc-P_2S_2 complex were performed in Sprague-Dawley rats and are summarized in table 2. The data suggests that this complex is cleared efficiently from the blood stream *via* the hepatobiliary and renal-urinary pathways (~0·98% ± 0·26% ID of ^{99m}Tc-P_2S_2 remaining in whole blood at 2 h post injection). The majority of the activity of this complex was excreted *via* the bile into the small intestine.

HPLC analysis of urine samples of the ^{99m}Tc activity excreted *via* the renal-urinary pathway indicated that there was no significant difference in the radiochromatograms of the injected samples and the ^{99m}Tc-species in urine, therefore, implying high *in vivo* stability of the ^{99m}Tc-P_2S_2 complex[21,22].

The carboxylate group in P_2S_2-COOH, **24** (scheme 10), can be activated using standard procedures. The activated ester of **24** undergoes conjugation reactions with $-NH_2$ groups of amines, amino acids and peptides. This feature has allowed the development of a novel bioconjugate using the –N terminus of bombesin (figure 2). Bombesin is a synthetic peptide and has the ability to target neoplastic cells that express gastrin releasing peptide (GRP) receptors (include., small cell lung cancers). Therefore, radiopharmaceuticals derived from this peptide or other biomolecular vectors will target specific cancer cells for potential applications in diagnosis and therapy of human cancer.

Acknowledgements

This work was supported by generous grants from the US Department of Energy and DuPont-Merck Pharmaceuticals, USA. Funding and facilities provided by the Departments of Radiology, Research Reactor and Chemistry at the University of Missouri are gratefully acknowledged. I am indebted to Professors W A Volkert and A R Ketring for their support and collaborations. The work reported in this account would not have been possible without the painstaking efforts by various students, post-doctoral scientists and technical staff whose names appear in the list of references. I also thank Professor

S Sarkar of the Indian Institute of Technology, Kanpur, India for the invitation to present our research work at the conference on "Modern Trends in Inorganic Chemistry".

References

1. Corbridge D E C 1990 In *Phosphorus – An outline of its chemistry, biochemistry and technology* (New York: Elsevier Science) Chap 4
2. Walsh E N, Griffith E J, Parry R N and Quinn L D (eds) 1992 *Phosphorus chemistry* ACS Symposium Series No. 486 (Washington, DC: Am. Chem. Soc.) pp 1–273
3. Holmes R R 1996 *Phosphorus, sulfur and silicon* **1** 109–110
4. Parshall G W (ed.) 1980 *Homogeneous catalysis. The application and chemistry of catalysis by soluble transition metal complexes* (New York: Wiley)
5. (a) Franz J E 1985 In *The herbicide glyphosate* (eds) E Grossbara, D Atkinson (Boston, MA: Butterworths) pp 3–17 (b) Franz J E, Mao M K and Sikorski J A 1997 In *Glyphosate: A unique global herbicide* Monograph No 189 (Washington, DC: Am. Chem. Soc.)
6. Kelly J D *et al.* 1990 In *Technetium and rhenium in chemistry and nuclear medicine* (eds) M Nicolini, G Bandoli and U Mazzi (New York: Raven) pp 405–412
7. Rosetti C, Vanoli G, Paganelli G, Kwiatkowski M, Zito F, Colombo F, Deutsch E and Fazio F 1991 *J. Nucl. Med.* **32** 1007
8. Forster A M, Storey A E, Archer C M, Nagle K R, Booker F S, Edwards B, Gill H K, Kelly J D and Mepartin M 1992 *J. Nucl. Med.* **33** 850
9. Reddy V S, Katti K V and Barnes C L 1995 *Inorg. Chim. Acta* **240** 367
10. Reddy V S, Katti K V, Berning D E, Volkert W A, Ketring A R and Barnes C L 1996 *Inorg. Chem.* **35** 1753
11. Smith C J, Reddy V S and Katti K V 1996 *J. Chem. Soc., Chem. Commun.* 2557
12. Smith C J, Reddy V S, Katti K V and Barbour L J 1997 *Inorg. Chem.* **36** 1786
13. Smith C J, Katti K V, Volkert W A and Barbour L J 1997 *Inorg. Chem.* **36** 3928
14. Reddy V S and Katti K V (unpublished results)
15. Katti K V 1996 *Curr. Sci.* **70** 219
16. Reddy V S, Katti K V and Barnes C L 1996 *J. Chem. Soc., Dalton Trans.* 1301
17. Berning D E, Katti K V and Barnes C L, Volkert W A and Ketring A R 1997 *Inorg. Chem.* **36** 2765
18. Berning D E, Katti K V, Barnes C L and Volkert W A 1997 *Chem. Ber.* **130** 190
19. Smith C J and Katti K V 1998 *J. Chem. Soc., Dalton Trans.* 1365
20. Berning D E, Katti K V, Singh P R, Reddy V S and Volkert W A 1996 *J. Nucl. Med. Biol.* **23** 617
21. Smith C J, Katti K V, Higginbotham C and Volkert W A 1997 *J. Labelled Compd. Radiopharmacol.* **40** 493
22. Smith C J, Li N, Katti K V, Higginbotham C and Volkert W A 1997 *Nucl. Med. Biol.* **24** 685