Copper(I) bis(diphosphine) complexes as a basis for radiopharmaceuticals for positron emission tomography and targeted radiotherapy

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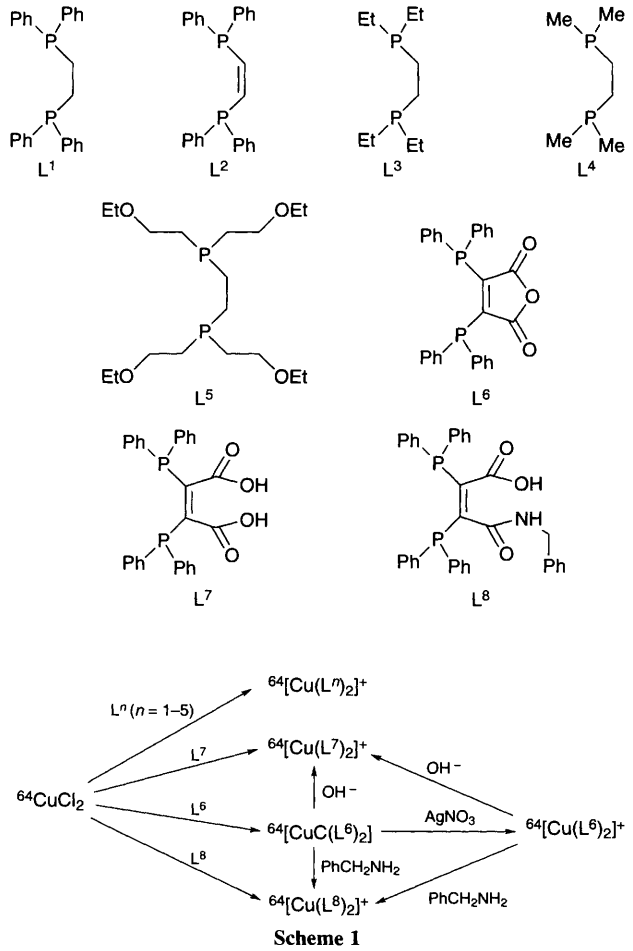
Copper(I) bis(diphosphine) complexes provide an excellent basis for development of short/medium-lived PET (positron emission tomography) imaging and therapy agents containing copper radioisotopes, because of their extreme facility of synthesis and scope for derivatisation and bioconjugate formation.

Positron-emitting (^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu) and β-emitting (^{64}Cu, ^{66}Cu, ^{67}Cu) radionuclides of copper can be produced on a scale that makes clinical application in positron emission tomography (PET) and targeted radiotherapy feasible. ^{62}Cu ($t_{1/2}$ = 9.74 min) can be used in hospitals remote from a cyclotron because it can be distributed in the form of a ^{62}Zn/^{62}Cu generator with a useful lifetime of two to three days.[1,2] ^{64}Cu ($t_{1/2}$ = 12.8 h) imparts a therapeutic radiation dose to tissues that is equivalent in cytotoxicity to ^{67}Cu ($t_{1/2}$ = 2.5 d),[3,4] despite its shorter half-life and different decay scheme. Moreover, its β^+ emission provides a means of monitoring its distribution during therapy. Despite these advantages, only two types of radiopharmaceutical containing copper radionuclides have been developed: monoclonal antibody conjugates with copper chelates,[5–7] and the pyruvaldehydebis(thiosemicarbazone) complex [Cu(ptsm)][8] [ptsm = pyruvaldehydebis(N^4-methylthiosemicarbazone)] which is non-selectively extracted into cells by a bioreductive mechanism.[9] There is a particular need for tracers that can be synthesised extremely quickly, that will be compatible with the short half-life of ^{60}Cu, ^{61}Cu and ^{62}Cu.

We have found that diphosphine complexes of copper(I) offer the necessary ease of synthesis, together with sufficient stability in biological media (serum) for meaningful *in vivo* targeting to be possible. Moreover, the targeting properties (cell uptake and selectivity) can be controlled by choice of functionality in the ligands. This is the first reported use of copper in oxidation state +1 as a basis for radiopharmaceutical development. Aqueous solutions of no-carrier-added $^{64}CuCl_2$ react instantly on addition of the 1,2-diphosphines L^1–L^8 (Scheme 1). The phosphines appear to act as both ligands and as reducing agents. No purification is required as yields are radiochemically quantitative. Ligands L^1–L^5 give mononuclear tetrahedral copper(I) complexes.[10] The radio-active complexes with L^1–L^4 were identified by comparison of their chromatographic properties with those of fully characterized (FABMS, elemental analysis, conductivity) non-radioactive analogues.[11]† The latter complexes with L^6–L^8 were prepared by the same route, or from copper(I) starting materials {*e.g.* $[Cu(MeCN)_4]^+$}; all behave as 1 : 1 electrolytes in acetonitrile.

In the case of the reaction of $^{64}CuCl_2$ or non-radioactive copper(I) chloride with the anhydride ligand L^6, the product is not the cationic complex but the brown uncharged $[CuCl(L^6)_2]$ in which a chloride is coordinated, as evidenced by the lack of conductivity and low solubility in polar solvents. The cationic species $[Cu(L^6)_2]^+$ was cleanly prepared both by the addition of $AgNO_3$ to $[CuCl(L^6)_2]$ and by treatment of $[Cu(MeCN)_4][BF_4]$ with L^6. Upon hydrolysis with aqueous sodium hydroxide both copper complexes of L^6 are cleanly converted to the yellow carboxylate derivative $[Cu(L^7)_2]^+$. In the case of $[CuCl(L^6)_2]$ this entails loss of Cl^- from the metal coordination sphere. The complex $[Cu(L^7)_2]^+$ can also be synthesised directly from $CuCl_2$ and the carboxylate form of the ligand, L^7. The anhydride complexes react with amines {*e.g.* benzylamine to give $[Cu(L^8)_2]^+$} and alcohols, offering the possibility of elaboration into more complex radiopharmaceuticals or bioconjugates.

All of the above chemistry can be conducted equally readily with radioactive $^{64}CuCl_2$ on the no-carrier-added scale. With the exception of the anhydride complex (which rapidly forms conjugates with serum proteins by aminolysis) the resulting complexes are degraded only slowly (< 5% loss over 24 h for L^1–L^5) in human serum at 37 °C. This timescale is compatible with all but the slowest *in vivo* targeting processes. The behaviour of the complexes in Chinese Hamster Ovary (CHO) cells grown in suspension (Fig. 1) suggests that cellular uptake is controlled by the lipophilicity of the complex, which is determined by the organic substituents at the phosphorus. The



Scheme 1

ethoxy-ethylene derivative $[Cu(L^5)_2]^+$ is a lipophilic cation with an octanol–water partition coefficient (log *P*) of 2.0 as the chloride salt. Despite its charge it enters cells almost as rapidly, and is as effectively retained, as the uncharged lipophilic complex [^{64}Cu(ptsm)], while the relatively hydrophilic complexes $[^{64}Cu(L^4)_2]^+$ (methyl) and $[^{64}Cu(L^7)_2]^+$ (carboxylate) are not taken up. With the exception of L^6 (data not shown) the complexes do not react with albumin or other components of the growth medium. A study of the biodistribution of selected ^{64}Cu–bis(diphosphine) complexes in rats showed rapid extraction of the tracer from blood into tissues (Fig. 2) consistent with the rapid cell uptake *in vitro* (Fig. 1), in contrast to free $CuCl_2$ which is cleared from blood more slowly.

Lipophilic cations [*e.g.* the ^{99m}Tc myocardial imaging agent technetium(I) hexakis(2-methoxyisobutylisonitrile] are known to enter cells passively by diffusion and to accumulate within cells, and more particularly within mitochondria, as a result of the electrical potential gradient across the respective membranes in actively metabolising cells.[12] They are also cytotoxic, and indeed copper bis(diphosphine) complexes have been investigated as anti-cancer agents because of this.[13] Complexes with these properties can act as substrates for the P-glycoprotein,[14] the active transport system responsible for resistance of tumour cells to chemotherapeutic agents ('multi-drug resistance'), and hence could be used to image expression of multi-drug resistance *in vivo*.

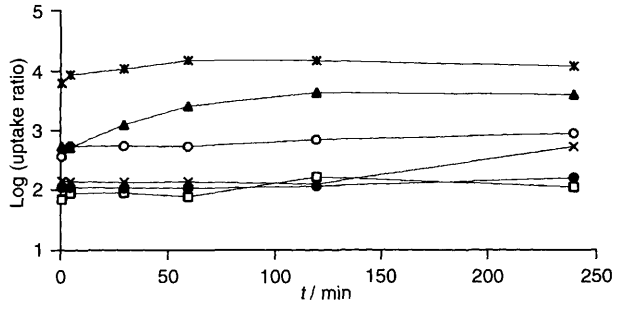


Fig. 1 Cellular uptake expressed as logarithmic concentration ratios of intracellular : extracellular uptake of ^{64}Cu-radiolabelled complexes into CHO cells with time; (○) L^3, (□) L^4, (▲) L^5, (×) L^7, (*) ptsm, (●) $CuCl_2$

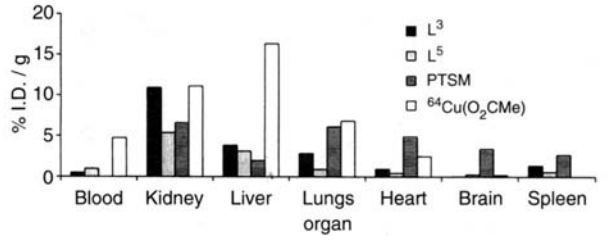


Fig. 2 Comparative 5 min post-injection biodistribution of $[^{64}Cu(L^3)_2]^+$, $[^{64}Cu(L^5)_2]^+$, (^{64}Cu(ptsm)] (blood data not shown) and $^{64}Cu(O_2CMe)_2$[15] (spleen data not shown) in 200 g rats

We conclude that bis(diphosphine) complexes of copper radioisotopes offer the advantages of extreme rapidity and facility of synthesis, *in vivo* stability, and the potential for control of biodistribution through ligand modification and bioconjugate formation. They can therefore serve as a basis for the development of copper radiopharmaceuticals for PET imaging and targeted radiotherapy.

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Footnote

† The compound with L^6 was produced by the direct addition of $^{64}CuCl_2$ to a Myoview™ kit which contains L^5 and is normally used for the purpose of the preparation of $[^{99m}TcO_2(L^5)_2]^+$, a myocardial imaging agent. The $[^{64}Cu(L^5)_2]^+$ complex was characterized by radio-chromatography [mobile phase: MeCN–0.9% saline (6:4)] and water–octanol partition coefficient values.

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