Tricarbonylrhenium(I) Complexes of Phosphine-Derivatized Amines, Amino Acids and Model Peptides: their Cytotoxicity and Potential Application in Nuclear Medicine

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Abstract: Modified Mannich reactions of amines, amino acids and a model peptide with Ph_2PH and CH_2O gave bis(diphenylphosphinomethyl)amines $(Ph_2PCH_2)_2NR$ [R= Ph (**1**), CH_2CH_2OH (**2**), CH_2COOCH_2Ph (**3**), $CH_2CONHCH_2COOCH_2Ph$ (**4**), CH_2COOH (**5**)] and $(Ph_2PCH_2)_2NCH_2CH_2N(CH_2PPh_2)_2$ (**6**). Reaction with $[ReBr_3(CO)_3]^{2-}$ under mild conditions led to $ReBr(CO)_3\{(Ph_2PCH_2)_2NR\}$ [R= Ph (**7**), CH_2CH_2OH (**8**), CH_2COOCH_2Ph (**9**), $CH_2CONHCH_2COOCH_2Ph$ (**10**), CH_2COOH (**11**)] and $[ReBr(CO)_3Ph_2PCH_2)_2NCH_2]_2$ (**12**). All new complexes have been characterized by NMR and IR spectroscopy and for **7**, **9** and **10,** single-crystal X-ray diffraction analyses. Stability studies by Electrospray Mass Spectrometry, showed that other than solvolysis, the complexes are fairly stable in neutral and acidic methanol. Cytotoxicity testing of **7**-**10** and **12** showed that all the complexes are active against specific tumour cell lines.

Introduction

There is much current interest in the development of radiopharmaceuticals through the radiolabeling of monoclonal antibodies with $^{186/188}Re$.[1-3] Although much emphasis had

been placed on the development of ligand systems for chelation to the Re(V) oxo core,[ref] the design of radiopharmaceuticals based on Re(I)-tricarbonyl complexes has gained considerable attention recently.[refs] The advantages of this organometallic approach and the methodology for generating the $[Re(CO)_3]^+$ synthon have also been established.[refs]

It has also been recognised that phosphines, by virtue of their versatile ligating capability with transition metals, can play a major role in the design of radiometal-antibody conjugates.[5,6,7] Work has been done in the area of direct introduction of phosphino groups onto peptides and proteins via the Mannich-type reaction of hydroxymethyl phosphines and the free amino groups on the protein.[refs] Such procedures, however, can potentially alter the structure and hence biological properties of the protein. The use of polyfunctional hydroxymethylphosphines such as $P(CH_2OH)_3$, for example, can potentially create undesirable cross-links within the protein structure,[refs] or link two separate peptides to the same radiometal atom (reducing the overall labelling efficiency). An alternative strategy is the "pre-formed chelate" approach, whereby the radiometal is first tightly bonded to a diphosphine chelate system, followed by the attachment of the chelate complex unit to the protein via an appropriate functional group on the diphosphine (Scheme 1).

Bis(diphenylphosphinomethyl)amines are good candidates for the pre-formed chelate approach. They are readily synthesised via the Mannich reaction between primary amines and secondary phosphines (with formaldehyde)[10] or bis(hydroxymethyl)phosphonium salts[15], and are known to form stable chelate complexes with palladium, platinum and rhodium[16]. In principle, any desired functional group (for protein conjugation or for increasing water-solubility) can be attached onto the

nitrogen atom by appropriate choice of the NH_2 group-containing starting material (Scheme 2). The fact that the functional group or molecular chain that links the chelate unit with the biomolecule is attached to the middle atom of the diphosphine backbone means that there should be minimal steric interaction between the radiometal's coordination sphere with the protein. The relative simplicity of the chelating system is also attractive.

In the course of our ongoing research on rhenium(I) carbonyl alkoxo complexes,[4 and more] we have found that the complexes $[Re_2(\mu\text{-}OR)_3(CO)_6]^-$ (R = H, Me, Et, Ph), $[Re_2(\mu\text{-}OH)(\mu\text{-}OPh)_2(CO)_6]^-$, $[Re_2(\mu\text{-}OR)_2(\mu\text{-}dppf)(CO)_6]$ [dppf = 1,1′-bis(diphenylphosphino)ferrocene; R = H, Me, Ph] and *fac*-$[Re(OPh)(\eta^2\text{-}dppf)(CO)_3]$ exhibit potent cytotoxicity against a number of cancer cell lines.[8b] It is of interest, therefore, to investigate the anti-tumour activity of bis(diphenylphosphinomethyl)amine tricarbonylrhenium(I) complexes as well. The possibility of having bis(diphenylphosphinomethyl)amine tricarbonylrhenium(I)-based radiopharmaceuticals serving dual functions as both chemo- and radiotherapeutic agents is appealing.

In this paper, we report the synthesis of tricarbonylrhenium(I) complexes of representative bis(diphenylphosphinomethyl)amines derived from aniline, glycine, and glycylglycine, and the study of their *in vitro* stability by electrospray mass spectrometry. Results of the cytotoxicity screening of these complexes against 18 cancer cell lines are also presented.

Experimental Section

All reactions were performed under pure dry nitrogen using standard Schlenk techniques. The solvents were purified and dried by standard methods and distilled under nitrogen prior to use. $[NEt_4]_2[Re(CO)_3Br_3]$ was prepared by the published procedure[9]. ^1H and ^{31}P-$\{^1H\}$ NMR spectra were recorded on a Bruker ACF 300 MHz spectrometer at ca. 300 K at operating frequencies of 300.0 and 121.5 MHz, respectively. ^1H and ^{31}P chemical shifts are quoted in ppm downfield of tetramethylsilane and external 80% H_3PO_4, respectively. 2-D ROESY NMR analysis was carried out on a Bruker DRX 500 spectrometer at an operating frequency of 500.23 MHz. IR spectra were taken in a KBr disc on a Perkin-Elmer 1600 FT-IR spectrophotometer. Elemental analyses were carried out in the Microanalytical Laboratory at the National University of Singapore. Cytotoxicity tests were carried out following previously-reported procedures.[8b]

Preparation of bis(diphenylphosphinomethyl)amines: Bis(diphenylphosphinomethyl)amines $(Ph_2PCH_2)_2NR$ [R=Ph (**1**), CH_2CH_2OH (**2**), CH_2COOCH_2Ph (**3**), $CH_2CONHCH_2COOCH_2Ph$ (**4**), CH_2COOH (**5**)] and $(Ph_2PCH_2)_2NCH_2CH_2N(CH_2PPh_2)_2$ (**6**) were generally prepared by the published procedures[10] with only slight modifications. ^{31}P-$\{^1H\}$ NMR ($CDCl_3$): **4** δ -27.3 (s); **5** δ -27.0 (s); **6** δ -28.2 (s).

Preparation of bis(diphenylphosphinomethyl)amine tricarbonylrhenium(I) bromide complexes: *General Method*. – $[NEt_4]_2[Re(CO)_3Br_3]$ (144 mg, 0.2 mmol) was dissolved in MeOH (30 ml) and an equimolar amount of bis(diphenylphosphinomethyl)amine added. The solution was stirred overnight at room temperature, after which the solvent

was evaporated under reduced pressure and the waxy residue was triturated with Et_2O (20 ml) to form a crystalline solid. After decantation, the product was extracted from the $[NEt_4]Br$ with THF (30 ml) and precipitated with hexane. Recrystallization of the solid from CH_2Cl_2/EtOH afforded colorless crystals of the product.

$ReBr(CO)_3\{(Ph_2PCH_2)_2NPh\}$ (7). The bis(diphenylphosphinomethyl)amine **1** (98mg, 0.2mmol) gave complex **7** as colorless crystals (120mg, 72%). **7** Found for $C_{35}H_{29}BrNO_3P_2Re$: C 50.21%, H 3.52%, N 1.60%. Calcd: C 50.06%, H 3.48%, N 1.67%. ^1H NMR ($CDCl_3$): δ 7.74-7.28 (m, 20H, P-Ph), 7.10 (t, 2H, N-Ph), 6.95 (t, 1H, N-Ph), 6.26 (d, 2H, N-Ph), 4.75 (m, 2H, H-CH), 4.14 (m, 2H, H-CH). $^{31}P-\{^1H\}$ NMR ($CDCl_3$): δ -21.1 (s). IR (cm^{-1}, KBr): 2031, 1950, 1901 (CO).

$ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2CH_2OH\}$ (8). The bis(diphenylphosphinomethyl)amine **2** (92mg, 0.2mmol) gave complex **8** as colorless crystals (83mg, 51%). **8** Found for $C_{31}H_{29}BrNO_4P_2Re$: C 46.05%, H 3.71%, N 1.79%. Calcd: C 46.10%, H 3.62%, N 1.73%. ^1H NMR ($CDCl_3$): δ 7.68-7.26 (m, 20H, Ph), 4.31 (m, 2H, H-CH), 3.73 (m, 2H, H-CH), 3.18 (t, 2H, CH_2), 2.71 (s, 2H, CH_2). $^{31}P-\{^1H\}$ NMR ($CDCl_3$): δ -19.6 (s). IR (cm^{-1}, KBr): 2031, 1944, 1899 (C≡O).

$ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2COOCH_2Ph\}$ (9). The bis(diphenylphosphinomethyl)amine **3** (110mg, 0.2mmol) gave complex **9** as colorless crystals (118mg, 65%). **9** Found for $C_{38}H_{33}BrNO_5P_2Re$: C 49.97%, H 3.67%, N 1.60%. Calcd: C 50.05%, H 3.65%, N 1.54%. ^1H NMR ($CDCl_3$): δ 7.66-7.26 (m, 25H, C_6H_5),

5.10 (s, 2H, CH_2), 4.63 (m, 2H, CH_2), 3.50 (m, 2H, CH_2), 3.31 (s, 2H, CH_2). ^{31}P-{^1H} NMR ($CDCl_3$): δ -21.7 (s). IR (cm^{-1}, KBr): 2031, 1952, 1896 (C≡O), 1734 (C=O).

$ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2CONHCH_2COOCH_2Ph\}$ (10). The bis(diphenylphosphinomethyl)amine **4** (124mg, 0.2mmol) gave complex **10** as colorless crystals (134mg, 69%). **10** Found for $C_{40}H_{36}BrN_2O_6P_2Re$: C 49.65%, H 3.81%, N 2.92%. Calcd: C 49.59%, H 3.75%, N 2.89%. ^1H NMR ($CDCl_3$): 7.44-7.25 (m, 25H, Ph), 5.50 (m, 1H, NH), 5.13 (m, 2H, O-CH_2-Ph), 4.37-4.36 (m, 2H, N-HCH-P), 3.47-3.37 (m, 2H, N-HCH-P), 3.33-3.30 (m, 4H, N-CH_2-C). ^{31}P-{^1H} NMR ($CDCl_3$): δ -18.1 (s). IR (cm^{-1}, KBr): 2032, 1954, 1904 (C≡O), 1735, 1673 (C=O).

$ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2COOH\}$ (11). The bis(diphenylphosphinomethyl)amine **5** (110mg, 0.2mmol) gave complex **11** as colorless crystals (87mg, 53%). **11** Found for $C_{31}H_{27}BrNO_5P_2Re$: C 45.51%, H 3.27%, N 1.73%. Calcd: C 45.32%, H 3.31%, N 1.70%. ^1H NMR ($CDCl_3$): δ 7.71-7.29 (m, 20H, Ph), 4.62 (m, 2H, H-CH), 3.47 (m, 2H, H-CH), 3.32 (s, 2H, N-CH_2-C). ^{31}P-{^1H} NMR ($CDCl_3$): δ -21.7 (s). IR (cm^{-1}, KBr): 2029, 1948, 1909 (C≡O), 1718 (C=O).

$[ReBr(CO)_3(Ph_2PCH_2)_2NCH_2]_2$ (12). $[Re(CO)_3Br_3][NEt_4]_2$ (144 mg, 0.2 mmol) was dissolved in MeOH (30 ml) and the bis(diphenylphosphinomethyl)amine **6** (85mg, 0.1mmol) added. The solution was stirred overnight at room temperature, the solvent evaporated under reduced pressure giving a waxy residue. The residue was separated, washed with THF and then with EtOH/H_2O, giving **12** as a colorless solid (97mg, 63%).

12 Found for $C_{60}H_{52}Br_2N_2O_6P_4Re_2$: C 46.51%, H 3.31%, N 1.76%. Calcd: C 46.40%, H 3.37%, N 1.80%. ^{31}P-{^1H} NMR ($CDCl_3$): δ -20.7 (s). IR (cm^{-1}, KBr): 2035, 1953, 1945, 1925, 1907 (C≡O).

Electrospray mass spectrometry. All ESMS spectra were recorded in positive-ion mode using a VG Platform II instrument employing nitrogen as both the drying and nebulizing gas. The spectra were typically obtained with an average of ten to twelve scans. A range of cone voltages, from 20-100 V were typically applied on each sample to investigate the fragmentation behavior. The analyte solution (Approx 0.1 mM) was delivered to the mass spectrometer source using a Spectra System P1000 HPLC pump, at a flow rate of 0.01 ml min^{-1}. Spectra were recorded in neutral, acidic and basic MeOH solutions, as well as in MeCN-H_2O solutions. Acidic solutions were prepared by dilution of 5 drops of 50% formic acid to 2mL with methanol. Two drops of this solution were added to the rhenium complex solutions, and the mixture allowed to stand for several weeks. Basic solutions were prepared by adding three drops NH_3 solution (2 M) to the rhenium complex solution, and the mixture allowed to age over one month. Assignment of major ions was aided by a comparison of the experimental and calculated isotope distribution patterns, the latter obtained using the Isotope program[11].

X-ray Crystallography.

Colorless crystals of **7** suitable for X- ray diffraction analysis were obtained from a $CH_2Cl_2/CHCl_3/C_2H_5OH$ solution at –20°C and colorless crystals of **9** and **10** were obtained from a CH_2Cl_2/C_2H_5OH solution at –20°C. Data collection was carried out on a

Siemens SMART CCD diffractometer using Mo-K_α radiation (λ 0.71073 Å). The data were corrected for absorption effects using the SADABS program.[12aa] Crystal and refinement data are summarised in Table 1.

The structures of the three complexes were solved by direct methods and difference Fourier maps. Full-matrix least-squares refinements (on F^2) were carried out with anisotropic thermal parameters for all non-hydrogen atoms, using all of the unique data. All hydrogen atoms were introduced in calculated positions and refined using the riding model. Computations were carried out using the SHELXTL software package.[12] Crystallographic data for the structural analyses have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. xxxxx for **7**, xxxxx for **9**, xxxxx for **10**. Selected geometric parameters of the complexes are given in Table 2.

Results and Discussion

Syntheses

The Mannich reactions of aniline, ethanolamine $H_2NCH_2COOCH_2C_6H_5$, $H_2NCH_2CONHCH_2COOCH_2C_6H_5$, H_2NCH_2COOH and ethylene diamine gave bis(diphenylphosphinomethyl)amines **1** - **6**, respectively. Reactions of $[NEt_4]_2[Re(CO)_3Br_3]$ and these bis(diphenylphosphinomethyl)amines give **7** - **12**, respectively (Scheme 1). Complex **7** - **10** are soluble in CH_2Cl_2, $CHCl_3$ and poorly soluble in H_2O, MeOH, EtOH. Complex **11** has increased solubility in H_2O due to its carboxylic group, which can potentially be functionalized. Unexpectedly complex **12** is virtually insoluble in all these solvents.

Crystal structures

In order to confirm the formation and geometry of the rhenium diphosphine complexes, X-ray structure determinations were carried out on **7**, **9** and **10**. Rhenium preferentially bonds to the phosphorus atoms of these bis(diphenylphosphinomethyl)amines as expected. The nitrogen atoms are uncoordinated and the six-membered chelate rings adopt chair conformations with the nitrogen and rhenium atoms out of the plane formed by the C_2P_2 unit. This is unlike the reported palladium, platinum and rhodium complexes of bis(diphenylphosphinomethyl)amines, in which the chelate rings adopt a flattened chair conformation with only the nitrogen atom substantially out of the plane formed by the C_2P_2M (M = Pd, Pt or Rh)[16] unit. The nitrogen atoms of compounds **7**, **9** and **10** are progressively more pyramidal [average C-N-C angles: 116.0(7)° for **7**, 112.7(3)° for **9**, and 109.6(2)° for **10**]; this is most likely a reflection of the decreasing steric hindrance at the nitrogen atom. The carbonyl groups are in the expected facial arrangement within the distorted octahedral coordination sphere of each of compounds **7**, **9** and **10**. The Re-Br, Re-CO and Re-P distances found for these structures are typical for analogous rhenium(I) tricarbonyl complexes[18].

NMR Spectroscopy

The ^{31}P-$\{^1H\}$ NMR spectrum of each rhenium complex consists of a single sharp resonance, indicating the formation of a single isomer with a plane of symmetry. The ^1H NMR spectra show a characteristic pattern for the methylene protons of the chelate ring. There are two distinct environments for the four protons, due both to the equatorial/axial distinction in the chair conformation, and to the position of the bromide ligand. The ^1H-

^{1}H ROESY spectrum of complex **7** is shown in Fig. 4 as a representative spectrum. The signal at 4.14 ppm is assigned to the equatorial protons because these protons are nearer to the *ortho* protons of the N-bonded phenyl ring (Fig. 1), and hence are expected to have stronger interaction with the latter (cross-peak B is stronger than cross-peak C).

Electrospray (ES) mass spectrometric characterisation

Positive-ion ES spectra of the rhenium bis(diphenylphosphinomethyl)amine complexes **7** – **10** and **12** were recorded in both methanol and acetonitrile-water solutions; ions observed in methanol solution are given in Table 3. Generally, spectra recorded in methanol were of better quality, probably due to improved solubility in this solvent, however spectra for **12** were unable to be obtained. At cone voltages of 50 - 60 V complexes **7**, **8**, **9** and **10** give both $[M + H]^+$ and $[M – Br]^+$ ions, with the relative intensity of the former decreasing with increasing cone voltage. The presence or absence of Br in an ion can be readily ascertained from the isotope pattern of the ion, due to the distinctive isotopic signature of bromine. At low cone voltages (e.g. 20 V) spectra are dominated by the $[M + H]^+$ ions (e.g. for **8** at *m/z* 808). For complex **7**, protonation can only be at the nitrogen atom, since the CO ligands are not expected to show any basicity, but for **8**, **9** and **10**, there are additional oxygen atoms which are also available for protonation. Aggregate ions of the type $[2M + H]^+$ were observed in some cases. Complex **10** showed a strong $[2M + H]^+$ ion (*m/z* 1940), presumably due to the ready ability of this species to engage in hydrogen bonding. Further increasing the cone voltage (to 80 V or higher) results in loss of CO ligands, e.g for **9**, where ions $[M – Br – CO]^+$ (*m/z* 804) and $[M – Br – 2CO]^+$ (*m/z* 776) ions were observed at 100 V.

In some cases several drops of pyridine were added to the analyte solutions, both to increase the solubility of the complexes (which in some cases were of low solubility) and to investigate whether the neutral pyridine ligand could replace the bromide ligand, leading to charged $[M - Br + pyridine]^+$ ions, as has been observed for a wide range of other transition metal halide complexes[19]. However, no pyridine-containing ions were observed. Finally, the negative-ion spectrum of complex **8** was recorded, but no ions (expected to be formed by deprotonation of the OH group) were observed.

Stability studies

The stability of the rhenium-phosphine complexes, in neutral, acidic and basic methanol solutions has also been qualitatively surveyed by ES mass spectrometry, by recording spectra of solutions aged for several weeks. In neutral solutions, spectra of complexes **7**, **8**, and **9** were generally similar to those of fresh solutions, giving $[M + H]^+$ and $[M - Br]^+$ ions. Comparison of the aged solution of **8** with a fresh solution indicated that the aged solution had a higher intensity $[M - Br]^+$ ion, together with additional low intensity ions $[M - Br + NH_3]^+$ and $[M - Br + MeOH]^+$. For complex **10**, the expected $[M + H]^+$ and $[M - Br]^+$ ions were observed, together with a weak ion at *m/z* 879, assigned to $[ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2C(O)NHCH_2CO_2H\} + H]^+$, formed by hydrolysis of the terminal benzyl group in **10**. These observations suggest that the complexes are generally stable in neutral methanol, and that ES mass spectrometry can be used to identify degradation products.

In aged acidic solutions (prepared by addition of a small quantity of formic acid to the Re complex solutions), complexes **7**, **8** and **9** again yielded similar spectra to the

neutral solutions, indicating that the complexes are quite stable in dilute acid solutions. The ion $[PhN\{CH_2P(O)Ph_2\}_2 + H]^+$ (*m/z* 522) was observed for **7**, which was not seen in fresh solutions, nor in the aged neutral solution. For complex **10** considerable decomposition appeared to occur, with one ion at *m/z* 760 assigned to $[Re(CO)_3(MeOH)\{(Ph_2PCH_2)_2NCH_2CO_2H\}]^+$ and the corresponding unsolvated ion at *m/z* 729. This seems to suggest that the C(O)NH linkage of **10** is susceptible to acid hydrolysis but the C(O)O linkage of **9** is less so. This observation may have implications on the choice of linker chains between the metal complex moiety and the antibody in the radiopharmaceutical conjugate. Ideally, the linker chain must be sufficiently stable to allow the conjugate to survive the journey to the target tumour site, but must be metabolizable to facilitate the clearance of radioactivity from non-target tissue.[17]

In aged basic solutions (formed by addition of dilute NH_3 solution) reactivity was much greater, and more hydrolysis was observed. For **7**, the diphosphine dioxide ion at *m/z* 522 was more intense, a weak $[M + H]^+$ ion was seen, together with major ions $[M – Br + NH_3]^+$ and $[M – Br + MeOH]^+$; complex **8** behaved similarly. For **9** considerable solvolylsis occurred, with the base peak at *m/z* 759 assigned to $[Re(CO)_3(MeOH)\{Ph_2PCH_2)_2CH_2CO_2H\}]^+$, plus other unidentified ions. This ion is formed by loss of the bromide ion (and coordination of methanol) together with solvolysis of the ester linkage. Solvolysis of **10** appeared to be even more severe, though interestingly, the ions formed under acidic conditions were not seen.

These preliminary studies suggest that the rhenium-bis(diphenylphosphinomethyl)amine complexes possess significant stability in neutral and mildly acidic solutions, but substantial degradation occurs in basic solution. It must

be emphasised, however, that the diphosphine-rhenium chelate unit remains largely intact in all the above studies, i.e. any degradation observed does not cleave the diphosphine from the rhenium atom. This is significant because one of the most important criteria for choosing a ligand system for the conjugation of radionuclides to antibodies for radioimmunotherapy is the stability of the conjugate. The solvolysis in basic medium may also represent a possible pathway for the interaction of the $[ReBr(CO)_3]$ moiety with blood proteins.

Cytotoxicity

Complexes **7**-**10** and **12** generally showed potent cytotoxicity in the suspended murine and human leukemias and lymphoma, as well as the HeLa suspended uterine carcinoma cells (Table 4). The compounds were however much more selective in inhibiting the growth of tumours derived from human solid cancers.

The pattern of cytotoxic activity shown by complexes **7**-**10** and **12** shows some similarities to that of the rhenium alkoxo complexes $[Re_2(\mu\text{-}OR)_3(CO)_6]^-$ (R = H, Me, Et, Ph), $[Re_2(\mu\text{-}OH)(\mu\text{-}OPh)_2(CO)_6]^-$, $[Re_2(\mu\text{-}OR)_2(\mu\text{-}dppf)(CO)_6]$ [dppf = 1,1′-bis(diphenylphosphino)ferrocene; R = H, Me, Ph] and *fac*-$[Re(OPh)(\eta^2\text{-}dppf)(CO)_3]$ [8b]. For example, all of these rhenium complexes show activity against L1210 mouse leukemia, P388 mouse lymphocytic leukemia, HuT-78 Lymphoma and HeLa-S^3 susp uterine cells, and all are inactive against KB nasopharynx cells. However, complexes **7**-**10** and **12** show much higher activity against MCF-7 breast cancer cells than the rhenium alkoxo complexes.

It is noteworthy that compounds **7**-**10** and **12** show no activity against normal human cells. This is an important property for any chemotherapeutic agent since harmful side-effects of the treatment will be minimised.

Conclusion

Bis(diphenylphosphinomethyl)amines are excellent chelating ligands to tricarbonylrhenium(I) fragments. Complex **11** has a free carboxyl group offering a potentially reactive site for functionalization and coupling to antibodies. Deprotection of complexes **9** and **10** by removal of the benzyl group by hydrogenation would also yield free carboxyl groups for protein conjugation. ESMS studies show that the rhenium-phosphine chelate unit is very stable, especially in neutral solution (similar pH to blood serum). These bis(diphenylphosphinomethyl)amine rhenium(I) complexes thus provide an ideal model for the labeling of antibodies or other amine-containing biomolecules with radioactive tricarbonylrhenium(I) fragments for radioimmunotherapy. Complexes **7**-**10** and **12** also show cytotoxic activity against several murine and human cancer cell lines, suggesting that bis(diphenylphosphinomethyl)amine rhenium(I) complexes with ^{186}Re or ^{188}Re radionuclides can potentially have dual functions, as both chemo- and radiotherapeutic agents, in cancer therapy.

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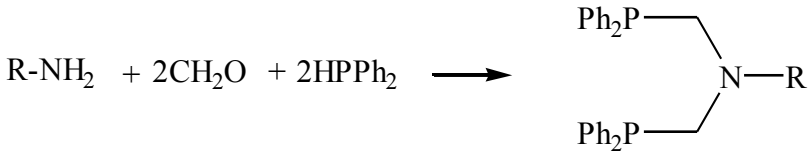
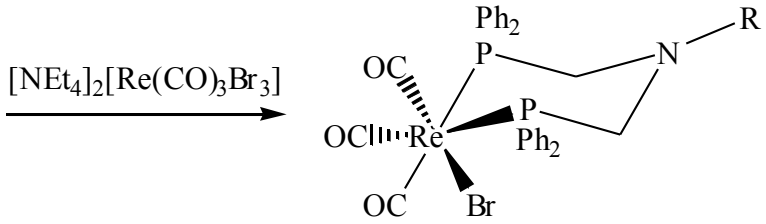
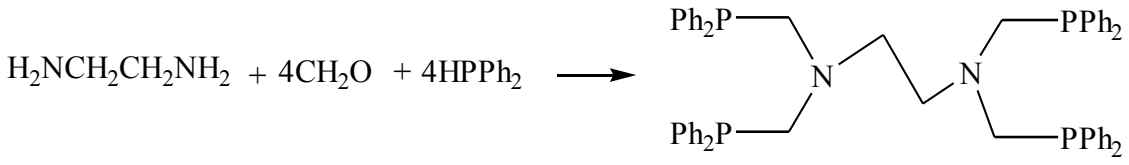
Scheme 1



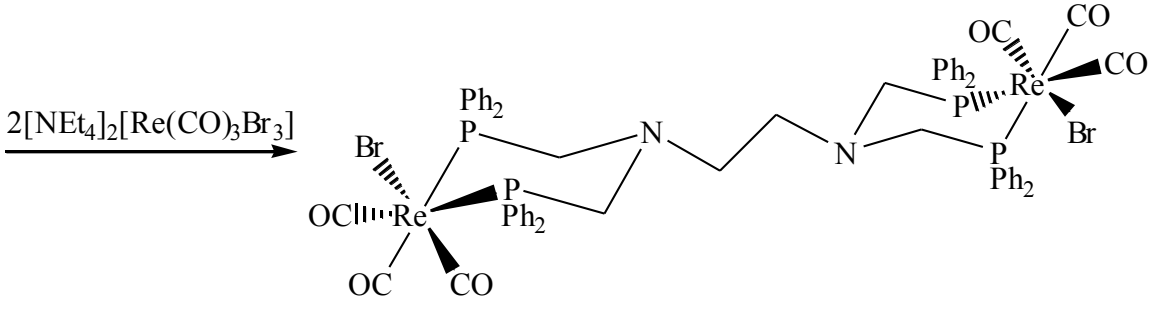
1: R=Ph
2: R=CH_2CH_2OH
3: R=CH_2COOCH_2Ph
4: R=CH_2CO-$NHCH_2COOCH_2Ph$
5: R=CH_2COOH



7: R=Ph
8: R=CH_2CH_2OH
9: R=CH_2COOCH_2Ph
10: R=CH_2CO-$NHCH_2COOCH_2Ph$
11: R=CH_2COOH



6



12

Table 1. Crystallographic Data

	7·$CHCl_3$	**9**	**10**
formula	$C_{36}H_{30}BrC1_3NO_3P_2Re$	$C_{38}H_{33}BrNO_5P_2Re$	$C_{40}H_{36}BrN_2O_6P_2Re$
fw	959.01	911.70	968.76
space group	P-1	Pbca	P-1
cryst syst	Triclinic	Orthorhombic	Triclinic
a (Å)	11.6694(5)	16.4076(3)	9.9690(1)
b (Å)	12.4671(6)	17.3407(4)	12.4920(2)
c (Å)	14.8036(7)	25.0776(5)	16.1088(2)
α (°)	113.358(1)	90	102.511(1)
β (°)	98.992(1)	90	96.301(1)
γ (°)	105.582(1)	90	94.926(1)
V ($Å^3$)	1818.5(2)	7135.1(3)	1934.14(4)
Z	2	8	2
D_{calcd} (Mg m^{-3})	1.751	1.697	1.663
F(000)	936	3584	956
μ(Mo Kα) (mm^{-1})	4.785	4.660	4.305
temp (K)	293(2)	223(2)	223(2)
no. of reflns collected	9064	42627	12688
no. of unique reflns	5965	9017	9000
goodness-of-fit on F^2	1.035	1.068	1.034
R_1[a]	0.0511	0.0332	0.0288
wR_2[b]	0.1111	0.0547	0.0643

[a] $R_1 = \Sigma||Fo| - |Fc||/\Sigma|F_o|$. [b] $wR_2 = [\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]]^{1/2}$.

Table 2. Selected bond lengths (Å) and bond angles (°) for **7**, **9** and **10** with estimated standard deviations in parentheses

	7	**9**	**10**
Re(1)-C(1)	1.900(11)	1.893(3)	1.898(4)
Re(1)-C(2)	1.941(11)	1.964(4)	1.951(4)
Re(1)-C(3)	1.942(12)	1.969(3)	1.949(4)
Re(1)-P(2)	2.461(2)	2.4669(8)	2.4561(8)
Re(1)-P(1)	2.468(2)	2.4848(8)	2.4690(9)
Re(1)-Br(1)	2.6460(10)	2.6604(4)	2.6537(4)
P(1)-C(4)	1.853(8)	1.853(3)	1.856(3)
P(2)-C(5)	1.852(8)	1.851(3)	1.849(3)
N(1)-C(4)	1.464(10)	1.474(4)	1.478(4)
N(1)-C(5)	1.452(10)	1.476(4)	1.471(4)
N(1)-C(1E)	1.416(10)		
N(1)-C(6)		1.462(4)	1.485(4)
C(1)-Re(1)-C(2)	89.5(4)	88.40(14)	89.25(16)
C(1)-Re(1)-C(3)	90.3(4)	89.95(14)	88.98(15)
C(2)-Re(1)-C(3)	89.3(4)	89.86(15)	89.18(18)
C(1)-Re(1)-P(2)	95.3(3)	94.56(10)	93.68(10)
C(2)-Re(1)-P(2)	91.2(3)	90. 19(11)	91.03(13)
C(3)-Re(1)-P(2)	174.4(3)	175.49(11)	177.33(11)
C(l)-Re(1)-P(1)	95.1(3)	97.31(10)	96.20(12)
C(2)-Re(1)-P(1)	175.3(3)	173.14(11)	174.44(11)
C(3)-Re(1)-P(1)	91.4(3)	93.92(10)	92.04(13)
P(2)-Re(1)-P(1)	87.66(7)	85.60(3)	87.50(3)
C(1)-Re(1)-Br(1)	175.7(3)	176.16(10)	177.74(11)
C(2)-Re(1)-Br(1)	86.6(3)	87.76(11)	91.29(11)
C(3)-Re(1)-Br(1)	91.6(3)	89.97(11)	88.83(l1)
P(2)-Re(1)-Br(1)	82.83(6)	85.52(2)	88.51(2)
P(1)-Re(1)-Br(1)	98.73(6)	86.52(2)	83.31(2)
C(4)-P(1)-Re(1)	115.2(3)	114.06(11)	114.41(11)
C(5)-P(2)-Re(l)	112.6(3)	113.35(11)	116.19(11)
C(1E)-N(1)-C(5)	116.0(7)		
C(1E)-N(1)-C(4)	118.8(7)		
C(6)-N(1)-C(4)		111.2(3)	109.0(2)
C(6)-N(1)-C(5)		111.8(2)	107.4(2)
C(5)-N(1)-C(4)	113.3(7)	115.2(3)	112.3(2)
N(1)-C(4)-P(1)	112.8(5)	113.9(2)	112.4(2)
N(1)-C(5)-P(2)	112.5(5)	113.7(2)	117.6(2)

Table 3. ESMS data for the rhenium bis(diphenylphosphinomethyl)amine complexes in methanol

Complex	Cone Voltage	Fragment assignment [m/z, relative intensity (%)]
7	20V	$[M+H]^+$ (*m/z* 840, 100%), $[M-Br]^+$ (*m/z* 760, 55%)
	50V	$[M+H]^+$ (*m/z* 840, 52%), $[M-Br]^+$ (*m/z* 760, 100%)
8	20V	$[M+H]^+$ (*m/z* 808, 100%), $[M-Br]^+$ (*m/z* 728, 5%), $[2M+H]^+$ (*m/z* 1615/1617, 5%)
	50V	$[M+H]^+$ (*m/z* 808, 100%), $[M-Br]^+$ (*m/z* 728, 53%), $[2M+H]^+$ (*m/z* 1615/1617, 3%)
9	20V	$[M+H]^+$ (*m/z* 912, 100%), $[M-Br]^+$ (*m/z* 832, 12%), $[2M+H]^+$ (*m/z* 1823/1825, 10%)
	50V	$[M+H]^+$ (*m/z* 912, 100%), $[M-Br]^+$ (*m/z* 832, 90%)
10	20V	$[M+H]^+$ (*m/z* 969, 100%), $[M-Br]^+$ (*m/z* 889, 5%), $[2M+H]^+$ (*m/z* 1937/1939, 10%)
	50V	$[M+H]^+$ (*m/z* 969, 100%), $[M-Br]^+$ (*m/z* 889, 73%), $[2M+H]^+$ (*m/z* 1615/1617, 18%)
12	50V	$[M+H]^+$ (*m/z* 1554), $[M-Br]^+$ (*m/z* 1473), plus several unidentified ions

Table 4. Cytotoxicity expressed as ED_{50} values ($\mu g\ ml^{-1}$)[a]

Tumor Cell Line	**7**	**8**	**9**	**10**	**12**
L1210 mouse Leukemia	2.36	1.72	1.92	2.80	2.90
P388 mouse lymphocytic Leukemia	2.72	2.91	2.30	3.61	2.02
HL-60 human Leukemia	3.42	3.33	2.14	3.37	2.05
$Tmolt_3$ T cell Leukemia	3.27	4.70	5.17	3.01	3.06
T $molt_4$ T cell Leukemia	4.52	4.36	4.32	3.64	6.16
HuT-78 Lymphoma	2.60	2.96	2.55	3.53	2.70
THP-1 Acute Monocytic Leukemia	2.10	3.28	3.30	5.21	3.87
HeLa-S^3 susp Uterine	2.69	2.32	2.68	2.85	1.45
KB Nasopharynx	6.88	4.88	4.34	6.45	5.49
Lung 549	8.02	4.02	8.51	3.24	6.82
Liver Hepe-2	4.76	5.07	4.02	4.49	3.74
Ovary 1-A9	7.71	3.39	8.92	3.72	7.39
Breast MCF-7	3.52	2.01	2.37	2.42	1.53
Glioma UM 86	8.99	6.49	9.05	6.67	7.78
Ileum HCT-8	5.45	10.69	3.95	4.55	5.65
Prostate PL	3.90	7.30	5.06	8.61	4.95
Osteosarcoma HSO	4.59	5.32	5.86	7.72	5.00
Melanoma SK2	6.11	4.18	3.45	13.3	2.87
Normal RMPI 1788	6.78	7.02	7.60	6.86	9.31

[a] ED_{50} values refer to the concentration of the compound inhibiting 50% of cell growth. A value of less than 4 $\mu g\ ml^{-1}$ is required for significant activity for inhibition of cell growth.

Figure 1. Crystal structure of $[ReBr(CO)_3\{(Ph_2PCH_2)_2NPh\}]$ **7** with 40% probability thermal ellipsoids. Hydrogen atoms on phenyl rings are omitted for clarity.

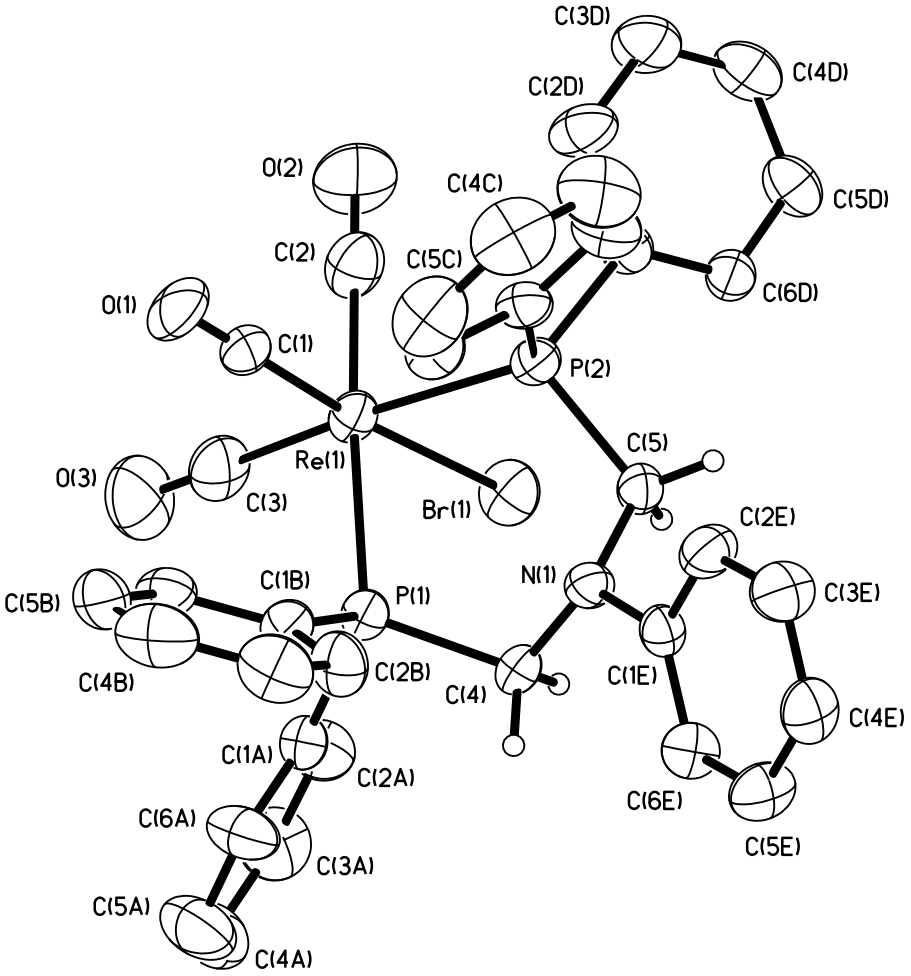


Figure 2. Crystal structure of $[ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2COOCH_2Ph\}]$ **9** with 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.

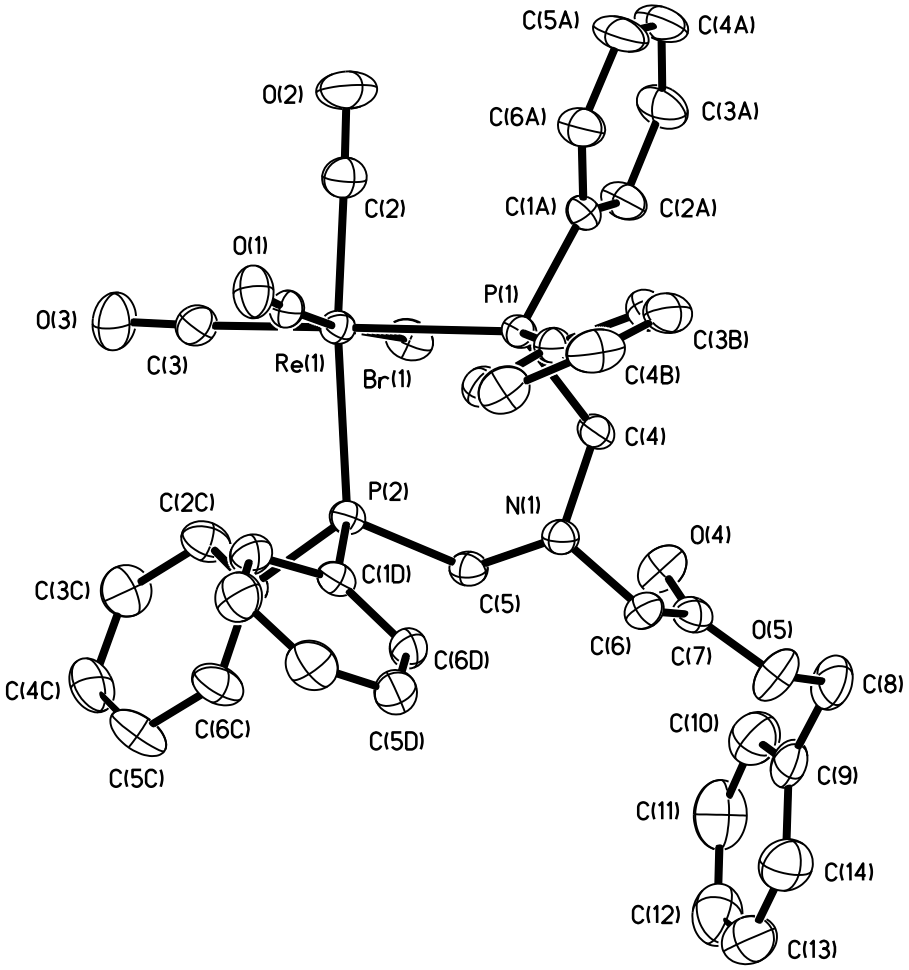


Figure 3. Crystal structure of $[ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2CONHCH_2COOCH_2Ph\}]$ **10** with 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.

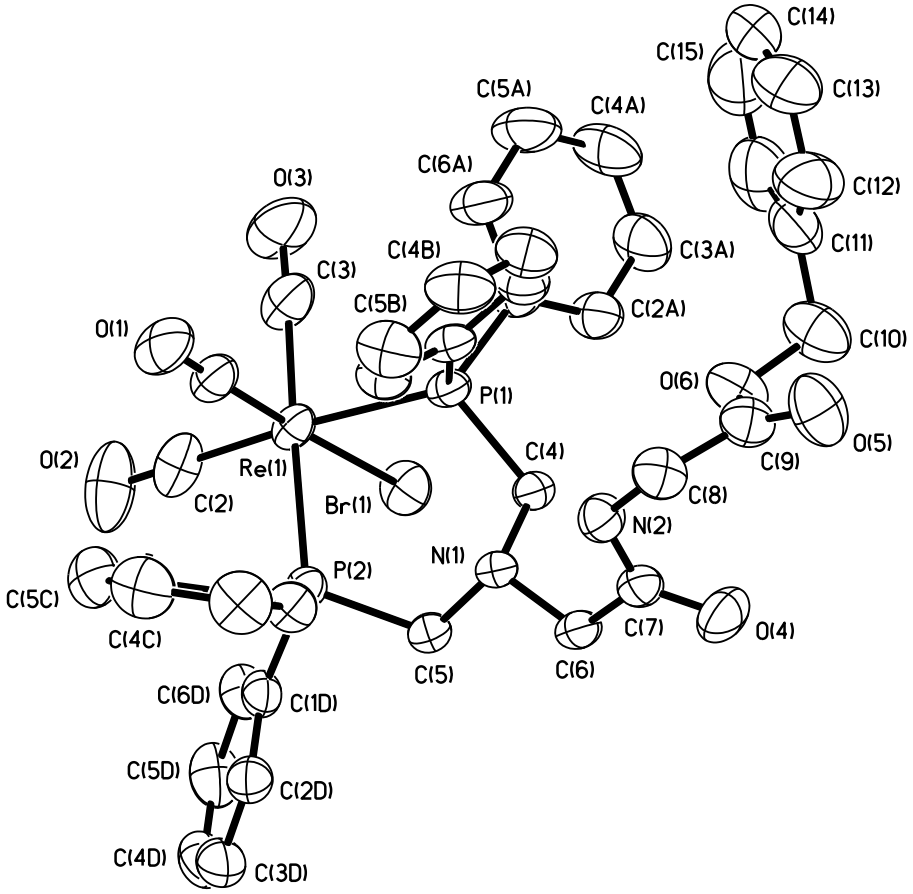


Figure 4. Two-dimensional ^{1}H ROESY NMR spectrum of complex **7** in $CDCl_3$ at 300.0 K. Cross-peaks: **A**, equatorial-CH_2 ··· axial-CH_2; **B**, equatorial-CH_2 ··· *p*-N-C_6H_5; **C**, axial-CH_2 ··· *o*-N-C_6H_5.