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Tricarbonylrhenium(I) complexes of phosphine-derivatized amines, amino acids and a model peptide: structures, solution behavior and cytotoxicity

Jianyong Zhang [a], Jagadese J. Vittal [a], William Henderson [b], Jessica R. Wheaton [c], Iris H. Hall [c], T.S. Andy Hor [a], Yaw Kai Yan [d,*]

[a] *Department of Chemistry, Faculty of Science, National University of Singapore, 3 Science Drive 3, Kent Ridge, Singapore 117543, Singapore*
[b] *Department of Chemistry, University of Waikato, Private Bag 3105, Hamilton, New Zealand*
[c] *Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7360, USA*
[d] *Natural Sciences, National Institute of Education, Block 7 (Science), Nanyang Technological University, 1 Nanyang Walk, Singapore 637616, Singapore*

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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 $[ReBr(CO)_3(Ph_2PCH_2)_2NCH_2]_2$ (**12**). All new complexes have been characterized by NMR and IR spectroscopy and for **7**, **9** and **10**, single-crystal X-ray diffraction analyses. Electrospray mass spectrometric studies show that the rhenium–phosphine chelates are very stable, especially in neutral methanolic solution. Hydrolysis of the ester and amide linkages slowly occur in acidic and basic solutions over several weeks; displacement of the bromide ligand also occurs in basic medium. Cytotoxicity testing of **7**–**10** and **12** showed that all the complexes are active against specific tumor cell lines, especially MCF-7 breast cancer and HeLa-S^3 suspended uterine carcinoma.

Keywords: Rhenium(I); Carbonyl; Bis(diphenylphosphinomethyl)amine; Cytotoxicity; Electrospray mass spectrometry

1. 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 [2], the design of radiopharmaceuticals based on Re(I)-tricarbonyl complexes has gained considerable attention recently [4]. The advantages of this organometallic approach and the methodology for generating the $[Re(CO)_3]^+$ synthon have also been described [4].

It has also been recognized that phosphines, by virtue of their versatile ligating capability with transition metals, can play a major role in the design of radiometal–antibody conjugates [5–7]. The direct introduction of phosphino groups onto peptides via the Mannich-type reaction of hydroxymethyl phosphines with the free amino groups on the peptide has been studied [7]. 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 [7], or link two separate peptides to the same radiometal atom (reducing the overall labeling efficiency). An alternative strategy is the "preformed 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.

* Corresponding author. Tel.: +65-790-3816; fax: +65-896-9432.
E-mail address: ykyan@nie.edu.sg (Y.K. Yan).

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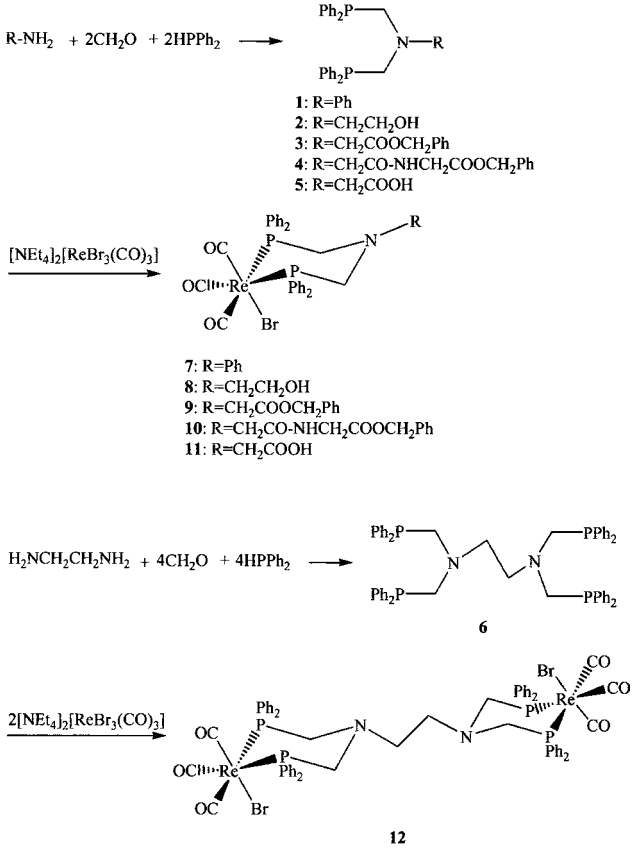
Bis(diphenylphosphinomethyl)amines are good candidates for the preformed chelate approach. They are readily synthesized via the Mannich reaction between primary amines and secondary phosphines (with formaldehyde) [8,9] or bis(hydroxymethyl)phosphonium salts [10], and are known to form stable chelate complexes with palladium, platinum and rhodium [11,12]. 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 1). When the functional group or molecular chain that links the chelate unit with the biomolecule is attached to the middle atom of the diphosphine backbone, the steric interaction between the radiometal's coordination sphere with the protein is minimized. The relative simplicity of the chelating system is also attractive.

In the course of our ongoing research on rhenium(I) carbonyl alkoxo complexes [13], 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)(η^2-dppf)$(CO)_3$] exhibit potent cytotoxicity against a number of cancer cell lines [14]. 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(diphenylphosphino-methyl)-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. Preliminary results of the cytotoxicity screening of these complexes against 18 cancer cell lines are also presented.



Scheme 1.

2. Experimental

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. $[NEt_4]_2[ReBr_3(CO)_3]$ was prepared by the published procedure [15]. ^{1}H- 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. ^{1}H 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 FTIR spectrophotometer. Elemental analyses were carried out in the Microanalytical Laboratory at the National University of Singapore.

2.1. Preparation of bis(diphenylphosphinomethyl)amines

Bis(diphenylphosphinomethyl)amines $(Ph_2PCH_2)_2NR$ {R = Ph [12] (**1**), CH_2CH_2OH [10] (**2**), CH_2COOCH_2Ph (**3**), $CH_2CONHCH_2COOCH_2Ph$ (**4**), CH_2COOH [8] (**5**)} and $(Ph_2PCH_2)_2NCH_2CH_2N(CH_2PPh_2)_2$ [12] (**6**) were generally prepared by the procedure in Ref. [10] with only slight modifications. $^{31}P\{^1H\}$-NMR ($CDCl_3$): **1** δ −27.4 (s); **2** δ −27.3 (s); **3** δ −26.9 (s); **4** δ −27.3 (s); **5** δ −27.0 (s); **6** δ −28.2 (s).

2.2. Preparation of bis(diphenylphosphinomethyl)amine tricarbonylrhenium(I) bromide complexes—general method

$[NEt_4]_2[ReBr_3(CO)_3]$ (144 mg, 0.2 mmol) was dissolved in MeOH (30 ml) and an equimolar amount of the appropriate 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.

2.2.1. $[ReBr(CO)_3\{(Ph_2PCH_2)_2NPh\}]$ (7)

The bis(diphenylphosphinomethyl)amine **1** (98 mg, 0.2 mmol) gave complex **7** (120 mg, 72%). Found for $C_{35}H_{29}BrNO_3P_2Re$: C, 50.2; H, 3.5; N, 1.6. Calc.: C, 50.1; H, 3.5; N, 1.7%. ^{1}H-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, CH_2P), 4.14 (m, 2H, CH_2P). $^{31}P\{^1H\}$-NMR ($CDCl_3$): δ −21.1 (s). IR (cm^{-1}, KBr): 2031vs, 1950vs, 1901vs (C≡O).

2.2.2. $[ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2CH_2OH\}]$ (8)

The bis(diphenylphosphinomethyl)amine **2** (92 mg, 0.2 mmol) gave complex **8** (83 mg, 51%). Found for $C_{31}H_{29}BrNO_4P_2Re$: C, 46.0; H, 3.7; N, 1.8. Calc.: C, 46.1; H, 3.6; N, 1.7%. ^{1}H-NMR ($CDCl_3$): δ 7.68–7.26 (m, 20H, Ph), 4.31 (m, 2H, CH_2P), 3.73 (m, 2H, CH_2P), 3.18 (t, 2H, CH_2O), 2.71 (br s, 2H, CH_2N). $^{31}P\{^1H\}$-NMR ($CDCl_3$): δ −19.6 (s). IR (cm^{-1}, KBr): 2031vs, 1944vs, 1899vs (C≡O).

2.2.3. $[ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2COOCH_2Ph\}]$ (9)

The bis(diphenylphosphinomethyl)amine **3** (110 mg, 0.2 mmol) gave complex **9** (118 mg, 65%). Found for $C_{38}H_{33}BrNO_5P_2Re$: C, 50.0; H, 3.7; N, 1.6. Calc.: C, 50.0; H, 3.6; N, 1.5%. ^{1}H-NMR ($CDCl_3$): δ 7.66–7.26 (m, 25H, C_6H_5), 5.10 (s, 2H, OCH_2Ph), 4.63 (m, 2H, CH_2P), 3.50 (m, 2H, CH_2P), 3.31 (s, 2H, NCH_2C). $^{31}P\{^1H\}$-NMR ($CDCl_3$): δ −21.7 (s). IR (cm^{-1}, KBr): 2031vs, 1952vs, 1896vs (C≡O), 1734m (C=O, ester linkage).

2.2.4. $[ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2CONHCH_2COOCH_2Ph\}]$ (10)

The bis(diphenylphosphinomethyl)amine **4** (124 mg, 0.2 mmol) gave complex **10** (134 mg, 69%). Found for $C_{40}H_{36}BrN_2O_6P_2Re$: C, 49.6; H, 3.8; N, 2.9. Calc.: C, 49.6; H, 3.8; N, 2.9%. ^{1}H-NMR ($CDCl_3$): 7.44–7.25 (m, 25H, Ph), 5.50 (m, 1H, NH), 5.13 (s, 2H, OCH_2Ph), 4.37 (m, 2H, CH_2P), 3.42 (m, 2H, CH_2P), 3.32 (m, 4H, NCH_2C). $^{31}P\{^1H\}$-NMR ($CDCl_3$): δ −18.1 (s). IR (cm^{-1}, KBr): 2032vs, 1954vs, 1904vs (C≡O), 1735m, 1673m (C=O, ester and amide linkages).

2.2.5. $[ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2COOH\}]$ (11)

The bis(diphenylphosphinomethyl)amine **5** (94 mg, 0.2 mmol) gave complex **11** (87 mg, 53%). Found for $C_{31}H_{27}BrNO_5P_2Re$: C, 45.5; H, 3.3; N, 1.7. Calc.: C, 45.3; H, 3.3; N, 1.7%. ^{1}H-NMR ($CDCl_3$): δ 7.71–7.29 (m, 20H, Ph), 4.62 (m, 2H, CH_2P), 3.47 (m, 2H, CH_2P), 3.32 (s, 2H, NCH_2C). $^{31}P\{^1H\}$-NMR ($CDCl_3$): δ −21.7 (s). IR (cm^{-1}, KBr): 2029vs, 1948vs, 1909vs (C≡O), 1718m(br) (C=O, carboxyl).

2.2.6. $[ReBr(CO)_3(Ph_2PCH_2)_2NCH_2]_2$ (12)

$[NEt_4]_2[ReBr_3(CO)_3]$ (144 mg, 0.2 mmol) was dissolved in MeOH (30 ml) and the bis(diphenylphosphinomethyl)amine **6** (85 mg, 0.1 mmol) added. The solution was stirred overnight at room temperature, and 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 (97 mg, 63%). Found for $C_{60}H_{52}Br_2N_2O_6P_4Re_2$: C, 46.5; H, 3.3; N, 1.8. Calc.: C, 46.4; H, 3.4; N, 1.8%. $^{31}P\{^1H\}$-NMR ($CDCl_3$): δ −20.7 (s). IR (cm^{-1}, KBr): 2035vs, 1953vs, 1945vs(sh), 1925vs, 1907vs (C≡O).

2.3. Electrospray mass spectrometry

ESMS spectra were recorded in the positive-ion mode (unless otherwise stated) using a VG Platform II instrument employing nitrogen as both the drying and nebulizing gas. The spectra were typically obtained with an average of 10–12 scans. A range of cone voltages, from 20–100 V were typically applied on each sample to investigate the fragmentation behavior. The analyte solution (ca. 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 five drops of 50% formic acid to 2 ml 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 of NH_3 solution (2 M) to the rhenium complex solution, and the mixture allowed to age over 1 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 [16].

2.4. 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. All the crystals take the form of rectangular blocks. 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 [17]. Crystal and refinement data are summarized 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

Table 1
Crystallographic data

	7·$CHCl_3$	**9**	**10**
Empirical formula	$C_{36}H_{30}BrCl_3NO_3P_2Re$	$C_{38}H_{33}BrNO_5P_2Re$	$C_{40}H_{36}BrN_2O_6P_2Re$
Formula weight	959.01	911.70	968.76
Space group	$P\bar{1}$	*Pbca*	$P\bar{1}$
Crystal system	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
Temperature (K)	293(2)	223(2)	223(2)
Crystal dimensions (mm)	$0.18 \times 0.10 \times 0.08$	$0.26 \times 0.24 \times 0.06$	$0.26 \times 0.14 \times 0.10$
μ(Mo–K_α) (mm^{-1})	4.785	4.660	4.305
Max. 2θ (°)	49.4	58.6	58.6
Number of reflections collected	9064	42 627	12 688
Number of unique reflections (R_{int})	5965 (0.0318)	9017 (0.0433)	9000 (0.0168)
Completeness of data to $2\theta_{max}$ (%)	95.9	92.5	85.2
Max/min transmission	0.7299, 0.5436	0.6040, 0.3850	0.7045, 0.5100
Number of parameters	425	433	469
R_1 [a]	0.0511	0.0332	0.0288
wR_2 [b]	0.1111	0.0547	0.0643

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

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 [18]. Selected geometric parameters of the complexes are given in Table 2.

2.5. Cytotoxicity tests

Complexes **7**–**10** and **12** were tested for cytotoxic activity by homogenizing the compounds as a 1 mg ml^{-1} solution in 0.05% Tween 80–H_2O. These solutions were sterilized by passing them through an acrodisc (0.45 μm). The cell lines investigated (Table 4) were maintained by literature techniques [19]. The NCI protocol was used to assess the cytotoxicity of the test compounds and standard drugs in each cell line [19]. The number of cells was determined by the trypan blue exclusion technique [19] and the percent inhibition of growth for each concentration of compound was calculated and averaged ($N = 4$). The percent inhibition was plotted against the log of the concentration of compound and the ED_{50} value (concentration of compound in μg ml^{-1} inhibiting 50% of cell growth) estimated. Solid tumor cytotoxicity was determined using crystal violet–MeOH and read at 580 nm (Molecular Devices) [20]. An ED_{50} value of less than 4 μg ml^{-1} was required for significant activity for inhibition of cell growth [19].

3. Results and discussion

3.1. Syntheses

The Mannich reactions of aniline, ethanolamine, $H_2NCH_2COOCH_2Ph$, $H_2NCH_2CONHCH_2COOCH_2$-Ph, H_2NCH_2COOH and ethylene diamine gave bis-(diphenylphosphinomethyl)amines **1**–**6**, respectively. The reaction involving the benzyl-protected glycine tended to be cleaner than the reaction involving glycine itself. Reactions of $[NEt_4]_2[ReBr_3(CO)_3]$ and these bis-(diphenylphosphinomethyl)amines gave the rhenium complexes **7**–**12**, respectively (Scheme 1). Complexes **7**–**10** are soluble in CH_2Cl_2 and $CHCl_3$, and slightly soluble in H_2O, MeOH and EtOH. Complex **11** has higher solubility in H_2O due to its carboxylic acid group, which can potentially also be functionalized for attachment to antibodies. Unexpectedly complex **12** is virtually insoluble in all these solvents.

3.2. 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** (Figs. 1–3). 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 conforma-

tions 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) [11,12] 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

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

	7	9	10
Bond lengths			
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)
Bond angles			
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)

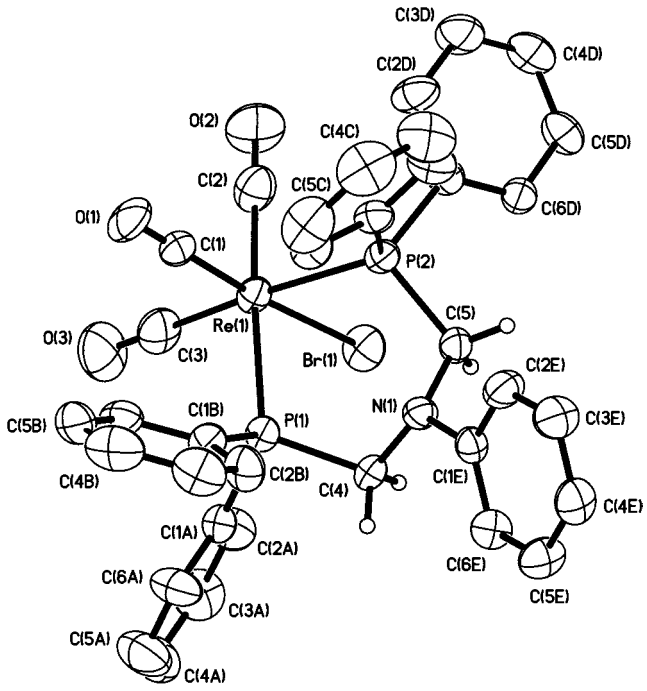


Fig. 1. Crystal structure of $[ReBr(CO)_3\{(Ph_2PCH_2)_2NPh\}]$ **7** with 40% probability thermal ellipsoids. Hydrogen atoms on phenyl rings are omitted for clarity.

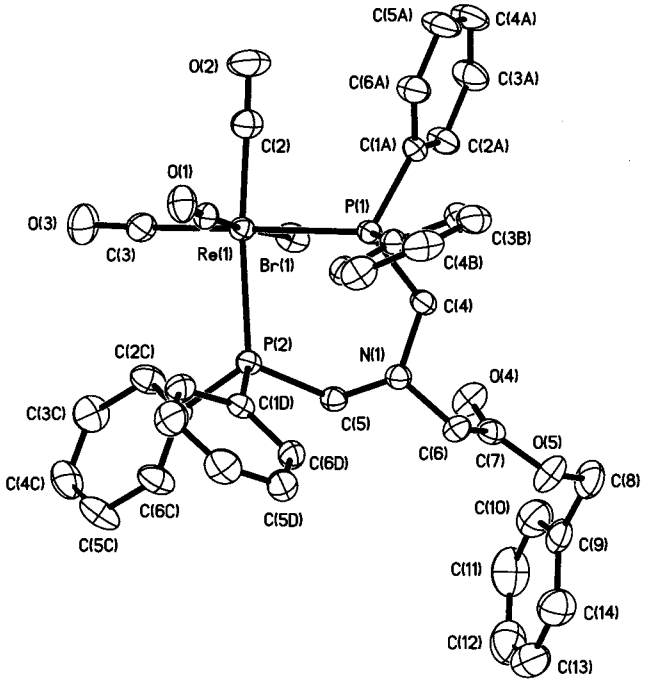


Fig. 2. Crystal structure of $[ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2COOCH_2Ph\}]$ **9** with 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.

Re–Br, Re–CO and Re–P distances found for these structures are typical for analogous rhenium(I) tricarbonyl complexes [21].

3.3. 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-^1H 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).

3.4. Electrospray (ES) mass spectrometric characterization

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. Complex **12**, however, did not give useful spectra, presumably due to its very poor solubility. 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.

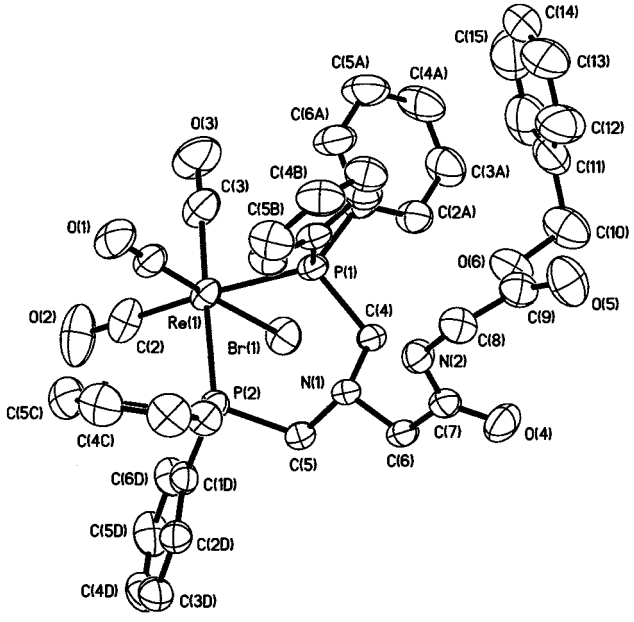


Fig. 3. Crystal structure of $[ReBr(CO)_3\{(Ph_2PCH_2)_2NCH_2CONHCH_2COOCH_2Ph\}]$ **10** with 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.

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 [22]. However, no pyridine-containing ions were observed.

The glycine complex **11**, containing a carboxylic acid group, gave the expected intense $[M - H]^-$ ion (m/z 820) as the base peak at low cone voltages. No Re-containing ions were observed in positive-ion mode. On increasing the cone voltage, fragmentation of the parent ion occurs, via loss of up to two CO ligands, decarboxylation of the CO_2^- group, and fragmentation of the phosphine ligand, giving a coordinated Ph_2P group in $[Ph_2PRe(CO)_3Br]^-$. This ion also undergoes loss of one, two or three CO ligands, giving the series of $[Ph_2PRe(CO)_nBr]^-$ions ($n = 0$–3). Ultimately, at a very high cone voltage of 160 V, the $[P(C_6H_4)ReBr]^-$ion (m/z 373) is observed, which can be distinguished from the isobaric Ph_2PReH by its isotope pattern. This species presumably contains a cyclometallated C_6H_4P moiety, with a four-membered Re–P–C–C ring. 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.

3.5. 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

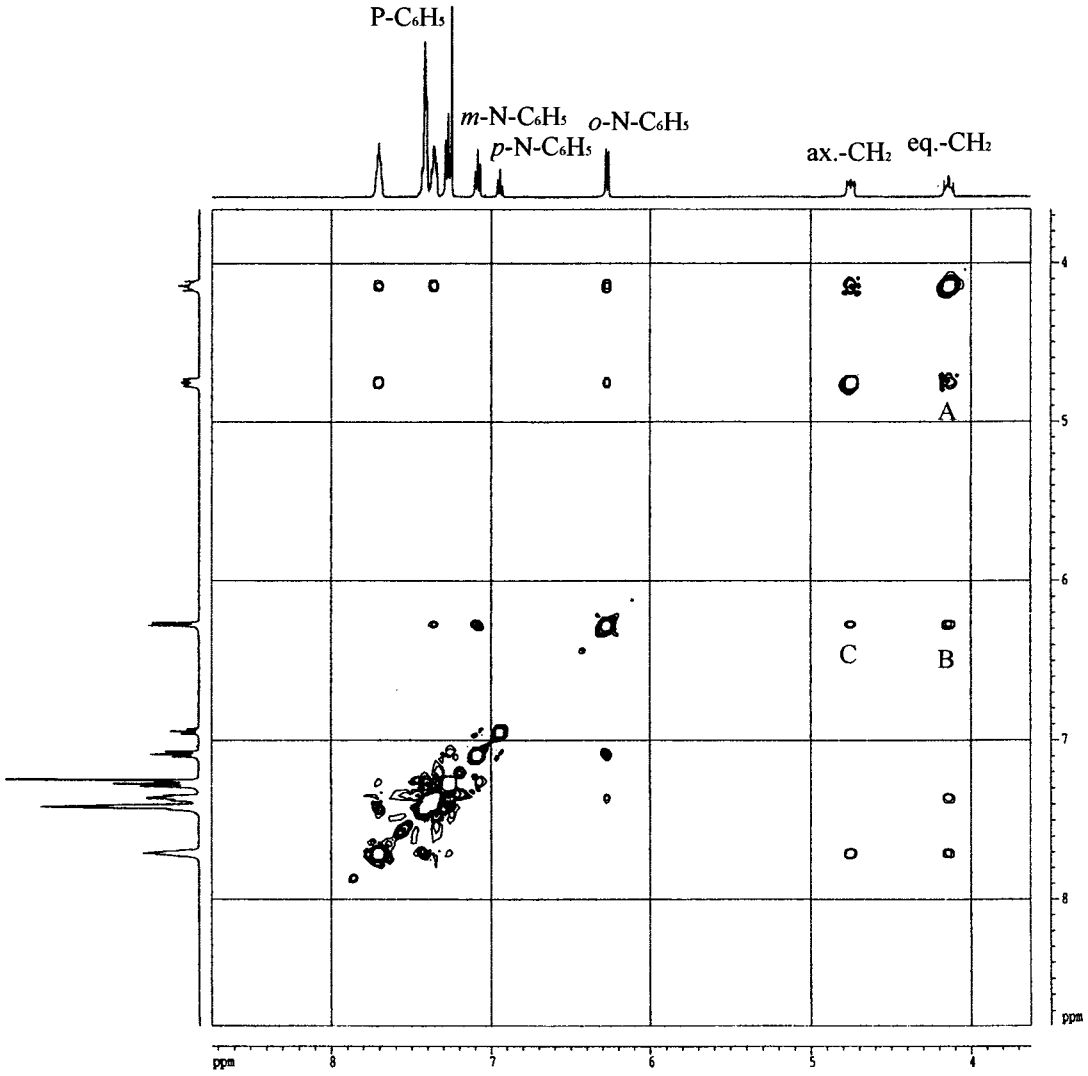


Fig. 4. Two-dimensional ^{1}H ROESY-NMR spectrum of complex **7** in $CDCl_3$ at 300.0 K. Selected NOE contacts: **A**, equatorial-CH_2···axial-CH_2; **B**, equatorial-CH_2···*o*-N–C_6H_5; **C**, axial-CH_2··· *o*-N–C_6H_5.

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

Complex	Cone voltage (V)	Fragment assignment [*m*/*z*, relative intensity (%)]
7	20	$[M+H]^+$ (*m*/*z* 840, 100%), $[M-Br]^+$ (*m*/*z* 760, 55%)
	50	$[M+H]^+$ (*m*/*z* 840, 52%), $[M-Br]^+$ (*m*/*z* 760, 100%)
8	20	$[M+H]^+$ (*m*/*z* 808, 100%), $[M-Br]^+$ (*m*/*z* 728, 5%), $[2M+H]^+$ (*m*/*z* 1615/1617, 5%)
	50	$[M+H]^+$ (*m*/*z* 808, 100%), $[M-Br]^+$ (*m*/*z* 728, 53%), $[2M+H]^+$ (*m*/*z* 1615/1617, 3%)
9	20	$[M+H]^+$ (*m*/*z* 912, 100%), $[M-Br]^+$ (*m*/*z* 832, 12%), $[2M+H]^+$ (*m*/*z* 1823/1825, 10%)
	50	$[M+H]^+$ (*m*/*z* 912, 100%), $[M-Br]^+$ (*m*/*z* 832, 90%)
10	20	$[M+H]^+$ (*m*/*z* 969, 100%), $[M-Br]^+$ (*m*/*z* 889, 5%), $[2M+H]^+$ (*m*/*z* 1937/1939, 10%)
	50	$[M+H]^+$ (*m*/*z* 969, 100%), $[M-Br]^+$ (*m*/*z* 889, 73%), $[2M+H]^+$ (*m*/*z* 1937/1939, 18%)
11 [a]	20	$[M-H]^-$ (*m*/*z* 820, 100%), $[2M-H]^-$ (*m*/*z* 1641, 5%)
	160 [b]	$[PC_6H_4ReBr]^-$ (*m*/*z* 373, 100%), $[Ph_2PReBr]^-$ (*m*/*z* 451, 18%), $[Ph_2PReBr(CO)]^-$ (*m*/*z* 479, 19%), $[Ph_2PReBr(CO)_2]^-$ (*m*/*z* 507, 21%), $[Ph_2PReBr(CO)_3]^-$ (*m*/*z* 535, 20%), $[M-H-2CO]^-$ (*m*/*z* 764, 17%), $[M-H-CO]^-$ (*m*/*z* 792, 16%), $[M-H]^-$ (*m*/*z* 820, 70%), $[2M-H]^-$ (*m*/*z* 1641, 50%)
12	50	$[M+H]^+$ (*m*/*z* 1554, trace), $[M-Br]^+$ (*m*/*z* 1473, trace), plus several unidentified ions

[a] Recorded in negative ion mode.
[b] Ions with relative intensity >10% only.

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 reaction appeared to have occurred, 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 tumor site, but must be metabolized eventually to facilitate the clearance of radioactivity from non-target tissue [23].

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 solvolysis occurred, with the base peak at m/z 759 assigned to $[Re(CO)_3(MeOH)\{(Ph_2PCH_2)_2NCH_2CO_2H\}]^+$, plus other unidentified ions of similar masses. The ion at m/z 759 is formed by loss of the bromide ion (and coordination of methanol) together with hydrolysis of the ester linkage. Solvolysis of **10** appeared to be even more severe, with no $[M + H]^+$ ion remaining. The base peak at m/z 831 is assigned to $[Re(CO)_3(NH_3)\{(Ph_2PCH_2)_2NCH_2C(O)NHCH_2C(O)NH_2\} + NH_3]^+$, with a hydrogen-bonded NH_3 molecule. Other major ions observed include $[Re(CO)_3\{(Ph_2PCH_2)_2NCH_2C(O)NHCH_2CO_2H\}]^+$ (m/z 798), $[Re(CO)_3(NH_3)\{(Ph_2PCH_2)_2NCH_2CO_2Me\} + NH_3]^+$ (m/z 805), and $[Re(CO)_3(H_2O)\{(Ph_2PCH_2)_2NCH_2C(O)NHCH_2CO_2H\}]^+$ (m/z 816). Since all these ions retain the amide linkage, it appears that the amide linkage is robust under mildly basic conditions.

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 emphasized, 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.

3.6. 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 tumors 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*-$[Re(OPh)(\eta^2\text{-}dppf)(CO)_3]$ [14]. For example, all of these rhenium complexes show activity against L1210 mouse leukemia, P388 mouse lymphocytic leukemia, HuT-78 Lymphoma and HeLa-S^3 suspended uterine carcinoma cells, and all are inactive against KB nasopharynx cells. However, complexes **7**–**10** and **12** (average ED_{50} for MCF-7 = 2.37 μg ml^{-1}) show much higher activity against MCF-7 breast cancer cells than the rhenium alkoxo complexes (average ED_{50} for MCF-7 = 9.96 μg ml^{-1}) [14]. It is noteworthy that compounds **7**–**10** and **12** show no activity against non-cancerous human fibroblast cells (Table 4). This is an important property for any chemotherapeutic agent since harmful side-effects of the treatment will be minimized.

Whilst it would be premature to draw any definite conclusions on the molecular mechanism responsible for the activity of compounds **7**–**10** and **12**, it seems probable that complexes **7**–**10** and **12** would bind to DNA bases or side-chains of amino acid residues in peptides and proteins after displacement of the bromide ligands; the feasibility of this process is illustrated by the ESMS studies in basic medium discussed earlier. The stable (as shown by ESMS) $[Re(CO)_3(diphosphine)]$ moiety most probably remains intact, with the

Table 4
Cytotoxicity of complexes **7**–**10** and **12**, expressed as ED_{50} values (μg ml^{-1}) [a]

Tumor cell line	**7**	**8**	**9**	**10**	**12**	Standards [b]	
						6-MP	Ara-C
Suspension cultured cells							
L1210 mouse leukemia	2.36	1.72	1.92	2.80	2.90	2.43	2.07
P388 mouse lymphocytic leukemia	2.72	2.91	2.30	3.61	2.02	2.04	0.79
HL-60 human leukemia	3.42	3.33	2.14	3.37	2.05	3.35	4.00
T $molt_3$ human T cell leukemia	3.27	4.70	5.17	3.01	3.06	1.62	2.67
T $molt_4$ human T cell leukemia	4.52	4.36	4.32	3.64	6.16	2.67	2.36
HuT-78 lymphoma	2.60	2.96	2.55	3.53	2.70	1.68	2.50
THP-1 acute monocytic leukemia	2.10	3.28	3.30	5.21	3.87	3.03	2.54
HeLa-S^3 suspended uterine	2.69	2.32	2.68	2.85	1.45	2.12	2.13
Solid tumor cultures							
KB nasopharynx	6.88	4.88	4.34	6.45	5.49	11.04	2.84
Lung 549	8.02	4.02	8.51	3.24	6.82	4.71	5.62
Ovary 1-A9	7.71	3.39	8.92	3.72	7.39	6.64	5.39
Breast MCF-7 effusion	3.52	2.01	2.37	2.42	1.53	8.84	12.45
Glioma UM 86	8.99	6.49	9.05	6.67	7.78	4.46	1.88
Ileum HCT-8	5.45	10.69	3.95	4.55	5.65	1.15	2.54
Osteosarcoma HSO	4.59	5.32	5.86	7.72	5.00	9.13	0.86
Melanoma SK2	6.11	4.18	3.45	13.3	2.87	6.86	10.53
Prostate PL	3.90	7.30	5.06	8.61	4.95	-	–
Liver Hepe-2	4.76	5.07	4.02	4.49	3.74	–	–
Non-cancerous human cells							
RMPI 1788 fibroblast	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 μg ml^{-1} is required for significant activity for inhibition of cell growth [19].
[b] 6-MP = 6-mercaptopurine, Ara-C = Cytosine β-D-arabinofuranoside.

OH, NH and C=O groups on the diphosphine ligands of complexes **8**, **9** and **10** contributing further to the binding with biomolecules via hydrogen bonding. It is worth mentioning that the related complexes $[Re_2(\mu\text{-}OH)_3(CO)_6]^-$, $[Re_2(\mu\text{-}OH)(\mu\text{-}OPh)_2(CO)_6]^-$, $[Re_2(\mu\text{-}OMe)_2(\mu\text{-}dppf)(CO)_6]$ and $[Re_2(\mu\text{-}OPh)_2(\mu\text{-}dppf)(CO)_6]$ have been shown to interfere with nucleic acid metabolism at multiple enzyme sites in L1210 lymphoid leukemia cells, and cause DNA strand scission after 60 min incubation [14].

4. Conclusions

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.

5. Supplementary material

Crystallographic data for the structural analyses have been deposited with the Cambridge Crystallographic Data Centre, CCDC nos. 173096 for **7**, 173097 for **9**, and 173098 for **10**. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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