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Research Article

Preparation and characterization of technetium-99m complexes with new diphosphines containing polyether groups

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Summary

We report the synthesis and characterization of three new diphosphine ligands with polyether groups of general formula $R(OCH_2CH_2)_nPPhCH_2CH_2PPh(CH_2CH_2O)_nR$: **1** (R = Me, $n = 3$), **2** (R = Bu, $n = 3$) and **3** (R = Bu, $n = 4$). *trans*-$[^{99m}TcO_2L_2]^+$ complexes were prepared by reaction between the diphosphine ligands and the ^{99m}Tc-gluconate precursor. The homologous rhenium complexes, *trans*-$[ReO_2L_2]Cl$ (L = **1**,**2**,**3**), were synthesized by ligand substitution reaction with $[ReO_2py_4]Cl$ and oxorhenium (V) gluconate, and characterized by IR, ESMS and ^1H, $^{13}C\{^1H\}$, $^{31}P\{^1H\}$ NMR spectroscopy. All these data are consistent with the formation of diphosphine complexes chelated to the *trans*-ReO_2 unit. The HPLC analyses of rhenium and ^{99m}Tc complexes display very similar profiles and retention times, consistent with the formation of the homologous complexes. Preliminary biodistribution studies with rats show significant differences between the behaviour of the three ^{99m}Tc complexes. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: diphosphine; ether phosphine; $^{99m}TcO_2$-core; biodistribution

Introduction

Technetium-99m complexes with diphosphine ligands have been widely studied. Some of them, such as the ligand (1,2-*bis*(*bis*-2-ethoxyethyl)phosphino)ethane (tetrofosmin), are used to prepare ^{99m}Tc radiopharmaceuticals. This diphosphine with ether groups is commercialized by Amersham as a

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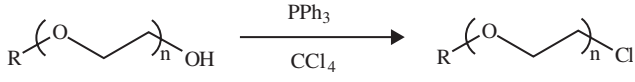
myocardial imaging agent under the name Myoview®.[1] Several studies have shown that the presence of ether groups in ligands designed for technetium myocardial imaging improves biodistribution properties such as background activity in blood and liver.[2,3] With this aim, the synthesis of new ether diphosphine ligands and the study of their potential use in myocardial perfusion imaging has been reported.[4] To study the effect on the imaging characteristics of several polyether chains and lipophilic groups linked to diphosphine ligands, we designed the synthesis of three new diphosphine ligands with polyether groups.[5] These new ether-substituted diphosphine ligands have a similar structure, but slight differences in the lengths of the polyether chain. The evaluation of their ^{99m}Tc complexes in animal studies may contribute to research into the association between the characteristics of the ether groups and biodistribution. In addition, the new ligands contain a butyl or a methyl group bonded to the end of the polyether chain. The different lipophilic characters of these ligands can supply information about the influence of the lipophilicity of their technetium complexes on uptake into the heart.[3]

In this paper, we report the preparation of the new polyether diphosphines and their respective ^{99m}Tc complexes. The homologous rhenium complexes were synthesized and used to characterize the ^{99m}Tc complexes. Preliminary biodistribution studies with rats are also reported.

Results and discussion

Preparation of ligands

Ligands **1**–**3** were synthesized from commercial polyethylene glycol monoalkyl ethers, $CH_3(OCH_2CH_2)_3OH$ and $CH_3(CH_2)_3(OCH_2CH_2)_nOH$. The first step consisted of the preparation of $CH_3(OCH_2CH_2)_3Cl$,[6] $CH_3(CH_2)_3(OCH_2CH_2)_3Cl$ and $CH_3(CH_2)_3(OCH_2CH_2)_4Cl$ by reaction of the alcohols with excess triphenylphosphine in carbon tetrachloride (Scheme 1). Distillation of the products obtained from reaction with $CH_3(CH_2)_3(OCH_2CH_2)_3OH$ (70%) led to $CH_3(CH_2)_3(OCH_2CH_2)_3Cl$ as a main product and to $CH_3(CH_2)_3(OCH_2CH_2)_4Cl$ as a minor product. Next, the reaction between the alkyl chlorides and $Li_2[PhPCH_2CH_2PPh]$[7] afforded ligands **1**–**3** (Scheme 2), which



n = 3; R = CH_3, $CH_3CH_2CH_2CH_2$
n = 4; R = $CH_3CH_2CH_2CH_2$

Scheme 1.

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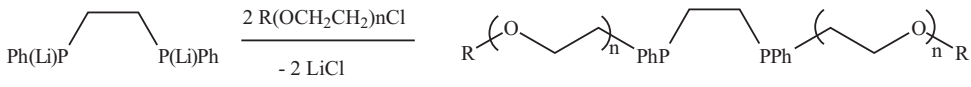

1 (R = CH_3, n = 3)
2 (R = $CH_3CH_2CH_2CH_2$, n = 3)
3 (R = $CH_3CH_2CH_2CH_2$, n = 4)

Scheme 2.

were obtained as oily products, although various attempts at crystallization were made. These products were characterized by NMR and mass spectroscopy. The $^{31}P\{^1H\}$ NMR spectra of **1–3** were nearly identical. Each compound showed two signals at −25.4 and −25.9 ppm, a chemical shift very similar to other $PhRP(CH_2CH_2O)_nR'$ phosphines,[6] and no other significant peaks were observed. This result reveals the absence of other phosphorus compounds and is consistent with the presence of a mixture of diastereoisomers (meso form and racemate). The ^1H and $^{13}C\{^1H\}$ NMR spectra are consistent with the proposed structures, showing the fragments $R(OCH_2CH_2)_n$ and $PhPCH_2CH_2PPh$. The most relevant data are the couplings and chemical shifts of methylene groups of the $R(OCH_2CH_2)_n$ fragment placed in alpha and beta positions with respect to the phosphorus atom. Thus, the signals assigned to the methylene groups in the alpha position were observed at 1.9–2.0 ppm as a triplet of doublets separated from the resonances of the other methylene groups. The methylene groups in the beta position could not be distinguished from the other methylene groups bonded to oxygen in the ^1H NMR spectra and were seen as a multiplet at 3.3–3.6 ppm. Yet, these methylene groups are properly recognized in the $^{13}C\{^1H\}$ NMR spectra as a triplet at nearly 68 ppm. This chemical shift is consistent with those reported for similar $PhRP(CH_2CH_2O)_nR'$ ligands.[6,8] The multiplicity of this signal is assigned to the virtual coupling of the carbon nucleus with the two phosphorus atoms, as described elsewhere.[9] This hypothesis was corroborated by $^{13}C\{^1H, ^{31}P\}$ NMR spectra, in which these signals are displayed as singlets, confirming the previous assignment of the coupling in the $^{13}C\{^1H\}$ NMR spectra to the phosphorus atoms. Finally, M + 1 ions gave the strongest signal in mass spectroscopy (CI).

Preparation of rhenium complexes

The cold rhenium complexes $[ReO_2L_2]^+$ (L = **1**,**2**,**3**) were prepared at the macroscopic level, in order to confirm the structure of the homologous $[^{99m}TcO_2L_2]^+$ (L = **1**,**2**,**3**) by comparison between the HPLC data. The rhenium complexes were prepared in two approaches, as shown in Scheme 3. In the first, excess ligand in a dichloromethane solution was added to an

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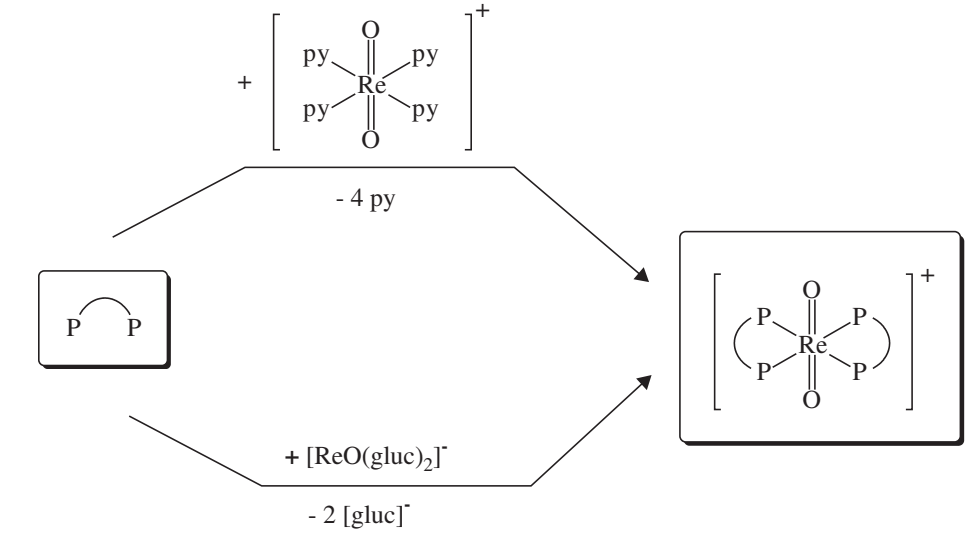

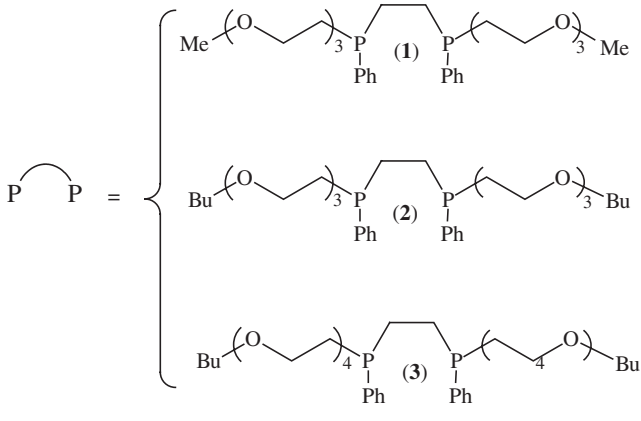

[gluc]- = gluconate

Scheme 3.

ethanol solution of the dioxorhenium (V) complex $[ReO_2py_4]Cl$. The resulting mixture was refluxed for 4 h, and the colour of the solution turned from orange to yellow. The residual oil obtained after evaporation was extracted with hexane to eliminate the ligand excess and other residual products. Unfortunately, no solid compounds could be obtained from these oily

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products, although a large number of attempts were made with different solvents and with substitution of chloride anion by other anions such as $[BF_4]^-$ and $[PF_6]^-$. This behaviour may be explained by the formation of a mixture of diasteroisomers because, as noted in the $^{31}P\{^1H\}$ NMR spectra of **1**, **2** and **3**, all these ligands are a mixture of the meso form and the racemate. Although solid compounds could not be isolated, NMR and mass spectroscopy showed that only products with the diphosphine chelated to the metal by means of the phosphorus atoms were obtained. Thus, the $^{31}P\{^1H\}$ NMR spectra of all reaction products only displayed a multiplet signal at 12.0–12.6 ppm. The position of this signal shows a significant downfield shift after coordination of the diphosphine to the metal fragment, as in other reported *trans*-$[ReO_2(diphosphine)_2]^+$ complexes.[10] The multiplicity of the signals at nearly 12 ppm is explained by the formation of a mixture of diasteroisomers. The IR spectra are consistent with the presence of the dioxorhenium (V) fragment in $[ReO_2L_2]Cl$ complexes (L = **1**,**2**,**3**). All prepared complexes had a strong IR band at 786–802/cm^{-1} that can be assigned to the characteristic ν (Re═O) stretch of the *trans*-ReO_2 fragment. Similar values have been reported for other $[ReO_2(diphosphine)_2]^+$ complexes.[10] The ^1H and $^{13}C\{^1H\}$ NMR spectra of $[ReO_2L_2]Cl$ complexes (L = **1**,**2**,**3**) are similar to those of the corresponding free ligands, but the signals are broader. Nevertheless, we want to highlight the changes observed after complexation in the resonance assigned to the carbon atom of the CH_2O group in the beta position with respect to the phosphorus atom. Though this signal was in a position similar to those of the free ligand, after complexation to the metal, multiplicity was reduced from a triplet to two peaks in the 64–66.0 ppm region. The same splitting was observed in $^{13}C\{^1H, ^{31}P\}$ NMR spectra, indicating that the peaks were due to distinct diastereoisomers.

Finally, electrospray mass spectra of dichloromethane solutions of $[ReO_2L_2]Cl$ complexes (L = **1**,**2**,**3**) in positive ion mode displayed the predominant signals of $[M]^+$ ions, with isotope patterns in concordance with the calculated distribution for the proposed stoichiometry, as shown in Figure 1 for the complex $[ReO_2(\mathbf{1})_2]Cl$.

The dioxorhenium $[ReO_2L_2]^+$ complexes (L = **1**,**2**,**3**) were also prepared by reaction at room temperature between an aqueous solution of the precursor oxorhenium (V) gluconate and a solution of excess diphosphine ligands in tetrahydrofuran (thf) (Scheme 3). This precursor and its homologous ^{99m}Tc complex are widely used as labile complexes for the preparation of target complexes by substitution of the gluconate ligands by more inert ligands.[11] The reaction products were studied by $^{31}P\{^1H\}$ NMR spectroscopy and the position and patterns of the peaks in the 12–13 ppm region were identical to the signals observed in the $^{31}P\{^1H\}$ NMR spectra of the $[ReO_2L_2]Cl$ complexes (L = **1**, **2**, **3**) prepared from $[ReO_2py_4]Cl$. This is consistent with

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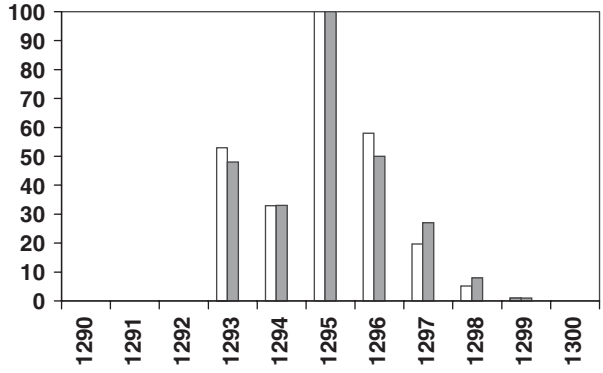
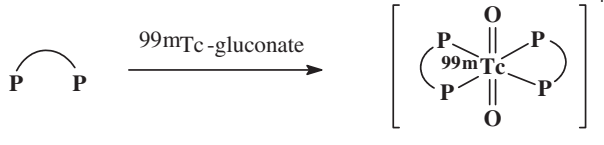


Figure 1. Positive-ion ES mass spectrum: calculated (white) and observed (grey) isotope pattern for the $[ReO_2(\mathbf{1})_2]^+$ ion

the formation of the same complexes on following the two approaches described in Scheme 3.

Preparation of ^{99m}Tc complexes

The $[^{99m}TcO_2L_2]^+$ complexes were prepared with the chelating diphoshine ligands **1**–**3** under no-carrier-added conditions by ligand substitution from the ^{99m}Tc-gluconate precursor, as shown in Scheme 4. The precursor was prepared by reduction of $[^{99m}TcO_4]^-$ with stannous chloride in the presence of D-gluconate and monitored by HPLC analysis. The addition of the diphosphine ligand to this solution at room temperature led to the formation of the expected complexes, as shown by HPLC analysis. The HPLC analysis of the three radiocomplexes showed the presence of a single broad peak with a retention time that was consistent with the formation of the $[^{99m}TcO_2L_2]^+$ complexes (L = **1**: 31.9 min; L = **2**: 36.8 min; L = **3**: 35.2 min), since the homologous rhenium complexes show similar retention times (L = **1**: 31.5 min; L = **2**: 34.9 min; L = **3**: 35.1 min) and signal profiles (Figure 2). The RCP of the technetium complexes, evaluated by HPLC chromatography, ranged between 85 and 90% for all complexes. All these data support the view that ^{99m}Tc complexes form structurally identical to the rhenium complexes described in the previous section.



Scheme 4.

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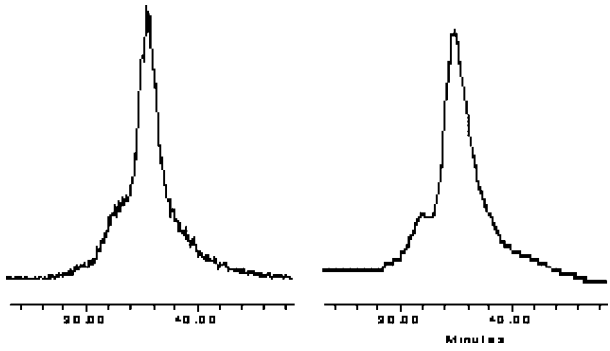

Figure 2. HPLC profiles for $[^{99m}TcO_2(3)_2]^+$ (left) and $[ReO_2(3)_2]^+$ (right)

Table 1. Biodistribution of $[^{99m}TcO_2L_2]^+$ in rats (% ID/g, $N = 3$)

Tissues	L = **1**	L = **2**	L = **3**
Heart	0.20 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
Liver	0.36 ± 0.03	0.29 ± 0.06	0.88 ± 0.11
Kidney	0.38 ± 0.04	0.25 ± 0.05	0.78 ± 0.08
Lung	0.15 ± 0.02	0.05 ± 0.01	0.08 ± 0.02
Muscle	0.06 ± 0.03	0.02 ± 0.01	0.03 ± 0.01

Biological distribution studies

The biodistribution of $[^{99m}TcO_2L_2]^+$ (L = **1**,**2**,**3**) complexes in rats is shown in Table 1. The three compounds have notable differences in their biodistribution, corroborating that small changes in the molecular structure of the complexes lead to large modifications in the biodistribution of radiocomplexes. Another point worthy of mention is that only complex with ligand **1** show significant heart uptake and that this is practically zero for complexes with ligands **2** and **3**. This may be linked to the presence of the butyl group in these complexes. Therefore, the increase in the lipophilic character of these cations due to butyl groups does not lead to higher heart uptake, but, on the contrary, uptake becomes virtually imperceptible. On comparing the results between ligands **2** and **3**, it is clear that the increase in the polyether chain in complex with ligand **3** involves greater liver and kidney uptake, but has no effect on heart uptake.

Experimental

Materials and methods

Diphosphine ligands and rhenium complexes were prepared under nitrogen by standard Schlenk tube techniques. Unless otherwise stated, NMR spectra were recorded on a Bruker AC250 instrument at the Nuclear Magnetic Resonance Service at the Autonomous University of Barcelona (UAB). All chemical shift values are expressed in ppm with respect to residual protons in the solvent for

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proton spectra, to solvent signals for ^{13}C spectra, and to external phosphoric acid for phosphorus spectra. Mass spectra were recorded at the Chemical Analysis Service of the UAB by a HP59-98X instrument (CI/NH_3) and at the Scientific-technical Service of the University of Barcelona (UB) with a VG-Quattro (Micromass) instrument (ES). The HPLC analysis of labelled reaction mixtures was performed on a Teknokroma nucleosil100 C-2 column (7 μm, 250 mm × 4 mm) at an isocratic flow rate of 1.0 ml/min of acetonitrile/water (8:2).

$CH_3(CH_2)_3(OCH_2CH_2)_3OH$ (70%), $NaReO_4$ and NH_4ReO_4 were purchased from Fluka, Alfa and Strem, respectively. The rhenium gluconate solution[11] and the compounds $CH_3(OCH_2CH_2)_3Cl$,[6] $PhHP(CH_2)_2PHPh$[7] and $[ReO_2py_4]Cl \cdot 2H_2O$[12] were prepared following the published procedures.

The experiments with rats complied with the relevant national laws on animal experiments. Male Sprague-Dawley rats weighing roughly 350 g were anaesthetized with an intraperoneal injection of ketamine and isofluorane. A solution of each ^{99m}Tc complex was prepared and two aliquots (300–400 μCi/300 μl) were drawn with insulin syringes. One aliquot was used for doses and the other one was diluted to 1000 ml. Three 1 ml portions of the resulting solution were used as standards. All doses were administered intravenously to rats via the tail vein. The animals ($N = 3$) were sacrificed by asphyxiation using carbon dioxide 60 min post-injection. The heart, liver, muscle, lungs and kidneys were removed and their activity was measured on a 1282 COMPUGAMMA CS gamma-counter, LKB Wallac. Results are reported as the percentage injected dose of organ per gram of the respective organ (% ID/g).

Preparation of the ligands

Synthesis of ***1***. Butyllithium 2.5 M (4.0 ml, 10.0 mmol) was dropwise added to a solution of $PhPHCH_2CH_2PHPh$ (0.90 g, 3.7 mmol) in thf (70 ml) cooled in an ice bath, giving rise to an orange solution that was stirred for a few minutes. The reaction mixture was kept in the ice bath and a solution of $CH_3(OCH_2CH_2)_3Cl$ (1.35 g, 7.4 mmol) in thf (20 ml) was slowly added. The ice bath was removed and the reaction mixture was allowed to warm to room temperature. At this point, some drops of water were added to eliminate excess butyllithium and the solvents were removed *in vacuo*. Water (50 ml) was added to the residual oil and the mixture was extracted with hexane (3 × 50 ml), which was subsequently dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo*. Diphosphine **1** was obtained as an almost colourless oil. Yield: 1.76 g (88%).

NMR data ($CDCl_3$). $^{31}P\{^1H\}$: −25.9 (s), −25.4 (s). ^1H: 1.5–1.8 (m, PCH_2CH_2P), 1.91 (td, $^3J_{H,H} = 7.3$ Hz, $^2J_{H,P} = 1.4$ Hz, PCH_2CH_2O), 3.28 (s, OCH_3), 3.3–3.6 (m, CH_2O), 7.2–7.5 (m, Ph). $^{13}C\{^1H\}$ (spectrum registered with a Bruker ARX500 instrument): 23.6 (s, PCH_2CH_2O), 27.6 (dd, $J_{PC} = 8.6$ Hz,

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J'_{PC} = 5.7 Hz, PCH_2CH_2P), 27.8 (dd, J_{PC} = 8.6 Hz, J'_{PC} = 4.8 Hz, PCH_2CH_2P), 58.7 (s, OCH_3), 67.8 (t, PCH_2CH_2O, J_{PC} = 10.5 Hz), 69.8–71.6 (m, CH_2O), 128.1 (b, Ph, C^3), 128.7 (s, Ph, C^4), 131.9 (b, Ph, C^2), 137.2 (b, Ph, C^1).

MS (CI/NH_3). m/z (M + 1) = 540.

Synthesis of ***2****. (a) Synthesis of* $CH_3(CH_2)_3(OCH_2CH_2)_3Cl$. Triphenylphosphine (100 g, 0.38 mol) was added to a solution of $CH_3(CH_2)_3(OCH_2CH_2)_3OH$ (59.8 g) in 300 ml of CCl_4. The alcohol $CH_3(CH_2)_3(OCH_2CH_2)_3OH$ was purchased as 70% w/w, and so the number of moles of $CH_3(CH_2)_3(OCH_2CH_2)_3OH$ was nearly 0.2. The other main product is $CH_3(CH_2)_3(OCH_2CH_2)_4OH$. The resulting mixture was refluxed for 2 h and during this period, $P(O)Ph_3$ precipitated as a white solid. The mixture was allowed to cool to room temperature, and pentane (150 ml) was added to complete the precipitation of $P(O)Ph_3$ and the remaining PPh_3. Solids were separated by filtration and washed with pentane (75 ml). The resulting solution was evaporated under vacuum, and a colourless oil was obtained, which was distilled at reduced pressure (5 Torr), b.p. 100–106°C. Yield: 41.7 g (≈ 90%). Analysis by gas chromatography revealed the purity to be higher than 97%.

NMR data ($CDCl_3$). ^{1}H: 0.74 (t, $^3J_{HH}$ = 7.3 Hz, CH_3), 1.19 (sext., $^3J_{HH}$ = 7.3 Hz, CH_2CH$_3$), 1.38 (quint., $^3J_{HH}$ = 7.3 Hz, CH_2CH$_2$CH$_3$), 3.28 (t, $^3J_{H,H}$ = 6.6 Hz, $CH_3CH_2CH_2CH_2O$), 3.3–3.7 (m, CH_2O, CH_2Cl). ^{13}C{^{1}H}: 13.6 (s, CH_3), 19.0 (s, CH_2CH_3), 31.4 (s, O$CH_2CH_2CH_2$), 42.3 (s, CH_2Cl), 69.8–71.1 (m, CH_2O).

MS (CI/NH_3). m/z (M + 18) = 242.

(b) Synthesis of $[CH_3(CH_2)_3(OCH_2CH_2)_3P(Ph)CH_2]_2$. This step was performed like the synthesis of **1**. Particular data for this synthesis are: $PhPHCH_2CH_2PHPh$ (0.90 g, 3.7 mmols), 2.5 M butyllithium (4.0 ml, 30 mmol), $CH_3(CH_2)_3(OCH_2CH_2)_3Cl$, (1.66 g, 7.4 mmol). Yield: 1.95 g (85%).

NMR data ($CDCl_3$). ^{31}P{^{1}H}: –25.8 (s), –25.4 (s). ^{1}H: 0.87 (t, $^3J_{HH}$ = 7.3 Hz, CH_3), 1.31 (sext., $^3J_{HH}$ = 7.3 Hz, CH_2CH$_3$), 1.50 (quint., $^3J_{HH}$ = 7.3 Hz, CH_2CH$_2$CH$_3$), 1.6–1.8 (m, PCH_2CH_2P), 1.95 (td, $^3J_{H,H}$ = 7.3 Hz, $^2J_{H,P}$ = 1.5 Hz, PCH_2CH$_2$O), 3.3–3.7 (m, CH_2O), 7.2–7.4 (m, Ph). ^{13}C{^{1}H}: 13.7 (s, CH_3), 19.0 (s, CH_2CH_3), 23.7 (s, PCH_2CH$_2$O), 28.2 (b, PCH_2CH_2P), 31.5 (s, O$CH_2CH_2CH_2$), 68.3 (t, PCH_2CH_2O, $^2J_{PC}$ = 10.5 Hz), 69.8–70.9 (m, CH_2O), 128.1 (b, Ph, C^3), 128.7 (s, Ph, C^4), 132.0 (b, Ph, C^2), 137.2 (b, Ph, C^1).

MS (CI/NH_3): m/z (M + 1) = 624.

Synthesis of ***3****. (a) Synthesis of* $CH_3(CH_2)_3(OCH_2CH_2)_4Cl$. This compound was obtained as a second fraction (b.p. 132–135°C) in the reduced pressure distillation of $CH_3(CH_2)_3(OCH_2CH_2)_3Cl$ described above. Yield: 11.7 g. Analysis by gas chromatography revealed a purity of ≈ 93%.

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NMR data ($CDCl_3$). ^1H: 0.72 (t, $^3J_{HH} = 7.3$ Hz, CH_3), 1.17 (sext., $^3J_{HH} = 7.3$ Hz, CH_2CH_3), 1.37 (quint., $^3J_{HH} = 7.3$ Hz, $CH_2CH_2CH_3$), 3.26 (t, $^3J_{H,H} = 6.6$ Hz, $CH_3CH_2CH_2CH_2O$), 3.3–3.6 (m, CH_2O, CH_2Cl). $^{13}C\{^1H\}$: 13.4 (s, CH_3), 18.7 (s, CH_2CH_3), 31.2 (s, $OCH_2CH_2CH_2$), 42.1 (s, CH_2Cl), 69.6–70.8 (m, CH_2O).

MS (CI/ NH_3). m/z (M + 18) = 286.

(b) Synthesis of $[CH_3(CH_2)_3(OCH_2CH_2)_4P(Ph)CH_2]_2$. This was performed like the synthesis of **1**. Particular data are: $PhPHCH_2CH_2PHPh$ (0.90 g, 3.7 mmol), butyllithium 2.5 M (4.0 ml, 10.0 mmol), $CH_3(CH_2)_3(OCH_2CH_2)_4Cl$ (2.00 g, 7.4 mmol). Yield: 2.15 g (82%).

NMR data ($CDCl_3$). $^{31}P\{^1H\}$: −25.9 (s), −25.4 (s). ^1H: 0.80 (t, $^3J_{HH} = 7.3$ Hz, CH_3), 1.25 (sext., $^3J_{HH} = 7.3$ Hz, CH_2CH_3), 1.45 (quint., $^3J_{HH} = 7.3$ Hz, $CH_2CH_2CH_3$), 1.5–1.8 (m, PCH_2CH_2P), 1.88 (td, $^3J_{H,H} = 7.3$ Hz, $^2J_{H,P} = 1.5$ Hz, PCH_2CH_2O), 3.3–3.6 (m, CH_2O), 7.2–7.4 (m, Ph). $^{13}C\{^1H\}$: 13.6 (s, CH_3), 18.9 (s, CH_2CH_3), 23.6 (s, PCH_2CH_2O), 28.1 (b, PCH_2CH_2P), 31.3 (s, $OCH_2CH_2CH_2$), 68.2 (t, PCH_2CH_2O, $J_{PC} = 10.5$ Hz), 69.7–70.7 (m, CH_2O), 128.0 (b, Ph, C^3), 128.6 (s, Ph, C^4), 131.9 (b, Ph, C^2), 137.1 (s, Ph, C^1).

MS (CI): m/z (M + 1) = 711.

Preparation of rhenium complexes

(a) Reactivity towards $[ReO_2py_4]Cl \cdot 2H_2O$. *Ligand* ***1***. A solution of **1** (0.260 g, 0.48 mmol) in dichloromethane (5 ml) was added to a solution of $[ReO_2py_4]Cl \cdot 2H_2O$ (0.100 g, 0.16 mmol) in EtOH (10 ml) and the reaction mixture was refluxed for 4 h. During this period, the solution changed colour from orange to yellow. The cold solution was evaporated to dryness and the oily residue was vigorously extracted with hexane (3 × 20 ml). The residual oil was dissolved in a few millilitres of dichloromethane and hexane was added dropwise until a cloudy liquid was formed. The resulting mixture was cooled to −10°C and a yellow oil was formed that was separated and evaporated under vacuum to yield a yellow oily product. Yield: 0.188 g (74%).

Analytically calculated for $C_{56}H_{88}ClO_{14}P_4Re \cdot 3CH_2Cl_2$: C, 44.69; H, 5.98. Found: C, 44.45; H, 5.79.

IR (KBr): ν (Re═O) 786/cm.

NMR data ($CDCl_3$). $^{31}P\{^1H\}$: 12.1, 12.3 and 12.5 ppm. ^1H: 1.5–2.8 (b, PCH_2), 3.3 (b, CH_2O, OCH_3), 5.3 (CH_2Cl_2), 6.9–7.4 (b, Ph). $^{13}C\{^1H\}$: 22.0 (b, PCH_2CH_2O), 26.3 (b, PCH_2CH_2P), 58.3 (s, OCH_3), 64.7 (s, PCH_2CH_2O), 65.4 (s, PCH_2CH_2O), 69.7 (b, CH_2O), 71.2 (b, CH_2O), 128.3 (b, Ph, C^3, C^4), 128.7 (s, Ph, C^4), 130.8 (b, Ph, C^2).

MS(ES): $m/z = 1295$. HPLC ($R_t = 31.5$ min).

The reactions with ligands **2** and **3** were performed following the procedure described for ligand **1** and the specific data for these preparations are as follows:

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Ligand 2. **2** (0.30 g, 0.48 mmol), $[ReO_2py_4]Cl \cdot 2H_2O$ (100 mg, 0.16 mmol). Yield: 0.210 g (79%).

Analytically calculated for $C_{68}H_{112}ClO_{14}P_4Re \cdot 2CH_2Cl_2$: C, 50.37; H, 7.01. Found: C, 49.88; H, 6.71.

IR (KBr): ν (Re = O) 802/cm.

NMR data ($CDCl_3$). $^{31}P\{^1H\}$: 12.2, 12.3 and 12.5 ppm. ^1H: 0.84 (b, CH_3), 1.29 (b, CH_2CH_3), 1.50 (b, $CH_2CH_2CH_3$), 1.9–2.8 (m, PCH_2), 3.5 (b, CH_2O), 5.3 (CH_2Cl_2), 7.1–7.6 (m, Ph). $^{13}C\{^1H\}$: 13.7 (s, CH_3), 19.0 (s, CH_2CH_3), 27.2 (b, PCH_2), 31.5 (s, $OCH_2CH_2CH_2$), 65.5 (b, PCH_2CH_2O), 69.8–70.9 (m, CH_2O), 128.8 (b, Ph, C^3, C^4), 131.2 (b, Ph, C^2).

MS(ES): $m/z = 1463$. HPLC ($R_t = 34.9$ min).

Ligand 3. **3** (0.34 g, 0.48 mmol), $[ReO_2py_4]Cl \cdot 2H_2O$ (100 mg, 0.16 mmol). Yield: 0.250 g (81%).

Analytically calculated for $C_{76}H_{128}ClO_{18}P_4Re \cdot 3CH_2Cl_2$: C, 49.16; H, 7.00. Found: C, 48.99; H, 6.65.

IR (KBr): *v* (Re=O) 797/cm.

NMR data ($CDCl_3$). $^{31}P\{^1H\}$: 12.1, 12.2 and 12.5 ppm. ^1H: 0.75–0.85 (m, CH_3), 1.23 (b, CH_2CH_3), 1.42 (b, $CH_2CH_2CH_3$), 1.9–2.8 (m, PCH_2), 3.3–3.6 (m, CH_2O), 5.3 (CH_2Cl_2), 7.0–7.5 (m, Ph). $^{13}C\{^1H\}$: 13.5 (s, CH_3), 18.8 (s, CH_2CH_3), 22.0 (b, PCH_2CH_2O), 26.7 (b, PCH_2CH_2P), 31.2 (s, $OCH_2CH_2CH_2$), 64.9 (s, PCH_2CH_2O), 65.6 (s, PCH_2CH_2O), 69.7–70.7 (m, CH_2O), 128.5 (b, Ph, C^3, C^4), 131.1 (b, Ph, C^2).

MS(ES): $m/z = 1640$. HPLC ($R_t = 35.1$ min).

(b) Reactivity towards rhenium (V) gluconate. *Ligand **1***. A solution of **1** (0.51 g, 0.95 mmol) in thf (5 ml) was added to a solution of rhenium gluconate (5.0 ml, 0.32 mmol) in water. After 4 days of stirring at room temperature, the initially dark blue mixture changed to pale yellow colour. The reaction mixture was evaporated *in vacuo* and an oily residue was obtained. $^{31}P\{^1H\}$ NMR ($CDCl_3$): 12.2, 12.4 and 12.6 ppm (signals of free ligand were also observed).

Reactions with ligands **2** and **3** were performed as described for **1** and the specific data are:

$[ReO_2(2)_2]^+$: **2** (0.59 g, 0.95 mmol), rhenium (V) gluconate (5.0 ml, 0.32 mmol).

$^{31}P\{^1H\}$ NMR ($CDCl_3$): 12.3, 12.5, and 12.7 ppm (signals of free ligand were also observed).

$[ReO_2(3)_2]^+$: **3** (0.67 g, 0.95 mmol), rhenium (V) gluconate (5.0 ml, 0.32 mmol).

$^{31}P\{^1H\}$ NMR ($CDCl_3$): 12.2, 12.4 and 12.7 ppm (signals of free ligand were also observed).

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Preparation of ^{99m}Tc complexes

Fifty microlitres of 0.1 M sodium gluconate and 25 µl of 0.005 M stannous chloride in HCl 0.05 M were added to a vial. Then, 1 ml of $^{99m}TcO_4$ (30–40 MBq) was added and the mixture was stirred for 10 min at room temperature. The complete reduction of the pertechnetate to ^{99m}Tc-gluconate was checked by ITLC-SG and HPLC. Afterwards, 50 µl of an ethanol solution of the appropriate ligand (0.013 M) was added and the resulting solution was stirred for 30 min at room temperature. Labelling yield: 85–90%.

HPLC: $[^{99m}TcO_2(\mathbf{1})_2]^+$ ($R_t = 31.9$ min), $[^{99m}TcO_2(\mathbf{2})_2]^+$ ($R_t = 36.8$ min), $[^{99m}TcO_2(\mathbf{3})_2]^+$ ($R_t = 35.2$ min).

Conclusions

Three new diphosphine ligands were synthesized with polyether groups for studying the influence of small changes in the structure of ligands in biodistribution. The $[^{99m}TcO_2L_2]^+$ complexes were prepared and their structure was established by comparison with studies performed with rhenium complexes. Biodistribution studies show that the presence of the *n*-butyl ether fragment leads to a practically zero heart uptake. It is only significant with the methyl ether fragment.

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