Citation for published version:

DOI:
10.1039/C4CC06367H

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

Link to publication

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Showcasing the research of the multidisciplinary team of Benny, Higham and Pascu.

Re and $^{99m}$Tc complexes of BodP₃ – multi-modality imaging probes

Shine a light! The fields of organometallic synthesis, fluorescence cell imaging and radiochemistry come together to develop complexes which are capable of probing biologically systems in two complementary ways.
Re and 99mTc complexes of BodP3 – multi-modality imaging probes†

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* Electronic supplementary information (ESI) available: Spectroscopic, crystallographic and photophysical data, cell imaging and cytotoxicity experiments. CCDC 849753 and 1001449–1001452. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4cc06367h

Abstract

A fluorescent tridentate phosphine, BodP3 (2), forms rhenium complexes which effectively image cancer cells. Related technetium analogues are also readily prepared and have potential as dual SPECT/fluorescent biological probes.

1 Introduction

99mTc–phosphine complexes are used in approved and emerging Single-Photon Emission Computed Tomography (SPECT) imaging agents due to the attractive nuclear properties of 99mTc (γ = 140 keV, t1/2 = 6 h) and to the facile formation of inert Tc–P coordinate bonds.1 Resolving the cellular fate of such radiopharmaceuticals remains challenging due to the spatial limitations of SPECT, thus there is a drive to develop novel probes with fluorescent tags in order to facilitate high-resolution imaging by fluorescence microscopy.2 Such a species has to be (i) kinetically inert, (ii) highly fluorescent upon metal ligation, and (iii) resistant to degradation by biological molecules. Here we report our work on developing phosphorus-based probes for this and related applications, including therapeutics. We recently described the synthesis of tridentate phosphine 2, BodP3, (Scheme 1) from air-stable 1, with both retaining the attractive photophysical properties common to Bodipy.3 Bianchini reported the synthesis of the single isomer cis,mer-[ReCl( militias-triphos-Me)] by refluxing mer-[ReCl(CO)3(PPh3)2] with 1,1,1-tris(diphenylphosphinomethyl)ethane.4 With this in mind, 2 was reacted with mer-[ReCl(CO)3(PPh3)2] under similar conditions; however, a mixture of three stereoisomers was generated, 3a–c.

These were separated via chromatography and all three were then characterised by X-ray crystallography (Fig. 1 and ESI†).

Scheme 1 The hydrophosphination reaction between primary phos- phine 1 and vinyl(diphenyl)phosphine, to produce the tridentate derivative 2, BodP3.

Reactions with Re5+ and [ReCl(CO)5][OTf] were performed in refluxing methylene chloride to give exclusively the cis,mer isomer, which was characterised by X-ray crystallography (Fig. 3). Substitution of the aqua ligands under aqueous or mixed polar organic conditions readily occurs, requiring the preparation of rhenium analogues under more polar conditions, to polarize the phosphorus bond.1

## Scheme 1

![Scheme 1](image1.png)

**Scheme 1** The hydrophosphination reaction between primary phophine 1 and vinyl(diphenyl)phosphine, to produce the tridentate derivative 2, BodP3.

**Fig. 1** The three isomers isolated from the reaction of the tridentate phosphine 2 and [ReCl(CO)3(PPh3)2] in refluxing toluene.

* Bis(diphenylphosphinoethyl)phenylphosphine (triphos-Ph) reacts with [ReCl(CO)5] in refluxing toluene to form the related isomers reported here, but on prolonged reaction times (> 24 h) at 170 °C, only the cis,mer (1) isomer was produced.5 Phosphine 2 was therefore reacted with mer-[ReCl(CO)3(PPh3)2] in refluxing mesitylene and after 4 h, 31P{1H} NMR spectroscopy showed only 3a. In comparison, the radioactive synthon fac-[99mTc(CO)3(OH2)3]⁺ is readily prepared in situ from an IsoLink kit.6 Substitution of the aqua ligands under aqueous or mixed polar organic conditions readily occurs, requiring the preparation of rhenium analogues under more polar conditions, to better correlate with the 99mTc experiments. Thus triphos-Ph was reacted with [Re(CO)5][OTf] in refluxing ethanol to give fac-[Re(CO)5(triphos-Ph)][OTf], 4; a sample suitable for X-ray crystallographic analysis was obtained by pentane diffusion into a tetrahydrofuran solution (Fig. 2). To synthesise a fluorescent analogue, 2 was reacted with [Re(CO)5][OTf] to give exclusively fac-[Re(CO)5(2)][OTf], 5, characterised by X-ray diffraction (Fig. 3).
The Re–P and Re–C bond lengths and angles are typical for such complexes.4,5 The photophysical properties of 2, 3a–c and 5 were then measured (Table 1). The absorption spectra of 2 and the complexes showed a strong S0–S1 (εabs = 90 000 M–1 cm–1 for 2 at 485 nm). A lower-intensity, broader absorption profile is seen at 527 nm in tetrahydrofuran for 2, and is shifted to lower wavelengths in dichloromethane and methanol. Upon complexation, no or very little change is observed in the absorption profiles are displayed in Fig. 4. Phosphine 2 and its complexes all exhibit emission at room temperature in tetrahydrofuran, dichloromethane and methanol, on excitation at 485 nm. The emission maximum (λem) is seen at 527 nm in tetrahydrofuran for 2, and is shifted to lower wavelengths in dichloromethane and methanol. Upon complexation, no or very little change is observed in the emission maxima. For all the complexes, when the solvent is changed from dichloromethane to methanol, the emission maxima are slightly blue-shifted. The Stokes shift for the complexes are small (14–15 nm in THF), suggesting negligible structural change on excitation. The fluorescence quantum yield (ΦF) for 2 is 0.34 in tetrahydrofuran, which is comparable to the parent primary phosphine 1 (ΦF = 0.33).3 This is important, as it shows that the two additional phosphorus groups in this tridentate derivative do not impact negatively on the fluorescence, and indicates reductive-PeT is not occurring.

On coordination, the fluorescence quantum yields are slightly lowered for all the complexes (Table 1). One explanation for this may be the heavy atom effect, causing spin–orbit coupling and giving rise to intersystem crossing to the triplet state.5

The ΦF values are high in comparison to tridentate quinoline-derived nitrogen-based rhenium complexes, which have ΦF values of 0.003–0.015, and therefore these phosphorus-based probes may be even more sensitive in vitro cell imaging agents.2

The corresponding technetium complexes were first investigated using triphos-Ph as a mimic for 2, to establish the general labelling conditions. Reacting fac[99mTc(CO)3(OH2)3]+ with 1 × 10−3 M triphos-Ph in a pH 7.2 sodium phosphate buffer–ethanol solution at 85 °C for 1 h resulted in quantitative radiochemical conversion to 6. A single HPLC peak for 6 closely matched that of fac-[Re(CO)3(triphos-Ph)]+ (Scheme 2 and Fig. 5). Under the same experimental conditions, a similar amount of radioactivity was observed for the technetium analogues of fac-[ReCl(CO)2(triphos-Ph)]+ and fac-[ReBr(CO)2(triphos-Ph)]+.

attributed to the SP2–S2 (π–π*) transition of the Bodipy core;7 the absorption profiles are displayed in Fig. 4. Phosphine 2 and its complexes all exhibit emission at room temperature in tetrahydrofuran, dichloromethane and methanol, on excitation at 485 nm. The emission maximum (λem) is seen at 527 nm in tetrahydrofuran for 2, and is shifted to lower wavelengths in dichloromethane and methanol. Upon complexation, no or very little change is observed in the emission maxima. For all the complexes, when the solvent is changed from dichloromethane to methanol, the emission maxima are slightly blue-shifted. The Stokes shift for the complexes are small (14–15 nm in THF), suggesting negligible structural change on excitation. The fluorescence quantum yield (ΦF) for 2 is 0.34 in tetrahydrofuran, which is comparable to the parent primary phosphine 1 (ΦF = 0.33).3 This is important, as it shows that the two additional phosphorus groups in this tridentate derivative do not impact negatively on the fluorescence, and indicates reductive-PeT is not occurring.

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Scheme 2. Synthesis of fac-[99mTc(CO)3(triphos-Ph)]+ 6 and fac-[99mTc(CO)3(Bodipy)]+ 7.

Table 1 Photophysical data for phosphine 2 and its Re complexes

<table>
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<tr>
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<th>λabs nm</th>
<th>εabs M–1 cm–1</th>
<th>λem nm</th>
<th>ΦF a,b</th>
<th>ΦF a,c</th>
<th>ΦF a,d</th>
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<td>527</td>
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<tr>
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<td>60 000</td>
<td>527</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
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</tr>
<tr>
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<td>0.24</td>
<td>0.24</td>
<td>0.24</td>
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a In degassed tetrahydrofuran at room temperature. b Measured with respect to 4,4-difluoro-8-phenyl-1,3,5,7-tetramethyl-2,6-diethyl-4-boradiazaisindacene; dyes were excited at 485 nm. c In degassed dichloromethane at room temperature. d In degassed methanol at room temperature. e Insufficient quantity of sample isolated for measurement.
conditions, the Bodipy phosphine, 2, produced fac-[99mTc(CO)3(2)2]+ in similar conversion, giving a single major HPLC peak that correlated with the rhonium analogue 5 (Fig. 5). The stabilities of 6 and 7 were examined by radio-HPLC using competitive amino acid challenge assays (1 mM histidine or cysteine) to simulate an in vivo environment ([PO4]3– 10 mM, pH 7.2, 37 °C). Analysis by HPLC indicated that both complexes remained >97% stable up to 18 h, no trans-chelation of the fac-[99mTc(CO)3]+ complex in 6 or 7 with either amino acid was observed during the study. The radiolabelling yields, purity and stability of 6 and 7 indicate that the tridentate phosphine ligand system is comparable for different; whereas [ReCl(CO)2(triphos-Ph)2]+ (4) at 22.9 min, fac-[99mTc(CO)3(triphos-Ph)2]+ (6) at 23.0 min, fac-[ReCl(CO)2(2)]+ (5) at 24.4 min and fac-[99mTc(CO)3(2)2]+ (7) at 24.5 min.

Radiolabelling of the complexes constitutes the first, crucial step towards their use in nuclear medicine for in vivo tissue imaging, but SPECT does not provide information at the sub-micron resolution level. Thus, in a preliminary screening, the rhenium complexes 3a and 5, Top: cis-mer-[ReCl(CO)2(2)]2+. 3a, 100 μM, 1% DMSO, 15 minutes. Bottom: fac-[ReCl(CO)2(2)]2+. 5, 50 μM, 2% ethanol, 15 minutes. (A) Brightfield image, (B) green channel λex = 460–500 nm, long pass filtered at 510 nm, (C) overlay of A and B.

Notes and references