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Two-photon absorption properties and $^1O_2$ generation ability of Ir complexes: an unexpected large cross section of [Ir(CO)$_2$Cl-(4-(*para*-di-*n*-butylaminostyryl)pyridine)]†‡

Alessia Colombo,*[a] Claudia Dragonetti,[a,b] Dominique Roberto,[a,b] Adriana Valore,[a] Camilla Ferrante,[c] Ilaria Fortunati,[c] A. Lorena Picone,§[c] Francesco Todescato[c] and J. A. Gareth Williams[d]

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The new complexes *cis*-[Ir(CO)$_2$Cl(4-(*para*-di-*n*-butylaminostyryl)pyridine)] (**1**) and [Ir(cyclometallated-2-phenylpyridine)$_2$(4,4′-(*para*-di-*n*-butylaminostyryl)-2,2′-bipyridine)][PF$_6$] (**3**) were synthesized and fully characterized along with the known complex Ir(cyclometallated-2-phenylpyridine)$_2$(5-Me-1,10-phenanthroline)][PF$_6$] (**2**). Remarkably, complex **1**, with an Ir(I) centre, displays fluorescence – as opposed to the phosphorescence typical of many Ir(III) complexes – with a modestly high quantum yield in solution, opening a new route for the design of iridium-based emitters which should not be limited to the +3 oxidation state. It is also characterized by an unexpectedly large two-photon absorption (TPA) cross section, an order of magnitude higher than that previously reported for Ir(III) or Pt(II) complexes. The great potential of cyclometallated Ir(III) complexes for photodynamic therapy was confirmed, with **2** and **3** showing a good singlet oxygen generation ability, coupled with a modest TPA activity for **2**.

Introduction

Molecular two-photon absorption (TPA), where a molecule absorbs simultaneously two photons, has attracted growing interest in recent years due to the wide range of interesting applications,[1,2] including 3D optical data storage[3,4] up-converted stimulated emission,[5,6] microscopy,[7,8] microfabrication,[9] photodynamic therapy,[10–12] localized release of bio-active species,[13] cell imaging,[14,15] and photo-uncaging of various therapeutic molecules including NO and neurotransmitters,[2] to name just a few examples. For this reason, molecular engineering directed towards TPA optimization has become very active, leading to a range of molecules of various symmetries including dipoles, quadrupoles, octupoles, and branched structures.[2,16] Most dipolar chromophores exhibit a smaller two-photon absorption cross-section in comparison with some quadrupolar and octupolar derivatives. However, combining dipolar branches within a three-branched octupolar structure can induce a very intense TPA response.[17] Therefore, there is a need for new dipolar chromophores with relatively high TPA ability. Among them, organometallic complexes and coordination compounds are particularly appealing due to a range of excited states not open to organic molecules, a large Stokes' shift, and facile colour tuning by an appropriate choice of the metal, its oxidation state and its coordination sphere. Another distinctive aspect lies in the longer lifetimes of emission, an aspect of particular relevance for example in bio-imaging. There is consequently increasing interest in metal complexes with TPA properties.[18–22]

A few years ago, some of us reported that the two-photon absorption enhancement upon coordination of a TPA-active ligand to a metal center may be larger in the dipolar rather than the corresponding octupolar complex, once normalized to the ligand unit.[18] In particular, it appeared that the TPA cross section (σ_{TPA}) of 4,4′-bis(*para*-di-*n*-butylaminostyryl)-2,2′-

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[a] *Dipartimento di Chimica dell'Università degli Studi di Milano, UdR- INSTM, Italy. E-mail: alessia.colombo@unimi.it*
[b] *ISTM-CNR, via Golgi 19, I-20133 Milano, Italy*
[c] *Dipartimento di Scienze Chimiche dell'Università di Padova, UdR INSTM di Padova, via Marzolo 1, 35131 Padova, Italy*
[d] *Department of Chemistry, Durham University, Durham, DH1 3LE, UK*

† Dedicated to Professor Renato Ugo, on the occasion of his 77th birthday.

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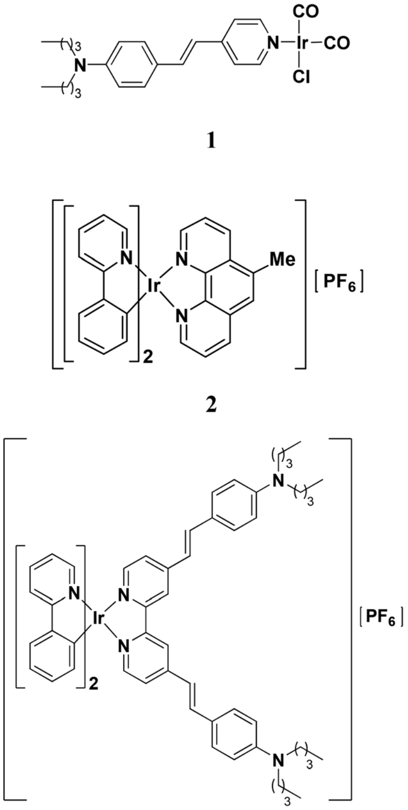
§ Current address: CEQUINOR (UNLP-CONICET), Departamento de Quimica, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 47 esq. 115, (1900) La Plata, Argentina.

bipyridine can be enhanced by a factor of *ca.* 2 upon co-ordination to a "$ZnCl_2$" moiety which increases the electron acceptor properties of the bipyridine fragment.[23] A similar enhancement was observed upon coordination to the same metal moiety of a π-delocalized terpyridine substituted by an electron donor amino group.[24] Interestingly, such complexes are also characterized by high second-order nonlinear optical (NLO) properties.[25,26] These results, and the observation that various dipolar *cis*-[Ir(CO)$_2$Cl(*para*-substituted pyridine)][27] and [Ir(cyclometallated-2-phenylpyridine)$_2$(substituted-1,10-phenanthroline)][PF$_6$][28] also exhibit large second-order NLO properties, prompted us to investigate the effect of coordination to an electron acceptor "Ir(CO)$_2$Cl" moiety on the TPA activity of the known 4-(*para*-di-*n*-butylaminostyryl)pyridine[29] and to study the TPA properties of the known complex [Ir(*N^C*-ppy)$_2$(5-Me-phen)][PF$_6$] (**2**), characterized by interesting emissive properties,[30] and of the new complex [Ir(*N^C*-ppy)$_2$(4,4′-(*para*-di-*n*-butylaminostyryl)-2,2′-bpy)][PF$_6$] (**3**) (ppy = 2-phenylpyridine, phen = 1,10-phenanthroline, bpy = bipyridine; Chart 1). In addition, we investigated the singlet oxygen ($^1O_2$) generation activity of **2** and **3** in order to verify the potential of such complex types for photodynamic therapy, a well-established therapeutic modality for cancer treatment where a photosensitizer generates highly reactive $^1O_2$ that causes cytotoxic effects to targeted cells.[10–12,31]



1

2

3

Chart 1 Structures of the iridium complexes investigated.

Experimental

General comments

All reagents were purchased from Sigma-Aldrich and were used without further purification. Reactions requiring anhydrous conditions were performed under nitrogen. The new complex *cis*-[Ir(CO)$_2$Cl(4-(*para*-di-*n*-butylaminostyryl)pyridine)] (**1**) was prepared as reported below by a reaction of [Ir(CO)$_2$Cl]$_2$ [32] with the corresponding substituted pyridine[29] (for NMR spectra see Fig. S1–S4 in the ESI‡). The known complex [Ir(*N^C*-ppy)$_2$(5-Me-phen)][PF$_6$] (**2**) was synthesized according to the literature,[30,33] and the new complex **3** was synthesised using a similar procedure as set out below.

^1H NMR spectra were recorded at 400 MHz on a Bruker AVANCE-400 instrument. Chemical shifts (δ) for ^1H and ^{13}C spectra are expressed in ppm relative to internal Me_4Si as the standard. Signals were abbreviated as s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Synthesis of *cis*-[Ir(CO)$_2$Cl(4-(*para*-di-*n*-butylaminostyryl)pyridine)] (1). In a three-necked flask [Ir(cyclooctene)$_2$Cl]$_2$ [34] (0.21 g; 0.23 mmol) was added to dehydrated CH_3CN (50 mL) under nitrogen. The suspension was stirred for 2 min, and then nitrogen was replaced by CO. A yellow solution was obtained immediately, due to the conversion of [Ir(cyclooctene)$_2$Cl]$_2$ into [Ir(CO)$_2$Cl]$_2$.[32] After 2 min under CO, 4-(*para*-di-*n*-butylaminostyryl)pyridine (0.14 g; 0.46 mmol) was added. After a further 5 min, all [Ir(CO)$_2$Cl]$_2$ had been converted into *cis*-[(CO)$_2$Cl(4-(*para*-di-*n*-butylaminostyryl)pyridine)], as shown by infrared spectroscopy. After 1 h, the solution was evaporated to dryness; the residue was dissolved in the minimum volume of dichloromethane, *n*-pentane was added, and the solution was cooled to 0 °C. The precipitate that subsequently formed was filtered and dried under vacuum, affording the analytically pure desired complex, which was stored in the dark under nitrogen.

IR (in CH_3CN): ν(CO) 2073(s), 1993(s) cm^{-1}.

^1H NMR (400 MHz CD_2Cl_2): δ(ppm) 8.55 (d, 2H, Py-H, J = 6.5 Hz), 7.48 (m, 4H, Ar-H, Py-H), 7.45 (d, 1H, CHPh, J = 16.2 Hz), 6.84 (d, 1H, PyCH, J = 16.2 Hz), 6.68 (d, 2H, Ar-H, J = 8.9 Hz), 3.36 (t, 4H, NCH_2, J = 7.6 Hz), 1.62 (m, 4H, CH_2), 1.40 (m, 4H, CH_2), 1.00 (t, 6H, CH_3, J = 7.6 Hz).

^{13}C NMR (100 MHz, CD_2Cl_2): δ(ppm) 151.6, 138.0, 129.4, 127.5, 121.7, 117.7, 110.5, 50.7, 29.1, 20.2, 13.7.

Anal. Calcd for $C_{23}H_{28}ClIrN_2O_2$: C, 46.65; H, 4.77; N, 4.73%. Found: C, 46.68; H, 4.76; N, 4.73%.

Synthesis of [Ir(cyclometallated-2-phenylpyridine)$_2$(4,4′-(*para*-di-*n*-butylaminostyryl)-2,2′-bipyridine)][PF$_6$] (3). A solution of the dimeric compound [Ir(*N^C*-ppy)$_2$(μ-Cl)]$_2$ (100 mg, 0.093 mmol) and 4,4′-(*para*-di-*n*-butylaminostyryl)-2,2′-bipyridine (115 mg, 0.187 mmol) in CH_2Cl_2–MeOH (18 mL, 2 : 1 v/v) was heated under reflux for 5 h. The red solution was cooled to room temperature and a 10-fold excess of ammonium hexafluorophosphate was added. The suspension was stirred for 15 min and then filtered to remove the insoluble inorganic salt. The filtrate was dried under reduced pressure, the residue was dissolved in CH_2Cl_2 and filtered to remove the residual traces of the inorganic salt. Diethyl ether was layered onto the filtrate and the product was separated in 80% yield as red crystals after 18 h.

^{1}H NMR (400 MHz, CD_2Cl_2): δ(ppm) 8.45 (s, 2H, bipy), 8.00 (d, J = 8.0 Hz, 2H, Py), 7.79 (d, J = 8.0 Hz, 4H, Py), 7.83 (d, J = 6.1 Hz, 2H, bipy), 7.66 (dd, J = 7.8 Hz, J = 0.8 Hz 2H, Ph), 7.55 (d, J = 8.6 Hz, 4H, C_6H_4), 7.53 (d, J = 16.0 Hz, 2H, =CH), 7.43 (dd, J = 6.1 Hz, J = 1.5 Hz, 2H, bipy), 7.12 (td, J = 7.8 Hz, J = 1.0 Hz, 2H, Py), 7.04 (td, J = 7.3 Hz, J = 1.1 Hz, 2H, Ph), 7.02 (d, J = 16.0 Hz, 2H, =CH), 6.96 (td, J = 7.3 Hz, J = 1.1 Hz, 2H, Ph), 6.74 (d, J = 8.6 Hz, 4H, C_6H_4), 6.38 (d, J = 7.3 Hz, 2H, Ph), 3.44 (m, 8H, CH_2), 1.62 (m, 16H, CH_2), 0.91 (m, 12H, CH_3).

^{13}C NMR (100 MHz, CD_2Cl_2): δ(ppm) 167.9, 156.9, 155.8, 150.9, 150.7, 149.9, 149.1, 149.9, 144.8, 137.9, 137.7, 131.7, 130.6, 129.4, 127.9, 124.8, 123.1, 122.4, 120.4, 119.7, 119.3, 117.7, 111.7, 27.3, 27.1, 22.7, 13.8.

Anal. Calcd for $C_{64}H_{70}F_6IrN_6P$: C, 60.98; H, 5.60; N, 6.67, found: C, 61.32; H, 5.58; N, 6.70.

Optical properties

Linear absorption and luminescence spectra were recorded with a Cary 5 and FluoroMax P (Jobin-Yvon), respectively. Quantum yields (QY) were determined in deaerated CH_2Cl_2 following the procedure of Demas and Crosby.[35] Fluorescein in water at pH > 10 (QY = 0.93)[36] and Coumarin 540A in ethanol (QY = 0.56) were used as reference standards.[37] The TPE technique was used to determine the TPA cross-section (σ_{TPA}) in the 730–930 nm excitation wavelength range employing ultrafast (150 fs) laser pulses.[38] The instrumental set-up has been previously described.[18] Absolute values of the TPA cross section were estimated following the procedure of Xu *et al.*[39] and using Fluorescein in water at pH > 10 as the reference standard. Time-resolved photoluminescence measurements were carried out by time-correlated single-photon counting (TCSPC), using the PicoHarp 300 electronics (PicoQuant) and a single-photon counting avalanche photodiode as the detector (SPAD, MPD, Italy). Excitation at 400 nm was provided by a frequency doubled Ti:sapphire fs laser system (Coherent, Mira900-F, 76 MHz). The fitting of the exponential decay curve was performed with the Symphotime software (PicoQuant). The lifetimes recorded for the Stern–Volmer analysis were obtained by TCSPC using a pulsed 405 nm laser diode as the excitation source in conjunction with a cooled Hamamatsu R928 photomultiplier tube for detection.

Singlet oxygen generation

The detection apparatus (InGaAs photodiode and Lock In) used to determine Φ_Δ allows one to record only an average signal and is not sensitive to the temporal decay of the $^1O_2$ emission. The measurements have been performed at a repetition rate of 200 Hz. For *meso*-tetraphenylporphyrin, used as the standard, and for complexes, the $^1O_2$ emission signal displays a linear dependence on incident power, as expected for a one-photon promoted process. All measurements of $^1O_2$ generation were performed in air and the molar fraction of oxygen present in $CHCl_3$ is 1.56×10^{-4} from the literature data.[40] The singlet oxygen quantum yield Φ_Δ is determined from the relative intensity of singlet oxygen luminescence, measured under identical conditions, by the relation:

$$\Phi_\Delta^{s} = \Phi_\Delta^{r} \frac{\beta_r}{\beta_s} \frac{S_s}{S_r}$$

where subscripts s and r refer to the sample and the standard, respectively. S_i (i = s, r) is the $^1O_2$ luminescence signal for the ith species and β_i is a correction factor accounting for the number of absorbed photons, which in turn depends on the absorbance of the solution at the wavelength of the incident laser beam (A_i) and on its intensity (I_0) through the relationship:

$$\beta_i = I_0\left(1 - 10^{-A_i}\right)$$

As previously reported,[41] a number of control experiments were performed in order to assign the recorded signal to $^1O_2$ emission. Specifically, singlet oxygen signals were not observed from sensitizer-free $CHCl_3$ nor from the degassed sample.[41]

The Stern–Volmer quenching experiments for complex **2** were performed by recording the emission spectra and luminescence lifetimes of this complex in solution in $CHCl_3$ at varying air pressures. The solution was contained within a degassing cuvette equipped with a Young's tap assembly for connection to a vacuum manifold. Data for a "fully degassed" solution was obtained using a high-vacuum rotary vane pump fitted to the manifold, monitoring the pressure with a Pirani gauge. Three freeze–pump–thaw cycles were employed such that the final pressure was $<10^{-2}$ mbar. The corresponding data at higher air pressures were subsequently obtained by means of a diaphragm pump, monitoring the pressure with a Vacuubrand DVR2 gauge. The solution was frozen prior to connection to the manifold in each case, to eliminate the partial pressure of the solvent. Assuming a concentration of oxygen of 2.4 mmol dm^{-3} in $CHCl_3$ under ambient conditions (1 atmosphere of air at 298 K),[42] the measured pressures were converted to concentrations of dissolved molecular oxygen using Henry's Law.

Results and discussion

The new complex *cis*-[$Ir(CO)_2Cl$(4-(*para*-di-*n*-butylaminostyryl)-pyridine)] (**1**) is readily prepared as previously reported for similar Ir(I) complexes,[27] by the reaction of $[Ir(CO)_2Cl]_2$ [32] with the corresponding substituted pyridine.[29] The known complex [Ir(*N^C*-ppy)$_2$(5-Me-1,10-phen)][PF_6] (**2**) and the new complex [Ir(*N^C*-ppy)$_2$(4,4′-(*para*-di-*n*-butylaminostyryl)-2,2′-bpy)][PF_6] (**3**) can be easily synthesized from $[Ir(N^\wedge C)_2(\mu\text{-}Cl)]_2$ according to well-established literature procedures.[30,33]

[Ir(CO)$_2$Cl(4-(*para*-di-*n*-butylaminostyryl)pyridine)]

One-photon absorption properties. The normalized one-photon absorption (OPA) and emission spectra of **1** dissolved in deaerated dichloromethane are shown in Fig. 1. The major absorption band at 447 nm ($\varepsilon = 2.93 \times 10^5$ M^{-1} cm^{-1}) can be

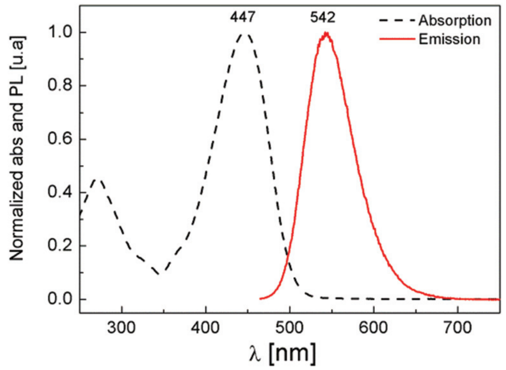


Fig. 1 Normalized one-photon absorption (dashed black line) and emission spectra (red line) of *cis*-[Ir(CO)$_2$Cl(4-(*para*-di-*n*-butylamino-styryl)pyridine)] in CH$_2$Cl$_2$ at 298 K.

attributed to the intramolecular charge transfer (ICT) transition from the donor *N*,*N*-di-*n*-butylamino group to the pyridine ring, red-shifted with respect to 4-(*para*-di-*n*-butylaminostyryl)-pyridine (λ_{max} = 387 nm),[29] as expected for the coordination to an "Ir(CO)$_2$Cl" moiety which increases the electron withdrawing properties of the pyridine ring.[27] The emission maximum of **1** (λ_{max} = 542 nm) is similarly red-shifted with respect to that of the free pyridine ligand[29] (λ_{max} = 467 nm). The Stokes' shift is similar to both the complex and free ligand (3900 and 4400 cm^{-1}, respectively), suggesting that the emission in the complex emanates from a spin-allowed excited state of similar ICT orbital parentage to the ligand, albeit one which is lowered in energy by coordination to the metal ion.

Complex **1** displays a quantum yield of 6.2% in CH$_2$Cl$_2$ at room temperature (details of measurement are given in the Experimental section), a value roughly double that reported for 4-(*para*-di-*n*-butylamino-styryl)pyridine[29] (2.9%). Interestingly, the emission lifetime under these conditions is very short, τ = 0.65 ns, and the value remains unchanged upon degassing the solution indicating no significant quenching by O$_2$. This short lifetime, coupled with the modest quantum yield, is indicative of a high radiative rate constant, k_r, of the order of 10^8 s^{-1}. Such a value is typical of spin-allowed singlet–singlet transitions – that is, of *fluorescence* – rather than the smaller k_r values and longer lifetimes that are typical of phosphorescence in many Ir(III) complexes, such as the very well-known family of complexes based on ppy ligands, of which **2** and **3** are examples. It should be emphasised that the mere presence of a heavy element like Ir in a complex does not necessarily lead to fast intersystem crossing and promotion of spin-forbidden S → T and T → S$_0$ transitions.[43] For the spin–orbit coupling pathways to be efficient, there must be a good match between the energies of metal and ligand orbitals and hence participation of the metal in the excited states, for example, as in the MLCT character of highly phosphorescent complexes like Ir(ppy)$_3$. In the present instance, it is likely that the excited state remains exclusively localised on the ligand, leading to fluorescence that is simply red-shifted with respect to the free ligand through the electron-withdrawing influence of the "Ir(CO)$_2$Cl" unit. Mismatching of metal and ligand orbitals also accounts for the fact that some complexes of Au(III)[44,45] and even of Pt(II)[46] {both of which are isoelectronic with Ir(I)} have been discovered that are fluorescent rather than phosphorescent.

In a frozen glass at 77 K, the fluorescence is strongly blue-shifted, as is typical for excited states having a high degree of intramolecular charge-transfer character, and some vibrational structure starts to emerge (see the ESI‡). The lifetime remains short at 1.1 ns, and there is no appearance of any phosphorescence bands, which supports the notion that S → T intersystem crossing fails to compete effectively with fluorescence in this complex.

These results are of particular interest because the luminescence studies of iridium(I) systems – with a d^8 electronic configuration – have been much less explored in solution, whereas a large number of photophysical and photochemical investigations have been carried out on iridium(III) complexes – with a d^6 Ir centre.[47–49] It is known that some Ir(I) complexes can luminesce in the solid state at room temperature and in frozen glass media,[50] but studies on their emission in fluid solution are rare. Duckett, Eisenberg *et al.* reported the first example of square-planar Ir(I) systems emitting in solution at room temperature.[51] They found that excitation near 450 nm of [Ir(CO)(PPh$_3$)(Et$_2$dtc)], [Ir(dppe)(Et$_2$dtc)], and [Ir(P(OPh)$_3$)$_2$(Et$_2$dtc)] (Et$_2$dtc = *N*,*N*′-diethyldithiocarbamate), dissolved in dichloromethane, leads to broad and featureless emissions centered near 500 nm but the quantum yields were not reported. To the best of our knowledge, luminescence studies of square planar Ir(I) complexes are still in their infancy perhaps due to the focus given to the Ir(III) systems. Remarkably, the quite high quantum yield observed in the present work for complex **1** demonstrates that a careful choice of the coordination sphere of Ir(I) can give interesting luminescent properties, opening a new route for the design of iridium-based emitters which should not be limited to the +3 oxidation state. It is pertinent to recall that Ir(I) is isoelectronic with Pt(II), and that over the past two decades a careful choice of the coordination sphere of square-planar Pt(II) complexes has allowed a huge increase of their quantum yields, from almost non-luminescent in solution at room temperature, to quantum yields approaching unity.[52,53]

Two-photon absorption properties

The two-photon absorption (TPA) spectra of complex **1** were collected using the two photon induced luminescence technique (see the Experimental section). OPA and TPA spectra in CH$_2$Cl$_2$ are depicted in Fig. 2, with the abscissa scale of the TPA spectra divided by two to have a direct comparison with the OPA spectra. The σ_{TPA} are expressed in Göppert-Mayer units (1 GM = 1 × 10^{-50} cm^4 s photon^{-1} molecule^{-1}). The strong overlap of the OPA and TPA spectra of **1** is clear evidence that the one- and two-photon allowed excited electronic state is the same in both cases. This was confirmed by collect-

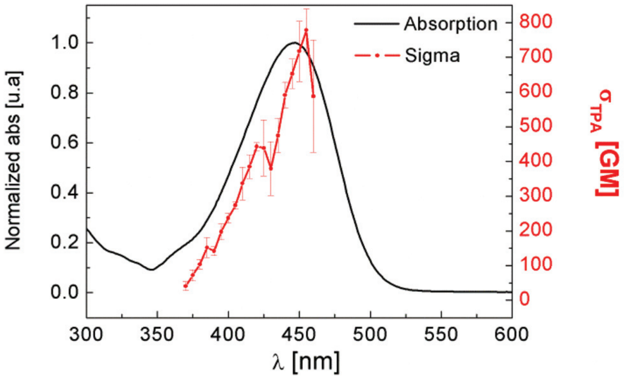


Fig. 2 One-photon absorption (black full line) and two-photon absorption (red line + symbol) spectra of *cis*-[Ir(CO)$_2$Cl(4-(*para*-di-*n*-butylaminostyryl)pyridine)] in CH_2Cl_2. The abscissa scale of the TPA spectrum is divided by two to have a direct comparison with the OPA spectrum.

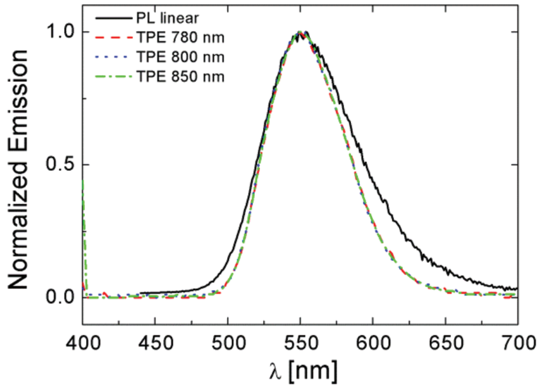


Fig. 3 Two-photon emission spectra of *cis*-[Ir(CO)$_2$Cl(4-(*para*-di-*n*-butylaminostyryl)pyridine)] in CH_2Cl_2 excited at various wavelengths.

ing the two-photon emission (TPE) spectra at various excitation wavelengths (Fig. 3) which are similar to that of the linearly-excited emission.

The TPA cross section maximum for complex **1** is 779 ± 61 GM, at a wavelength of 910 nm, by using fluorescein as the standard. This σ_{TPA} value is four times higher than that reported for 4-(*para*-di-*n*-butylaminostyryl)pyridine[29] (190 ± 34 GM), showing that coordination to a Ir(I) metal center is a convenient way to enhance the efficiency of two-photon absorption. Remarkably, this enhancement is much higher than that previously observed upon coordination of π-delocalized ligands to a Zn(II) metal centre.[23,24]

[Ir(*N^C*-ppy)$_2$(5-Me-phen)][PF$_6$] (2) and [Ir(*N^C*-ppy)$_2$(4,4′-(*para*-di-*n*-butylaminostyryl)-2,2′-bpy)][PF$_6$] (3)

Two-photon absorption properties. Iridium(III) complexes exhibit rich photophysical properties, high luminescence quantum yields, tunable emission colours (from blue to red), and relatively long lifetimes. These properties have resulted in their utilization, for example, in organic light-emitting diodes,[38,54,55] phosphorescent chemosensing systems,[56,57] light-emitting electrochemical cells,[58–60] solar cells,[61] luminescence sensitizers,[62] and recently in bio-imaging.[15,63,64] Concerning the latter application, two-photon excitation is an important tool for non-invasive imaging of live cells and tissues because it involves excitation of the light-absorbing molecule in the red or near-infra-red region using simultaneous absorption of two photons of low-energy light in the range of 600–1100 nm, the region of highest transparency in tissues.[7,65] The efficiency of this nonlinear process relies on the energy density at the excitation point, and thus it is possible to focus on, and image, a specific small volume deep inside a relatively large object. This property enables *z*-axis imaging depths of hundreds of micrometers, and the production of 3D imaging maps. Compounds with high two-photon absorption are desirable for optimal results.[2]

These observations prompted us to study the two-photon absorption efficiency of the known luminescent complex [Ir(*N^C*-ppy)$_2$(5-Me-phen)]PF$_6$, which emits at 559 nm and is characterized by a good quantum yield (38% in CH_2Cl_2; phosphorescence lifetime = 3.17 μs).[30] Its TPA spectrum in CH_2Cl_2 is shown in Fig. 4, for which fluorescein was used as the standard. Clearly the efficiency of two photon absorption for complex **2** is much lower than that for complex **1**. The σ_{TPA} value is 2.9 ± 0.5 GM at 910 nm whereas the highest value, 8.0 ± 1.4 GM, is observed at 750 nm.

In a similar way, while this work was in progress, Beeby *et al.* found that [Ir(*N^C*-ppy)$_3$], [Ir(*N^C*-ppy)$_2$(acac)] (acac = acetylacetonate), and [Ir(*N^C*-2-phenyl-4-(phenylethynyl) pyridine)$_2$(acac)] exhibit TPA cross-sections of 20, 17 and 44 GM, respectively, at 800 nm.[20] The much higher σ_{TPA} value obtained for complex **1** suggests that the use of push–pull π-delocalized chelating ligands might also lead to an enhancement of the TPA efficiency of Ir(III) complexes.

Unfortunately the TPA activity of complex **3** could not be determined by our experimental set-up because it is poorly luminescent at room temperature (the quantum yield in oxygen free CH_2Cl_2 = 0.46%), as expected due to the known

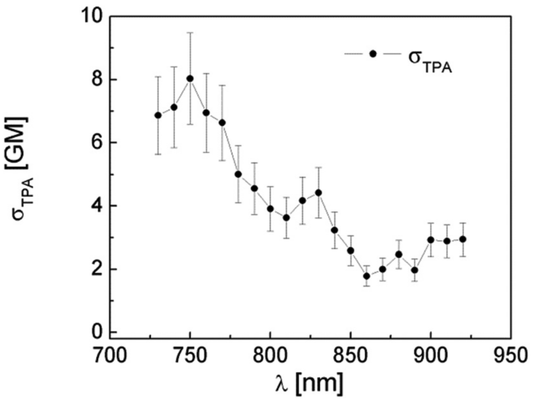


Fig. 4 Two-photon absorption spectrum of [Ir(cyclometallated-2-phenylpyridine)$_2$(5-Me-1,10-phenanthroline)][PF$_6$] in CH_2Cl_2.

trans–cis isomerization of bipyridines linked to an Ir(III) center.[33] In any case, although the magnitude of the TPA cross-sections measured up to now for Ir(III) complexes are relatively low, they are still appreciable when compared with those reported for other coordination and organometallic compounds which have found their application in bio-imaging. For example, platinum *N^C^N* complexes that could be successfully used in time-resolved two-photon cell imaging have values that range between 4 and 20 GM.[20,66,67]

Singlet oxygen generation

The benefits that TPA brings to microscopy also translate to photodynamic therapy (PDT).[2,68,69] One-photon PDT is widely used to treat cancers of the skin and hollow organs, as well as the eye disease macular degeneration. PDT employs a photosensitizer, which is harmless in the absence of light, to induce damage upon optical irradiation. The phototoxicity of these photosensitizers is primarily due to singlet oxygen ($^1O_2$), which is generated by energy transfer from the excited state of the sensitizer to the ground-state (triplet) of oxygen. Two-photon PDT should confine excitation of the photosensitizer to the focal volume. The longer wavelength (near-IR) light required for two-photon excitation also penetrates deeper into living tissues than visible light. These advantages make TPA-PDT of particular interest in neurology and ophthalmology, where there is a need to improve therapeutic targeting whilst simultaneously minimizing invasiveness. Therefore photosensitizers with high σ_{TPA} and that are able to generate $^1O_2$ are needed to allow a significant reduction in irradiation intensities and treatment times, and make TPA-PDT an attractive therapeutic strategy.

These observations prompted us to investigate the singlet oxygen generation activity of complexes **2** and **3** following the method previously reported by some of us.[12,41] Thus, the quantum yield of one-photon sensitized $^1O_2$ formation (Φ_Δ) of both complexes was estimated through measurements of the 1270 nm emission intensity of $^1O_2$ upon excitation at 403 nm, working in $CHCl_3$ and using *meso*-tetraphenylporphyrin as the reference.[70] It turned out that both complexes **2** and **3** have a good singlet oxygen generation activity ($\Phi_\Delta = 0.30 \pm 0.03$ and 0.35 ± 0.03 for **2** and **3** respectively), as recently reported for other Ir(III) complexes.[31,71]

In parallel to the $^1O_2$ production, the quenching of the luminescence of complex **2** by molecular $^3O_2$ could be monitored. The luminescence spectra and lifetimes of the complex in solution in $CHCl_3$ (10^{-5} M) were recorded at varying pressures of air at 298 K, and converted to corresponding O_2 concentrations as described in the Experimental section (Fig. 5). The intensity of emission at 565 nm was used to generate a Stern–Volmer plot (Fig. 6a). The Stern–Volmer quenching constant K_{SV} is obtained from the gradient of the plot of $I(0)/I$ against $[O_2]$, where $I(0)$ is the intensity in the absence of a quencher. A value of 11 800 M^{-1} was obtained in this way.

The luminescence lifetime in the degassed solution was measured to be 1340 ns. Since

$$K_{SV} = k_Q{}^{O_2} \times \tau(0)$$

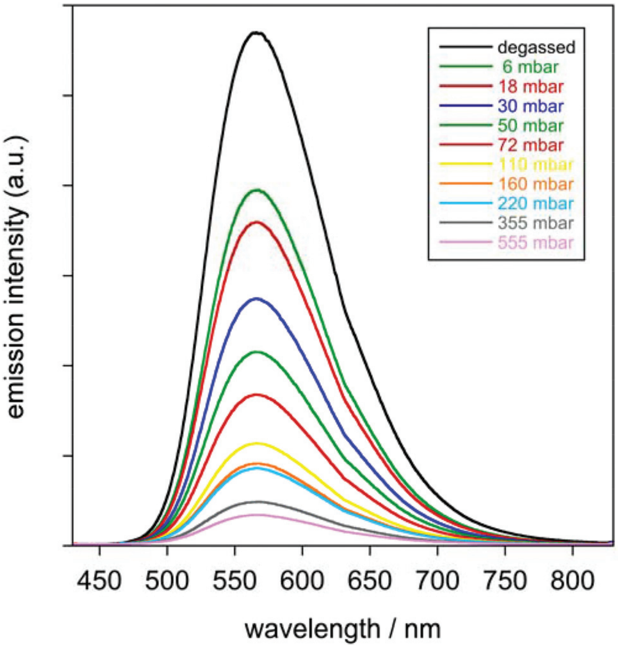


Fig. 5 Emission spectra of complex **2** in $CHCl_3$ at 298 K, recorded in degassed solution ($p < 10^{-2}$ mbar) (black line) and at variable pressures of air as indicated in the inset. λ_{ex} = 400 nm.

where $\tau(0)$ is the lifetime in the absence of the quencher, the value of the bimolecular oxygen quenching constant $k_Q{}^{O_2}$ can be estimated to be $8.8 \times 10^9\ M^{-1}\ s^{-1}$. This figure is comparable to values measured previously for other bis- and tris-cyclometallated iridium(III) complexes.[72,73]

The quenching constant $k_Q{}^{O_2}$ can also be estimated more directly from the lifetime data, being the gradient of a plot of the observed decay rate constant ($= \tau^{-1}$) as a function of $[O_2]$:

$$\tau^{-1} = \tau^{-1}(0) + k_Q{}^{O_2}[O_2]$$

A value of $1.1 \times 10^{10}\ M^{-1}\ s^{-1}$ is obtained in this way (Fig. 6b), which is comparable to that obtained from the intensity data, within the uncertainty in the measurements. The efficient quenching of emission by $^3O_2$ is consistent with the observed high efficiency of $^1O_2$ formation.

Conclusions

This work demonstrates how a careful choice of the coordination sphere of square-planar Ir(I) can give interesting luminescent properties in solution, potentially opening a new route for the design of iridium emitters which should not be limited to the +3 oxidation state. Indeed, the new complex *cis*-[Ir(CO)$_2$Cl(4-(*para*-di-*n*-butylaminostyryl)pyridine)] (**1**) is characterized by a quantum yield of 6.2%, as determined in CH_2Cl_2, a value roughly double that of uncoordinated 4-(*para*-di-*n*-butylamino-styryl)pyridine. Moreover, it is remarkable that coordination to an "Ir(CO)$_2$Cl" moiety allows for the two-photon absorption cross section of the π-delocalized pyridine to be

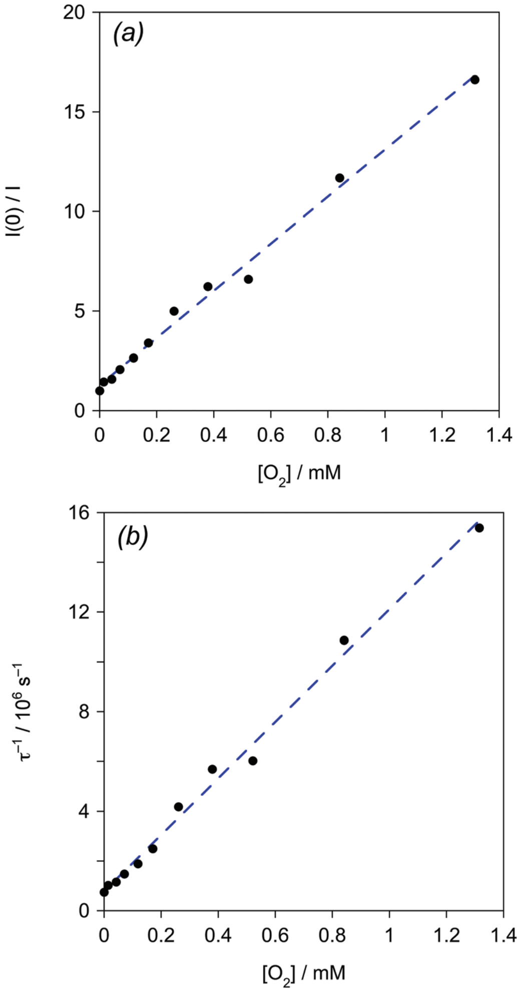


Fig. 6 (*a*) Stern–Volmer plot using the intensity of emission at 565 nm (from spectra shown in Fig. 5) in the absence, *I*(0), and presence, *I*, of oxygen at varying concentrations in oxygen. (*b*) Plot of the observed decay rate constant (= reciprocal of luminescence lifetime) as a function of $[O_2]$. The blue dashed lines show the least-squares linear fit to the data.

increased by a factor of 4, reaching a σ_{TPA} value an order of magnitude higher than that previously reported for Ir(III) or Pt(II) complexes. This observation is of particular relevance and could pave the way to the use of square planar Ir(I) complexes for many applications including iridium-based bio-imaging which was previously restricted to +3 complexes with a d^6 electronic configuration. In addition, our work confirms the potential of cyclometallated Ir(III) compounds for photodynamic therapy (PDT).[63] Both the new complex $[Ir(N^\wedge C\text{-ppy})_2(4,4'\text{-}(para\text{-di-}n\text{-butylaminostyryl})\text{-}2,2'\text{-bpy})]PF_6$ (**3**) and the known complex $[Ir(N^\wedge C\text{-ppy})_2(5\text{-Me-phen})]PF_6$ (**2**) have a good singlet oxygen generation activity (Φ_Δ = 0.30–0.35). Clearly, it will be necessary to seek for design systems in the future that combine the augmented TPA activity associated with complex **1**, with the singlet-oxygen generating ability of complexes of type **2**.

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