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Analytical and preparative enantioseparation and main chiroptical properties of Iridium(III) bis(4,6-difluorophenylpyridinato)picolinato

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We dedicate this work to Ettore Castiglioni who, with his great experience in the field of chromatography and chiroptical spectroscopies, gave important contributions to scientific research.

Keywords:
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ABSTRACT

Almost all Iridium(III) complexes employed both as dopants in PhOLEDs and as pharmaceuticals and fluorescence bioprobes are racemic mixtures. In this study the single enantiomers of the most stable diastereomeric form *fac-trans-N–N*, bis[2-(4,6-difluorophenyl)pyridinato-C^2,*N*](picolinato)iridium(III) (FIrpic) were separated and analysed. The data obtained showed that the complex can be separated into stable optically active Λ and Δ isomers employing cellulose based chiral stationary phase both in normal and polar phase mode. Their chirality was confirmed and their absolute configuration assigned employing several methods (DFT and TDDFT calculations, CD and VCD). The CPL spectroscopy of the isolated enantiomers of FIrpic was also recorded due to its possible value in the OLEDs field. The chromatographic method was applied for a semipreparative purpose demonstrating that polar organic solvent chromatography (POSC) could be used to avoid the low-solubility issues associated with these Iridium(III) complexes. Finally, the chemical and stereochemical stability of the single isomers was evaluated under thermal stress by liquid chromatography coupled to high-resolution mass spectrometry (LC-QTOF) on both chiral and achiral columns. No racemization and/or isomerization was observed; however, the dissociation of the ancillary ligand was demonstrated employing LC-QTOF.

1. Introduction

In the last decade cyclometalated iridium(III) (Ir^{III}) complexes have attracted great attention both from a photophysical point of view and for their wide range of biological applications. They currently represent the emitters of choice for the fabrication of high-efficiency organic blue light emitting devises (OLEDs) due to their electroluminescent properties [1]. From a biological point of view, for many years Ir^{III} complexes have been studied for their applications both as intracellular fluorescent probes and as anticancer and antibacterial agents [2–4]. Recently, the rich luminescent properties of cyclometalated Ir^{III} probes have been employed in oligonucleotide-based sensing [5–7]. Some of them show a higher potency compared to Pt(II) metallo-drugs, such as cisplatin, which are the most widely used anticancer drugs. In particular, they seem to possess the ability to modulate their reactivity and sensitivity (ROS increase, DNA damage, protein disruption) by changing the chelating ligand [8]. In general, the cytotoxicity of cyclometalated Ir^{III} compounds is related to their cellular uptake efficiency, which in turn correlates with their lipophilicity [9]. As anticancer agents, they can act as effective photoinduced singlet oxygen producers ($^1O_2$) leading to cell death [10–12] or inhibitors of the tumor necrosis factor-α (TNF-α), a cytokine involved in systemic inflammation and other biological processes [13]. More

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recently, it has been demonstrated that cyclometalated Ir^{III} complexes possess a potent broad-spectrum antibacterial activity [14]. This is of considerable interest to counteract the resistance to common carbon chain based antibiotics.

Metal complexes contain bidentate organic ligands bound to the metal center in a precise three-dimensional arrangement [15]. Depending on the type of ligands surrounding the central iridium atom, these organometallic complexes can be categorized as homoleptic and heteroleptic. Both homoleptic and heteroleptic complexes can exist as geometric isomers often referred to as meridional (*mer*) and facial (*fac*) isomers. Geometric isomers display a wide range of photophysical and chemical properties, which can affect the performance and lifetime stability of OLED devices and the biological activity of the Ir^{III} complex [16–18]. Each geometric isomer can exist as a couple of two enantiomers, named delta (Δ) and lamda (Λ). The donor atoms of each chelate ring define a line. Two such lines for a pair of chelate rings in the same complex define a helix, one line being the axis of the helix and the other a tangent of the helix at the normal common to the skew lines. The tangent describes a right-handed (Δ) or a left-handed (Λ) helix with respect to the axis and thereby defines the chirality of that configuration. In this context, the stereochemistry of the cyclometalated Ir^{III} complexes gains great importance.

Only very few studies in the literature regarding the single enantiomers of cyclometalated Ir^{III} complexes have been reported. In particular, Coughlin et al. [19], Mazzeo et al. [20] and Li et al. [21] have described the different stereo-optical properties of the single enantiomers of Ir^{III} complexes. In the literature, only a pharmacological evaluation of the single enantiomers of an Ir^{III} complex has been reported by Leung et al. [13]. The lack of research studies regarding the single enantiomers of cyclometallated Ir^{III} complexes is most likely due to the difficulty to find an appropriate method for their enantioseparation. Only supercritical phase chromatography based methods with the use of amylose-based chiral stationary phases (CSPs) have been reported.

Since the most widely employed Ir^{III} complex is bis[2-(4,6-difluorophenyl)pyridinato-C^2,*N*](picolinato)iridium(III) (FIrpic) (Fig. 1), a chromatographic method for the enantioseparation of its most stable geometric isomer has been developed in the present work. FIrpic can exist as four geometric isomers (Fig. 1), but only the one with *cis*-C—C and *trans*-N—N disposition in either the Λ (left) or Δ (right) isomers (Fig. 2) of the two cyclometallated ligands is present in the commercially available product [1]. Once the two enantiomers have been isolated, the absolute configuration was assigned by time-dependent density functional theory (TDDFT) calculations, circular dichroism (CD) and vibrational circular dichroism (VCD). In view of a subsequent application to OLEDs fabrication, circularly polarized luminescence (CPL) spectroscopy of the isolated enantiomers of FIrpic was recorded and thermal stability studies were conducted by chiral and achiral liquid chromatography coupled to high-resolution mass spectrometry (LC-QTOF).

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents, except those specifically noted, were purchased from Sigma- Aldrich (Milan, Italy). LC/MS grade solvents were purchased from Sigma- Aldrich (Milan, Italy).

2.2. Chiral separation

The chromatographic apparatus was an Agilent 1200 series liquid chromatograph (LC) interfaced to an Agilent 1260 photodiode array detector (DAD), to a Jasco 1595 circular dichroism (CD) detector and to an Agilent 6540 Q-TOF mass spectrometer equipped with an electrospray ionization source (ESI) operating in positive ion mode. The optimized source parameters for MS analysis were: drying gas temperature 350 °C and gas flow 11 L/min, nebulizer gas flow pressure 35 psi and capillary voltage 3500 V, fragmentor 110 V. MS spectra were acquired in the positive ionization mode with an acquisition rate of 1 spectra/s over a mass range of 100–1700 *m*/*z*. Mass calibration was enabled using reference masses of *m*/*z* 922.010 and 1521.972 (ESI-TOF tuning mix G1969-85000, Agilent Technologies, Milan, Italy). An automatic rheodyne six-port valve installed post-column permitted to waste ESI non-compatible mobile phases. MS and DAD chromatograms were acquired and analysed using Agilent MassHunter Qualitative Analyses version B.06.00 data processing software. CD chromatograms were recorded with a Jasco J-700 program (Jasco Europe, Italy, Milan). The columns used were a Chiralcel® OB-H (cellulose tribenzoate, 150 × 4.6 mm I.D., 5 μm), Chiralcel® OJ-H (cellulose tris(4-methylbenzoate), 150 × 4.6 mm I.D., 5 μm) and Chiralcel® OD (cellulose tris(3,5-dimethylphenylcarbamate), 250 × 4.6 mm I.D., 5 μm). Different mobile phase combinations and temperatures were screened in order to find the optimal conditions for the chiral separation: 100% methanol at 25 °C, 100% acetonitrile at 25 °C, 100% 2-propanol at 25 °C, hexane:2-propanol 70:30 (v/v) at 25 °C and 5 °C, hexane:2-propanol 50:50 (v/v) at 25 °C, hexane:2-propanol 30:70 (v/v) at 25 °C and 5 °C, and hexane:2-propanol 10:90 (v/v) at 25 °C.

Pure enantiomers of FIrpic were obtained by semipreparative HPLC on Chiraspher OD column (cellulose tris(3,5-dimethylphenylcarbamate), 250 × 10 mm I.D., 10 μm) with fraction collection of the respective peaks. The mobile phase consisted of 100% 2-propanol. The compound was dissolved in mobile phase at final concentration of 10 mg/mL. The injection volume was 100 μL and the flow 2.5 mL/min. The detector was set at 250.4 nm. The collected fractions corresponding to the enantiomers were analysed by injection on Chiralcel OD analytical column with a 100% 2-propanol as mobile phase with a 0.5 mL/min flow rate. HPLC-grade 2-propanol was obtained from Baker.

2.3. Chromatographic parameters

The separation factor (α) was calculated as k_2/k_1 and retention factors (k_1 and k_2) as $k_1 = (t_1 - t_0)/t_0$, where t_1 and t_2 refer to the retention times of the first and second eluted enantiomers respectively. The resolution factor (R_s) was calculated by the formula $R_s = 2(t_2 - t_1)/(w_1 + w_2)$, where w_1 and w_2 are the peak widths at base for the first and second eluted enantiomer respectively.

2.4. HPLC-DAD-HRMS (thermal stability)

The analytical apparatus employed to evaluate the thermal stability was the same described for the chiral separation. The experimental parameters were set as follows: the capillary voltage was 3500 V, the nebulizer (N_2) pressure was 35 psi, the drying gas temperature was 350 °C, the drying gas flow was 11 L/min and the skimmer voltage was 40 V. Data were acquired by Agilent Mass Hunter system software (version 6.2). The mass spectrometer was operated in the full-scan mode in the *m*/*z* range 100–1700. MS^2 spectra were automatically performed with nitrogen as the collision gas in the *m*/*z* range 50–1700, using the auto MS/MS function and a collision energy of 20 eV. Extracted ion chromatograms (EICs) were obtained with an accuracy of 10 ppm *m*/*z* from total ion chromatogram (TIC). The chiral column used was a Chiralcel® OD (cellulose tris(3,5-dimethylphenylcarbamate), 250 × 4.6 mm I.D., 5 μm), 100% 2-propanol as mobile phase at a flow rate of 0.5 mL/min. The achiral columns used were a C18 Poroshell 120