



Article

Arene Ruthenium Metalla-Assemblies with Anthracene Moieties for PDT Applications

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Abstract: The synthesis and characterization of three metalla-rectangles of the general formula $[\text{Ru}_4(\eta^6\text{-}p\text{-cymene})_4(\mu_4\text{-clip})_2(\mu_2\text{-L}_{\text{anthr}})_2][\text{CF}_3\text{SO}_3]_4$ (L_{anthr} : 9,10-bis(3,3'-ethynylpyridyl)anthracene; clip = oxa: oxalato; dobq: 2,5-dioxido-1,4-benzoquinonato; donq: 5,8-dioxido-1,4-naphthoquinonato) are presented. The molecular structure of the metalla-rectangle $[\text{Ru}_4(\eta^6\text{-}p\text{-cymene})_4(\mu_4\text{-oxa})_2(\mu_2\text{-L}_{\text{anthr}})_2]^{4+}$ has been confirmed by the single-crystal X-ray structure analysis of $[\text{Ru}_4(\eta^6\text{-}p\text{-cymene})_4(\mu_4\text{-oxa})_2(\mu_2\text{-L}_{\text{anthr}})_2][\text{CF}_3\text{SO}_3]_4 \cdot 4 \text{ acetone}$ ($\text{A}_2 \cdot 4 \text{ acetone}$), thus showing the anthracene moieties to be available for reaction with oxygen. While the formation of the endoperoxide form of L_{anthr} was observed in solution upon white light irradiation, the same reaction does not occur when L_{anthr} is part of the metalla-assemblies.

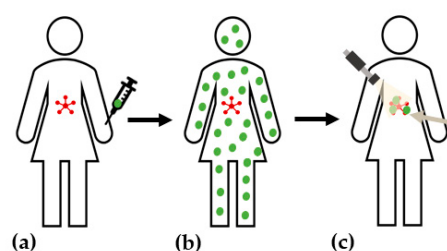
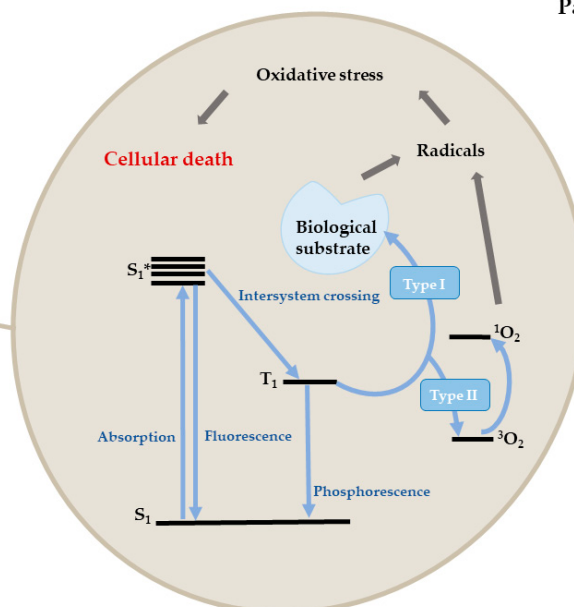
Keywords: photodynamic therapy; singlet oxygen; anthracene; arene ruthenium; metalla-assemblies

1. Introduction

Nowadays, one of the challenges in treating cancers is increasing the drug efficacy while limiting or even annihilating side effects. Photodynamic therapy (PDT) can possibly achieve these goals [1,2]. Although still emerging and under development, this method is already used in the clinic or under clinical trials for skin disorders [3–5], infections [6,7], superficial cancers [3,8–10] and for deeper cancers [3,11–14] (see Table 1). PDT involves three main elements which are individually harmless: a light-absorbing molecule called photosensitizer (PS), oxygen and light. [15] For medical treatment, the PS is administered either systemically or topically and often intravenously. After a period of systemic distribution, the PS accumulates in the tumor, thanks to both selective accumulation and selective retention [16]. Finally, the tumor is irradiated by light, which activates the PS (Figure 1, part A). At that point, the combination of the PDT elements becomes dangerous for the cells. The PS, at its singlet ground state, is excited and reaches an unstable singlet state S_1^* . Then, the PS reemits this energy and undergoes an intersystem crossing process leading to a triplet state T_1 , which is lower in energy (i.e., a longer-living state) than S_1 . The PS subsequently interacts with the biological environment through two kinds of photochemical reactions (Figure 1, part B) [17]. Both pathways (type I and type II) involve oxidative stress, mainly due to the formation of reactive oxygen species (ROS), and accordingly need oxygen to be effective [18].

Table 1. List of photosensitizers approved by the FDA (Food and Drug Administration) or under clinical trials [14].

Photosensitizers	Approved	Trial	Cancer Types
Porfimer sodium (hematoporphyrin, Photofrin)	Worldwide	-	Lung, esophagus, bile duct, bladder, brain, ovarian
ALA (5-aminolevulinic acid, Levulan)	Worldwide	-	Skin, bladder, brain, esophagus
ALA esters	Europe	-	Skin, bladder
Temoporfin (<i>m</i> -tetrahydroxy phenylchlorin, Foscan)	Europe	USA	Head, neck, lung, brain, skin, bile duct
Verteporfin	Europe	UK	Ophthalmic (age-related macular degeneration), pancreatic, skin
HPPH (2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-a)	-	USA	Head, neck, esophagus, lung
SnEt ₂ (tin ethyl etiopurpurin, Purlytin)	-	USA	Skin, breast
Talaporfin (mono-(L)-aspartylchlorin-e6)	-	USA	Liver, colon, brain
Ce6-PVP (chlorin e6-polyvinylpyrrolidone, Fotolon), Ce6 derivatives (photodithazine, Radachlorin)	-	Belarus, Russia	Nasopharyngeal, sarcoma, brain
Silicon phthalocyanine	-	USA	Cutaneous T-cell lymphoma
Padoporfin (pallado-porphyrin, TOOKAD)	-	USA	Prostate
Motexafin lutetium (Lutex)	-	USA	Breast

Part A**Part B****Figure 1.** **A**—Standard PDT treatment: administration of PS **(a)** systemic PS distribution into the body; **(b)** PS accumulation in the tumor and irradiation **(c)**; **B**—Jablonski diagram describing the PDT process.

PDT is considered a promising treatment as it possesses several benefits in comparison to common cancer therapies. Traditionally, the advantages attributed to PDT are to be less invasive, with a short treatment time and a double selectivity (combination of PS and light); it can be applied several times at the same location (contrary to traditional radiations); and it has a lower overall cost (no surgery involved). Nevertheless, the PDT technique is still limited and its use in routine treatments remains restricted. Limitations are generally associated with a residual photosensitivity after PDT treatment, difficulty to treat metastases (because of limited laser focalization), the need of light at a specific wavelength to be able to reach deeper tumors and finally to an adequate tissue oxygenation [2,14,19].

Unfortunately, hypoxia is a fundamental physiological feature in human solid cancers. This characteristic is absent in the healthy tissue and it corresponds to a low oxygen concentration in a determined cancerous region [20]. The first observation of this phenomenon was made by Thomlinson and Gray in the 1950s. They realized the importance of oxygen in cancerous tissues for obtaining a good efficiency of radiation treatments [21]. Later, they showed the existence of hypoxic and necrotic regions in cancerous areas, which are at 150 μm from the irregular blood vessels. Consequently, they were able to deduce the effective diffusion distance of oxygen in cancer tissue [22]. In the 1990s, the concentration of oxygen in human tumors was precisely determined by Vaupel et al. Using the “Eppendorf” electrode: the oxygen amount is very heterogeneous, and the median values are lower than in healthy tissues [23]. Since then, researchers undoubtedly confirmed the negative impact of hypoxia on cancer treatments. Hypoxia generally drives to drug resistance and failure of the radio- and chemotherapy [24,25], since cancerous cells exploit numerous mechanisms to survive. Here comes a non-exhaustive list of hypoxia consequences in a cancerous environment: an imperfect exposition of anti-cancerous drugs [26], a decrease of the DNA-breaking drugs toxicity [27,28], an upregulation of some genes [29,30], a selection of cells with genetic alterations carrying out the decrease or the suppression of apoptosis [31,32], a creation of genetic instability with an increased number of mutations [33], an adaptation of normal pathways to tumors for their survival [34,35], a dysfunction of RTK (receptor tyrosine kinase) and endocytosis [36], a tumor progression [37] and a restauration of the irradiation-damaged vasculature by vasculogenesis [38]. Thus, to apply the promising PDT treatment on human solid cancer, there is an indispensable need to overcome the problem of hypoxia, since oxygen remains a *sine qua non* condition for effectiveness.

Therefore, to carry oxygen to cancer cells, coordination chemistry can be a solution. In fact, several ruthenium-based complexes have been tested as photoactivated compounds and they appear to have good biological activity [39–53]. Moreover, we have demonstrated ten years ago the potential of arene ruthenium complexes in PDT [54–59], and recently we showed that the octonuclear metalla-cube $[\text{Ru}_8(\eta^6\text{-}p\text{-cymene})_8(\text{tpvb})_2(\text{donq})_4]^{8+}$ (tpvb: 1,2,3,4-tetrakis{2-(4-pyridyl)vinyl}benzene; donq: 5,8-dioxido-1,4-naphthoquinonato) can entrap porphyrin (a photosensitizer) into its cavity [60]. When inside the hydrophobic cavity of the cage, porphyrin is not reactive to light, and the overall assembly is relatively harmless to cells. After internalization, porphyrin is released within the cells, and, after an irradiation at an appropriate wavelength, the system shows an excellent phototoxicity. Therefore, we describe here a new strategy that consists of using arene ruthenium metalla-assemblies to transport one or two molecules of singlet oxygen. For that purpose, the literature gives several examples of oxygen carriers [61,62], but we have focused our attention to anthracene units [63–65]. Actually, Aubry and coworkers have described the ability of aromatic compounds to permit a reversible binding of oxygen [66]. Thus, the basic idea is to attach anthracene units on metalla-assemblies, either on the clips or on the linkers, and ultimately to be able to deliver $^1\text{O}_2$ and a PS to cells for PDT optimization.

In this conceptual paper, we have synthesized an anthracene-based linker, 9,10-bis(3,3'-ethynylpyridyl) (L_{anthr}), which form in combination with three arene ruthenium clips, $[\text{Ru}_2(\eta^6\text{-}p\text{-cymene})_2(\mu_4\text{-oxa})_2\text{Cl}_2]$, $[\text{Ru}_2(\eta^6\text{-}p\text{-cymene})_2(\mu_4\text{-dobq})_2\text{Cl}_2]$ and $[\text{Ru}_2(\eta^6\text{-}p\text{-cymene})_2(\mu_4\text{-donq})_2\text{Cl}_2]$ (oxa: oxalato; dobq: 2,5-dioxido-1,4-benzoquinonato; donq: 5,8-dioxido-1,4-naphthoquinonato), three metalla-rectangles $[\text{Ru}_4(\eta^6\text{-}p\text{-cymene})_4(\mu_4\text{-oxa})_2(\mu_2\text{-L}_{\text{anthr}})_2][\text{CF}_3\text{SO}_3]_4$ (A_1), $[\text{Ru}_4(\eta^6\text{-}p\text{-cymene})_4$

(μ_4 -dobq) $_2$ (μ_2 -L_{anthr}) $_2$ [(CF₃SO₃)₄] (A₂), and [Ru₄(η^6 -*p*-cymene)₄(μ_4 -donq) $_2$ (μ_2 -L_{anthr}) $_2$][(CF₃SO₃)₄] (A₃), respectively. Then, the potential of the linker L_{anthr} and the arene ruthenium metalla-rectangles to act as oxygen carriers was evaluated by various spectroscopic methods.

2. Results and Discussion

The synthesis of the metalla-rectangles is straightforward. First, the bis-pyridyl linker, 9,10-bis(3,3'-ethynylpyridyl) (L_{anthr}), is prepared according to a modified version of the previously published method [67]. It starts with a palladium-catalyzed Sonogashira reaction between 1 equivalent of 9,10-dibromoanthracene and 2.2 equivalents of 3-ethynylpyridine (63% yield). Crystals of L_{anthr} are obtained by the slow vapor diffusion of toluene into a solution of L_{anthr} in dichloromethane at room temperature and they have permitted to confirm the molecular structure of L_{anthr}. The compound crystallized in the non-centrosymmetric space group *P*2₁, a monoclinic crystal system. In the solid state, the anthracene unit is nearly planar with the root mean square deviation of the 12 carbon atoms of the plane being 0.024 Å. The angles between the anthracene plane and the pyridyl rings are 1.6° and 2.9°, respectively. As illustrated in Figure 2, the occupancy of the nitrogen atoms of the pyridyl rings is poorly defined, showing the rotating flexibility around the ethynyl axes despite the conjugated aromatic system.

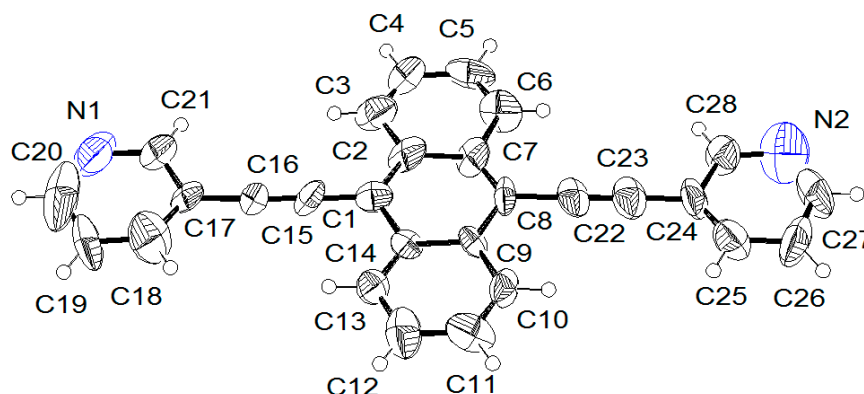
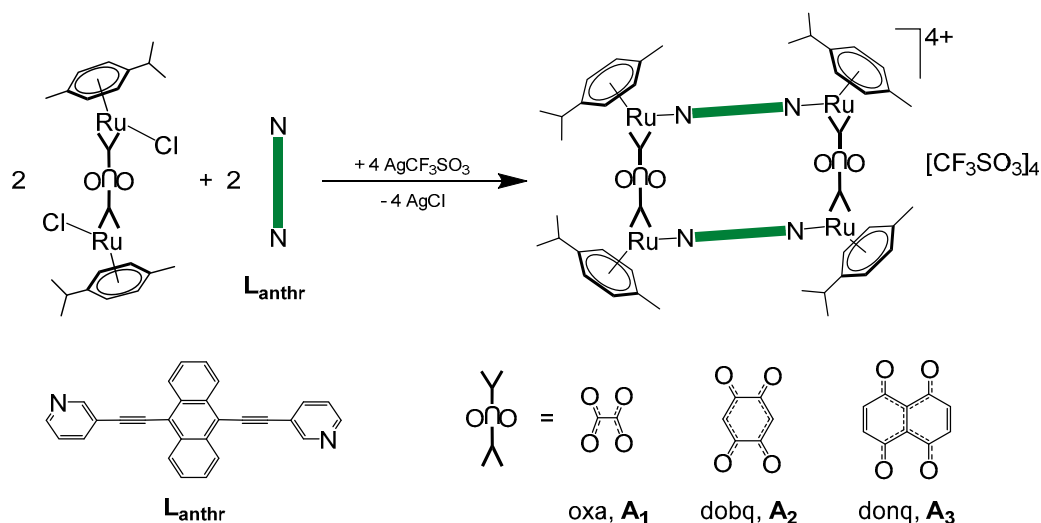


Figure 2. Ortep drawing of L_{anthr} at 50% probability level ellipsoids.

Then, the dinuclear arene ruthenium clips were prepared according to published methods: [Ru₂(η^6 -*p*-cymene) $_2$ (μ_4 -oxa)Cl₂] [68], [Ru₂(η^6 -*p*-cymene) $_2$ (μ_4 -dobq)Cl₂] [69] or [Ru₂(η^6 -*p*-cymene) $_2$ (μ_4 -donq)Cl₂] [70]. These dinuclear complexes react with two equivalents of silver trifluoromethanesulfonate in dichloromethane to afford a reactive intermediate (not isolated), and after filtration of AgCl, 1 equivalent of L_{anthr} is added to obtain the corresponding metalla-rectangles (Scheme 1). The resulting cationic *p*-cymene metalla-assemblies are isolated as their trifluoromethanesulfonate (CF₃SO₃[−]) salts in good yields (between 68% and 82%). The solubility of the metalla-rectangles depends on the nature of the dinuclear clip: A₁ is soluble in ethanol but not in dichloromethane, while A₂ and A₃ show the opposite, being soluble in dichloromethane but not in ethanol. Moreover, only A₁ is slightly soluble in water, but they are all soluble in acetone. These metalla-rectangles were fully characterized by various spectroscopic techniques (see the Supplementary Materials).



Scheme 1. Synthesis of L_{anthr} and metalla-rectangles A_1 , A_2 and A_3 .

The formation of the metalla-rectangles A_1 , A_2 and A_3 was first confirmed by electrospray ionization mass spectrometry (ESI-MS). All spectra show the typical pattern of arene ruthenium metalla-assemblies with trifluoromethanesulfonate as counterions: a monocationic peak corresponding to $[\text{M}-\text{CF}_3\text{SO}_3^-]^+$ (respectively at $m/z = 2323.2$ and $m/z = 2423.0$; not observed for A_3) and a dicationic peak for $[\text{M}-2(\text{CF}_3\text{SO}_3^-)]^{2+}$ (respectively at $m/z = 1088.9$, $m/z = 1139.2$ and $m/z = 1188.1$). They also present a peak which corresponds to $[\text{M}-2\text{L}_{\text{anthr}}-2(\text{CF}_3\text{SO}_3^-)]^+$ at respectively $m/z = 708.0$, $m/z = 759.3$ and $m/z = 809.0$. The experimental results perfectly correlate with the calculated isotopic distributions of the different species.

The ^1H NMR spectra of A_1 , A_2 and A_3 in acetone- d_6 present a chemical shift of the L_{anthr} protons, as compared to the non-coordinated material (Figure 3). The pyridyl protons show a mixed behavior; those adjacent to the Ru–N bond are shifted upfield, while the other two are shifted downfield. However, the protons of the anthracene units (marked by an orange triangle and a red dot) are strongly upfield shifted in all metalla-rectangles because of the shielding effect of the anthracene–anthracene parallel arrangement. In view of trapping singlet oxygen, these protons will be the most affected by the formation of the endoperoxide derivative.

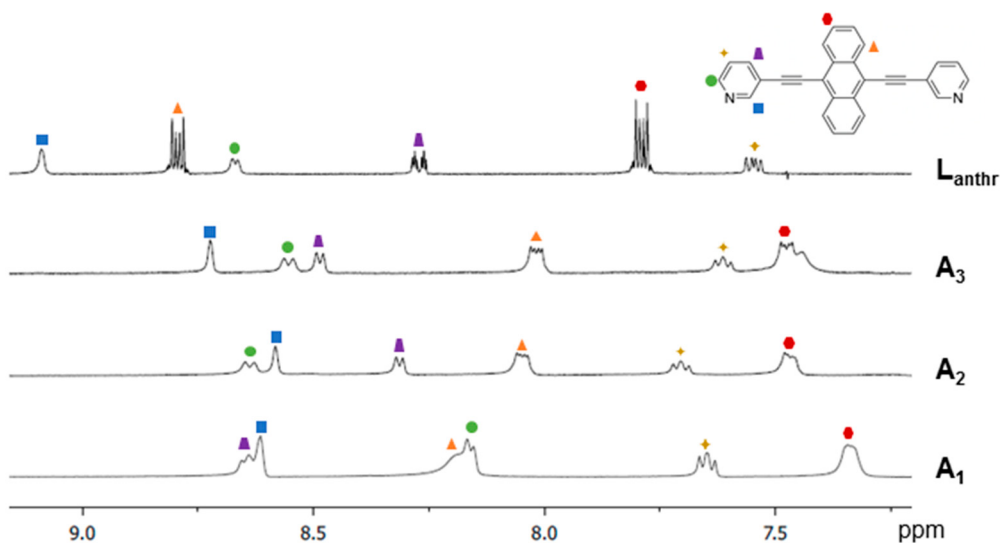


Figure 3. ^1H NMR spectra of the aromatic region of L_{anthr} , A_3 , A_2 and A_1 (acetone- d_6 , 23 °C).

In addition, typical peak multiplicities and chemical shifts of the arene ruthenium units are observed in the ^1H NMR spectra of **A**₁, **A**₂ and **A**₃. The aryl protons of the *p*-cymene ligands are observed between ~6.85 and ~6.35 ppm, the isopropyl protons at ~1.40 ppm, the septuplet of the –CH from the isopropyl groups at ~3.00 ppm and the methyl groups at ~2.30 ppm (see the Experimental section). Interestingly, the spectrum of **A**₁ suggests the formation of two isomers due to the presence of broad signals in the aromatic region and due to additional splitting of some protons of the *p*-cymene ligands. On the other hand, the ^1H NMR spectra of **A**₂ and **A**₃ do not show this phenomenon and their overall signals are rather well-defined as compared to those of **A**₁. These elements support the presence of *cis* and *trans* isomers, in which the pyridyl units are pointing to the same or opposite sides of the metalla-rectangle: The free rotation of the pyridyl units being restricted upon formation of the metalla-rectangles. In fact, in the oxalato derivative **A**₁, the short distance between the ruthenium atoms within the dinuclear clip (5.4 Å) forces the two **L**_{anthr} panels to be in close proximity [71]. Therefore, the chemical environment of the protons associated with the *cis* and *trans* isomers is more different. Accordingly, π – π stacking interactions between the two **L**_{anthr} units of the metalla-rectangles are stronger in **A**₁ than in **A**₂ and **A**₃, where the Ru–Ru distances are estimated to be at 7.9 and 8.4 Å, respectively [71,72].

The diffusion coefficient (*D*) of **A**₁, **A**₂ and **A**₃ was determined by Diffusion-Ordered Spectroscopy (DOSY) NMR experiments, recorded in acetone-*d*₆ at 23 °C. The presence of a single diffusion line suggests, for each metalla-rectangle, the formation of one discrete species (isomers being undistinguishable). Interestingly, **A**₁ and **A**₃ have a similar value, at, respectively, $6.67 \times 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$ and $6.80 \times 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$, while **A**₂ is slightly different ($6.04 \times 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$). Generally, the bigger the assembly is, the smaller its diffusion coefficient. Herein, the size of the metalla-rectangles is increasing from **A**₁ to **A**₃. However, the results are not consistent with their theoretical molecular size since **A**₂ has the smallest value of *D*. However, they might possess different arrangement in solution. Then, according to these *D* values and by applying the Stokes–Einstein equation, the hydrodynamic radius *r*_H or the metalla-rectangles was calculated (viscosity of 0.31 mPa·s for acetone and a temperature of 298 K): the *r*_H values are 10.5 Å for **A**₁ and 10.4 Å for **A**₃, while for **A**₂ it is 11.7 Å. These values are consistent with the formation of the expected metalla-assemblies [59,60,71–76].

Indeed, the molecular structure of **A**₁ was further confirmed by single-crystal X-ray structure analysis (Figure 4). Crystals were obtained by slow diffusion of a mixture of ether–benzene (99:1) into an acetone solution of the corresponding metalla-rectangle. The salt crystallizes in the triclinic space group *P*-1. The asymmetric unit includes the tetra-cationic metalla-rectangle (*trans* isomer), four CF₃SO₃[−] anions, and four acetone molecules. The size of the rectangle defined by the four Ru–Ru edges is $5.4 \times 17.7 \times 5.4 \times 17.9 \text{ Å}$, which correlates well with the *r*_H determined by the DOSY experiment.

As mentioned before, π – π stacking interactions play an important role in the formation of these metalla-rectangles [77]. In the crystal structure of **A**₁ · 4 acetone, both anthracene groups are positioned in a “parallel fashion” that maximize π – π stacking interactions. The distance between the two centroids of the anthracene moieties is only 3.8 Å. However, as compared to **L**_{anthr}, the pyridyl groups are far from co-planarity with the anthracene unit (Figure 4). Two pyridyl rings are observed at an angle of approximately 7°, while the two others are rotated by 34°, from the idealized plane of their anthracene group. This distortion is probably imposed by the arene ruthenium oxalato clips as well as by an optimization of the anthracene–anthracene π – π interactions.

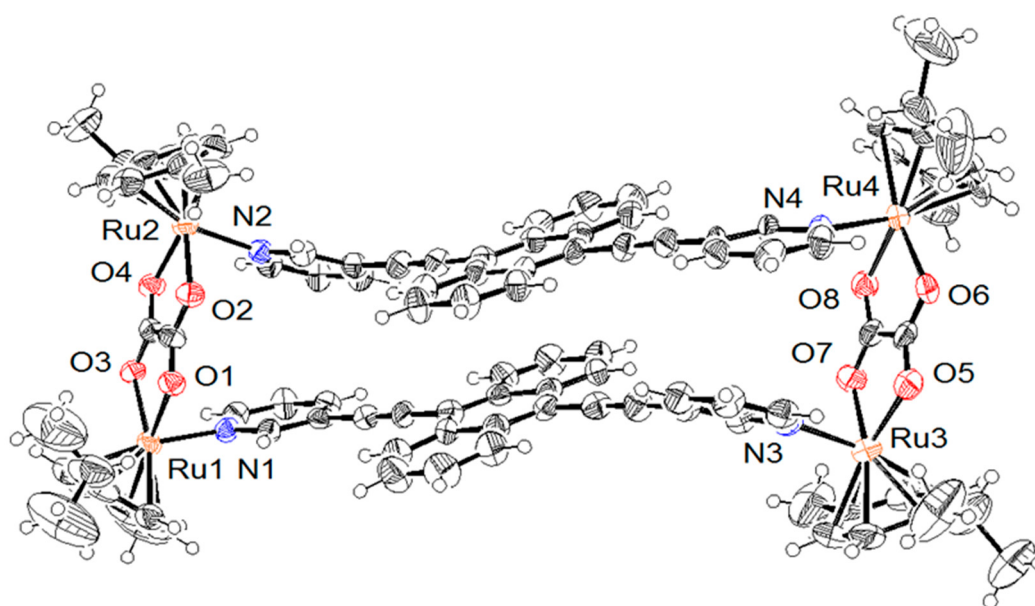


Figure 4. Molecular structure of $A_1 \cdot 4$ acetone at 50% probability level ellipsoids. Trifluoromethanesulfonate and acetone molecules omitted for clarity.

The electronic absorption spectra have been measured at room temperature in dichloromethane for L_{anthr} , A_2 and A_3 and in ethanol for A_1 (Figure 5). The intense high energy band centered at 270 nm is assigned to ligand-localized or ligand $\pi \rightarrow \pi^*$ transition and the broad low-energy band corresponds to metal-to-ligand charge transfer (MLCT). Typical anthracene prints in the UV–visible spectra are observed around 400–500 nm. These bands are different in the three metalla-assemblies. In fact, A_1 displays a quite similar pattern as L_{anthr} , while A_2 and A_3 show broader band and a bathochromic shift of the bands associated with the anthracene located between ~ 400 –550 nm. Moreover, A_2 presents a wider band, from which it is not possible to distinguish in the visible region of the spectrum the two bands of the anthracene moiety.

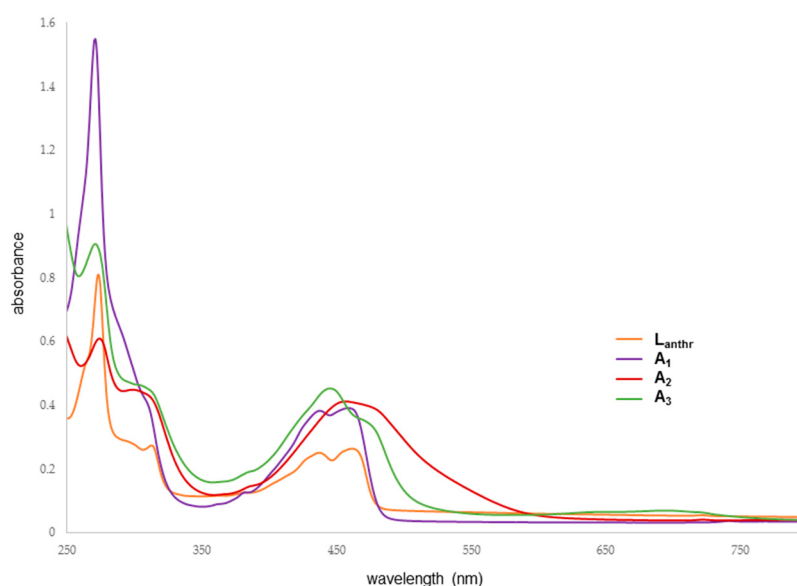


Figure 5. UV–visible spectra of L_{anthr} , A_1 , A_2 and A_3 .

In order to evaluate the ability of the ligand and the metalla-rectangles to form endoperoxide derivatives, several experiments were carried out in solution (NMR, UV–visible and fluorescence).

Comparisons were made between the results obtained under an inert-atmosphere to those obtained with an O₂-saturated atmosphere, as well as before or after light irradiation.

First, UV–visible spectroscopy was used to visualize the trapping of singlet oxygen by the metalla-rectangles. When dealing with anthracene derivatives, a visible-light excitation can be employed to trap singlet oxygen, and upon oxygen addition to the anthracene moiety the intensity of the absorption bands decreases over time [78,79]. Therefore, solutions of all compounds were prepared at a concentration of 10^{−5} M (EtOH for A₁; CH₂Cl₂ for A₂ and A₃), and kept in the dark before starting the measurements. Then, multiple spectra were recorded: under nitrogen, after 15 min of oxygen bubbling into the solution, and also after one hour of visible light irradiation (cool white light, Hg, 8 W). However, in all cases, no spectroscopic changes were observed under these conditions.

We then turn our attention to fluorescence spectroscopy (Figure 6). Oxygen is known to be a common quencher of the fluorescence of aromatic compounds, since the formation of endoperoxide disrupts the electron delocalization of aromatic molecules [80–82]. Therefore, fluorescence spectroscopy was also used to study the behavior of the metalla-rectangles in the presence of O₂. Each compound (5 × 10^{−8} M concentrations; EtOH for A₁; CH₂Cl₂ for A₂ and A₃) was irradiated at a specific wavelength, where the maximal absorbance was detected by the fluorimeter (L_{anthr}, 271 nm; A₁, 457 nm; A₂, 273 nm; A₃, 459 nm). At first sight, the spectra show different bands and intensities before and after placing the compounds under O₂ (30 min of bubbling). In an inert atmosphere, A₂ displays six bands: one at ~270 nm, two between 300 and 400 nm, two between 450 and 520 nm and one at ~550 nm; while with O₂, there are seven bands, one additional band at ~620 nm (Figure 6). In the ~270 nm and ~550 nm regions, the intensity of the bands is decreasing with O₂, whereas it is increasing in the other parts of the spectrum. Moreover, there is a slight hypsochromic shift of the bands located between 300 and 400 nm.

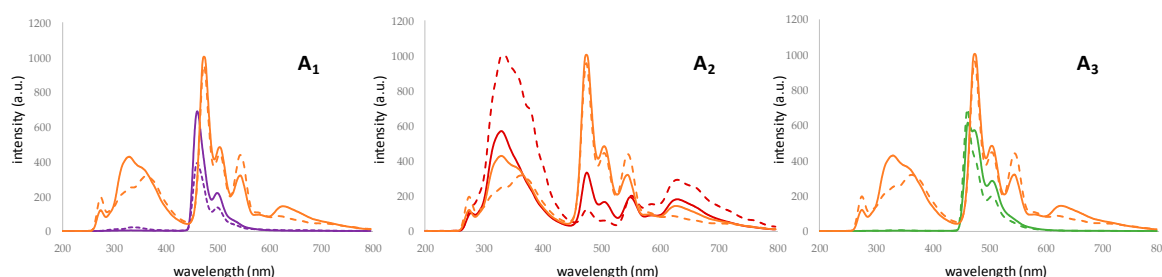


Figure 6. Fluorescence spectra of metalla-rectangles A₁ (purple), A₂ (red), A₃ (green) and L_{anthr} (orange) at a concentration of 5 × 10^{−8} M (in CH₂Cl₂ or EtOH), under a N₂ (dotted lines) or O₂ atmosphere (solid line).

For A₁ and A₃ (Figure 6), no band before 450 nm is observed, and all their bands are hyperchromic. Only a very small decrease in the intensity of the band at ~470 nm is observed for A₃. In comparison with L_{anthr}, the behavior of A₂ follows an opposite trend: when the intensity is increasing for L_{anthr}, it is decreasing for A₂. The only different variation is the intensity of its bands: in the UV part and at the end of the visible domain, A₂ has a more intense fluorescence, and a less intense one between 450 and 550–570 nm. All together, these results show no evidence for an endoperoxide formation on the metalla-rectangles A₁–A₃. Therefore, we focused our attention to L_{anthr} alone, in order to determine if the bis-pyridyl anthracene ligand interacts with O₂.

A series of UV–visible spectra have been recorded (Figure 7), in which an air-opened solution of L_{anthr} was continuously irradiated under white light. After a week, the intensity of the bands associated with the anthracene moiety was significantly reduced, and the solution appeared to have reached an equilibrium. Two isosbestic points were observed, at ~330 nm and ~390 nm, respectively. The formation of a new compound was confirmed by ¹H NMR spectroscopy, in which a new set of signals was observed. Two new doublet of doublets, slightly downfield shifted as compared to

the anthracene protons of **L_{anthr}** (8.7 and 7.7 ppm), are observed at 8.8 and 7.9 ppm, respectively: thus suggesting the formation of the endoperoxide derivative.

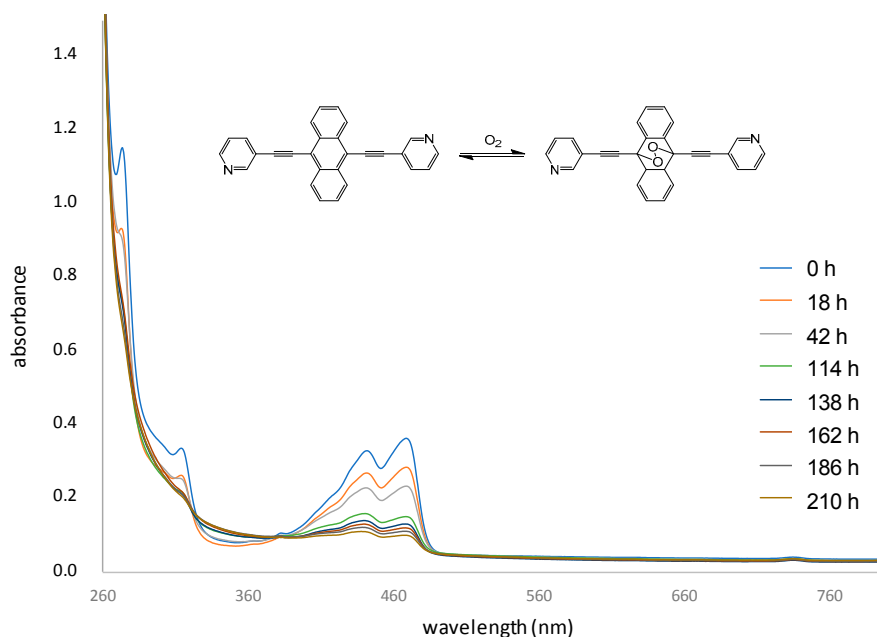


Figure 7. UV–visible spectra of **L_{anthr}** upon white light irradiation (10^{-5} M in DMSO).

3. Experimental Section

3.1. Material and Methods

All solvents were dried before use according to standard procedures and all reactions were performed under an inert atmosphere. The ruthenium precursors $[Ru_2(\eta^6\text{-}p\text{-cymene})_2(\mu_4\text{-oxa})Cl_2]$ [68], $[Ru_2(\eta^6\text{-}p\text{-cymene})_2(\mu_4\text{-dobq})Cl_2]$ [69] and $[Ru_2(\eta^6\text{-}p\text{-cymene})_2(\mu_4\text{-donq})Cl_2]$ [70] were prepared according to published methods. All other reagents were commercially available (Sigma-Aldrich, Brunschwig, Basel, Switzerland) and used as received. NMR spectra were recorded with a Bruker Avance II 400 spectrometer (Karlsruhe, Germany). UV–vis absorption spectra were recorded with a PerkinElmer UV–vis spectrophotometer (Waltham, MA, USA). IR spectra were recorded with a ThermoScientific Nicolet iS5 spectrometer (Waltham, MA, USA). Electrospray ionization mass spectrometry (ESI-MS) spectra were obtained in positive mode with a LCQ Finnigan mass spectrometer (San Jose, CA, USA). Microanalyses were carried out by the Mikroelementaranalytisches Laboratorium, ETH Zurich, Switzerland. Fluorescence spectra were recorded on a PerkinElmer LS 50 B luminescence spectrophotometer (Waltham, MA, USA). Irradiation studies were performed using a Luzchem LZC-ORG photoreactor (Montreal, QC, Canada) equipped with the corresponding lamp (cool white lamp, Hg, 8 W, Sylvania® F8T5/CW).

3.2. Synthesis and Characterization

9,10-bis(3,3'-ethynylpyridyl)anthracene (L_{anthr}**):** In a Schlenk flask, a mixture of 9,10-dibromoanthracene (400 mg, 1.19 mmol) and 3-ethynylpyridine (270 mg, 2.62 mmol) was dissolved in a solution of toluene:trimethylamine (1:1, 25 mL) and let under a nitrogen atmosphere during 15 min. Then, a mixture of palladium(II)acetate (5 mg, 0.024 mmol), copper(I)iodide (6 mg, 0.030 mmol) and triphenylphosphine (17 mg, 0.065 mmol) was added to this solution. The reaction mixture was stirred at reflux for 24 h. The solvent was removed under vacuum. The residue was dissolved in water and stirred for 2 h at RT, to eliminate the triethylammonium salt. The solid was filtered off and dried under vacuum. Recrystallization was done in toluene and the product was obtain

as orange needles, which were dried under vacuum (285 mg, 63%). ^1H NMR (CDCl_3 , 400 MHz): δ 9.02 (s, 2H, CH_{pyr}), 8.66 (m, 6H, CH_{pyr} and CH_{anthr}), 8.06 (d, $J = 7.6$ Hz, 2H, CH_{pyr}), 7.69 (dd, 4H, CH_{anthr}), 7.42 (m, 2H, CH_{pyr}). ^{13}C NMR (CDCl_3 , 400 MHz): δ 152.4 (2C, CH_{pyr}), 149.2 (2C, CH_{pyr}), 138.6 (2C, CH_{pyr}), 132.3 (4C, C_{anthr}), 127.4 (4C, CH_{anthr}), 127.3 (4C, CH_{anthr}), 123.4 (2C, CH_{pyr}), 120.7 (2C, C_{pyr}), 118.4 (2C, C_{anthr}), 99.12 (2C, $\text{C}_{\text{ethynyl}}$), 89.77 (2C, $\text{C}_{\text{ethynyl}}$). IR (cm^{-1}): 3078 (m; C–H aromatic), 2200 (w; $\text{C}\equiv\text{C}$), 1557 and 1477 (m; C=C aromatic), 799, 763 and 697 (s, sharp; C–H aromatic). ESI–MS (+); $m/z = 381.2$ $[\text{M}+\text{H}]^+$. UV–vis [5.0×10^{-6} , CH_2Cl_2 ; λ_{max} , nm (ϵ , $\text{M}^{-1}\cdot\text{cm}^{-1}$): 277 (1.2×10^5), 313 (5.5×10^4), 437 (5.0×10^4), 462 (5.3×10^4), 722 (1.1×10^4). Anal. calc. for $\text{C}_{28}\text{H}_{16}\text{N}_2$ (380.46): C 88.38, H 4.25, N 7.37; Found: C 88.26, H 4.14, N 7.26.

General procedure for the synthesis of metalla-rectangles A_1 , A_2 and A_3 : A mixture of metalla-clip (oxa: 200 mg, 0.32 mmol; dobq: 200 mg, 0.29 mmol; donq: 200 mg, 0.27 mmol) and silver trifluoromethanesulfonate (2 eq.) was dissolved in dichloromethane and was stirred for 3 h at RT. The mixture was filtrated in order to eliminate silver chloride. The resulting solution was added to a dichloromethane solution of L_{anthr} (1 eq.). Then, the mixture was refluxed overnight and consequently concentrated under vacuum. The concentrated solution was slowly poured into cold diethyl ether to induce precipitation. After filtration, the metalla-rectangles were filtered off and dried under vacuum.

$[\text{Ru}_4(\eta^6\text{-p-cymene})_4(\mu_4\text{-oxa})_2(\mu_2\text{-L}_{\text{anthr}})_2][\text{CF}_3\text{SO}_3]_4$ (A_1): Yellow solid. Yield, 74%. ^1H NMR ($(\text{CD}_3)_2\text{CO}$, 400 MHz): δ 8.64 (m, 8H, CH_{pyr}), 8.16 (m, 12H, CH_{anthr}), 7.65 (t, $J = 6.6$ Hz, 4H, CH_{pyr}), 7.34 (m, 8H, CH_{anthr}), 6.20 (dd, $J = 11.6$ Hz, $J = 6.0$ Hz, 8H, CH_{cym}), 6.05 (m, 8H, CH_{cym}), 3.04 (sept, $J = 6.6$ Hz, 4H, CH_{cym}), 2.35 (s, 12H, $\text{CH}_{3\text{cym}}$), 1.45 (d, $J = 6.8$ Hz, 12H, CH_{cym}), 1.43 (d, $J = 6.8$ Hz, 12H, CH_{cym}). ^{13}C NMR ($(\text{CD}_3)_2\text{CO}$, 400 MHz): δ 172.14 (2C, C_{oxa}), 172.03 (2C, C_{oxa}), 154.65 (4C, C_{pyr}), 152.54 (4C, C_{pyr}), 143.77 (4C, C_{pyr}), 137.19 (4C, C_{anthr}), 131.42 (4C, C_{anthr}), 128.15 (8C, C_{anthr}), 127.65 (4C, C_{pyr}), 127.17 (8C, C_{anthr}), 123.78 (4C, C_{pyr}), 120.90 (4C, C_{anthr}), 117.87 (4C, C_{otf}), 103.43 (4C, C_{cym}), 98.98 (4C, C_{cym}), 97.40 (4C, $\text{C}_{\text{C}\equiv\text{C}}$), 92.88 (4C, $\text{C}_{\text{C}\equiv\text{C}}$), 83.34 (2C, C_{cym}), 83.26 (2C, C_{cym}), 83.14 (4C, C_{cym}), 82.85 (2C, C_{cym}), 82.79 (2C, C_{cym}), 82.30 (2C, C_{cym}), 82.28 (2C, C_{cym}), 31.96 (4C, C_{cym}), 22.69 (4C, C_{cym}), 22.35 (4C, C_{cym}), 18.21 (4C, C_{cym}). IR (cm^{-1}): 3079 (w; C–H aromatic), 2211 (w; $\text{C}\equiv\text{C}$), 1625 (s; $\text{C}=\text{O}$ oxa), 1482 (m; C=C aromatic), 765 (m, sharp; C–H aromatic), 636 (s; C–H aromatic). ESI–MS (+); $m/z = 708.0$ $[\text{M}-2\text{L}_{\text{anthr}}-2\text{OTf}^-]^+$, 1088.9 $[\text{M}-2\text{OTf}^-]^2+$, 2323.2 $[\text{M}-\text{OTf}^-]^+$. UV–vis [5.0×10^{-6} , EtOH; λ_{max} , nm (ϵ , $\text{M}^{-1}\cdot\text{cm}^{-1}$): 271 (3.1×10^5), 312 (7.5×10^4), 437 (7.6×10^4), 459 (7.8×10^4), 742 (7.3×10^3). D ($(\text{CD}_3)_2\text{CO}$): 6.67×10^{-10} $\text{m}^2\cdot\text{s}^{-1}$. Anal. calc. for $\text{C}_{104}\text{H}_{88}\text{F}_{12}\text{N}_4\text{O}_{20}\text{Ru}_4\text{S}_4$ (2474.48): C 50.48, H 3.59, N 2.27; Found: C 50.56, H 3.68, N 2.22.

$[\text{Ru}_4(\eta^6\text{-p-cymene})_4(\mu_4\text{-dobq})_2(\mu_2\text{-L}_{\text{anthr}})_2][\text{CF}_3\text{SO}_3]_4$ (A_2): Red solid. Yield, 68%. ^1H NMR ($(\text{CD}_3)_2\text{CO}$, 400 MHz): δ 8.64 (d, $J = 7.6$ Hz, 4H, CH_{pyr}), 8.58 (s, 4H, CH_{pyr}), 8.31 (d, $J = 6.0$ Hz, 4H, CH_{pyr}), 8.05 (dd, $J = 6.8$ Hz, $J = 3.2$ Hz, 8H, CH_{anthr}), 7.70 (t, $J = 6.8$ Hz, 4H, CH_{pyr}), 7.47 (m, 8H, CH_{anthr}), 6.34 (d, $J = 6.0$ Hz, 8H, CH_{cym}), 6.11 (d, $J = 6.0$ Hz, 8H, CH_{cym}), 5.96 (s, 4H, CH_{dobq}), 3.04 (sept, $J = 6.6$ Hz, 4H, CH_{cym}), 2.33 (s, 12H, $\text{CH}_{3\text{cym}}$), 1.42 (d, $J = 6.8$ Hz, 24H, CH_{cym}). ^{13}C NMR ($(\text{CD}_3)_2\text{CO}$, 400 MHz): δ 184.40 (8C, C_{dobq}), 155.33 (4C, C_{pyr}), 153.20 (4C, C_{pyr}), 142.77 (4C, C_{pyr}), 131.27 (8C, C_{anthr}), 128.27 (8C, C_{anthr}), 127.32 (4C, C_{pyr}), 127.06 (8C, C_{anthr}), 123.96 (1C, C_{anthr}), 123.30 (1C, C_{anthr}), 120.76 (4C, C_{pyr}), 117.69 (2C, C_{anthr}), 104.84 (4C, C_{cym}), 102.37 (4C, C_{dobq}), 100.09 (4C, C_{otf}), 97.03 (4C, C_{cym}), 92.11 (4C, $\text{C}_{\text{C}\equiv\text{C}}$), 90.63 (4C, $\text{C}_{\text{C}\equiv\text{C}}$), 84.60 (8C, C_{cym}), 82.62 (8C, C_{cym}), 32.12 (4C, C_{cym}), 22.48 (8C, C_{cym}), 18.24 (4C, C_{cym}). IR (cm^{-1}): 3075 (w; C–H aromatic), 2198 (w; $\text{C}\equiv\text{C}$), 1574 (m; C=C aromatic), 817 and 764 (m; C–H aromatic) and 693 (s; C–H aromatic). ESI–MS (+); $m/z = 759.3$ $[\text{M}-2\text{L}_{\text{anthr}}-2\text{OTf}^-]^+$, 1139.2 $[\text{M}-2\text{OTf}^-]^2+$, 2423.0 $[\text{M}-\text{OTf}^-]^+$. UV–vis [5.0×10^{-6} , CH_2Cl_2 ; λ_{max} , nm (ϵ , $\text{M}^{-1}\cdot\text{cm}^{-1}$): 274 (1.2×10^5), 314 (8.3×10^4), 457 (8.2×10^4), 722 (8.2×10^3). D ($(\text{CD}_3)_2\text{CO}$): 6.04×10^{-10} $\text{m}^2\cdot\text{s}^{-1}$. Anal. calc. for $\text{C}_{112}\text{H}_{92}\text{F}_{12}\text{N}_4\text{O}_{20}\text{Ru}_4\text{S}_4$ (2572.46): C 52.24, H 3.61, N 2.18; Found: C 52.45, H 3.81, N 2.22.

$[\text{Ru}_4(\eta^6\text{-p-cymene})_4(\mu_4\text{-donq})_2(\mu_2\text{-L}_{\text{anthr}})_2][\text{CF}_3\text{SO}_3]_4$ (A_3): Green solid. Yield, 82%. NMR ($(\text{CD}_3)_2\text{CO}$, 400 MHz): δ 8.72 (s, 4H, CH_{pyr}), 8.55 (d, $J = 6.8$ Hz, 4H, CH_{pyr}), 8.48 (d, $J = 5.6$ Hz, 4H, CH_{pyr}), 8.01 (dd, $J = 6.4$ Hz, $J = 2.8$ Hz, 8H, CH_{anthr}), 7.62 (t, $J = 6.8$ Hz, 4H, CH_{pyr}), 7.48 (dd, $J = 6.8$ Hz, $J = 3.2$ Hz, 8H, CH_{anthr}), 7.44 (s, 8H, CH_{donq}), 6.09 (d, $J = 6.0$ Hz, 8H, CH_{cym}), 5.86 (d, $J = 6.0$ Hz, 8H, CH_{cym}), 3.04 (sept, $J = 6.8$ Hz, 4H, CH_{cym}), 2.26 (s, 12H, $\text{CH}_{3\text{cym}}$), 1.42 (d, $J = 6.8$ Hz, 24H, CH_{cym}).

^{13}C NMR ($(\text{CD}_3)_2\text{CO}$, 400 MHz): δ 154.93 (4C, C_{pyr}), 152.73 (4C, C_{pyr}), 142.40 (4C, C_{pyr}), 138.58 (8C, C_{donq}), 132.98 (4C, C_{donq}), 131.79 (8C, C_{anthr}), 128.31 (8C, C_{anthr}), 127.03 (8C, C_{anthr}), 126.82 (4C, C_{pyr}), 122.85 (4C, C_{pyr}), 117.66 (4C, C_{anthr}), 112.43 (8C, C_{donq}), 109.28 (4C, C_{Otf}), 104.41 (4C, C_{cym}), 100.94 (4C, C_{cym}), 97.22 (4C, $\text{C}_{\text{C}\equiv\text{C}}$), 91.66 (4C, $\text{C}_{\text{C}\equiv\text{C}}$), 85.47 (8C, C_{cym}), 83.72 (8C, C_{cym}), 31.62 (4C, C_{cym}), 22.48 (8C, C_{cym}), 17.43 (4C, C_{cym}). IR (cm^{-1}): 2971 (w; C–H aromatic), 2204 (w; $\text{C}\equiv\text{C}$), 1531 (m; C=C aromatic), 967, 852 and 763 (m; C–H aromatic), 635 (s, sharp; C–H aromatic). ESI–MS (+); m/z = 809.0 $[\text{M}-2\text{L}_{\text{anthr}}-2\text{Otf}^-]^+$, 1188.1 $[\text{M}-2\text{Otf}^-]^2+$. UV–vis [5.0×10^{-6} , CH_2Cl_2 ; λ_{max} , nm (ϵ , $\text{M}^{-1}\cdot\text{cm}^{-1}$): 271 (1.8×10^5), 310 (9.0×10^4), 445 (9.1×10^4), 476 (6.7×10^4), 694 (1.9×10^4). D ($(\text{CD}_3)_2\text{CO}$): $6.80 \times 10^{-10} \text{ m}^2\cdot\text{s}^{-1}$.

X-ray crystallography: Crystals were mounted on a Stoe Image Plate Diffraction system equipped with a Φ circle goniometer, using Mo $\text{K}\alpha$ graphite monochromated radiation (λ = 0.71073 Å) with Φ range 0–200°. The structures were solved by direct methods using the program *SHELXS*–97 [83], while the refinement and all further calculations were carried out using *SHELXL*–97. The H-atoms were included in calculated positions and treated as riding atoms using *SHELXL*–97 default parameters. The non-H atoms were refined anisotropically using weighted full-matrix least-square on F^2 . In both structures, relatively high R factors are observed due to disorder within the crystals. Crystallographic details are summarized in Table 2, and Figures 2 and 4 were drawn with *ORTEP*–32 [84].

Table 2. Crystallographic data and structure refinement parameters for L_{anthr} and $\text{A}_1 \cdot 4$ acetone.

Parameters	L_{anthr}	$\text{A}_1 \cdot 4$ acetone
chemical formula	$\text{C}_{28}\text{H}_{16}\text{N}_2$	$\text{C}_{116}\text{H}_{112}\text{F}_{12}\text{N}_4\text{O}_{24}\text{Ru}_4\text{S}_4$
formula weight	380.43	2706.61
crystal system	monoclinic	triclinic
space group	$P2_1$	$P-1$
crystal size (mm^3)	$0.23 \times 0.13 \times 0.12$	$0.25 \times 0.18 \times 0.14$
crystal color and shape	colorless rod	yellow plate
a (Å)	5.0015(6)	16.0557(11)
b (Å)	16.944(2)	20.7694(15)
c (Å)	11.5374(15)	21.3136(14)
α (°)	90	115.639(5)
β (°)	92.433(10)	98.267(5)
γ (°)	90	101.194(6)
cell volume ($\text{\AA}^3$)	976.9(2)	6073.6(8)
T (K)	293(2)	203(2)
Z	2	2
scan range (°)	$1.77 < \theta < 29.30$	$1.48 < \theta < 29.33$
ρ_{calcd} (g cm^{-3})	1.293	1.480
μ (mm^{-1})	0.076	0.644
unique reflections	5272	32944
reflections used [$I > 2\sigma(I)$]	1378	9666
R_{int}	0.1715	0.2498
final R indices [$I > 2\sigma(I)$] ^[a]	0.0799, wR_2 0.1714	0.0911, wR_2 0.2228
R indices (all data) ^[b]	0.2499, wR_2 0.2388	0.2609, wR_2 0.2939
GOF ^[c]	0.805	0.836
max, min $\Delta\rho/e$ ($\text{\AA}^{-3}$)	0.268, -0.196	1.627, -1.213

^[a] $R_1 = \sum |F_o| - |F_c| / \sum |F_o|$; ^[b] $wR_2 = \{\sum [w(F_o^2 - F_c^2)^2] / \sum [w(F_o^2)^2]\}^{1/2}$; ^[c] GOF = $\{\sum [w(F_o^2 - F_c^2)^2] / (n - p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined.

CCDC-1853676 (L_{anthr}) and 1853677 ($\text{A}_1 \cdot 4$ acetone) contain the supplementary crystallographic data for this paper. This can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Center, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk).

4. Conclusions

Three metalla-rectangles containing anthracene-derived linkers have been synthesized and characterized. Upon formation of the metalla-assembly, the propensity of the anthracene moiety to react with oxygen to form endoperoxide derivatives was lost, probably due to electronic or steric constraints. Nevertheless, the introduction of anthracene groups on metalla-assemblies remains an interesting avenue to transport oxygen to cancer cells; however, in view of this study, the anthracene moiety should be anchored elsewhere to allow the formation of metalla-assemblies with endoperoxide groups.

Supplementary Materials: All NMR and mass spectra are available online at <http://www.mdpi.com/2304-6740/6/3/97/s1>. Cif and Check cif files of $C_{28}H_{16}N_2$ (**L_{anthr}**) and $C_{116}H_{112}F_{12}N_4O_{24}Ru_4S_4$ (**A₁** · 4 acetone).

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “conceptualization, B.T.; methodology, M.G., F.N. and T.C.; validation, M.G., F.N., T.C. and B.T.; formal analysis, M.G., T.C. and F.N.; investigation, M.G., T.C. and F.N.; writing—original draft preparation, M.G. and B.T.; writing—review and editing, M.G. and B.T.; supervision, B.T.; project administration, B.T.”, please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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