Photochemistry and DNA photocleavage by a new unsupported dirhodium(II,II) complex

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The new complex \([\text{Rh}_2(\text{phen})_2(\text{CH}_3\text{CN})_6](\text{BF}_4)_4\) (1) was synthesized and characterized in solution and its crystal structure was determined. Irradiation of 1 with visible light (\(\lambda_{\text{irr}} > 590\,\text{nm}\)) in water results in the release of two equatorial CH$_3$CN ligands, CH$_3$CNeq, as well as in the formation of mononuclear radical Rh(II) fragments stemming from the homolytic photocleavage of the metal–metal bond. The photoproducts, identified by electrospray ionization mass spectrometry, include \([\text{Rh}(\text{phen})(\text{CH}_3\text{CN})(\text{OH})]^+\) and \([\text{Rh}(\text{phen})(\text{CH}_3\text{CN})(\text{H}_2\text{O})_3(\text{BF}_4)]^+\).

The quantum yield for the photochemical transformation of 1 in H$_2$O exceeds unity (\(\Phi_{550\,\text{nm}} = 1.38\)) indicative of dark reactions following the initial photoprocess. DNA photocleavage was observed for 1 (\(\lambda_{\text{irr}} > 590\,\text{nm}\)), whereas the complex is unreactive in the dark. This feature makes 1 a promising photodynamic therapy agent that does not operate via the production of singlet oxygen, $^1\text{O}_2$.

1. Introduction

The therapeutic properties of cisplatin, cis-[Pt(NH$_3$)$_2$Cl$_2$], were discovered by Barnett Rosenberg in the 1960s [1–3], and the drug received approval for the treatment of metastatic testicular and ovarian cancer in 1978 [4]. The biologically active forms of the drug, cis-[Pt(NH$_3$)$_2$Cl(OH$_2$)]$^+$ and cis-[Pt(NH$_3$)$_2$(OH$_2$)$_2$]$^{2+}$ generated through ligand exchange with water molecules, covalently bind to DNA forming intrastrand cross-links [5–15]. The
Pt–DNA adducts are recognized by proteins in the cell, inhibiting processes that include transcription and DNA replication, and inducing apoptosis [5–15]. A significant drawback of cisplatin is the systemic rather than targeted distribution in the body which causes significant damage of healthy cells, in some cases leading to severe nephrotoxicity and neurotoxicity [4]. The ligand exchange process that produces the active species of cisplatin and related platinum complexes occurs in rapidly proliferating cells that are both healthy and malignant, a fact that reduces the selectivity of the drug [4].

Photodynamic therapy (PDT) takes advantage of light activation to achieve spatio-temporal selectivity for cancerous tissue, thus reducing the systemic toxicity to healthy cells [16–19]. PDT has recently emerged as an alternative, and, in some cases, a superior approach to conventional dermatological therapies and for the treatment of endoscopically accessible tumours [20–26]. To date, the only agents approved for PDT are organic molecules that sensitize 3\textsuperscript{O}_2 to produce singlet oxygen, 1\textsuperscript{O}_2, a reactive oxygen species (ROS) that can effect direct cell damage, vascular shutdown and activation of immune responses [27]. These organic photosensitzers are activated with light in the ‘PDT window’, 600–850 nm, and systemic toxicity is avoided because the cytotoxic activity is confined to the irradiated tissues [28–30]. Inorganic complexes have also been investigated for the potential use as PDT agents, and various polypyridyl and porphyrin compounds that sensitize the formation of cytotoxic 1\textsuperscript{O}_2 upon excitation with visible light have been reported [31–50]. Of greater interest are photoactive transition metal complexes that operate via alternative mechanism of action and which do not require O\textsubscript{2} for activity; these agents have the potential to be active against cisplatin-resistant cell lines and hypoxic tumours.

In the present work, the new complex \([\text{Rh}_2(\text{phen})_2(\text{CH}_3\text{CN})_6](\text{BF}_4)_4\) (1) was synthesized and characterized which contains a single Rh–Rh bond that is not supported by bridging ligands. The photochemistry of the related complex \([\text{Rh}_2(\text{CH}_3\text{CN})_{10}](\text{BF}_4)_4\) (2) was previously reported, and it was found that irradiation of \([\text{Rh}_2(\text{CH}_3\text{CN})_{10}]^{4+}\) in CH\textsubscript{3}CN \((\lambda_{\text{irr}} \geq 435\text{ nm}, 60\text{ min})\) resulted in the homolytic cleavage of the Rh–Rh bond and formation of paramagnetic Rh(II) radicals [51]. Several dark reactions involving the initially generated \([\text{Rh}(\text{CH}_3\text{CN})_5]^{2+}\) fragments were also reported [51]. A number of ROS are formed by traditional PDT photosensitzers, including superoxide (\(\bullet\text{O}_2^-\)), peroxide (\(\bullet\text{O}_2^2\)) and hydroxyl radical (\(\bullet\text{OH}\)) [52], such that any reaction that results in the generation of one or more of these ROS has the potential to act as a therapeutic agent. It is expected that reactive mononuclear Rh(II) radicals generated through the photolysis of 1 may react with biomolecules themselves or can produce ROS. The photochemistry and DNA photocleavage of 1 were investigated and the data reveal that it possesses the desired qualities of a PDT agent.

2. Experimental

(a) Materials

\(\text{Rh}_2(\text{OAc})_4\cdot2\text{MeOH}\) was purchased from Pressure Chemical Company and 1,10-phenanthroline was obtained from Sigma-Aldrich, both being used as received. Acetonitrile and dichloromethane used for the synthesis were obtained from Sigma-Aldrich, with the former distilled over 3 Å molecular sieves under N\textsubscript{2} and later over P\textsubscript{2}O\textsubscript{5} under N\textsubscript{2}. Benzene was obtained from Fisher, acetonitrile was procured from Mallinckrodt, and CD\textsubscript{3}CN and D\textsubscript{2}O were purchased from Sigma-Aldrich. All solvents, except for the distilled acetonitrile used for electrochemistry experiments, were used as received. Electrochemistry-grade tetrabutylammonium hexafluorophosphate (TBAPF\textsubscript{6}, Fluka) was used without further purification. Boric acid, 1 kb DNA ladder in buffered solution, EDTA, ethidium bromide (EtBr), electrophoresis-grade agarose, gel loading buffer, Tris base, sodium acetate trihydrate, sodium phosphate and sodium hydroxide were purchased from Sigma-Aldrich and used as received. Water for photolysis and biological experiments was deionized to a resistivity of 18 M\(\Omega\) cm using a Barnstead B-pure filter system. The pUC18 plasmid DNA was purchased from Bayou Biolabs, QIAprep Spin Miniprep and Gel Extraction Kits were obtained from Qiagen.
(b) Instrumentation and methods

Electronic absorption measurements were performed on a Hewlett-Packard (HP) diode array spectrophotometer interfaced with a computer running HP 8453 WinSystem software. Cyclic voltammetry experiments were performed in a three-electrode cell with a Pt working electrode, a Pt wire auxiliary electrode and a saturated Ag/AgCl reference electrode using a BAS CV-50W voltammetric analyzer (v. 2.3). Photolysis experiments were performed using a fan-cooled, 150 W Xe short arc lamp (USHIO) in a Milliarc lamp housing unit (PTI) powered by a LPS-220 power supply (PTI) equipped with a LPS-221 igniter (PTI). A 10 cm long water cell, placed between the lamp and the sample, was used to absorb infrared irradiation and the desired wavelength range was attained using bandpass filters (Thorlabs, FWHM approximately 10 nm) and/or 3 mm thick Schott colour glass long-pass filters (CVI MellesGriot). Electrospray ionization mass spectrometry (ESI-MS) data were acquired on a Bruker MicroTOF spectrometer with a CH$_3$OH mobile phase and $^1$H NMR spectra were collected using a Bruker 400 MHz DPX—Ultrashield system. Gel electrophoresis was conducted with an EC-105 unit (EC-Apparatus Corporation) and stained gels were imaged using a GelDoc 2000 transilluminator (Bio-Rad Laboratories) and visualized with QUANTITY ONE v. 4.6.9 software (Bio-Rad Laboratories).

Positive ion ESI-MS data for samples dissolved in CH$_3$CN or H$_2$O were referenced to a sodium formate internal standard. $^1$H NMR spectroscopy was performed in CD$_3$CN or D$_2$O, containing benzene (25 mM) for quantification, and referenced to the residual CHD$_2$CN or H$_2$O solvent peaks [53]. Electrochemical measurements were performed on samples dissolved in distilled CH$_3$CN containing 0.1 M TBAPF$_6$ as the supporting electrolyte, and bubbled for 5 min with N$_2$ prior to data collection. Cyclic voltammetry data were recorded at a scan rate of 100 mV s$^{-1}$, and after collecting data for each complex, ferrocene was added to the samples to serve as an internal standard [54].

The quantum yields, $\Phi$, for photoinduced ligand exchange were determined for 1 in H$_2$O by irradiation with 500 and 550 nm light, filtered using appropriate bandpass filters with 10 nm bandwidths [55]. Electronic absorption spectroscopy was used to quantitate the decrease in concentration of the reactant as a function of irradiation time (moles reacted per second), and Reinecke's salt actinometry was used to determine the intensity (einstins per second) of the Xe arc lamp at the two wavelengths [55,56]. Owing to the low extinction coefficient and solubility of 1 in H$_2$O, optically dense ($A > 4$) solutions could not be prepared for quantum yield determinations and a modified actinometry procedure was developed. Actinometry was performed with various concentrations of the actinometer with absorbances matched to the initial absorbance of 1 at the two irradiation wavelengths. A working curve of actinometer absorption versus lamp intensity was then constructed which was used to correlate the absorption of 1 to the moles of photons absorbed per minute.

DNA photocleavage gels were performed on samples of 20 $\mu$l total volume in 0.5 ml transparent Eppendorf tubes containing 50 $\mu$M linearized pUC18 plasmid, 5 mM Tris buffer (50 mM NaCl, pH 7.5), and 250 $\mu$M 1. Following irradiation or dark incubation, 3 $\mu$l of gel loading buffer was added to each tube, and the samples were loaded into the wells of a 1 per cent agarose gel in TBE buffer (Tris-borate/EDTA buffer, 0.09 M Tris-borate, 0.002 M EDTA, pH = 8.0) which had 5 $\mu$g ml$^{-1}$ EtBr, the dye used to image the DNA, dispersed in it. The gel was submerged in TBE buffer and gel electrophoresis was carried out at 59 V for 90 min. After the electrophoresis, the gel was soaked in 50 ml of water for 30 min to remove excess EtBr.

Geometry optimization of 1 was performed with a GAUSSIAN 09 program package using the density functional theory (DFT) method [57]. The MPW1PW91 correlation and exchange functionals [58] were used with the Stuttgart RSC 1997 Electron Core Potential (ECP) [59] basis set for Rh atoms, 6–31G (d') basis set for C, N and H atoms [60,61]. Geometric parameters from the single crystal structure without the [BF$_4$]$^-$ anions were used as the starting point for the simulations, followed by a frequency calculation to confirm the full optimization. The TD-DFT calculations were conducted under both a gas phase and solvation model, with the first 30 singlet-to-singlet excitations included.
X-ray data for 1 were collected on a Bruker charge-coupled device Gadds diffractometer with graphite monochromated Cu Kα radiation (λ = 1.54096 Å). A hemisphere of crystallographic data was collected and the frames were integrated with the Bruker AXS SAINT software package [62], and the data were corrected for absorption using the SADABS program in the same software package [63]. The structure was solved and refined using X-SEED, a graphical interface to SHELX97 [64]. Crystal parameters and information pertaining to data collection and refinement for all the structures are summarized in the electronic supplementary material, table S1.

3. Results and discussion

(a) Synthesis and characterization

The compound [Rh₂(phen)₂(CH₃CN)₆](BF₄)₄ (1) was synthesized by the reaction of cis-[Rh₂(μ-OOCCCH₃)₂(phen)₂(CH₂CN)₂](OOCCH₃)₂ [65] with HBF₄•Et₂O. A sample of cis-[Rh₂(μ-OOCCCH₃)₂(phen)₂(CH₂CN)₂](OOCCH₃)₂ (0.1004 g, 0.125 mmol) was added to 30 ml of dry CH₂Cl₂ and stirred for 30 min which resulted in a green-coloured solution with a small amount of undissolved starting material being present. A 0.8 ml aliquot of dry CH₃CN was added into the solution, followed by the addition of 1.2 ml of HBF₄•Et₂O (8.82 mmol), resulting in the immediate formation of a red-coloured solution. Stirring under N₂ flow for 2 h resulted in the precipitation of the desired product, which was washed three times each with dry CH₂Cl₂ (10 ml) and dry diethyl ether (10 ml). The recovered product was 0.11 g which is an overall yield of 75 per cent based on rhodium. Recrystallization of [Rh₂(phen)₂(CH₃CN)₆](BF₄)₄ was accomplished by the slow diffusion of diethyl ether into a dilute CH₃CN solution containing the product at 4°C. The ¹H NMR spectrum of 1 in CD₃CN exhibits four peaks in the aromatic region at 8.50 (d), 8.43 (d), 7.93 (s), and 7.65 (dd) ppm that correspond to two bound phen ligands, and a peak at 2.94 ppm that corresponds to the four equatorial CH₃CN ligands, CH₃CNeq (see the electronic supplementary material, figure S1). No resonances are observed for the axial CH₃CN ligands, CH₃CNax, since these ligands are rapidly displaced by solvent molecules and appear as free CH₃CN. Similarly, a ¹H NMR spectrum of 1 in D₂O exhibits four aromatic resonances, two of which are coincident, and a peak at 2.98 that represents the CH₃CNeq ligands (see the electronic supplementary material, figure S2). Four mass clusters are observed in the ESI-MS of 1 in CH₃CN (see the electronic supplementary material, figure S3). The peaks at m/z = 203.56 and 182.55 correspond to [Rh₂(phen)₂(CH₃CN)₆]⁺ and [Rh₂(phen)₂(CH₃CN)₄]⁴⁺, respectively, and the peaks at m/z = 339.02 and 352.05, which are the dominant features in the ESI-MS of 1 in H₂O (see the electronic supplementary material, figure S4), represent [Rh₂(phen)₂(CH₃CN)(CH₃OH)(F)₂]²⁺ and [Rh₂(phen)₂(CH₃CN)(H₂O)(F)₂]²⁺, respectively.

Compound 1 crystallizes in the P1 space group, containing one unsupported dirhodium centre with each metal centre coordinated by one phen ligand and three CH₃CN ligands to fulfil the pseudo-octahedral geometry and four outer-sphere [BF₄]⁻ anions (figure 1a). The Rh–Rh bond length is 2.658(2) Å, which is significantly longer than the distance of 2.559(1) Å in the starting material cis-[Rh₂(μ-OAc)₂(phen)₂(C₅H₅N)₂](PF₆)₆ [66], but similar to the 2.634(1) Å bond distance for [Rh₂(CH₃CN)₁₀]⁴⁺, a related unsupported dirhodium(II,II) metal–metal bonded complex [67]. The Rh–CH₃CNax bond distances, 2.23(1) and 2.19(1) Å, are longer than those of the Rh–CH₃CNeq bonds, which range from 1.998(8) to 2.026(9) Å. The Rh–N (phen) distances range from 2.01(1) to 2.024(9) Å, comparable to those in cis-[Rh₂(μ-OAc)₂(bpy)₂(CH₃CN)₂]²⁺ [65] and cis-[Rh₂(μ-OAc)₂(dppz)₂(CH₃OH)(OAc)]⁺ [68].

The crystal structure reveals intramolecular π-stacking interactions between the two phen ligands which are offset with respect to each other, with N1-Rh1-Rh2-N3 and N2-Rh1-Rh2-N4 dihedral angles of 45.3(4)° and 44.7(4)°, respectively (see the electronic supplementary material, table S2). These dihedral angles are greater than the corresponding values in cis-[Rh₂(μ-OAc)₂(bpy)₂(CH₃CN)₂]²⁺ [65] and cis-[Rh₂(μ-OAc)₂(dppz)₂(CH₃OH)(OAc)]⁺ [68], 6.2(4)°, 6.2(4)° and 15.8(2)°, 15.1(2)°, respectively, owing to the absence of the bridging ligands in compound 1. The four equatorial CH₃CN ligands adopt a staggered conformation with...
the dihedral angles defined by N5-Rh1-Rh2-N7 and N6-Rh1-Rh2-N8 measured to be 44.5(4)° and 43.0(4)°, respectively (see the electronic supplementary material, table S2), similar to the unbridged complex 2 [67]. Owing to the distortion of the phen ligands, the best least-squares planes of the two phen ligands in compound 1 are not exactly parallel to each other, and thus no unique distance between them can be defined. The shortest contact between two atoms in the two phen ligands is between N2 and N3, with a distance of 3.185 Å.

As can be seen in figure 1b, there are also intermolecular π–π stacking interactions between two phen ligands in two adjacent molecules of compound 1. No unique distance can be defined either owing to the same reason as previously stated. In this case, the closest distance between two atoms in the phen ligands is 3.501 Å, which is defined by C15 and C19. In addition, owing to the intermolecular π–π stacking interactions, one of the CH3CN ligands in the axial position is bent, with Rh–N–C bond angle of 143(1)°, as shown in figure 1b. Selected bond distances and dihedral angles are listed in the electronic supplementary material, table S2.

(b) Electrochemistry, electronic absorption spectroscopy, and calculations

Complex 1 undergoes an irreversible reduction at −0.096 V versus Ag/AgCl in CH3CN (0.1 M TBAPF6, electronic supplementary material, figure S4). It is noted that compound 2 was found to be irreversibly reduced at −0.05 V versus Ag/AgCl [51]. No other redox processes were observed for 1 up to +2.0 V, as previously reported for 2, probably owing to the high positive charge on the metal centres. Two absorption bands with maxima at 364 nm (ε = 4450 M$^{-1}$ cm$^{-1}$) and 480 nm (ε = 480 M$^{-1}$ cm$^{-1}$) were observed in the electronic absorption spectrum of 1 in CH3CN (figure 2). Similarly, 2 absorbs at 365 nm (1200 M$^{-1}$ cm$^{-1}$) and 468 nm (570 M$^{-1}$ cm$^{-1}$) in CH3CN. In H$_2$O, the lowest energy absorption band of 1 red-shifts to 540 nm (300 M$^{-1}$ cm$^{-1}$), while the higher energy band remains at a similar energy with maximum at 366 nm (4500 M$^{-1}$ cm$^{-1}$, figure 2). The lowest energy transition in d$^7$–d$^7$ dirhodium(II,II) complexes is known to involve promotion of an electron to the unoccupied metal–metal σ$^*$ orbital; the energy of this molecular orbital (MO) is sensitive to the identity of the axial ligands [66,68]. Therefore, a shift in the lowest energy band is expected as a function of solvent.

DFT calculations were performed on the cation [Rh$_2$(phen)$_2$(CH$_3$CN)$_6$]$^{4+}$ (1a) in a polarizable continuum model that mimicked the solvation effects of CH3CN. The bond lengths and angles from the optimized structure agree well with those from the single-crystal X-ray diffraction structure (see the electronic supplementary material, table S2). The calculated MO diagram is

![Figure 1. X-ray crystal structure of 1 showing (a) the single molecule and (b) extended structure (thermal ellipsoids drawn at 50% probability level; anions and hydrogen atoms were omitted for clarity). (Online version in colour.)](https://rsta.royalsocietypublishing.org/article/10.1098/rsta.20120128)
shown in figure 3. The highest occupied MO of 1a has contributions from both of the phen ligands (62%) and the dirhodium core (29%), the latter with significant metal–metal $\sigma$-bonding character (figure 3). In contrast, the lowest unoccupied molecular orbital (LUMO) is predominantly Rh–Rh $\sigma^*$ in character (68%), possessing only minor contributions from other moieties (figure 3). The two lowest energy electronic transitions predicted using DFT methods appear at 467 nm ($f = 0.0003$) and 457 nm ($f = 0.0002$) and are in good agreement with the experimental data, which revealed an absorption maximum at 480 nm in CH$_3$CN (see the electronic supplementary material, table S3). Transitions 3–5 are calculated at 373 nm ($f = 0.0076$), 368 nm ($f = 0.0000$) and 338 nm ($f = 0.0925$) consistent with the strong absorption features observed in the spectrum of 1 in the ultraviolet (UV) and near-UV spectral regions (figure 2). The calculations indicate that excitation in the visible

\[ \text{Figure 2. Electronic absorption spectra of 1 in CH$_3$CN (solid) and H$_2$O (dashed). Inset: expanded view of the visible region.} \]

\[ \text{Figure 3. MO diagram calculated for 1 and electron densities of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). (Online version in colour.)} \]
region results in the placement of the promoted electron in the LUMO with significant Rh–Rh σ*
character. Population of this orbital is expected to lead to homolytic metal–metal bond cleavage
and dissociation, resulting in two mononuclear fragments.

(c) Photochemistry

The changes to the electronic absorption spectrum of 1, monitored as a function of irradiation
time in H₂O (λ_{irr} ≥ 590 nm), are displayed in figure 4a in which it can been seen that
rapid disappearance of the peak at 540 nm, corresponding to starting material, occurs with a
concomitant increase in absorption at λ < 470 nm with a maximum at 355 nm (see the electronic
supplementary material, figure S8). As shown in the electronic supplementary material, figure
S9, no changes are observed in the absorption spectrum of 1 in water when kept in the dark
at room temperature for 60 min. The changes to the ¹H NMR spectrum during the photolysis
of 1 in D₂O are shown in figure 4b with 25 mM of benzene as an internal standard to obtain
relative peak integrations, which appears as a singlet at 7.38 ppm. The initial spectrum collected
prior to irradiation shows the characteristic signals from the phen ligands in the aromatic region,
as well as the four equatorial CH₃CN ligands, CH₃CNeq, and two equivalents of free CH₃CN
from the axial ligands that results from the exchange with D₂O (figure 4b). Irradiation with
visible light (λ_{irr} ≥ 590 nm) results in a decrease in intensity of the peaks associated with the
starting material and an increase of the resonance of free CH₃CN, resulting in an additional two
equivalents at t_{irr} = 60 min (figure 4b). Interestingly, no new resonances that could be used to
identify a photoproduct appeared in the aromatic region throughout the photolysis (figure 4b).

The disappearance of the ¹H NMR signal can be explained by the formation of paramagnetic
species, such as Rh(II) mononuclear complexes, with a d⁷ electron configuration. The homolytic
cleavage of the Rh–Rh bond to yield mononuclear Rh(II) fragments was previously observed
upon irradiation of 2 with visible light in CH₃CN (λ_{irr} ≥ 435 nm, 60 min) [51]. The disappearance
of the peak at 540 nm observed here for 1 is consistent with metal–metal bond cleavage, since
that transition involves the Rh–Rh σ* orbital (LUMO). The lack of new ¹H NMR signals after
irradiation is also consistent with the formation of paramagnetic mononuclear Rh(II) species.

ESI-MS was used to characterize the identity of the products following irradiation of
1 mM of 1 in H₂O (λ_{irr} ≥ 590 nm, t_{irr} = 60 min). Three major peaks are observed at m/z =
299, 341 and 465, which correspond to [Rh(phen)(OH)]⁺, [Rh(phen)(CH₃CN)(OH)]⁺ and
[Rh(phen)(CH₃CN)(H₂O)₃(BF₄)]⁺, respectively (see the electronic supplementary material,
figure S7). These peaks are markedly different from those of the dinuclear starting material in
H₂O at m/z = 339 and 352 (see the electronic supplementary material, figure S4). The quantum
yields for disappearance of 1 measured using 500 and 550 nm irradiation were determined to be
1.8 and 1.4, respectively, indicating that the photoproduct itself or another reactive species derived
from the initial product also reacts with the starting material, generating additional species.

(d) DNA photocleavage

The photocleavage of 50 μM pUC18 plasmid by 200 μM of 1 is shown in figure 5, where in the
absence of complex (lane 1) the plasmid appears in the supercoiled form (type I) with a small
amount of nicked impurity (type II). Lane 2 shows that incubation of the plasmid with 200 μM of
1 in the dark for 15 min does not result in DNA cleavage. Irradiation of 200 μM of 1 in the presence
of DNA with λ_{irr} ≥ 590 nm for 15 min (lane 3) results in depletion of nearly all of the undamaged
supercoiled DNA (form I) and a significantly greater amount of nicked plasmid (form II). Lane
4 in figure 5 shows that for irradiation of 1 (λ_{irr} ≥ 590 nm, 15 min) in the presence of sodium
azide (NaN₃), a ¹O₂ and radical scavenger, DNA cleavage is still operative but it appears to be
reduced when compared with that in the absence of azide (lane 3). Lanes 5 and 6 show that the
DNA photocleavage is still operative in the absence of oxygen in solution after six freeze–pump–
thaw cycles, and conducting the experiment in D₂O, which enhances the reactivity of ¹O₂, does
not appear to have an effect on the DNA photocleavage. These results are consistent with the
**Figure 4.** Photolysis of 0.5 mM of 1 as a function of irradiation time (0 to 60 min) monitored by the changes in the (a) electronic absorption spectrum in H₂O and (b) ¹H NMR spectrum in D₂O (λ_{irr} ≥ 590 nm).

**Figure 5.** Ethidium bromide-stained agarose gel showing the photocleavage of 50 μM pUC18 plasmid by 200 μM of 1 in 5 mM Tris, 50 mM NaCl, pH = 7.5. Lane 1: DNA only; lane 2: DNA and 1 incubated in the dark (15 min); lane 3: DNA and 1 irradiated; lane 4: DNA and 1 irradiated in the presence of 2 mM NaN₃; lane 5: DNA and 1 irradiated after freeze–pump–thaw; and lane 6: DNA and 1 irradiated in D₂O (λ_{irr} ≥ 590 nm, 15 min).

The generation of reactive radical species upon irradiation that are able to cleave DNA, but that are not derived from oxygen present in solution. The sensitized production of singlet oxygen can also be ruled out because 1 releases the excited energy through homolytic Rh–Rh bond cleavage; further work is currently ongoing in order to establish the species involved in the reactivity with DNA.
4. Conclusions

The new complex \([\text{Rh}_2(\text{phen})_2(\text{CH}_3\text{CN})_6](\text{BF}_4)_4\) (1) was synthesized and characterized in solution and its crystal structure was determined. Irradiation of 1 with visible light (\(\lambda_{\text{irr}} > 590\ nm\)) in water results in the release of two equatorial \(\text{CH}_3\text{CN}\) ligands, \(\text{CH}_3\text{CNeq}\), as well as in the formation of mononuclear radical \(\text{Rh(II)}\) fragments stemming from the homolytic photocleavage of the metal–metal bond. The photoproducts, identified by ESI-MS, include \([\text{Rh}(\text{phen})(\text{CH}_3\text{CN})(\text{OH})]^+\) and \([\text{Rh}(\text{phen})(\text{CH}_3\text{CN})(\text{H}_2\text{O})_3(\text{BF}_4)]^+\). The quantum yield for the photochemical transformation of 1 in \(\text{H}_2\text{O}\) exceeds unity (\(\Phi_{\text{550 nm}} = 1.38\)) indicative of dark reactions following the initial photoprocess. DNA photocleavage was observed for 1 (\(\lambda_{\text{irr}} > 590\ nm\)), whereas the complex is unreactive in the dark. This feature makes 1 a promising PDT agent that does not operate via the production of singlet oxygen, \(^1\text{O}_2\).

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