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LIGHT CLEAVAGE OF SOME POTENTIAL LINKERS FOR DRUG CARRIERS

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Abstract. The study of photocleavage of different substrates as potential linkers for drug carrier compounds is reported. The role of singlet oxygen and the kinetic rates implied in the photoreactions were determined. The study envisaged the use of two olefins: 7-(4-chlorophenyl)hept-6-enoic acid and 7-(ferrocene)hept-6-enoic acid as potential linkers for the controlled release of a specific drug, by singlet oxygen induced cleavage. The rate constants for quenching of the singlet oxygen by these olefins were determined. The photoproducts were analyzed by FTIR spectroscopy.

Key words: olefins, singlet oxygen generation, quenching rate constant.

1. INTRODUCTION

Photo-activated drug carriers systems and light activated drugs are options taken into account in advanced delivery by controlled release at the target of the active form of the drug. This can be important in order to optimize the medication and reduce side effects. The ability to manipulate light in terms of its wavelength, intensity, site of application and duration allows to accurately control the place, dose and time at which a therapeutic agent is released, if the liberation of drug is linked to a photochemical process that takes place in the biological target [1].

The combination of a cytostatic drug with optical radiation may have potential use in cancer therapy [2] and finds extended applications in fighting against bacteria that are resistant to treatment with one or more antibiotics [3]. The

UV light triggering of 5-Fluorouracil activation has been reported [4]. However, the poor tissue penetration of UV radiations restricts their clinical application. In general, the visible/NIR light between 600–800 nm is more suitable for such studies due to its deeper penetration in the tissue. This can be used for the release of a drug linked to a photosensitizer (PS). The light irradiation of the linked compound in the absorbing range of PS generates singlet oxygen that chemically breaks the linker between the PS and the drug. In this way the drug is released and becomes free and active at the desired site. As photocleavable groups, nitrobenzil [5] and cyclodextrin dimers [6] were used in light triggered porphyrin anticancer drugs. In [7] is reported a recipe for site specific drugs release from complexes of compounds using visible light. The used linkers between PS and the drugs were different substituted olefins. Under visible light the PS generates singlet oxygen, this cleavages the linker by 2+2 cyclo-addition reaction and the drug is released [8]. In [9], the photo-oxidation reaction yields for different types of di-hetero-substituted olefins under low energy visible light in the presence of a porphyrin derivative are reported. Among the tested olefins, 1,2-cis-diphenoxyethylene seems to be a good linker for the singlet oxygen-cleavable drug complexes with respect to reaction kinetics and side reactions. An extended compilation of the rate constants values for the reactions of singlet oxygen with several organic compounds, including olefins, is reported in [10].

In this paper, the study of photo-cleavage of different substrates as potential linkers for drug delivery compounds is reported. The singlet oxygen quenching rate constants for some substituted olefins were determined. Before using active ingredients that are expensive, preliminary studies for the feasibility of the cleavage reactions should be envisaged. The mixtures of the studied olefins with a Cresyl Violet derivative (used as PS) were exposed to low energy visible light. The irradiated solutions were investigated by FTIR spectroscopy and potential photoproducts were suggested.

2. MATERIALS AND METHODS

The chemical structures of the studied olefins are depicted in Fig. 1: 7-(4-chlorophenyl)hept-6-enoic acid (L1) and 7-(ferrocene)hept-6-enoic acid (L4) are two olefins which are analyzed in mixtures with a Cresyl Violet Acetate derivative, 9-(6-bromohexanamido)-6,6-dihydrobenzophenoxazin-5-iminium acetate (L3), used as PS which generates singlet oxygen when excited with visible light. The generation of singlet oxygen was also studied for 9-(7-(4-chlorophenyl)hept-6-enamido)-6,6-dihydrobenzophenoxazin-5-iminium (labeled as L2) which consists

in L1 linked with L3. The compounds L1, L2, L3 and L4 were home synthesized. The studied solutions were prepared in dichloromethane (DCM) (99.9 %, Merck).

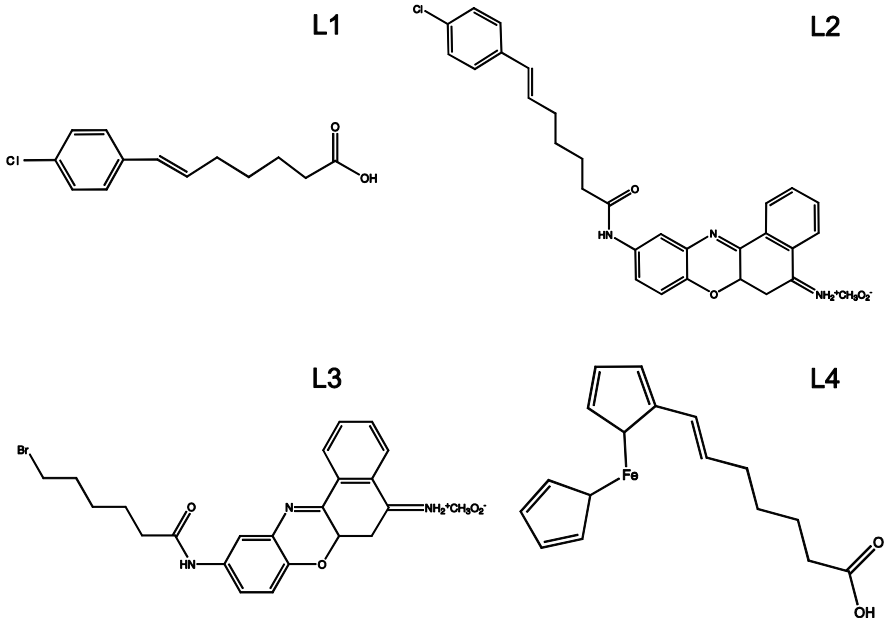

Fig. 1 – Chemical structures of L1, L2, L3, and L4.

The laser radiation used for the L3 photosensitization was provided by the second harmonic generation (SHG) of a Nd:YAG (Continuum, Excel Technology) pulsed laser, model Minilite II emitting pulses with 10 Hz frequency, 6 ns duration, and 2 mJ average energy at 532 nm. The singlet oxygen was generated by energy transfer from the excited L3 molecules. The time-resolved phosphorescence of singlet oxygen (λ = 1,270 nm) was detected by a cooled NIR photomultiplier (Hamamatsu H-10330), whose output was fed to a digital oscilloscope (Tektronix DPO 7254). The experimental set-up is described in detail elsewhere [11].

The lifetime of singlet oxygen phosphorescence was measured for the compounds L1 and, respectively, L4 at varied concentrations between 5 mM and 25 mM mixed with the dye L3 always kept at 25 μM in DCM. The rate constants for singlet oxygen quenching by the studied olefins were determined from the Stern-Volmer plots of $1/\tau$ versus the olefin concentration by using the following equation:

$$\frac{1}{\tau} = \frac{1}{\tau_0} + k[c], \tag{1}$$

where τ is the singlet oxygen excited state lifetime in the presence of the quencher, τ_o is the excited state lifetime in the absence of the quencher, k is the $^1O_2$ quenching rate constant and [c] is the concentration of the tested compound.

Volumes of 1 mL solutions of the dye L3 and, respectively, linker (L1 or L4) were irradiated with a Xe high pressure lamp (Hamamatsu, model L2273). The lamp radiation was filtered against UV light by a long pass filter type FGL495 (Thorlabs) and the total beam power was 145 mW in the 500–800 nm spectral range. The total light power was measured using an optical power system PM 120 (Thorlabs). The light intensity of the lamp relative to wavelength, measured by an Ocean Optics spectrometer (type HR4000), is shown in Fig. 2. The irradiation was made in a standard absorption cuvette having 1 cm absorption path length and the lamp beam was focused to have 1 cm diameter. The irradiation time was 1 hour for all the studied solutions.

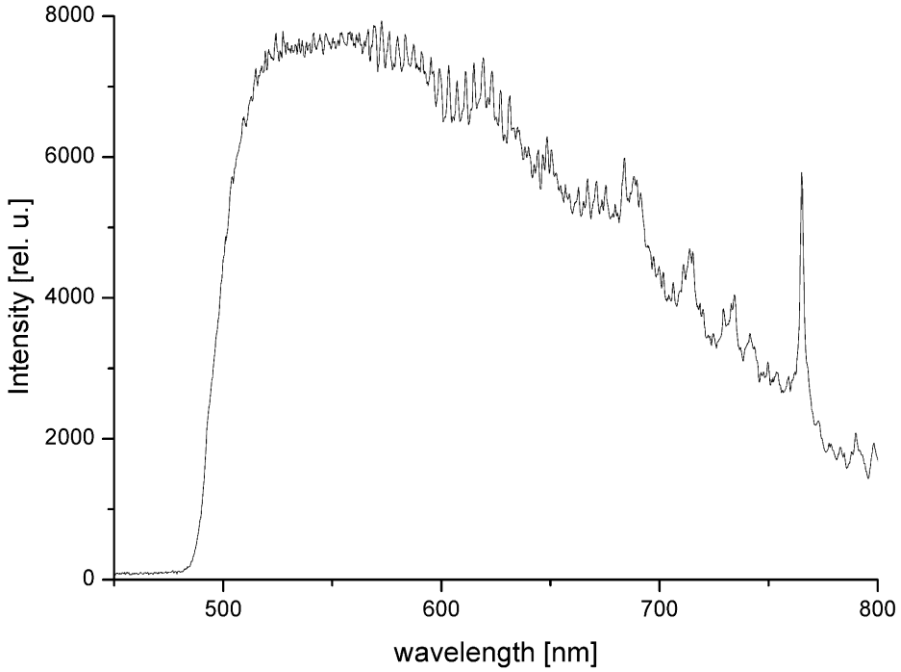

Fig. 2 – Filtered emission spectrum of the Xe lamp.

The IR absorption spectra of unirradiated and irradiated solutions were recorded using a FTIR Nicolet™ iS™ 50 spectrophotometer in the range 750–4,000 cm^{-1} at a 4 cm^{-1} resolution and using KBr cells (0.05 mm absorption path length). From all the spectra, the background due to DCM was subtracted.

3. RESULTS AND DISCUSSIONS

The interaction of the olefins with singlet oxygen generated by the interaction of L3 molecules with the laser beam emitted at 532 nm was investigated by time-resolved phosphorescence. The absorption spectra of all the compounds implied in this study are given in Fig. 3, showing a peak at 492 nm for L3, one at 362 nm for L4, and another at 448 nm for L2.

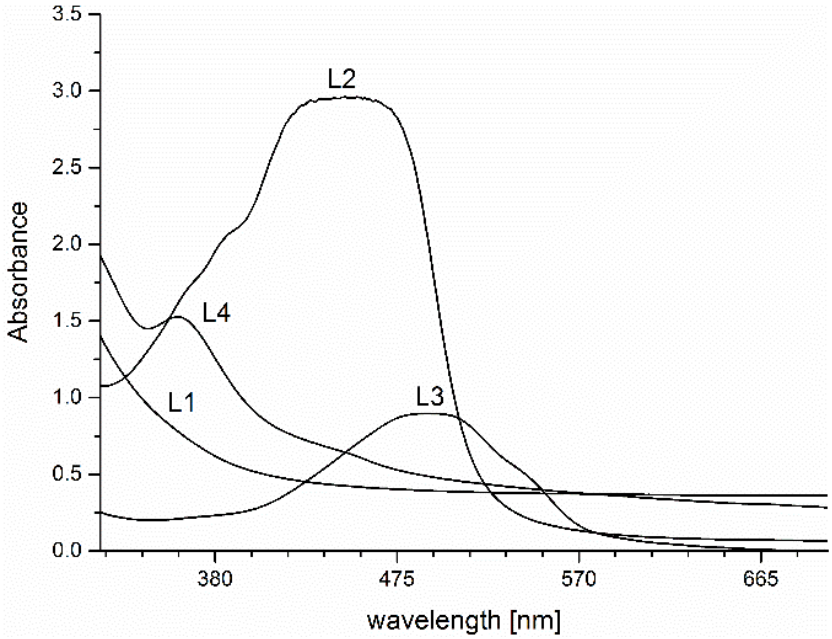

Fig. 3 – Absorption spectra of L1 (50 mM), L2 (100 μM), L3 (50 μM) and L4 (5 mM) solutions in DCM.

The rate constants of L1 and, respectively, L4 reactions with singlet oxygen were determined. The lifetime of singlet oxygen generated by the laser excited L3 molecules was determined for mixtures of L3 at 25 μM in DCM with L1 or L4 at varied concentrations in the range 2 mM–25 mM. The corresponding Stern-Volmer plots are shown in Fig. 4. From the slope of the two lines, the oxygen quenching rate constant was obtained. This is 2.9×10^4 $M^{-1}s^{-1}$ for L1 and 2.4×10^5 $M^{-1}s^{-1}$ for L4. Comparing the two values of the rate constants and the chemical structures of L1 and L4, it can be noticed that the presence in the olefin structure of the two cyclopentadiene rings bounded with Fe (L4 structure) produces a singlet oxygen quenching one order of magnitude larger than in the case of the substitution with chloro-benzene (L1 structure).

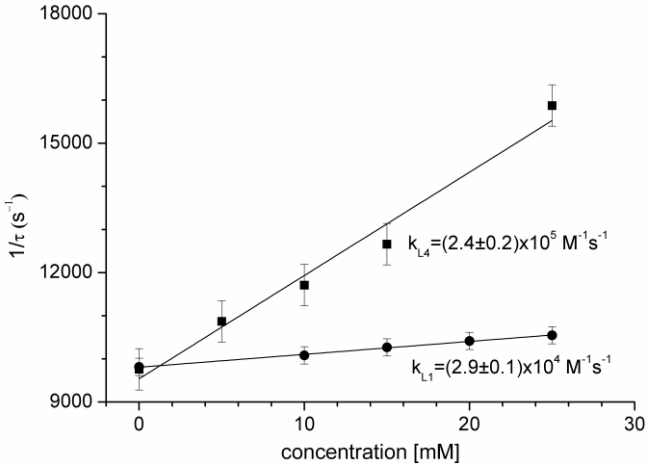

Fig. 4 – Stern-Volmer plots for L1, L2, and L4 compounds.

The compound labeled L2 and obtained from olefin L1 linked to the photosensitizer L3 was also studied. The lifetime of the singlet oxygen generated by the linked compound L2 was compared to that obtained only for L3. The time dependences for the phosphorescence of the singlet oxygen for L2 and L3 at 25 μM concentration in DCM are shown in Fig. 5. The singlet oxygen lifetime was obtained from the phosphorescence transients averaged over 1,000 laser pulses. The decaying part of the averaged phosphorescence transient fitted with a mono-exponential function (sketched in the inset of Fig. 5) gives the time constant, representing the lifetime of the singlet oxygen population.

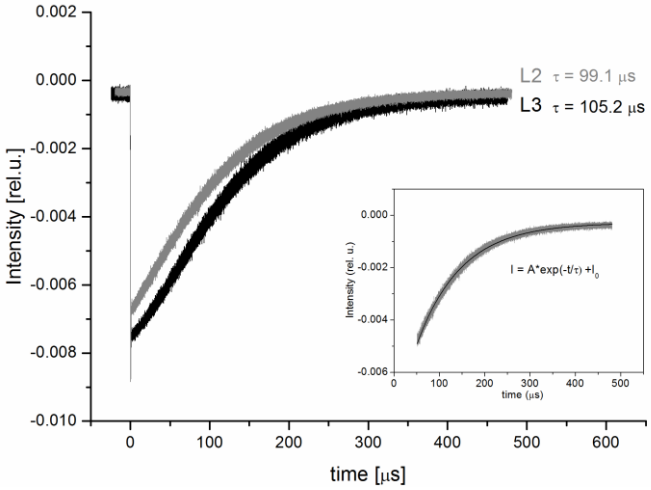

Fig. 5 – Phosphorescence of singlet oxygen as a function of time for L2 and L3 at 25 μM concentration in DCM (The inset shows the fitting procedure with a mono-exponential function).

The singlet oxygen lifetime for L3 and L2 was 105.2 μs and 99.1 μs, respectively. The close values of τ and the signals intensity for the two compounds indicate that there is not a singlet oxygen static quenching, in the case of the linked compound, and that adding the linker to the photosensitizer structure generates the same amount of singlet oxygen as in the case of their unbounded structure.

One mL mixture solutions of L3 at 25 μM with L1 or L4 at 25 mM were irradiated one hour with visible light emitted by the Xe lamp. The irradiation resulting products were analyzed by FTIR spectroscopy. For the mixture of L1 and L3, no significant modifications were observed in the spectrum of irradiated solution compared to that of the unirradiated one.

The mixture of L3 and L4 solutions irradiated one hour shows in the NIR spectra modifications in comparison with the unirradiated compounds (Fig. 6).

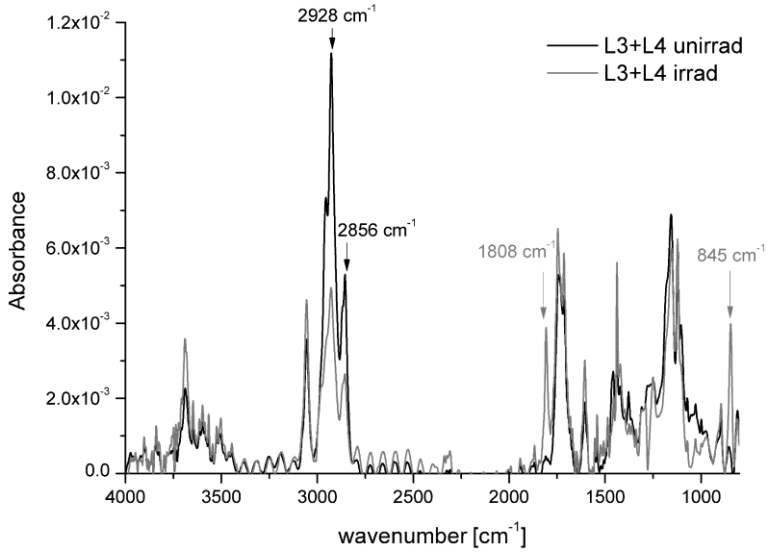

Fig. 6 – FTIR spectra for unirradiated and 1h irradiated solution of L3 (25 μM) mixed with L4 (25 mM) in DCM.

The new peak that shows up at 1,808 cm^{-1} in the spectrum of the irradiated solution can be attributed to the stretch of a C=O bond found in the neighborhood of the cyclopentadiene ring [12]. This can be an indication of a carbonyl (C=O) formation via (2+2) cyclo-addition reaction due to the C=C bond cleavage in the olefine structure [9].

On the other hand, the occurrence of a new band at 845 cm^{-1} can be assigned to a peroxide (O-O) formation as a result of ene reaction competitive to cyclo-addition in the alkene oxidation [13]. The chemical formulas for the two expected reactions are depicted in Fig. 7.

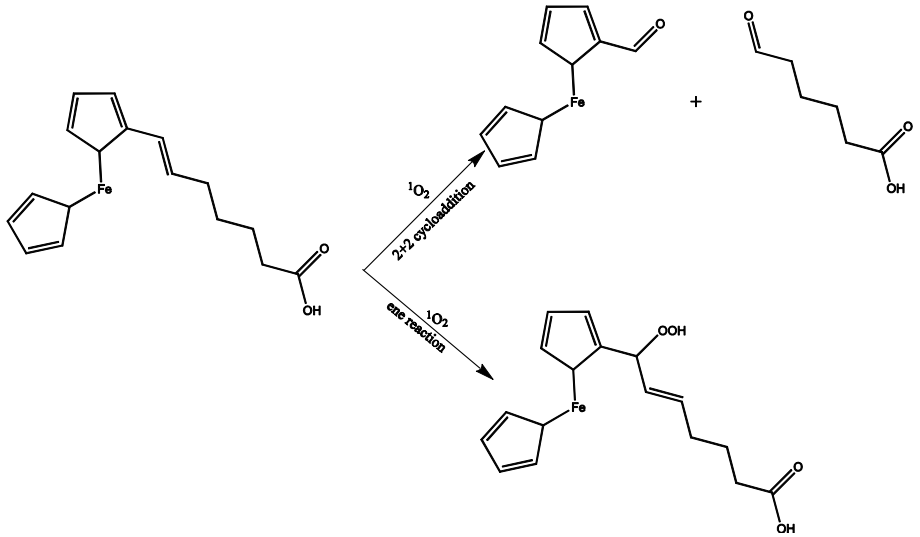

Fig. 7 – Reaction pathways for the two expected reactions.

Another indication of ene reaction can be the decrease of the peaks at 2,856 cm^{-1} and 2,928 cm^{-1} (symmetrical and asymmetrical stretching of C-H from CH_2) with 50 % and, respectively, 55 % (decreasing of the CH_2 specific vibration is a result of the replacement of a C-C bond with a C=C in the ene reaction).

The possible molecules formed after photodegradation of L1 or L4 are aldehydes which result following the reaction of cleavage of double bond C=C. Moreover, it is probable that these aldehydes are oxidized in their corresponding carboxylic acids.

4. CONCLUSIONS

The reported results described the photo-cleavage of potential linkers such as olefins for targeted drug delivery complexes. The role of the photo-generated singlet oxygen and the kinetic rates of the reactions were determined.

The quenching rate constants of singlet oxygen were determined for two members of the olefin class. The presence in the olefin structure of the two cyclopentadiene rings bounded with Fe (L4) yields a singlet oxygen quenching rate one order of magnitude larger than in the case of the substitution with chloro-benzene (L1). This suggests that L4 could be a better linker to be used for drug delivery by cleavage via singlet oxygen.

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