SUMMARY

Ultraviolet radiation is able to induce changes in the chemical composition of DNA that have been closely related to skin cancer. Among the most relevant lesions, damage to pyrimidine bases leading to bipyrimidine units (cyclobutane dimers and (6,4) photoproducts) are found. Therefore numerous studies have been performed to understand the photochemistry involved in their formation. However, despite the extensive mechanistic knowledge achieved, there are still some aspects that need to be clarified. The overall thesis objective is to clarify some of the DNA photoreaction mechanisms, with special attention to bipyrimidinic lesions formation.

First of all, the aim of the work was to determine the influence of C5 substitution on the photophysical properties of 2-thiopyrimidines. For this purpose, 2-thiouracil (TU), 5-*tert*-butyl-2-thiouracil (BTU) and 2-thiothymine (TT) were selected as target thionucleobases for the experimental studies and, in parallel, for DFT theoretical calculations. The UV spectra displayed by the three compounds were very similar to each other. They showed a maximum around 275 nm and a shoulder at ca. 290 nm. The three 2-thiopyrimidines exhibited a strong phosphorescence emission; from the recorded spectra, triplet excited state energies of ca. 307, 304 and 294 kJ/mol were determined for TU, BTU and TT, respectively. Transient absorption spectra displayed after excitation at 308 nm gave rise to a broad band ranging from 500 nm to 700 nm, which was in principle assigned to triplet–triplet absorption. This assignment was confirmed by energy transfer experiments using biphenyl as an acceptor. The triplet lifetimes were 70 ns, 1.1 μs and 2.3 μs, for TU, BTU and TT, respectively. The obtained photophysical data, both in phosphorescence and transient absorption measurements, point to significantly different properties of the TT triplet excited state in spite of the structural similarities. Theoretical calculations at the B3LYP/aug-ccpVDZ/PCM level agree well with the experimental range of excited state energies and support the ππ* nature of the lowest triplet states.

Secondly, the influence of steric hindrance on the formation of bipyrimidine lesions was analyzed by introducing a bulky substituent at C5 position of uracil. Thus, the reactivity of 5-*tert*-butiluracil methyl ester (**1c**) was compared to its thymine analogue (**2c**) after BP, xanthone and acetone photosensitization. As a general trend, **1c** reacted slower than **2c**. Benzophenone photoreaction led exclusively to oxetanes while acetone gave rise to uracil-5-*tert*-butiluracil heterodimers (**1e-1** and **1e-2**), an acetonyl derivative (**1e-3**) and to a dehydrogenation photoproduct (**1e-4**). In the case of xanthone only one oxetane (**1f**) was observed. However, parallel irradiations performed with **2c**, revealed the formation of cyclobutane dimers in all cases. The obtained results indicated that the presence of a bulky group in C5 prevents the formation of cyclobutane dimers.

Then, the photochemistry of thymine from its upper nπ* triplet excited states, through the Norrish-Yang photocyclation was explored. This required designing a dyad that consisted of a 5-*tert*-butiluracil moiety covalently linked by an aliphatic amide to BP chromophore. Benzophenone was chosen as a photosensitizer because it has a T_n with a suitable energy

and lifetime (~ 400 kJ/mol and 37 ps, respectively) to participate in a triplet-triplet energy transfer, and populate T_2 of thymine by selective irradiation. The multiphotonic excitation of the dyad was performed using a laser beam at high power (Nd: YAG, 355 nm, 40 mJ/pulse) and then the reaction mixture was analyzed by UPLC-MS/MS. The results showed that the Norrish- Yang photocyclation was produced by the formation of expected pyrimidone compound. The chemical yield was 0.003% in concordance with other processes that occur from higher excited states. Finally, relating the Norrish-Yang photoproduct yield *vs* laser power used, the biphotonic nature process was confirmed.

The last chapter of this thesis addresses the possibility that the photoproduct (6,4) once formed in DNA, could act as an endogenous photosensitizer. This lesion is capable of absorbing light in the UVB-UVA region because of the presence in its structure of the 5-methyl-2-pyrimidone moiety. The equivalent synthesized desoxyribonucleoside (Pyo) was irradiated in the presence of DNA to analyze the photoinduced damage in the biomacromolecule. This was performed by agarose gel migration in the presence and the absence of T4 endonuclease V, a specific cyclobutane dimers recognition enzyme. The experiments established that Pyo acts as a photosensitizer in DNA, and that approximately 20% of the damage was from cyclobutane dimers. Indeed, photophysical studies conducted with Pyo confirmed that it can participate in energy transfer and oxidative processes since its triplet excited state has higher energy than thymine (both isolated and in the DNA), and it is able to generate hydroxyl radicals and singlet oxygen.

Since the photochemical properties of the whole lesion may differ from the isolated chromophore Pyo, the 6,4 PP potential to act as a photosensitizer was also considered. Irradiation of 6,4 PP in the presence of DNA revealed that in fact, it can act as a photosensitizer too, although their photophysical properties are not entirely coincident. Thus, the final chapter of this thesis has served to establish that (6,4) photoproduct can act as a Trojan horse and extend the active fraction of UV radiation, causing the formation of pyrimidine dimers and oxidative damage, making it potentially more dangerous than estimated so far.