Bipyridyl ligands as photoactivatable mono- and bis-alkylating agents capable of DNA cross-linking†

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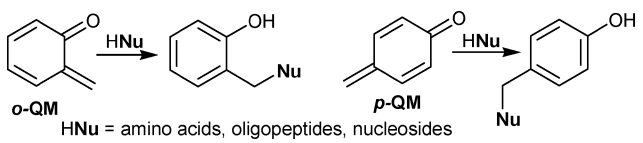
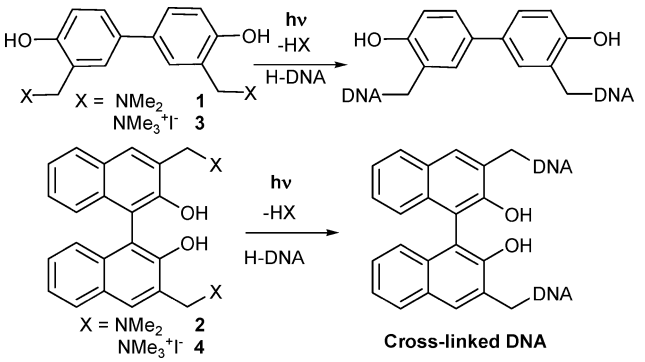
The photoactivation of 6,6′-bis-CH_2X-[2,2′]bipyridinyl-5,5′-diol ligands as mono and bis-alkylating agents has been investigated, detecting transient heterocyclic quinone methides.

In the recent past, a wide variety of photochemically activated DNA reactive agents have been discovered and investigated. Although a great deal of attention has been devoted to those acting as cleavers involving radical reactive intermediates,[1] fairly limited examples of photochemically activated DNA alkylating agents have been reported.[2] In fact, to date, the psoralens are the only commercial class of drugs known to induce DNA or RNA cross-linking upon photolysis.[3] Among reactive electrophilic intermediates generated by photochemical activation starting from stable precursors, quinone methides (QMs) have attracted considerable attention. QMs, particularly those with an *ortho* geometry (***o*-QMs**), have been successfully used to accomplish amino acid, oligopeptide, and nucleoside alkylations (Scheme 1).[4] These reactive intermediates have been trapped by nucleophiles and detected by nanosecond laser flash photolysis (LFP).[4a,5,6] More recently, Saito, Zhou and our group[7] exploited the photogeneration of bifunctional QMs, starting from bifunctional Mannich bases of biaryls (**1**) and binaphthyls (**2**) and their quaternary ammonium salts (**3** and **4**, respectively) in water, to achieve DNA-crosslinking with promising potency (Scheme 2). Among the mild activation protocols recently exploited for selective DNA-alkylation, metal cation complexation plays a key role.[8] Photoactivation and tunable reactivity of the resulting alkylating species by metal binding could be achieved by the heterocyclic analogues of the biaryls **1** and **3**, such as 6,6′-bis-CH_2X-[2,2′]bipyridinyl-5,5′-diols, bearing additional ligand sites on the 6,6′-bis-CH_2X arms.[9] Bipyridyl functionality should also allow a more tunable reactivity not only by metal coordination but also by pH.



Scheme 1 Alkylation reactions by quinone methides (QMs).

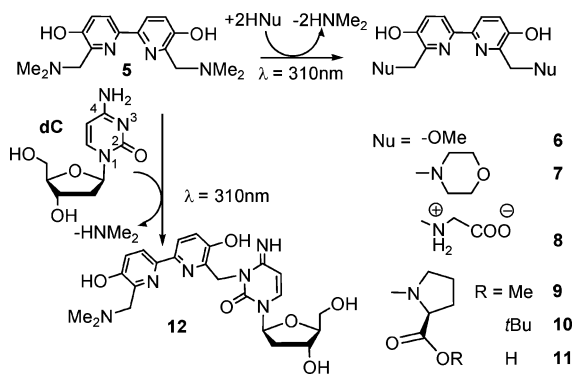
The present study is a preliminary investigation on the phototriggerable mono- and bis-alkylating properties of these bipyridyl ligands, through the transient generation of heterocyclic QMs.



Scheme 2 Biaryls (**1**,**3**) and binaphthyls (**2**,**4**) as phototriggerable DNA cross-linking agents.

We will address general mechanistic aspects concerning the photogeneration, LFP detection and reactivity of the transient heterocyclic QMs toward amines and amino acids, in potential competition with water in aqueous solution.

In more detail, here we report (i) the synthesis of a new bipyridyl, the 6,6′-bis-dimethylaminomethyl-[2,2′]bipyridinyl-5,5′-diol (**5**, Scheme 3), (ii) the investigation of its photoreactivity as a mono- and bis-alkylating agent towards amines, amino acids and deoxycytidine (**dC**) in aqueous solution, and (iii) its DNA cross-linking ability. The bifunctional amine **5** has been synthesized starting from [2,2′]bipyridinyl-5,5′-diol[10] by Mannich reactions using *N*,*N*-dimethylmethyleneiminium chloride in anhydrous $CHCl_3$ at r.t. for 12 hrs. To test the possibility of using **5** and a few of its derivatives as water soluble precursors of bis-alkylating QMs, we explored their photoreactivity in the presence of nucleophiles. Photolysis of **5** (10^{-3} M, in a photoreactor with 4 lamps 15 W,



Scheme 3 Photoreactivity of the 5,5′-dihydroxy-2,2′-bipyridyl **5**, as a mono- and bis-alkylating agent in water.

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310 nm; *ca.* 25 °C, 60 min.) in MeOH in the absence and in the presence of L-proline-OMe and -O*t*Bu esters (at 5×10^{-2} M concentration) gave the corresponding bis-alkylated adducts **6**, **9** and **10** (Scheme 3), in quantitative yields. Photolysis of **5** in a water solution containing morpholine, glycine, and L-proline ([HNu] = 5×10^{-2} M, pH 8, phosphate buffered) similarly afforded adducts **7**, **8** and **11**, in 70% yields, accompanied by a not well characterized oligomeric adduct, together with unreacted starting material (20%). Photolysis of the water soluble adducts **8** and **11** in the presence of morpholine gave the corresponding bis-alkylated adduct **7**, suggesting that the photoreactivity is a general feature of these alkylation adducts. Noteworthy, the hydration adduct (Nu = OH, Scheme 3) and monoalkylated adducts were not detected by HPLC under the conditions described above (*i.e.* large excess of nucleophile), and therefore, if they formed at all, their yields were very low (*i.e.* <5%). Unlike the photoalkylation experiments with small nucleophiles, the photolysis of **5** in the presence of a much bulkier and less nucleophilic substrate such as deoxycytidine (under pH 7.5) afforded only the monoalkylated adduct **12** with lower yield (11%). ^{1}HNMR analysis of adduct **12** suggested that N3 rather than N4 or O2 (Scheme 3 for numbering) reacts with the quinone methide. The N3-alkylation was indicated by the chemical shift of CH_2 (5.4 ppm), which is more deshielded than in reagent **5** (4.6 ppm), or in the other N-alkylated adducts **7–11** (4.6–4.9 ppm). Formation of **12** is also consistent with N3-alkylation of deoxycytidine by a prototype ***o*-QM** (CH_2 chemical shift: 5.0 ppm), as proposed by Rokita.[4e]

Wan,[5a,b] Kresge[5c] and our group[4a,6] had shown that LFP provides an effective method for direct detection of QMs, using benzylic alcohols and Mannich bases as precursors. Therefore we decided to use LFP to detect any transient species photochemically generated from **5**. Indeed, LFP of **5** at both 266 (4 mJ/pulse) and 354 nm (8 mJ/pulse, Nd:YAG laser), in aqueous solutions (pH 7.0, buffered conditions) yielded a transient absorbance centered at λ_{max} 460 nm (Fig. 1, 50 nm red shifted compared to the prototype ***o*-QM**), quite similar to that obtained flashing a solution of a pyridoxine derivative which was assigned to a neutral heterocyclic QM by Wan.[5b] The profile of such a transient absorbance follows a single second order decay (Fig. 1, inset a), with an observed second order constant $k_2 = 4.5 \times 10^{-5}$ M^{-1} $s^{-}1$, and it is not fitted by a double exponential decay. Therefore the transient spectrum shown in Fig. 1 has to be assigned to a single transient species. The quenching of such a reactive intermediate is probably due to a dimerization process, in the absence of added nucleophiles, in agreement with the product distribution analysis which displays no formation of the hydration adduct.

The decay trace becomes a single exponential upon addition of 2-mercaptoethanol, a well known QM trap (Fig. 1, insets b–d). This transient was assigned to the bipyridyl-quinone methide **BiPy-QM** on the basis of (a) the similarity of the spectroscopic properties with the structurally related QM, photo-generated from a pyridoxine derivative,[5b] and (b) trapping experiment results. In fact, after 40–50 laser shots at 354 nm, **12** was detected by HPLC in flashed aqueous solutions of **5** in the presence of deoxycytidine.

The DNA cross-linking (XL) ability of compounds **5** and **11** was investigated using a negatively supercoiled plasmid DNA (pBR322) in an alkaline agarose gel assay (Fig. 2). DNA XL experiments were carried out in 50 mM phosphate buffer at pH 7.5. Samples were irradiated at 310 nm for 5 min at 120 W. Irradiated

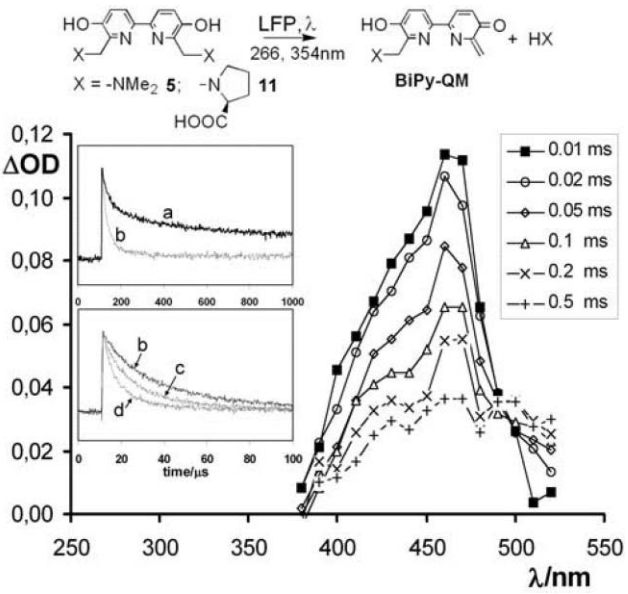

Fig. 1 Transient absorptions upon flashing **5** (and **11**) in water. Inset: decay of the absorption at 460 nm for **BiPy-QM**, in the absence (a) and in the presence of 2-mercaptoethanol [(b) 0.024, (c) 0.048 and (d) 0.12 M].

DNA without substrates (lane b) or with psoralen (**PS**, lane a) were used as controls. XL concentration dependence for compounds **5** and **11** are depicted in Fig. 2. The non-reacted plasmid presents three bands corresponding to the circular (C), linear (L) and open-circular (OC) forms. The diamine **5** induces detectable cross-links of all three plasmid forms (XL-C, XL-L, and XL-OC) at concentrations as low as 5 μM. The L-prolino derivative **11** is at least 16-fold less potent than **5** since no detectable XL was recorded up to an 80 μM concentration (Fig. 2, lanes i–n).

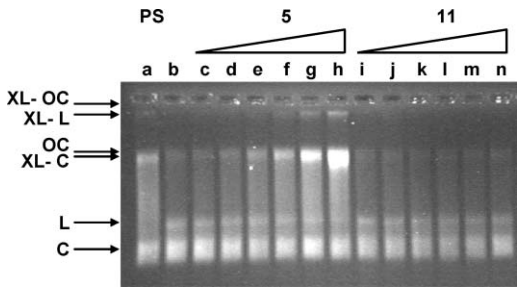

Fig. 2 DNA cross-linking (XL) activity: concentration dependent activity (2.5, 5, 10, 20, 40, 80 μM) of compounds **5** (lanes c–h) and **11** (lanes i–n).

In conclusion, we have shown the formation of a new bis-alkylating heterocyclic *o*-QM (**BiPy-QM**) which is a reactive electrophile toward amines and amino acids, but unlike the prototype *o*-QM, does not react with water. Moreover, the potential metal–ligand binding properties and the general photoreactivity of the bipyridyl precursors **5–12** may allow preconcentration of the precursor on the target and tunable reactivity by cationic coordination.[9]

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