Photochemical cleavage of duplex DNA by *N*-benzoyloxy-2-thiopyridone linked to 9-aminoacridine

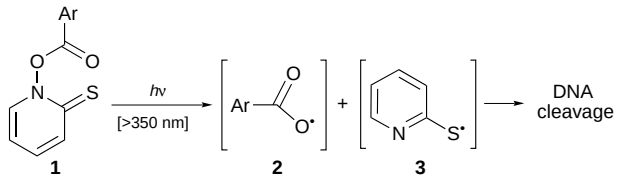
Emmanuel A. Theodorakis,* Xin Xiang and Petra Blom

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0358, USA

Upon illumination *N*-aroyloxy-2-thiopyridones induce non-specific single-strand nicks in duplex DNA at micromolar concentrations.

Synthetic reagents that cleave DNA are of great interest as tools in biochemical sciences. The design of such molecules constitutes a timely and challenging research topic and has led to the development of both sequence-specific DNA cleavers[1] and DNA footprinting reagents.[2]

Our studies are directed towards the development of conceptually new approaches to DNA cleavage, induced by photoactivation of *N*-aroyloxy-2-thiopyridone derivatives, such as **1** (Scheme 1).[3,4] To this end, we were guided by the operational advantages offered by the photoinducible DNA cleavage using the *N*-benzoyloxy-2-thiopyridone **4**.[5] These

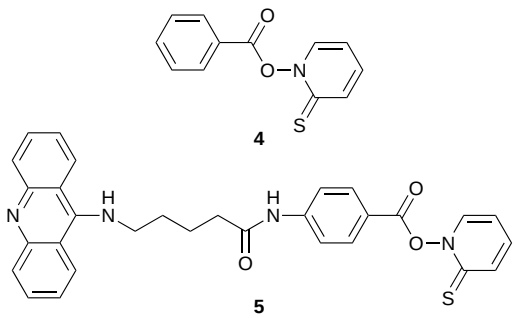


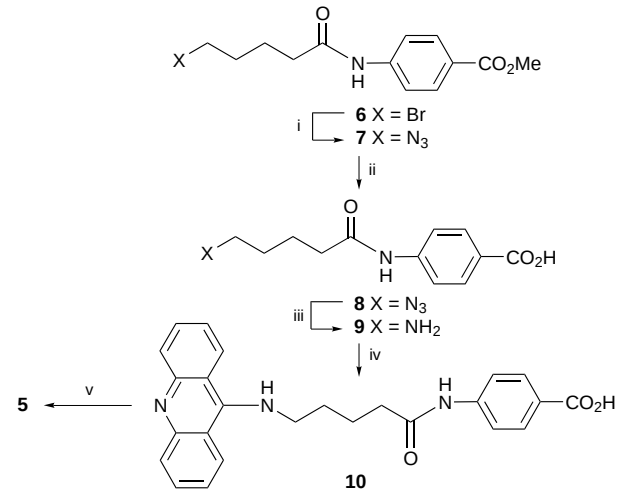
Scheme 1

molecules could be ideal nucleic acid cleavers since they possess the following characteristics: a purely organic structure, facile preparation, prolonged stability in the absence of light, and well documented radical chemistry.[3] The observed nucleic acid strand scission occurs upon a simple irradiation (λ > 350 nm), presumably *via* the generation of aroyloxyl radicals **2** and without the need of a metal or external oxidants.

Herein we describe our preliminary data on a novel family of photoactivated DNA-cleavers represented by **5**. The reagent design is based on linking a DNA photocleaving ligand to the 9-aminoacridine *via* a polymethylene chain. The aminoacridinyl group could assure high affinity for duplex DNA *via* intercalation,[6] while the thiopyridone entity could account for the DNA cleavage. Indeed, upon irradiation (λ > 350 nm), compound **5** produces single strand breaks in duplex DNA with no intrinsic sequence selectivity.

The synthesis of compound **5** commences with bromide **6**, itself readily available by the condensation of 5-bromovaleroyl chloride with methyl *p*-amino benzoate. Azidation of **6**,





Scheme 2 *Reagents and conditions*: i, NaN_3 (2.0 equiv.), 18-crown-6 (0.05 equiv.) DMF, 25 °C, 3 h, 91%; ii, KOH (2.0 equiv.), THF–H_2O (1 : 1), 25 °C, 24 h, 98%; iii, 10% Pd/C (0.1 equiv.), H_2, MeOH, 24 h, 89%; iv, 9-chloroacridine (1.0 equiv.), PhOH, 110 °C, 1 h, 90%; v, 2-mercaptopyridine *N*-oxide (1.0 equiv.), EDC (1.0 equiv.), DMF, 25 °C, 1 h, 43%

(Scheme 2), followed by saponification of the methyl ester moiety and reduction of the azido group gave rise to amino acid **9** in 79% overall yield, through intermediates **7** and **8**. Coupling of **9** with 9-chloroacridine followed by esterification with 2-mercaptopyridine *N*-oxide using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) afforded conjugate **5** in 38% overall yield.

We further compared the visible light photolysis of **5** and **4** in the presence of supercoiled circular φX174 DNA (Fig. 1). Control experiments indicated that both light and the thiopyridone derivatives **5** or **4** are necessary for the DNA cleavage (lanes 1–3). Complete inhibition of the cleavage by glutathione (lanes 11, 12) further supports the notion that free radicals are responsible for the DNA damage. Moreover, compound **5**

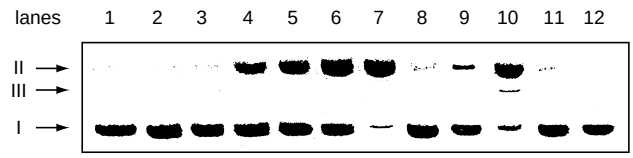


Fig. 1 Concentration-dependent photocleavage of φX174 DNA induced by **4** and **5**. The φX174 DNA (50 μM/base pair) was incubated for 1 h at 25 °C with 4 or 5 (30 mM Tris-HCl, 20 mM NaCl), then subjected to irradiation at 4 °C (lanes 1 and 4–12) with one lamp (GE 300 W) placed at approximately 20 cm from the samples. The results were analysed on 1% agarose gel (Tris-acetate buffer) stained with ethidium bromide. Lane 1: φX174DNA (control); lane 2: DNA and 60 μM of **5** (no *hν*); lane 3: DNA and 1 mM of **4** (no *hν*) lanes 4–7: DNA and **5** at concentrations of 5: 5, 10, 30 and 60 μM, respectively; lanes 8–10: DNA and **4** at concentrations of **4**: 30, 60 and 1.0 mM, respectively; lane 11: DNA, 60 mM of **5**, and 3.0 mM of glutathione; lane 12: DNA, 1.0 mM of **4** and 3.0 mM of glutathione.

cleaves DNA at concentrations as low as 5×10^{-6} M, while at 6×10^{-5} M complete conversion of the form I to form II is observed. In comparison, derivative **4** produces similar results at 1×10^{-3} M concentration. This substantial increase in efficiency of cleavage is attributed to the intercalating properties of 9-aminoacridine.[6]

An autoradiogram illustrating the results obtained upon irradiation of **5** in the presense of the 5′-^{32}P labelled 93-mer duplex DNA is shown in Fig. 2. Our data show that both **4** and **5** generate identical DNA ladders upon irradiation and the photocleavage is indisputably neither base- nor sequence-specific. Furthermore, comparison of lanes 8–12 indicate that **5** is more efficient than **4** in cleaving DNA and can accurately cut the duplex at concentrations as low as 5×10^{-6} M. This increase in efficiency of cleavage is attributed to the presence of the 9-aminoacridinyl group. We ruled out the possibility that the 9-aminoacridinyl group enhances cleavage by altering the conformation of the DNA, since less cleavage was detected when 9-aminoacridine was added as an external intercalator (compare lines 12,13). Interestingly, the cleavage is more enhanced upon subsequent treatment with piperidine at 90 °C for 30 min without any change in sequence or base specificity (lines 7,8). Based on the above data we believe that in the case of **5** the DNA cleavage is performed by the intercalation complex and is probably mediated by aroyloxyl radicals.

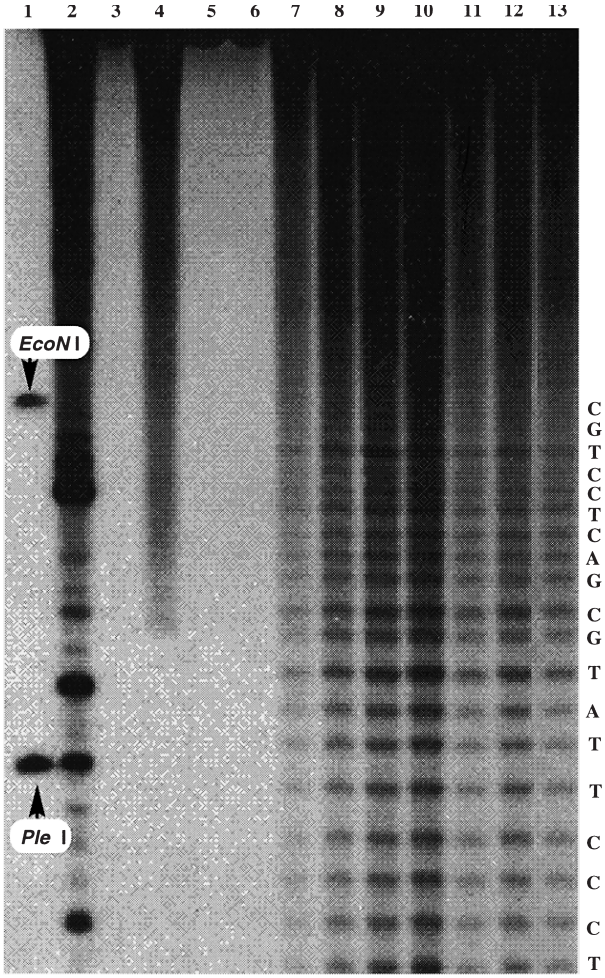


Fig. 2 Autoradiogram of a 10% denaturing polyacrylamide gel showing photocleavage of 5′-32P end-labeled Sal I / Sph I restriction fragment of pBR322 duplex DNA (93-mer), induced by **4** and **5**. DNA was incubated for 1 h at 25 °C with compounds **4** or **5** in buffered solution (30 mM Tris-HCl, 20 mM NaCl) and then irradiated (with one GE 300 W lamp placed 20 cm from the samples) for another 2 h at 4 °C (lanes 3,4 and 7–13). The resulted solution was treated with piperidine (1 M) at 90 °C for 30 min, followed by EtOH precipitation (lanes 8–13). Lane 1: DNA cut by PIe I (14 base pairs) and EcoN I (28 base pairs); lane 2: DNase footprinting; lane 3: DNA (control); lane 4: DNA irradiated and piperidine treated without **4** or **5**; lane 5: DNA and 200 μM of **4** (no *hν*); lane 6: DNA and 60 μM of **5** (no *hν*); lane 7: DNA and 5 μM of **5** (no piperidine treatment); lanes 8–10: DNA and **5** at concentrations of **5**: 10 and 20 μM respectively; lanes 11,12: DNA and **4** at concentrations of **4**: 40 and 60 μM respectively; lane 13: DNA, **4** (60 μM) and 9-aminoacridine (30 μM).

It is evident from the above studies that the *N*-aroyloxy-2-thiopyridones **1** can induce non-specific single strand nicks in duplex DNA in a light-dependent reaction. The efficiency and/or selectivity of the cleavage could be tuned by the proper choice of the DNA recognition element. In addition the light intensity that is responsible for the photoactivation could be tuned by structurally modifying the thiopyridone core. Thus, the *N*-benzoyloxy-2-thiopyridone moiety can be used for the design of new DNA photocleaving reagents with potential use as 'photofootprinting reagents' or as 'site-directed photonucleases'. Studies across these lines are now under investigation in our laboratories.

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Footnote

* E-mail: etheodor@ucsd.edu

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