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DNA cross-linking by a phototriggered pyrrolic progenitor developed from monocrotaline

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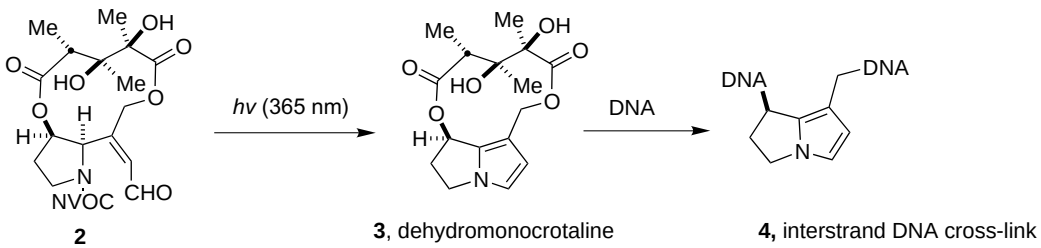
Abstract—A wide variety of pyrrolizidine alkaloids, such as monocrotaline, exert their cytotoxicity through the formation of DNA–DNA interstrand cross-links and DNA–protein cross-links. These pyrrolizidine alkaloids are oxidatively activated in vivo forming a highly reactive pyrrolic-type intermediate, which is responsible for the DNA cross-linking reaction. The oxidative pathway of activation leads to undesired toxicity. Based on a previously reported photochemically triggered progenitor of monocrotaline, we describe here the semi-synthesis and DNA cross-linking of a dicarbamate analogue of the original phototriggered progenitor.

Pyrrolizidine alkaloids (PAs) are potent hepatotoxins and carcinogens isolated from a wide variety of plants.[1,2] Natural members of this large family, such as monocrotaline **1**, exert their cytotoxicity through the formation of DNA–DNA and DNA–protein cross-links.[3] Pyrrolizidine alkaloids are activated in vivo by liver cytochrome P450 mixed-function oxidases.[4,5] Oxidation of the dihydropyrrole moiety results in the in situ generation of electrophilic pyrrolic intermediates that have been shown to cross-link DNA primarily at the $^{5'}$CpG$^{3'}$ site in the minor groove of DNA.[6] The pyrrolic pyrrolizidine alkaloids are potent DNA–DNA and DNA–protein cross-linking agents and are therefore of interest as potential antitumor and antibacterial agents.[3a,7–9] The clinical utility of such substances has, unfortunately, been obviated by the acute hepatotoxicity that these compounds display; a direct manifestation of the mechanism for their activation in vivo.

In 1999, Tepe and Williams reported the first photochemically triggered progenitor of a pyrrolizidine alkaloid (compound **2**, Scheme 1), that generated dehydromonocrotaline upon photochemical activation.[8] In this system, photochemical cleavage of the NVOC group on the tetrahydropyrrole ring nitrogen atom, lead to the formation of dehydromonocrotaline, which induced DNA–DNA cross-link formation in linear pBR322 DNA. However, this original phototriggered compound (**2**) displayed poor solubility in water, requiring the DNA interstrand cross-linking experiments to be conducted in 10% DMSO/water solutions. In an attempt to improve the water solubility of such substances, we have endeavored to remove the macrocyclic diester backbone and replace this substituent with primary carbamates. The carbamate groups were envisioned to impart good water-solubility as well as providing for good leaving groups in the covalent DNA interstrand cross-linking reactions, as illustrated by the antitumor agent mitomycin C and the FR-class of antitumor antibiotics.[10,11]

The synthesis of the photoactivated dicarbamate derivative **8** was accomplished from commercially available monocrotaline (Scheme 2).[12] Monocrotaline was



2 **3**, dehydromonocrotaline **4,** interstrand DNA cross-link

Scheme 1.

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condensed with 9-fluorenylmethyl chloroformate in the presence of KI in acetonitrile to afford the allylic iodide **5**.[8,9] The allylic iodide was oxidized to the corresponding aldehyde under Kornblum conditions (DMSO and $AgBF_4$). The aldehyde was subsequently protected as the ethylene glycol acetal. The 9-fluorenylmethyl protecting group was cleaved with piperidine in THF (1:1 mixture) and the free amine was acylated with 6-nitroveratryl chloroformate, to give the NVOC-derivative **6**. The diester backbone was cleaved with KCN in 95% ethanol to give the diol **7**. The carbamate moieties were installed using trichloroacetylisocyanate, followed by removal of the trichloroacetyl residues over neutral alumina.[13] Finally, the acetal was deprotected with 1% aqueous HCl in acetone to give the masked pyrrolizidine derivative **8**.

The DNA–DNA cross-linking ability of dicarbamate derivative **8** was investigated using linear pBR322 DNA by denaturing alkaline agarose gel electrophoresis according to the protocol reported by Cech.[14] Compound **8** (various concentrations of a 10 mM stock solution) and 0.5 μg DNA (EcoR1 linearized pBR322) in a DMSO–water solvent mixture (10 μL, 0.001% $DMSO/H_2O$) was exposed to 365 nm light at 23°C for 1 h, followed by incubation at 37°C for 9 h. The crude reaction mixture was loaded onto a denaturing alkaline agarose gel and provided the results shown in Fig. 1.

Lambda DNA-BstE II was employed as a molecular weight standard (lane 1). Control reactions were performed with NVOC-protected piperidine (10 μM, 1 μM, 100 nM) to assure that the photocleaved side product, 6-nitroveratryl aldehyde was not responsible for the observed cross-linked DNA. An authentic specimen of dehydromonocrotaline, **3** (10 μM) was also used as a DNA cross-link standard. As illustrated in Fig. 1, incubation of compound **8** with the DNA duplex in the dark leads to no detectable cross-linked product (lanes 9, 11, 13). Incubation of compound **8** with linear pBR322 at 10 and 1 μM with exposure to 365 nm light for 1 hour results in DNA cross-link formation (lanes 10 and 12). As shown, only the reactions depicted in lanes 10 and 12 produced the interstrand DNA–DNA cross-link product similar to that observed from treatment with dehydromonocrotaline (lane 4).

These studies suggest that cleavage of the diester backbone does not adversely affect the cross-linking potential of the pyrrolic substrate. In addition, this work further demonstrates the viability that more structurally diverse masked DNA-reactive pyrrolizidine progenitors

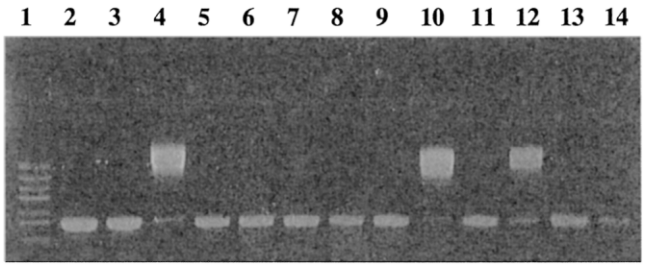
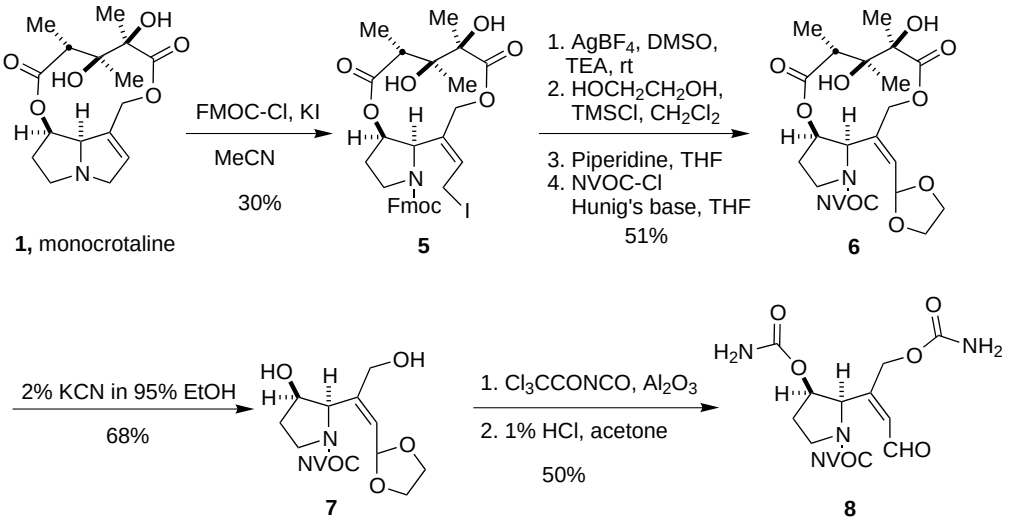


Figure 1. All dark (control) reactions were incubated at 37°C for 10 hours. The reactions exposed to UV radiation (1 hour) were incubated an additional 9 hours at 37°C. Lane (1) 0.5 μg lambda DNA-BstE II digest (molecular weight standard); lane (2) 0.5 μg pBR322 (control); lane (3) 0.5 μg pBR322+*hv*, 1 hour (light control); lane (4) 0.5 μg pBR322+10 μM dehydromonocrotaline; lane (5) 0.5 μg pBR322+10 μM NVOC-piperidine (dark control); lane (6) 0.5 μg pBR322+10 μM NVOC-piperidine+*hv*, 1 hour (light control); lane (7) 0.5 μg pBR322+1 μM NVOC-piperidine+*hv*, 1 hour (light control); lane (8) 0.5 μg pBR322+100 nM NVOC-piperidine+*hv*, 1 hour (light control); lane (9) 0.5 μg pBR322+10 μM compound **8** (dark control); lane (10) 0.5 μg pBR322+10 μM compound **8**+*hv*, 1 hour; lane (11) 0.5 μg pBR322+1 μM compound **8** (dark control); lane (12) 0.5 μg pBR322 +1 μM compound **8**+*hv*, 1 hour; lane (13) 0.5 μg pBR322+100 nM compound **8** (dark control); lane (14) 0.5 μg pBR322+100 nM compound **8**+*hv*, 1 hour.



Scheme 2.

should be capable of being designed and synthesized for more selective oligonucleotide modifications. Such agents hold promise as useful tools to gain insight into the mechanism of DNA–DNA and DNA–protein cross-linking. It has been demonstrated that dehydromonocrotaline undergoes rapid polymerization that generates a structure capable of cross-linking several fragments of DNA and that the cross-linked adducts are structurally complex.[16] As such, an attempt to ascertain the sequence selectivity of interstrand DNA cross-linking for compound **8** was not undertaken. It might be further noted that Hincks et al. concluded that pyrrolizidine alkaloids require both a macrocyclic necic ester and an α,β-unsaturated ester function for potent cross-linking.[17,18] Our preliminary results with compound **8** that lacks both such functionalities, suggests that additional structure–activity data needs to be acquired and considered. With the increasing demand for new and less cytotoxic antitumor agents, and the recent success of the clinically significant photopheresis technologies, these agents can provide a conceptual framework for the development of new pyrrolizidine-type pro-drugs.[15] Studies towards these ends are under investigation in these laboratories and will be reported on in due course.

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