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# DNA Cross-Linking by a Phototriggered Dehydromonocrotaline Progenitor

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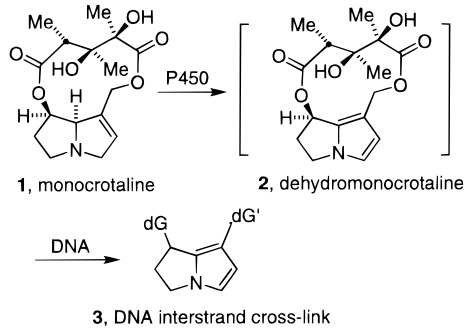
**Abstract:** A wide variety of pyrrolizidine alkaloids, such as monocrotaline, and the clinically significant mitomycins and the related FR-900482, FK 973, and FR-66979 exert their cytotoxicity through the formation of DNA−DNA interstrand cross-links and DNA−protein cross-links. These naturally occurring antitumor antibiotics are generally either oxidatively or reductively activated in vivo forming a highly reactive pyrrolic-type intermediate, which is responsible for the ultimate DNA cross-linking reaction. These oxidative and reductive pathways, however, often lead to a variety of undesirable toxic events. With the increasing demand for new and less cytotoxic antitumor agents and the recent success of clinically significant photopheresis technologies, we describe here the semisynthesis and DNA cross-linking of the first photochemically triggered progenitor of dehydromonocrotaline.

## Introduction

Pyrrolizidine alkaloids (PAs), such as monocrotaline, **1**, are potent hepatotoxins and carcinogens isolated from a wide variety of plants.[1,2] The mode of action of these toxic agents is through a cytochrome P450 mediated oxidation forming the highly reactive dehydropyrrolizidine **2** (dehydromonocrotaline, Scheme 1) which subsequently mediates the interstrand cross-linking of DNA, forming the bis-alkylated adduct **3**.[3,4] This oxidation results in the electrophilic activation of the C-7 and C-9 positions, via conjugation with the pyrrole nitrogen lone pair, which are prone to nucleophilic attack by DNA. The DNA cross-linking specificity of this reaction has been elucidated and demonstrated to occur at $^{5'}$CpG$^{3'}$ sites via the exocyclic amine of dG residues in the minor groove.[5] The pyrrolic PAs are potent

**Scheme 1**




(1) Mattocks, A. R. *Chemistry and Toxicology of Pyrrolizidine Alkaloids*; Academic Press: London, UK; 1986.
(2) Smith, L. W.; Culvenor, C. C. J. *J. Nat. Prod.* **1981**, *44*, 129.
(3) Niwa, H.; Ogawa, T.; Yamada, K. *Tetrahedron Let.* **1991**, *32*, 927.
(4) For excellent general reviews pertaining to the biological relevance of DNA interstrand cross-linking, see: (a) Kohn, K. W. In *Topics in Molecular and Structural Biology 3. Molecular Aspects of Anti-cancer Drug Action*; Neidle, S., Waring, M., Eds.; Verlag Chemie GmbH: D-6940, Weinheim, 1994; p 315. (b) Lawley, P. D. *BioEssays* **1995**, *17*, 561. (c) Paustenbach, D. J.; Finley, B. L.; Kacew, S. *Proc. Soc. Exp. Biol. Med.* **1996**, *211*, 211. (d) Gniazdowski, M.; Cera, C. *Chem. Rev.* **1996**, *96*, 619. (e) Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723.
(5) (a) Weidner, M. F.; Sigurdsson, S. T.; Hopkins, P. B. *Biochemistry* **1990**, *29*, 9225. (b) Woo, J.; Sigurdsson, S. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 3407.

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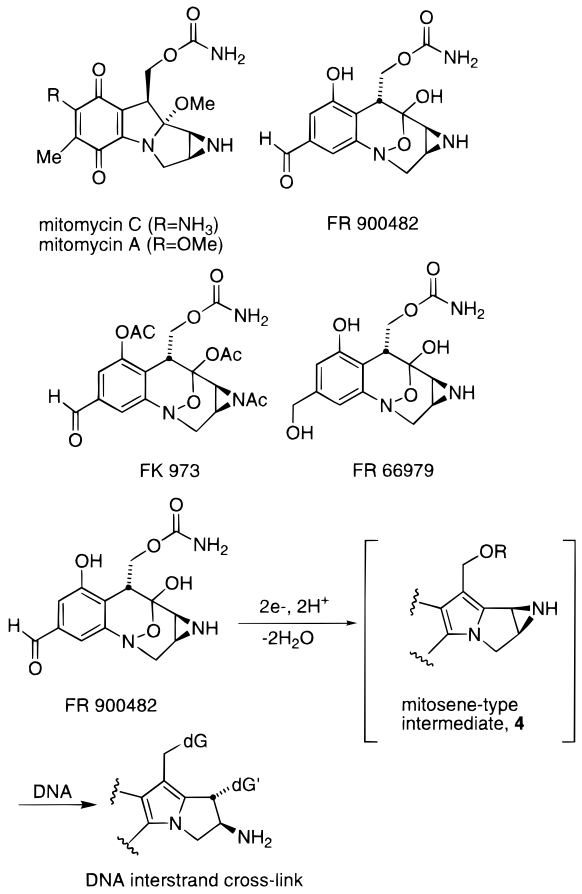



**Figure 1.**

DNA−DNA and DNA−protein cross-linking agents and are therefore of interest as potential antitumor and antibacterial agents.[6,7] The clinical utility of such substances has, unfortunately, been obviated by the acute hepatotoxicity that these compounds display.

Similar to the pyrrolizidine alkaloids, the mitomycins and the related FR-900482, FK973, and FR-66979 also exert their cytotoxicity through the formation of DNA−DNA interstrand cross-links and DNA−protein cross-links.[4e,8] However, the irreversible formation of these DNA cross-links in these systems, are the result of an in vivo reductive activation, forming the highly reactive mitosene intermediate (**4**, Figure 1), which is structurally similar to the aforementioned pyrrolic PAs.

Most families of naturally occurring antitumor antibiotics are activated in vivo by either reduction or oxidation.[4] Reductive activation pathways often lead to adventitious production of superoxide and related oxygen radical species which manifest in a variety of undesirable toxic species and events. Oxidative activation, which requires liver cytochrome P450 enzymes, is typically accompanied by undesirable liver toxicity where the reactive drug molecule is initially formed.[4] As part of a program aimed at diversifying the repertoire of chemical reactions that can be applied to selectively activating antitumor anitbiotics,[9,10] we describe here the semisynthesis and DNA cross-linking reactivity of the first photochemically triggered progenitor of dehydromonocrotaline. The masked dehydromonocrotaline described herein, provides the first example of a new class of pyrrolizidine alkaloids that can be activated photochemically.

## Results and Discussion

The synthesis of the photoactivated dehydromonocrotaline derivative **10** is shown in Scheme 2. Commercially available[11] monocrotaline was condensed with trichloroethylchloroformate (TrocCl) in the presence of KI in acetonitrile, furnishing the ring-cleaved product **5**.[12] Substitution at the tertiary allylic bridgehead position was not observed, presumably due to steric interference. A more direct approach using 4,5-dimethoxy-2-nitrobenzylchloroformate (6-nitroveratrylchloroformate or NVOC-Cl) proved to be unsuccessful under a wide variety of conditions and was consequently abandoned. Thus, the allylic iodide **5** was oxidized with DMSO and $AgBF_4$ to afford the unsaturated aldehyde **6** in excellent yield. Protection of the aldehyde **6** as the corresponding ethylene glycol acetal (**7**) followed by removal of the 2,2,2-trichloroethyl carbamate with zinc in hot ethanol (**8**) and acylation with 6-nitroveratryl chloroformate provided the corresponding NVOC derivative (**9**).

Finally, deprotection of the acetal with 1% aqueous HCl in acetone afforded the masked dehydromonocrotaline derivative **10**. As a control experiment the "pro-dehydromonocrotaline" (**10**) was dissolved in a DMSO-$d_6$−$D_2O$ mixture (pH = 7.0) and was exposed to 365-nm UV irradiation. The reaction was monitored by $^1$H NMR, and after 5 h, analysis of the reaction mixture indicated that compound **10** was cleanly converted to dehydromonocrotaline (**2**). The cleaved aldehyde intermediate derived from **10** was not observed by $^1$H NMR analysis during the reaction. The dehydromonocrotaline (**2**) produced in this experiment was isolated (purified by PTLC silica gel), and the spectral data for this substance was identical to an authentic sample of dehydromonocrotaline prepared by oxidation of monocrotaline as previously described.[13]

The DNA−DNA cross-linking ability of this pro-dehydromonocrotaline (**10**) was investigated using linear plasmid

(6) Kim, H. Y.; Stermitz, F. R.; Coulombe, R. A., Jr. *Carcinogenesis* **1995**, *16*, 2691.

(7) Hurley, L. H. *J. Med. Chem.* **1989**, *32*, 2027.

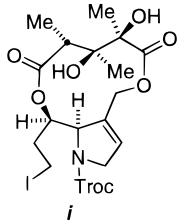
(8) (a) Williams, R. M.; Rajski, S. R.; Rollins, S. B. *Chem. Biol.* **1997**, *4*, 127. (b) Williams, R. M.; Rajski, S. R. *J. Am. Chem. Soc.* **1998**, *120*, 2192.

(9) Rollins, S. B.; Williams, R. M. *Tetrahedron Lett.* **1997**, *38*, 4033.

(10) For some relevant examples of photochemically activated DNA-reactive agents, see: (a) Nicolaou, K. C.; Dai, W. M.; Wendeborn, S. V.; Smith, A. L.; Torisawa, Y.; Maligres, P.; Hwang, C. K. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1032. (b) Wender, P. A.; Zercher, C. K.; Beckham, S.; Haubold, E.-M. *J. Org. Chem.* **1993**, *58*, 5867. (c) Wender, P. A.; Beckham, S.; O'Leary, J. G. *Synthesis* **1994**, 1278. (d) Nakatani, K.; Isoe, S.; Maekawa, S.; Saito, I. *Tetrahedron Lett.* **1994**, *35*, 605. (e) Funk, R. L.; Young, E. R. R.; Williams, R. M.; Flanagan, M. A.; Cecil, T. R. *J. Am. Chem. Soc.* **1996**, *118*, 3291−3292. DNA photocleavage by dynemicin A involves an initial photoreduction step, see: (f) Shiraki, T.; Sugiura, Y. *Biochemistry* **1990**, *29*, 9795. DNA photocleavage by esperamicin and neocarzinostatin chromophore has also been reported, see: (g) Sugiura, Y.; Kuwahara, J.; Vesawa, Y. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 903. The latter undergoes a Norrisch type II cleavage to produce a fulvene derivative which may be responsible for the DNA cleavage, see: (h) Hirama, M.; Nehira, T.; Fujiwara, K.; Gomibuche, T. *Tetrahedron Lett.* **1993**, *34*, 5753.

(11) Monocrotaline used in this study was purchased from Aldrich Chemical Co.

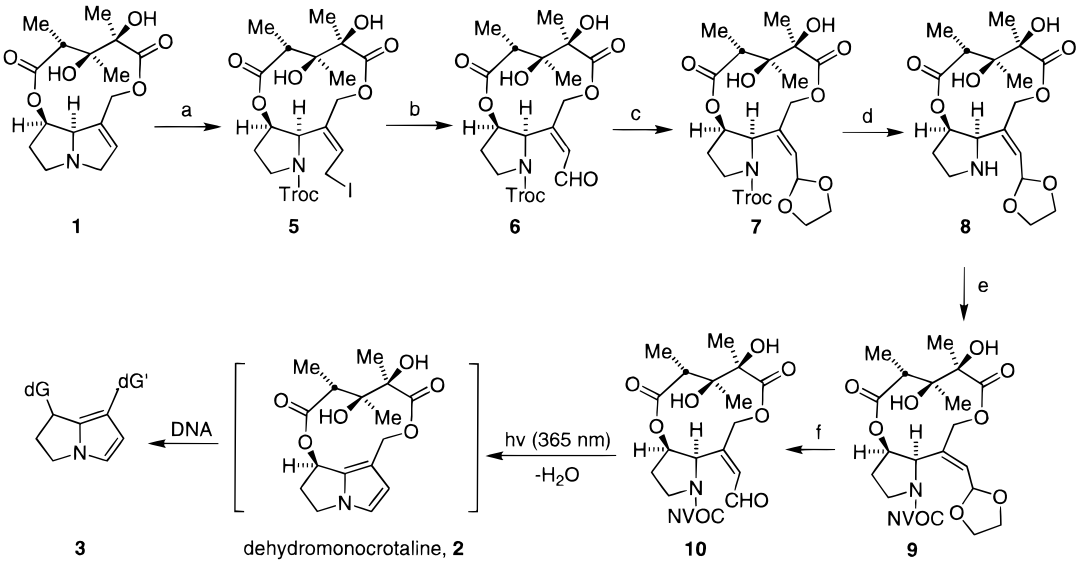
(12) Cooley, J. H.; Evain, E. J. *Synthesis* **1989**, 1. Compound **5** was obtained as a 1:2 mixture with the alternate regioisomer *i*:




*i*

(13) An authentic specimen of dehydromonocrotalin (**2**) was prepared by oxidation of commercially available monocrotalin (**1**) with *o*-chloranil, as previously described in ref 6.

**Scheme 2**[a]




[a] Reagents and conditions: (a) TrocCl, KI, $CH_3CN$, rt, 10 h (30%); (b) $AgBF_4$, DMSO, TEA, rt, 20 min (95%); (c) TMSCl, $HOCH_2CH_2OH$, $CH_2Cl_2$, rt, 5 h (95%); (d) $Zn^0$, EtOH, reflux, 8 h, (63%); (e) NVOCCl, Hunig's base, $CH_2Cl_2$, rt, 30 min (88%); (f) 1% aqueous HCl, acetone, rt, 5 h (75%).

DNA by denaturing alkaline agarose gel electrophoresis according to Cech.[14] The duplex DNA substrate employed in this study was pBR322 plasmid DNA which was linearized by restriction endonuclease digestion with *Eco*R1. The amount of linearized pBR322 was quantitated by UV analysis at 260 nm as described by P. N. Borer.[15] Due to the poor water solubility of compound **10**, DNA cross-linking experiments were performed in a dilute DMSO−$H_2O$ solvent mixture (10% DMSO). Compound **10** (various concentrations of a 10 mM stock solution made from 2.6 mg of **10** dissolved in 460 mL of DMSO) and 0.5 mg of DNA (*Eco*R1 linearized pBR322) in a DMSO/$H_2O$ solution (10 mL, 10% DMSO/$H_2O$ final volume) was exposed to 365-nm irradiation at 23 °C for 2 h (longer UV exposure times did not result in an increased amount of cross-linking), followed by 3 h incubation at 37 °C. The crude reaction mixture was loaded onto a denaturing 1.2% alkaline agarose gel[14] and provided the results shown in Figure 2.

Lambda Hind III was employed as a molecular weight standard (lane 1). Control reactions were performed with monocrotaline (10 $\mu$M, **1**) in the dark (lane 4) and with an authentic sample of dehydromonocrotaline (10 $\mu$M, **2**)[13] irradiated for 2 h at 365 nm (lane 6) as well as in the dark (lane 5).

As illustrated in Figure 2, incubation of compound **10** with the DNA duplex in the dark leads to no detectable cross-linked product (lane 7 and 9). Lanes 7 and 9 contain mixtures of 0.5 mg linearized pBR322 with compound **10** at 10 and 1.0 mM concentrations, respectively and were not exposed to UV irradiation. Lanes 8 and 10 contain mixtures of 0.5 mg linearized pBR322 with compound **10** at 10 mM and 1.0 mM concentrations, respectively and were exposed to UV irradiation for 2 h. As shown, only the reactions depicted in lanes 5, 6, 8, and 10 produced the interstrand DNA−DNA cross-link product produced by dehydromonocrotaline (lane 5 and 6).

These preliminary studies demonstrate the viability of masked DNA-reactive pyrrolizidine alkaloid progenitors that are capable of photochemical activation. Such agents hold promise as tools to further gain insight into the mechanism of DNA−DNA and

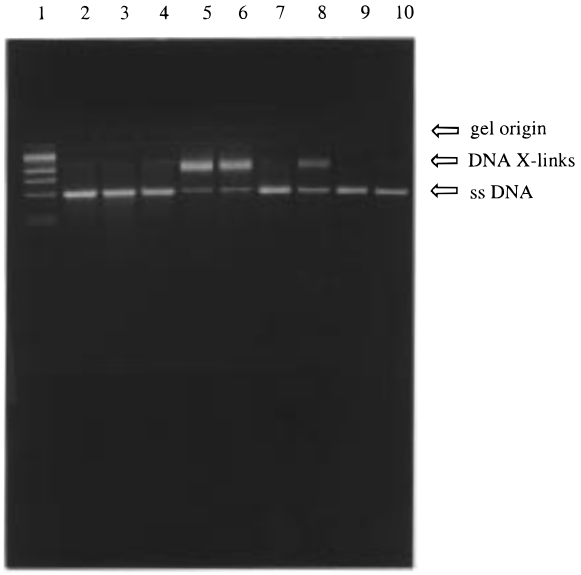



**Figure 2.** All dark (control) reactions were incubated at 37 °C for 5 h. The reactions exposed to UV irradiation (2 h) were incubated an additional 3 h after UV irradiation. Lane 1 0.5 $\mu$g lambda Hind III (molecular weight standard); lane 2 0.5 $\mu$g pBR322 (control); lane 3 0.5 $\mu$g pBR322 + h$\nu$, 2 h (light control); lane 4 0.5 $\mu$g pBR322 + 10 $\mu$M monocrotaline (**1**) (dark control); lane 5 0.5 $\mu$g pBR322 + 10 $\mu$M dehydromonocrotaline (dark reaction control); lane 6 0.5 $\mu$g pBR322 + 10 $\mu$M dehydromonocrotaline + h$\nu$, 2 h, (light control); lane 7 0.5 $\mu$g pBR322 + 10 $\mu$M compound **10** (dark control); lane 8 0.5 $\mu$g pBR322 + 10 $\mu$M compound **10** + h$\nu$, 2 h; lane 9 0.5 $\mu$g pBR322 + 1.0 $\mu$M compound **10** (dark control); lane 10 0.5 $\mu$g pBR322 + 1.0 $\mu$M compound **10** + h$\nu$, 2 h.

DNA−protein cross-linking. In addition, these agents may also provide a conceptual framework upon which the design and synthesis of a wide variety of pyrrolic PA prodrugs with potential clinical applications, such as in clinically significant photopheresis technologies,[16] may be examined. It is important to note that the currently used photodynamic therapies based on psoralen derivatives target AT-rich sequences in DNA, whereas the system described herein targets GC-rich regions

(14) Cech T. R. *Biochemistry* **1981**, *20*, 1431.
(15) Borer, P. N. *Handbook of Biochemistry and Molecular Biology*, CRC Press: 1975.

of duplex DNA; this difference in chemical selectivity coupled with selective chemical activation is being examined as a cellular targeting vehicle. Studies toward these ends are under investigation in these laboratories and will be reported on in due course.

## Experimental Section

$^1$H NMR and $^{13}$C spectra were obtained using a Varian 300 at 300 MHz. NMR spectra were recorded at room temperature unless otherwise noted. All compounds were further analyzed by HMQC and/or APT spectra obtained using a Varian 400 MHz spectrometer. All chemical shifts are reported in ppm. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F-254 plated and were visualized with a phosphomolybdic acid/ethanol solution. All solvents were distilled prior to use.

**Dehydromonocrotaline 2.** Chloranil (0.05 mmol, 12 mg) was dissolved in chloroform (2.0 mL) in a separatory funnel. Monocrotaline (Aldrich Chemical Co., 0.031 mmol, 10 mg) in 2.0 mL of chloroform was added, and the solution was gently stirred for 2 min. A mixture of $NaBH_4$ (0.08 mmol, 3 mg) and an excess of NaOH (700 mg) in 2.0 mL of $H_2O$ were added. A green precipitate formed, and the organic layer was drained through a filter of decolorizing charcoal into an Erlenmeyer flask. The organic layer was dried over $Na_2SO_4$, filtered, and concentrated to yield 10 mg of pure dehydromonocrotaline (100%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.62 (d, $J$ = 2.4 Hz, 1H), 6.32 (d, $J$ = 3.0 Hz 1H), 6.13 (dd, $J$ = 3.0 Hz, 7.8 Hz, 1H), 5.72 (d, $J$ = 12 Hz, 1H), 4.61 (d, $J$ = 12 Hz, 1H), 4.20 M, 1H), 4.06 (m, 1H), 3.03 (m, 3H), 2.56 (m, 1H), 2.45 (m, 1H), 1.52 (s, 3H), 1.49 (s, 3H), 1.37 (d, $J$ = 6.9 Hz, 3H).

**Ring Opening of Monocrotaline. Compound 5.** KI (0.675, 112 mg) was added to a suspension of monocrotaline (0.135 mmol, 44 mg) in freshly distilled acetonitrile (2.0 mL). The suspension was stirred for 10 min at room temperature after which 2,2,2-trichloroethylchloroformate (0.162 mmol, 22 $\mu$L) was added. The suspension slowly turned yellow and was allowed to stir overnight. The mixture was diluted with ether (10 mL) and was extracted with saturated $NaHCO_{3(aq)}$ (10 mL) and brine (10 mL). The ether layer was dried over $Na_2SO_4$ and filtered, and the product was purified by column chromatography on silica gel (eluted with ether) ($R_f$ = 0.4 for compound **5**) to yield 26 mg of compound **5**, (30%) as a white foam, and 58 mg of compound **i**, (68%) as a white solid. The NMR spectrum of product **5** revealed that this substance was as a mixture of rotamers at room temperature on the NMR time scale. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.14 (t, $J$ = 9.3 Hz, 1H), 5.68 (bm, 1H), 5.43 (dd, $J$ = 11.7 Hz, 12 Hz, 1H), 5.0−4.5 (m, 3H), 4.17−3.95(m, 6H), 3.91 (bs, 1H, *OH*), 3.66 (m, 1H), 2.93 (q, $J$ = 7.2 Hz, 14.1 Hz, 1H), 2.75 (bs, 1H, *OH*), 2.07 (m, 1H), 1.98 (m, 1H), 1.48 (s, 3H), 1.32 (s, 3H), 1.17 (d, $J$ = 6.9 Hz, 3H). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta$ 175, 174, 153, 136, 133, 95, 79, 76, 75, 74, 63, 61, 46, 43, 31, 21, 19, 14, −3. IR (NaCl, neat) 3485, 3017, 2987, 1721, 1273, 1161, 1111 cm$^{-1}$; exact mass calcd for $C_{19}H_{26}NO_8Cl_3I$ $m/z$ 627.976877, found $m/z$ 627.975475.

**Oxidation of the Allylic Iodide. Compound 6.** $AgBF_4$ (0.234 mmol, 46 mg) was added to a solution of the allylic iodide **5** (0.213 mmol, 134 mg) in 1.0 mL of dry DMSO. After 10 min triethylamine (1.065 mmol, 108 mL) was added, and the suspension was stirred at room temperature for 20 min. The suspension was diluted with ether (10 mL) and extracted with saturated $NH_4Cl_{(aq)}$ (10 mL), $NaHCO_3$ (10 mL), and brine (10 mL). The ether layer was dried over $Na_2SO_4$, filtered, and purified by column chromatography on silica gel (eluted with 1:1 ether/ethyl acetate) ($R_f$ = 0.42) to yield 105 mg (95%) of a white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.99 (d, $J$ = 3.0 Hz, 1H), 6.50 (d, $J$ = 5.1 Hz, 1H), 5.77 (m, 1H), 5.64 (m, 1H), 5.54 (m, 1H), 4.80 (m, 2H), 4.26 (dd, $J$ = 11.7 Hz, 17.4 Hz, 1H), 4.02 (m, 1H), 3.86 (bs, 1H, *OH*), 3.72 (m, 1H), 2.89 (q, $J$ = 7.2 Hz, 14.1 Hz, 1H), 2.76 (bs, 1H, *OH*), 1.47 (s, 3H), 1.23 (s, 3H), 1.17 (d, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (300 MHz, $CDCl_3$) $\delta$ 189, 175, 173, 153, 149, 133, 95, 79, 76, 76, 75, 63, 62, 47, 43, 32, 21, 19, 14. IR (NaCl, neat) 3482, 3012, 2959, 2916, 1726 (broad signal), 1678, 1411, 1171, 1109 cm$^{-1}$; exact mass calcd for $C_{19}H_{25}NO_9Cl_3$ $m/z$ 516.059490, found $m/z$ 516.058233.

**Protection of the Unsaturated Aldehyde. Compound 7.** An excess ethylene glycol (0.34 mmol, 19 mL) was added to a solution of the aldehyde **6** (0.0677 mmol, 35 mg) in 1.0 mL of dry $CH_2Cl_2$. After 5 min TMSCl (0.20 mmol, 26 $\mu$L) was added, and the solution was stirred for 5 h at room temperature. The mixture was diluted with ether (10 mL) and was extracted with saturated $NaHCO_{3(aq)}$ (10 mL) and brine (10 mL). The ether layer was dried over $Na_2SO_4$ and filtered, and the product was purified by column chromatography on silical gel (eluted with 1:1 ether/ ethyl acetate) ($R_f$ = 0.42) to yield 36 mg of **7** as a colorless oil (95%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.99 (d, $J$ = 3.0 Hz, Hz, 1H), 5.66 (m, 1H), 5.31 (d, $J$ = 8.7 Hz, 1H), 4.98 (m, 1H), 4.87−4.50 (AB, $J$ = 9 Hz, 2H), 4.10 (m, 1H), 4.00−3.88 (m, 3H), 3.86(m, 2H), 3.62 (m, 1H), 2.85 (q, $J$ = 5.1 Hz, 10.5 Hz, 1H), 2.72 (bs, 1H, *OH*), 2.05 (m, 1H), 1.88 (m, 1H), 1.45 (s, 3H), 1.20 (s, 3H), 1.12 (d, $J$ = 5.4 Hz, 3H); exact mass calcd for $C_{21}H_{29}NO_{10}Cl_3$ $m/z$ 560.085705, found $m/z$ 560.082972.

**Deprotection of the 2,2,2-Trichloroethylene Carbamate. Compound 8.** A suspension of the acetal **7** (0.0642 mmol, 36 mg) in dry ethanol (2.0 mL) containing an excess (0.642 mmol, 42 mg) of zinc was refluxed for 8 h. The suspension was cooled to room temperature and was filtered through a Celite plug. The solvent was evaporated off under reduced pressure, and the product was purified by column chromatography on silica gel (eluted with 5% diisopropylamine in ethanol) ($R_f$ = 0.37) to yield 16 mg of **8** (63%) as a white solid; mp 55−57 °C (recryst ether). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 5.87 (d, $J$ = 3.9 Hz, 1H), 5.57 (m, 2H), 5.33 (t, $J$ = 4.2 Hz, 1H), 4.13 (d, $J$ = 8.4 Hz, 1H), 3.99−3.84 (m, 6H), 3.36 (m, 1H), 2.84 (m, 1H), 2.83 (q, $J$ = 5.7 Hz,11.2 Hz, 1H), 2.13 (m, 1H), 1.75 (m, 1H), 1.44 (s, 3H), 1.25 (s, 3H), 1.09 (d, $J$ = 5.7 Hz, 3H). $^{13}$C NMR and APT (400 MHz, $CDCl_3$) $\delta$ 175.05 (C), 173.50 (C), 136.41 (C), 136.42 (CH), 98.95 (CH), 78.96 (C), 77.33 (CH), 75.63 (C), 65.02 (2 × $CH_3$), 64.88 ($CH_2$), 62.33 (CH), 44.45 ($CH_2$), 43.63 (CH), 33.75 ($CH_2$), 21.81 ($CH_3$), 18.40 ($CH_3$), 13.87 ($CH_3$). IR (NaCl, neat) 3421, 2983, 1732, 1115 cm$^{-1}$; exact mass calcd for $C_{18}H_{28}NO_8$ $m/z$ 386.181492, found $m/z$ 386.181998.

**NVOC Protection of the Pyrrole. Compound 9.** Hunig's base (0.065 mmol, 11 $\mu$L) was added to a solution of the pyrrole **8** (0.013 mmol, 5 mg) in dry $CH_2Cl_2$ (2.0 mL). The solution was cooled to 0 °C, and NVOCCl (0.026 mmol, 7 mg) was added. The solution was slowly warmed to room temperature. The solution was stirred for 30 min, and the mixture was diluted with ether (10 mL) and was extracted with saturated $NaHCO_{3(aq)}$ (10 mL) and brine (10 mL). The ether layer was dried over $Na_2SO_4$ and filtered, and the product was purified by column chromatography on silica gel (eluted with 1:1 ether/ethyl acetate) ($R_f$ = 0.27) to yield 7 mg of **9** (88%) as a light yellow solid; mp 94−96 °C (recryst ether). $^1$H NMR (400 MHz, DMSO-$d_6$, 75 °C) $\delta$ 7.64 (s, 1H), 7.10 (s, 1H), 5.73 (d, $J$ = 6.0 Hz, 1H), 5.51 (d, $J$ = 6.0 Hz, 1H), 5.33−5.29 (m, 3H), 5.09 (s, 1H, *OH*), 4.92−4.88 (m, 2H), 4.06−3.98 (m, 1H), 3.89 (s, 3H), 3.84 (s, 3H), 3.82−3.68 (m, 5H), 3.43 (m, 1H), 3.05 (s, 1H, *OH*), 2.97 (q, $J$ = 5.4, 10.5 Hz, 1H), 2.05 (m, 1H), 1.75 (m, 1H), 1.30 (s, 3H), 1.08 (s, 3H), 0.99 (d, $J$ = 5.4 Hz, 3H). $^{13}$C NMR and APT (400 MHz, DMSO-$d_6$, 75 °C) d 173.70 (C), 173.58 (C), 170.23 (C), 153.99 (C), 153.57 (C), 148.37 (C), 140.15 (C), 136.57 (C), 134.64 (CH), 111.85 (CH), 108.88 (CH), 98.33 (CH), 79.09 (C), 76.02 (CH), 74.88 (C), 64.38 (2 × $CH_2$), 63.41 ($CH_2$), 61.42 (CH), 59.74 ($CH_2$), 56.52 ($CH_2$), 56.47 ($CH_3$), 45.35 ($CH_2$), 43.10 (CH), 30.83 ($CH_2$), 21.67 ($CH_3$), 19.44 ($CH_3$), 13.88 ($CH_3$). IR (NaCl, neat) 3484, 2987, 1731, 1706, 1518, 1324, 1274, 1111 cm$^{-1}$; exact mass calcd for $C_{28}H_{37}N_2O_{14}$ $m/z$ 625.224479, found $m/z$ 625.223328.

**Deprotection of the Acetal. Compound 10.** An aqueous solution of 1% HCl (0.5 mL) was added to a solution of acetal **9** (0.010 mmol, 6 mg) in 2.0 mL of acetone and was stirred at room temperature for 5 h. The clear solution was diluted with ether (10 mL) and was extracted with saturated $NaHCO_{3(aq)}$ (10 mL) and brine (10 mL). The ether layer was dried over $Na_2SO_4$ and filtered, and the product was purified by column chromatography on silica gel (eluted with 1:1 ether/ethyl acetate) ($R_f$ = 0.42) to yield 4 mg of **10** (75%) as a light yellow solid; mp 68−70 °C (recryst ether). $^1$H NMR (400 MHz, DMSO-$d_6$, 75 °C)

(16) (a) Dougherty, T. J.; Gomer, C. J.; Henderson, B. w.; Jori, G.; Kessel, D.; Korbelik, M.; Moan, J.; Peng, Q. *J. Natl. Cancer Inst.* **1998**, *90*, 889. (b) Gollnick, H. P. m.; Owsianoswki, M.; Ramaker, J.; Chun, S. C.; Orfanos, C. E. *Recent Results Cancer Res.* **1995**, *139*, 409. (c) Lucvigsson, J. *Diabetes-Metab. Rev.* **1993**, *9*, 329. (d) Rook, A. H.; Cohen, J. H.; Lessin, S. R.; Vowels, B. R. *Dermatol. Clinics* **1993**, *11*, 339.

$\delta$ 10.08 (d, $J$ = 6 Hz, 1H), 7.63 (s, 1H), 7.10 (s, 1H), 6.16 (d, $J$ = 6.0 Hz, 1H), 5.52 (s, 2H), 5.35 (d, $J$ = 13.6 Hz, 1H), 5.33 (d, $J$ = 13.6 Hz, 1H), 5.18 (s, 1H, *OH*), 5.01 (d, $J$ = 11.6 Hz, 1H), 4.24 (d, $J$ = 11.6 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.78 (m, 1H), 3.52 (m, 1H), 3.05 (s, 1H, *OH*), 2.98 (q, $J$ = 6.8, 14.5 Hz, 1H), 2.15 (m, 1H), 1.87 (m, 1H), 1.30 (s, 3H), 1.06 (s, 3H), 0.99 (d, $J$ = 57.6 Hz, 3H). $^{13}$C NMR and APT (400 MHz, DMSO-$d_6$, 75 °C) $\delta$ 191.35 (CH), 173.86 (C), 173.44 (C), 170.25 (C), 154.11 (C), 153.52 (C), 150.97 (C), 148.46 (C), 140.15 (C), 134.41 (CH), 111.85 (CH), 108.91 (CH), 79.07 (C), 76.20 (CH), 74.95 (C), 63.73 ($CH_2$), 61.83 ($CH_2$), 61.83 (CH) 56.53 ($CH_3$), 56.50 ($CH_3$), 45.62 ($CH_2$), 43.02 (CH), 31.19 ($CH_2$), 21.94 ($CH_3$), 19.10 ($CH_3$), 14.15 ($CH_3$). IR (NaCl, neat) 3505, 3027, 2915, 1736, 1705, 1685, 1522, 1279, 1106 cm$^{-1}$; exact mass calcd for $C_{26}H_{33}N_2O_{13}$ *m*/*z* 581.198265, found *m*/*z* 581.198011.

**General Procedure for Linearization of Plasmid pBR322 by *Eco*R1.** Supercoiled pBR322 (30 $\mu$L, 30 $\mu$g) was incubated with *Eco*R1 (New England Biolabs) (10 $\mu$L), *Eco*R1 buffer (10×, 20 $\mu$L), and 144 $\mu$L of $H_2O$ (sterile) for 1 h and 20 min at 37 °C. NaOAc (20 mL, 3M) and ethanol (440 $\mu$L) were added, and the solution was cooled at −70 °C for 10 min. The mixture was centrifuged for 15 min, and the ethanol was decanted off. The remainder of the ethanol was evaporated off in vacuo, and the remaining linearized DNA was suspended in 60 $\mu$L of sterile $H_2O$. The amount of linearized pBR322 was quantitated by UV analysis as described by P. N. Borer in the *Handbook of Biochemistry and Molecular Biology*, CRC Press, 1975, in conjunction with the optical density measurements obtained by UV absorption at 260 nm. For this plasmid DNA substrate the conversion factor was determined to be an $OD_{260}$ of 1 = 50 $\mu$g/mL.

**General Protocol for Alkaline Agarose Gel Electrophoresis.** The agarose gels were prepared by adding 50 mL of a 50 mM NaCl/2 mM EDTA (at pH = 8) to 0.6 g of agarose. The suspension was heated in a microwave oven until all of the agarose was dissolved (45 s). The gel was poured and was allowed to cool and solidify for 1 h at room temperature. After 1 h the gel was soaked in an alkaline running buffer (25 mL of 2 N NaOH, 2 mL of 0.5 M EDTA in 1 L of $H_2O$) for an additional hour. The comb was removed, and the buffer was refreshed prior to electrophoresis. Agarose loading dye (5 $\mu$L) was added to the samples (10 $\mu$L), and the samples were loaded into the wells. The gel was run for 4 h at 40 mAmps/90 V which resulted in a travelling distance of approximately 8 cm. The gel was then neutralized for 45 min in a 1 M Tris pH = 7/1.5 M NaCl solution, which was refreshed every 15 min. The gel was subsequently stained in an ethidium bromide solution (200 $\mu$L of a 10 mg/mL ethidium bromide solution in 1 L of 1 M Tris/ 1.5 M NaCl buffer at pH = 7.5) for 1 h. The background staining was then removed by soaking the gel in a solution of 50 mM $NH_4OAc$ and 10 mM $\beta$-mercaptoethanol. Gels were visualized on a UV transilluminator and photographed using Polaroid black and white film No. 667.

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