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Synthesis and DNA Cross-Linking of a Phototriggered FR900482 Mitosene Progenitor

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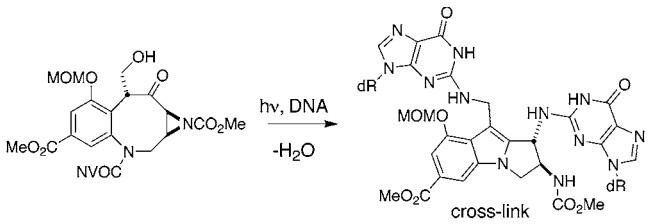
ABSTRACT



The synthesis and biochemical reactivity of the first photoactivated mitosene-based DNA interstrand cross-linking agent is described.

DNA cross-linking agents have played a significant role in the discovery of clinically useful antineoplastic agents.[1] FR900482 (**1**) and FR-66979 (**2**), which are natural antitumor antibiotics obtained from the fermentation harvest of *Streptomyces sandaensis* No. 6897 at the Fujisawa Pharmaceutical Co. in Japan, have shown highly promising activity in this area (Figure 1).[2−6] The clinical candidates FK973 (**3**)[5,7] and, most recently, FK317 (**4**),[6] both semisynthetically derived from FR900482, have shown highly promising antitumor

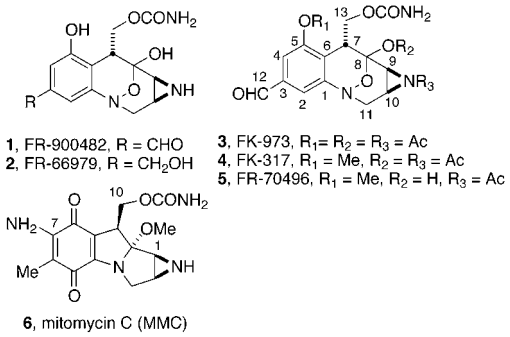


Figure 1.

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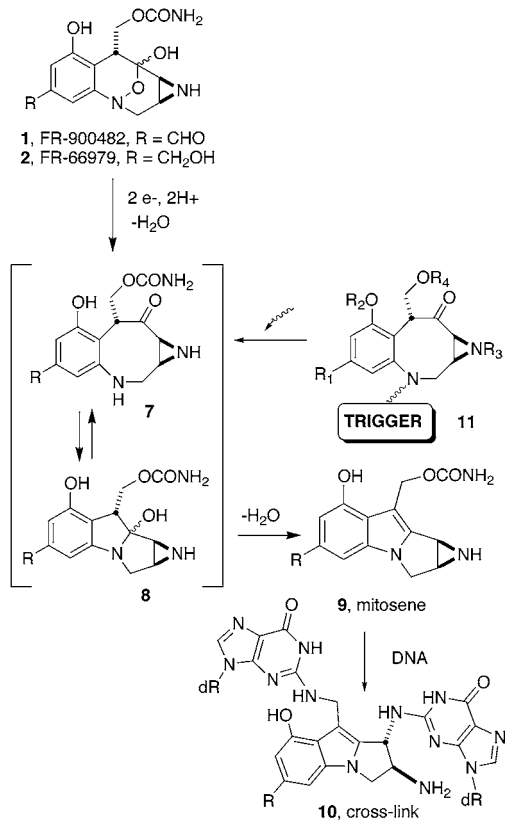
activity in human clinical trials. FK317 is now in advanced human clinical trials in Japan[6] and holds significant promise for replacing the structurally related and widely used antitumor drug mitomycin C (MMC, **6**).[8] Additionally, FK317 does not induce vascular leak syndrome, a serious side effect that precipitated the withdrawal of FK973 (**3**) from development.[6]

Natural products FR900482 and FR-66979 (and by analogy, FK973 and FK317) have been demonstrated to be activated by a two-electron reduction of the N−O bond[9] to give the intermediate ketone **7**, which is in equilibrium with carbinolamine **8** (Scheme 1). Elimination of water and

Scheme 1



tautomerization culminates in the production of the reactive mitosene species **9**, which preferentially cross-links duplex DNA at 5′CpG′3 steps in the minor groove.[10,11] It should be noted that the semisynthetic derivatives FK317 and FK973 must be monodeacetylated in vitro and in vivo to display cytotoxic activity and the capacity to mediate interstrand cross-link formation.[6b]

Our laboratory has focused on the design and synthesis[12,13] of pro-mitosenes that are activated by alternative chemical signals to the obligate reductive activation pathway necessary for FR900482 and congeners.[14] The Fujisawa series drugs and MMC all function at the limit of endogenous reducing equivalents in the hypoxic environment of the tumor. Synthesis of masked mitosene progenitors with the general structure **11** was envisioned to afford opportunities for the efficient, controlled release of the highly reactive, bis-electrophilic mitosene that should be useful for improving the potency and selectivity of this family of agents. Efforts toward this approach have been previously reported[14] that demonstrated the viability of accessing a mitosene core from a structure similar to **11** but lacked the second electrophilic site (the carbamoylmethyl residue at C-13). We describe here the synthesis of a fully functional masked mitosene progenitor based on structure **11** that cross-links DNA upon photochemical activation at concentrations down to 1.0 μM.

Optically active aziridine **13**, prepared as previously described,[14,15] was condensed with **12** in the presence of sodium methoxide to afford the secondary alcohol **14** in 90% yield as a mixture of epimers (Scheme 2).[14] Protection with diethylisopropylsilyl chloride (DEIPSCl) followed by oxidative removal of the *para*-methoxybenzyl ether afforded **15** in 72% yield.

Dess−Martin oxidation[16] afforded aldehyde **16** in 90% yield. Reduction of the nitro function by catalytic hydrogenation and subsequent cyclization of the amino aldehyde to the eight-membered ring substance under dilute conditions (∼1.0 mM) was effected with $MgSO_4$ and 4 Å sieves furnishing the corresponding cyclic imine. Reduction of the imine with $NaCNBH_3$ and AcOH gave **17** in 55∼75% yield.

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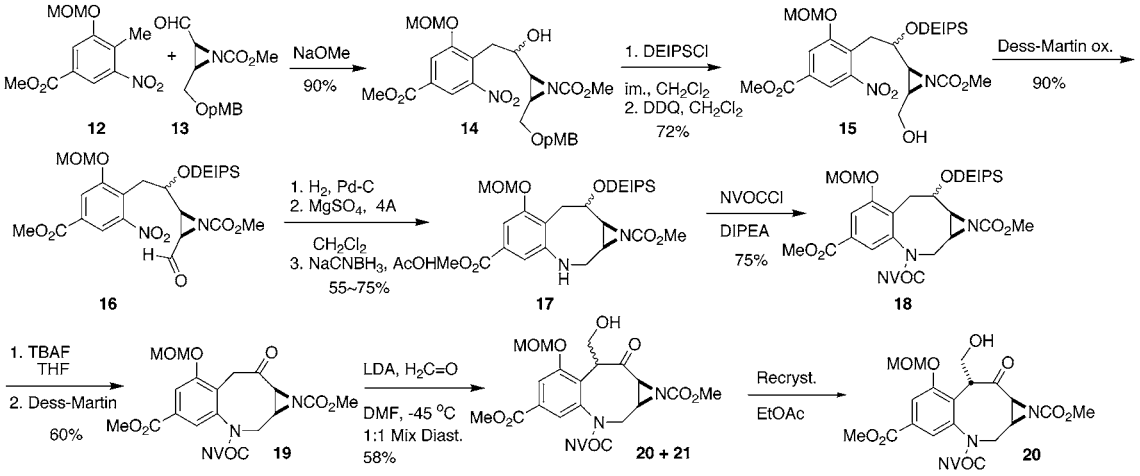
Scheme 2



Acetylation with 6-nitroveratryl chloroformate (NVOC−Cl) (75%), followed by removal of the DEIPS group with TBAF and Dess−Martin oxidation, gave ketone **19** in 60% yield (two steps). Introduction of the crucial hydroxymethyl group was accomplished by reaction of **19** with LDA in dry DMF at −45 °C followed by addition of an anhydrous solution of formaldehyde in THF[17] to give the desired aldol adduct **20** and epimer **21** as a 1:1 mixture of diastereomers in 58% yield. Recrystallization from EtOAC gave suitable crystals for X-ray analysis, which revealed that the desired anti-configuration had been secured (see Supporting Information).

While we were initially not expecting a substrate such as **20**, which lacked the carbamoyl group and had protection of both the aziridine nitrogen atom (carbomethoxy) and the phenolic hydroxyl groups (MOM ether), to lead to interstrand cross-link formation, it is instructive to compare the structure of compound **20** to that of both FK317 (**4**) and FR70496 (**5**).[6b] Workers at Fujisawa recently reported that FK317 must first be deacylated to FR70496 to form DNA−DNA interstrand cross-links.[6b] FR70496 (**5**) has the phenolic hydroxyl blocked as a methyl ether, and the aziridine nitrogen is acylated with an acetyl group. The only other difference is the presence of a carbomethoxy group in the aromatic ring for **20** versus the aldehyde for FR70496. The methoxy group of FR70496 apparently provides sufficient electron-donating power to activate the aziridine for ring-opening to an electrophilic species that is first monoalkylated and subsequently leads to cross-link formation.

The capacity of **20** to cross-link DNA was evaluated using linearized pBR322 plasmid DNA by denaturing alkaline agarose gel electrophoresis according to Cech.[18] Plasmid pBR322 was linearized by restriction endonuclease digestion with *Eco*R1 and quantitated by UV analysis at 260 nm. To compensate for the low solubility of **20** in H_2O, reactions were conducted in ≤1% DMSO/H_2O solutions. Varying concentrations of compound **20** were made by dilution of a 10 mM stock solution of **20** in DMSO with the appropriate amount of H_2O. Reaction of **20** was prepared by addition of the appropriate amount of stock solution to a total volume of 10 μL containing 0.5 μg of linearized DNA buffered to pH 8 with 10 mM Tris, 1 mM EDTA. The reaction was then exposed to 350 nm irradiation with a Rayonet lamp for 1 h followed by incubation at ambient temperature for 12 h. Alkaline agarose (1.2%) gel electrophoresis at 40 V/80 mA for 3.5 h provided the results shown in Figure 2.

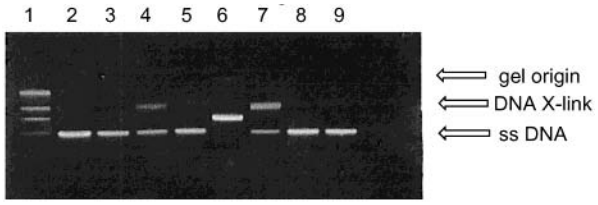


Figure 2. Photoactivated cross-link of pBR322 by analogue **20**. Reaction mixtures were prepared by addition of appropriate stock solutions to a total volume of 10 μL containing 0.5 μg of linearized DNA buffered to pH 8 with 10 mM Tris, 1 mM EDTA. Irradiations were performed in clear Eppendorfs at 25 °C with a Rayonet lamp equipped with four 350 nm bulbs. All samples were incubated for 24 h prior to analyses. Mixtures were analyzed on a 1.2% agarose gel at 80 V for 3.5 h. Lane 1: Lambda Hind III. Lane 2: DNA, dark. Lane 3: DNA, *hν*, 60 min. Lane 4: 1.0 mM FR900482 + 1.0 mM 2-mercaptoethanol. Lane 5: 10 uM **20**, dark, 60 min. Lane 6: 10 uM **20**, *hν*, 60 min. Lane 7: 1 uM **20**, *hν*, 60 min. Lane 8: 100 nM **20**, *hν*, 60 min. Lane 9: 10 nM **20**, *hν*, 60 min.

Lambda Hind III was used as a molecular weight standard (lane 1). The natural product, FR900482 at 1.0 mM was used as a control standard and activated with 1 mM 2-mercaptoethanol in the presence of 0.5 μg of DNA duplex producing the interstrand cross-link (lane 4).[10a]

Interstrand cross-link formation was evident following 1 h of irradiation of compound **20** at 10 and 1 μM concentrations with 0.5 μg of DNA duplex (lanes 6 and 7, respectively).[19] Incubation of **20** at a 10 μM concentration with

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0.5 mg of DNA duplex for 12 h in the dark (lane 5) did not lead to detectable cross-link formation.

It is further significant that **20** cross-links a synthetic 33 bp DNA duplex at the same $^{5'}$CpG$^{3'}$ step as that observed for FR900482.

Photolysis of compound **20** (10 mM) with the 5′-^{32}P-labeled duplex shown in Figure 3 (in a 5:1 H_2O/CH_3CN mixture) with 350 nm light in a Rayonet equipped with four bulbs for 1 h followed by a 12 h of incubation in the dark led to cross-link formation. Following ethanol precipitation and purification by 20% DPAGE, the major cross-link band was isolated (see Supporting Information). Following Fe(II)−EDTA digestion and Maxam−Gilbert reactions, all samples were ethanol precipitated and subjected to 20% DPAGE at 40 W for 3.5 h. Autoradiography of the gel electrophoresis produced the image shown above in Figure 3. As expected for a mitosene, cross-link formation appears to occur at the exocyclic amine of the dG residue in the minor groove. Corroboration of the footprinting data was secured by substitution of the 2'-deoxyguanosine base with 2'-deoxyinosine at the $^{5'}$CpG$^{3'}$ steps. Reactions with the 2'-deoxyinosine-modified duplex did not result in any observable cross-link, while the use of the 2'-deoxy-7-deazaguanosine showed cross-links in the same manner as the unmodified 2'-deoxyguanosine-containing duplex. These results strongly implicate the N-2 exocyclic amine of 2'-deoxyguanosine as the site of alkylation in the same manner as the natural products **1** and **2**.

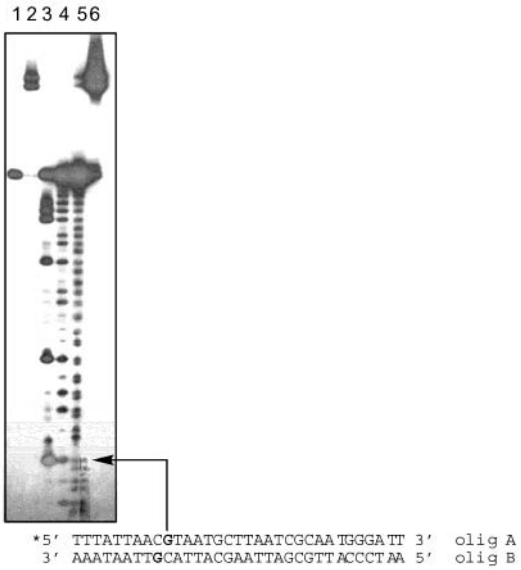
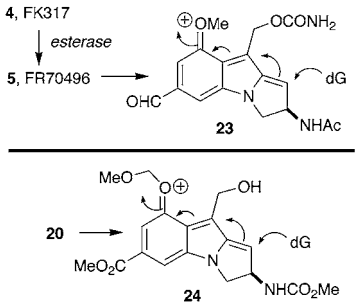


Figure 3. Autoradiogram of Fe(II)−EDTA footprinting of cross-linked A/B duplex (labeled at the 5′-terminus of oligo A). Lane 1: DNA standard (oligo A). Lane 2: cross-linked **1**. Lane 3: Maxam−Gilbert G. Lane 4: Maxam−Gilbert G + A. Lane 5: 1 mM Fe(II)−EDTA digestion of oligo A (control). Lane 6: 1 mM Fe(II)−EDTA digestion of cross-linked **1**.

These studies demonstrate the viability of photoactivated pro-mitosenes based on the FR70496 framework to lead to the efficient generation of interstrand DNA cross-link formation. The implication of this study is that compound **20**, upon photochemical activation, most likely generates the reactive mitosene intermediate **24** (Scheme 3), which upon successive monoalkylation followed by cross-linking appears to be very similar to the presumed FR70496-derived mitosene (**23**). In both cases, the respective phenolic alkoxyl groups in the aromatic ring are apparently sufficiently electron-rich to activate the acylated aziridine species for DNA adduction.

Scheme 3



Studies to elucidate the precise molecular structure of the interstrand cross-link derived from the synthetic agents **20**, as well as on the naturally derived congeners **4** and **5**, are presently under way. Additionally, efforts to synthesize related compounds with alternative chemical triggers are under study in these laboratories and will be reported in due course.

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Supporting Information Available: X-ray structure for **20**, spectroscopic and analytical data for all new compounds, and 20% DPAGE of 33 bp DNA duplex cross-linked by **20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) The faster mobility of the cross-linked DNA at the 10 μM concentration (lane 6, Figure 1) relative to that for the 1 μM concentration (lane 7 Figure 1) reaction is attributed to extensive (multiple) cross-links at the higher concentration.