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Synthetic Studies on FR 900482. Synthesis of a Photo-triggered Pro-Mitosene‡

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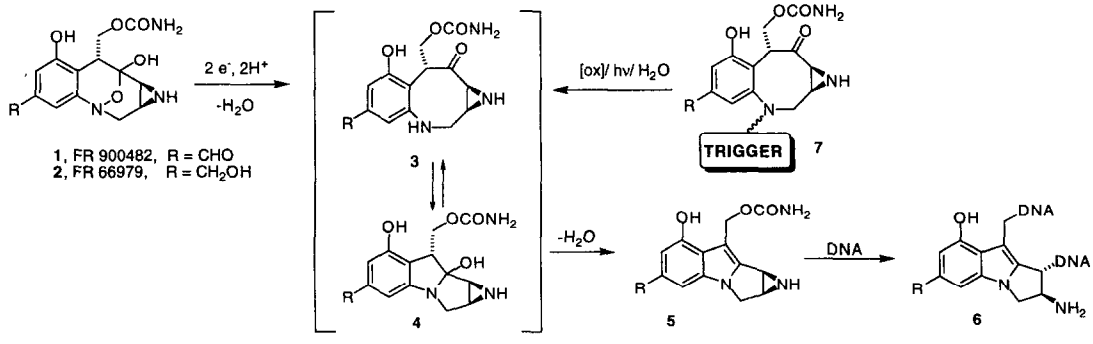
Abstract: A stereocontrolled synthesis of an eight-membered ring precursor to a photo-triggered mitosene is described. © 1997 Elsevier Science Ltd.

In 1987 the Fujisawa Pharmaceutical Co. in Japan isolated[1] a new anti-tumor antibiotic,[2] FR 900482 (**1**), from the fermentation broth of *Streptomyces Sandaenis* No. 6897. Two years later, the dihydroderivative, FR 66979 (**2**), was isolated from the same strain.[3] The semi-synthetic triacetyl derivative of FR 900482, FK 973, possesses promising activity against various transplanted murine and human tumors.[4] These substances are structurally related to mitomycin C (MMC) but lack the quinone moiety of MMC and contain a novel hydroxylamine hemi-ketal.

These substances behave similarly to MMC in that they are reductively activated *in vitro* and *in vivo* resulting in DNA cross-links.[5] Studies of the *in vitro* DNA-DNA interstrand cross-linking reaction of FR 66979 and FR 900482 have determined the *in vitro* site of cross-linking (5'-CpG) and sequence selectivity.[6] In addition, several studies have provided strong evidence[6] that FR 900482 undergoes a two electron reduction[7] cleaving the N-O bond to give amine **3** which cyclizes to **4**. Subsequent dehydration yields the mitosene **5** (Scheme 1) which cross-links[8] double-stranded DNA (**6**). Thus, the FR 900482 series of compounds are "latent" reductively-activated mitosenes.



Scheme 1

‡**Dedicated to Professor Yoshito Kishi on the occasion of his 60th birthday**

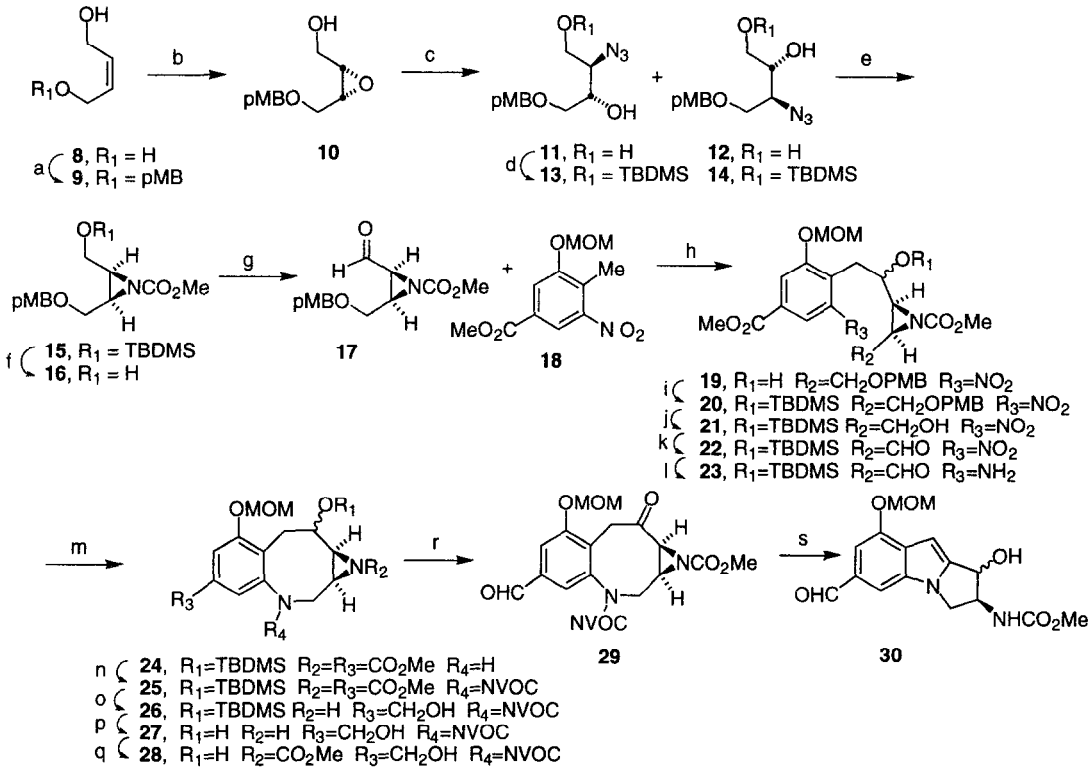
The unique structure of **1** and its extraordinary antitumor activity have made it an attractive synthetic target. Several different approaches to the core nucleus of **1** have been published,[9] and three groups have successfully completed the total synthesis.[10] In an attempt to design and synthesize molecules that mimic or combine the cross-linking activity of FR 900482, synthetic efforts in our labs have been focused on constructing a natural product analog (ie., **7**) that is not reductively activated but that could, in principle, be triggered photochemically, oxidatively, or hydrolytically to form a reactive mitosene. To test this hypothesis, the synthesis of the first light-activated pro-mitosene is described below.

The aliphatic portion of the prodrug was prepared from commercially available *cis*-2-butene-1,4-diol (**8**) (Scheme 2). Formation of the cyclic acetal with *p*-anisaldehyde and $LiAlH_4/AlCl_3$ reduction of the acetal gave the mono-protected *cis*-diol **9** (45%, 2 steps). Sharpless epoxidation of the allylic alcohol gave epoxide **10** (75%) in approximately 87% *ee*. Non-selective ring opening of **10** with sodium azide gave a mixture of 1,3- and 1,2-diols **11** and **12** in a 3:2 ratio (the mixture was not purified except for characterization purposes). Selective protection of the primary alcohols of **11** and **12** gave a mixture of TBS ethers **13** and **14** (90%, 2 steps). Reduction of the azides with triphenylphosphine under anhydrous conditions and carbomethoxylation of the resulting aziridine [10b] afforded **15** (92%, 2 steps, ~ 87% *ee*). Removal of the TBS ether from **15** with tetra-*n*-butyl ammonium fluoride gave alcohol **16** (86%) which was converted to the corresponding aldehyde (**17**) with Dess-Martin periodinane [11] in 92% yield.

Following literature procedures, commercially available 3,5-dinitro-*p*-toluic acid was transformed into methyl 3-methoxymethyloxy-4-methyl-5-nitrobenzoate (**18**).[9b,12] Deprotonation of nitro toluene **18** and nucleophilic addition [9a] to aldehyde **17** afforded the secondary alcohol **19** as a 4:1 mixture of diastereomers (85%) which were separated by chromatography and subsequently processed individually. The secondary alcohol was protected as a TBS ether to afford **20** (96%). The oxidative removal of the O-*p*-methoxybenzyl group [13] gave primary alcohol **21** (93%) which was subjected to Dess-Martin oxidation to afford aldehyde **22** (82%). Reduction of the nitro group with H_2 over Pd/C to the unstable aniline **23** set the stage for ring closure.

As expected, cyclization of **23** to the eight-membered ring substance **24** proved difficult. It was found that cyclization was best accomplished by prior dehydration to the imine in the presence of $MgSO_4$ and 4 Å mol. sieves under dilute conditions (~0.002 M). After 24 hrs., the crude imine was reduced with $NaCNBH_3$ to give **24** (60%, 3 steps). Acylation of **24** with 6-nitroveratryl chloroformate produced carbamate **25** (88%) as a mixture of conformational isomers (^1H nmr analysis). Reduction of the methyl ester and removal of the carbomethoxy group in one step with DIBAH gave **26** (61%). [10b] It was observed that the TBS ether of **26** could be removed only with the aziridine unprotected. Thus, following decarbomethoxylation of the aziridine, the TBS ether was smoothly removed with TBAF to afford diol **27** (85%). Selective reprotection of the aziridine gave **28** (89%). Finally, Dess-Martin oxidation of the primary and secondary alcohols produced keto-aldehyde **29** (83%).

With the "pro-mitosene"(**29**) in hand, we examined removal of the NVOC group photochemically under various conditions. This was best effected by treating **29** (λ_{max} = 345 nm, ε = 6,800; 295 nm, ε = 7,740; 238 nm, ε = 17,300; 217 nm, ε = 18,500, CH_3CN) with UV radiation for 24 hrs. at room temperature in a 3:1 solution of CH_3CN/H_2O. [14] The sole isolable product was the ring-opened mitosene **30** as a 1:1 mixture of secondary alcohol diastereomers (38%).



Scheme 2[15]

Reagents and conditions: a) i. *p*-anisaldehyde, *p*-TsOH, benzene, reflux, 58%; ii. $LiAlH_4/AlCl_3$, THF, 0° --> rt, 78% b) $Ti(OiPr)_4$, L-(+)-DET, *t*BuOOH, CH_2Cl_2 , -20 °C, 75% c) NaN_3 , NH_4Cl, $CH_3OCH_2CH_2OH$, reflux d) TBDMSCl, Et_3N, DMAP, CH_2Cl_2 , 4 °C, 90% for 2 steps e) i. Ph_3P, THF, reflux; ii. $ClCO_2Me$, Py, 92% for 2 steps f) TBAF, THF, rt, 86% g) Dess-Martin, CH_2Cl_2 , rt, 92% h) NaOMe/MeOH, DMF, 0 °C, 85% i) TBDMSCl, Im, DMF, rt, 96% j) DDQ, CH_2Cl_2/H_2O, rt, 93% k) Dess-Martin, CH_2Cl_2 , rt, 82% l) 5% Pd/C, H_2 (1 atm), MeOH, rt m) i. MgSO4 , 4A mol sieves, CH_2Cl_2 , reflux; ii. $NaCNBH_3$, CH_2Cl_2/MeOH, 0 °C, 60% for three steps n) NVocCl, iPr_2EtN, DMAP, CH_2Cl_2 , 88% o) DIBAH, CH_2Cl_2, -78 °C, 61% p) TBAF, THF, 0 °C --> rt q) *N*-((methoxy)carbonyloxy)succinimide, Py, rt, 89% (two steps) r) Dess-Martin, CH_2Cl_2 , rt, 83% s) 350 nm, CH_3CN/H_2O, rt, 38%.

Synthesis of **29** and the selective production of **30** from this material demonstrates the viability of constructing novel "pro-mitosene" derivatives which may find utility as new and selectively activated DNA-DNA and DNA-protein cross-linking agents and probes. Studies towards the synthesis of fully funtionalized photoactivated mitosenes and other non-reductively activated "pro-mitosene" and related derivatives is under intensive investigation in these laboratories and will be reported on in due course.

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14. A control experiment where, incubation of **29** in the dark for 24 h in 3 : 1 CH_3CN : H_2O at room temperature led to no detectable loss of the starting material.

15. All new compounds exhibited satisfactory ^1H nmr, ^{13}C nmr, ir, mass spectrum and / or combustion analytical data consistent with the assigned structures.

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