DISSERTATION

CONCISE ENANTIOSELECTIVE SYNTHESIS OF (+)-FR66979 AND (+)-FR900482 AND THE SYNTHESIS AND BIOCHEMICAL STUDIES OF A PHOTO-TRIGGERED FR900482 MITOSENE PROGENITOR

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In partial fulfillment of the requirements
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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY TED CHARLES JUDD ENTITLED CONCISE ENANTIOSELECTIVE SYNTHESIS OF (+)-FR66979 AND (+)-FR900482 AND THE SYNTHESIS AND BIOCHEMICAL STUDIES OF A PHOTO-TRIGGERED FR900482 MITOSENE PROGENITOR BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS OF THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Advisor

Department Head

ABSTRACT OF DISSERTATION

CONCISE ENANTIOSELECTIVE SYNTHESIS OF (+)-FR66979 AND (+)-FR900482 AND THE SYNTHESIS AND BIOCHEMICAL STUDIES OF A PHOTO-TRIGGERED FR900482 MITOSENE PROGENITOR

The total synthesis of the natural products (+)-FR66979 and (+)-FR900482 is presented. The synthesis features a novel dimethyldioxirane-mediated reaction for construction of the hydroxylamine hemiketal functionality and represents the shortest total synthesis reported to date.

The synthesis of a photo-triggered mitosene progenitor based on the natural products (+)-FR66979 and (+)-FR900482 has also been described. Biochemical studies demonstrate the capacity of the mitosene progenitor to form DNA interstrand cross-links upon photo-activation. Furthermore, the cross-links have been shown to share the identical sequence specificity as the natural products.

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For Mom, Dad, and Tim

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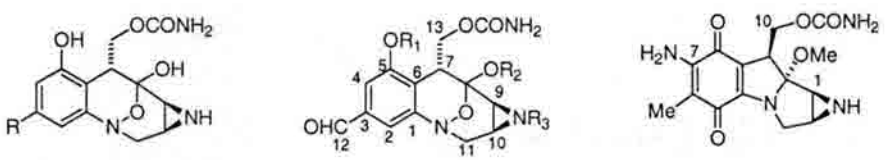
Chapter 1

FR900482 and the Mitosene Family of Compounds

1.1 Introduction

DNA cross-linking agents have played a significant role in the discovery of clinically useful antineoplastic agents.[1] FR900482 (**1**) and FR66979 (**2**), 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.[2,3,4,5,6] The clinical candidates FK973 (**3**),[5,7] and more recently FK317 (**4**),[6] both semi-synthetically derived from FR900482, have shown highly promising antitumor activity in human clinical trials (Figure 1). FK317 is now in advanced human clinical trials in Japan and holds significant promise to replace the structurally related and widely used antitumor drug mitomycin C (MMC, **6**).[8]



1 FR900482, R = CHO
2 FR66979, R = CH_2OH

3 FK973, $R_1 = R_2 = R_3$= Ac
4 FK317, R_1 = Me, $R_2 = R_3$ = Ac
5 FR70496, R_1 = Me, R_2 = H, R_3 = Ac

6 Mitomycin C

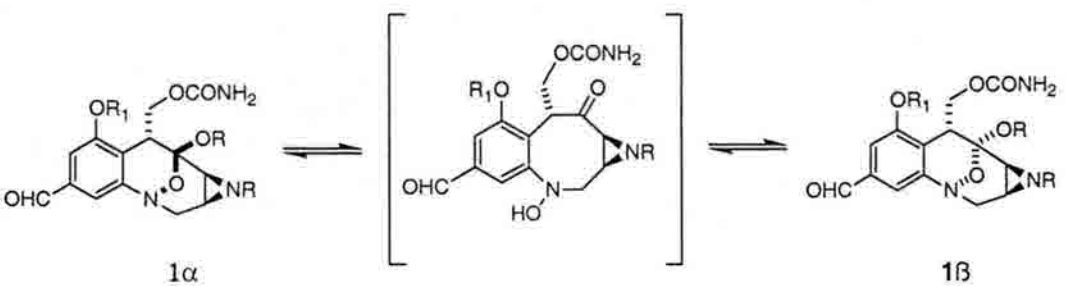
Figure 1.1. FR-series of compounds and mitomycin C.

Since their isolation in 1987 and 1989 respectively, the natural products FR900482 (**1**) and FR66979 (**2**) have provided formidable challenges for bioorganic and synthetic chemists alike, both in the elucidation of their biological modes of action and in

the synthesis of the various complex functionalities inherent to this family of compounds. Following several attempts to elucidate the structure using NMR analysis, the complete structure of the natural product was finally determined by X-ray crystallographic analysis of the triacetalylated derivative FK973.[2c] The absolute configuration was later confirmed by total synthesis in 1996 by Terashima *et al.*[18c-h]

Structurally related to the mitomycin family of molecules, the FR series of compounds share several features, including the hydroxymethyl carbamoyl moiety and the aziridine ring. Compounds **1** and **2**, however, lack the quinone moiety of the mitomycins and additionally, they posses a hydroxylamine hemiketal moiety unique to this family of natural products. This characteristic functionality results in FR900482 existing as mixture of equilibrating anomeric diastereomers **1α** and **1β**. In the natural product, this distribution favors the **1α** tautomer in an approximate ratio of 2.3:1 at neutral pH, allowing for intramolecular hydrogen bonding between the aziridine hydrogen and the bridgehead oxygen of the hydroxylamine hemiketal bond. Acylation of the aziridine reverses this ratio to 4:1 favoring the β-anomer, with the change in preference based on steric considerations (Scheme **1.1**).

Scheme 1.1. Interconversion of FR900482 and FR70496 diastereomers.



1α 1β

R = H, 2.3:1 Mixture
R = Ac, 1:4 Mixture
R_1 = H or Me

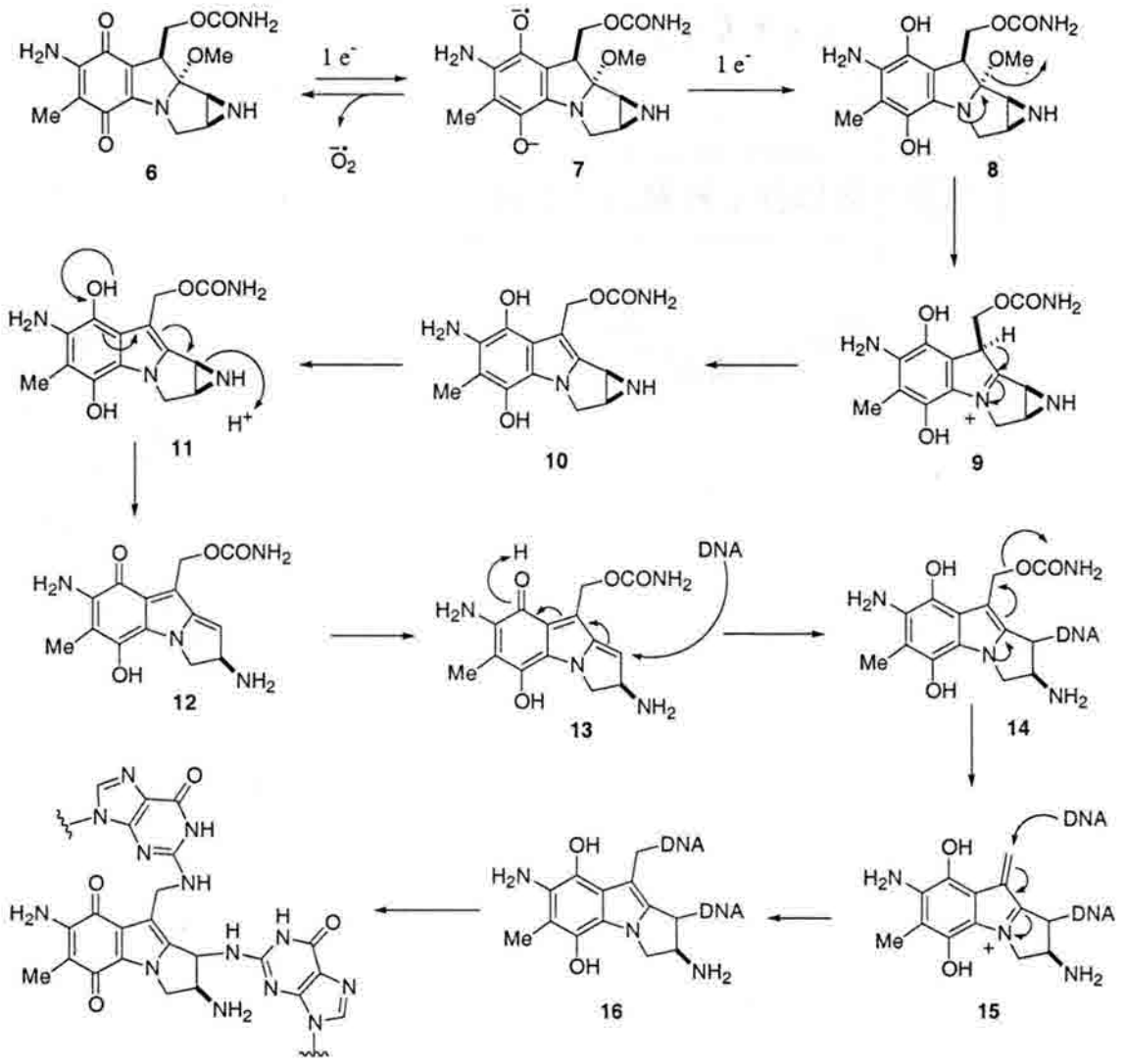
As with Mitomycin C,[8] initial *in vivo* studies demonstrated the ability of FR900482 (**1**), FR66979 (**2**), and congeners to form both DNA-DNA interstrand cross-links and DNA-protein cross-links following reductive activation.[9,10] Additionally, both MMC and FR66979 have since been demonstrated to monoalkylate DNA in addition to the bisalkylated cross-links, although this manifold seems more prevalent with MMC. The origin of the cytotoxic effects of these compounds arises from their ability to form covalent interstrand cross-links within duplex DNA. This event in turn prevents DNA synthesis and cell replication, ultimately leading to cell death. Although the alternative monalkylation event gives rise to some cytotoxicity with MMC, the ease of cellular repair of such a lesion compared to the bisalkylated cross-link precludes its effectiveness as a therapeutic mode of action.

The requirement for an exogenous reducing agent for the activation of FR900482 (**1**) and FR66979 (**2**), in the same manner as MMC and other mitomycins, has been demonstrated by past efforts from Rajski and Williams [9a,c,d] and by Hopkins *et al.*[10a-d] The dependence on reductive activation allows both the mitomycins and FR compounds to be selectively activated in environments with low oxygen content. Solid tumors and similar cancer masses are frequently characterized as hypoxic environments, allowing the aforementioned cross-linking agents to be used to discriminate between neoplastic and normal healthy cells, thereby giving rise to their therapeutic value.

The reductive pathway by which MMC is activated to cross-link DNA has been well established.[8] Initial direct or stepwise two electron reduction of the quinone by either enzymatic or chemical means leads to the formation of hydroquinone **8** followed by the

expulsion of the methoxyl group. Subsequent tautomerization affords the intermediate leuco-aziridinomitosene **10**, whereupon electron donation from the hydroquinone indole core opens the aziridine ring to the intermediate quinone methide (**12**).

Scheme 1.2. Proposed mechanism of DNA cross-linking by reductive activation of mitomycin C.

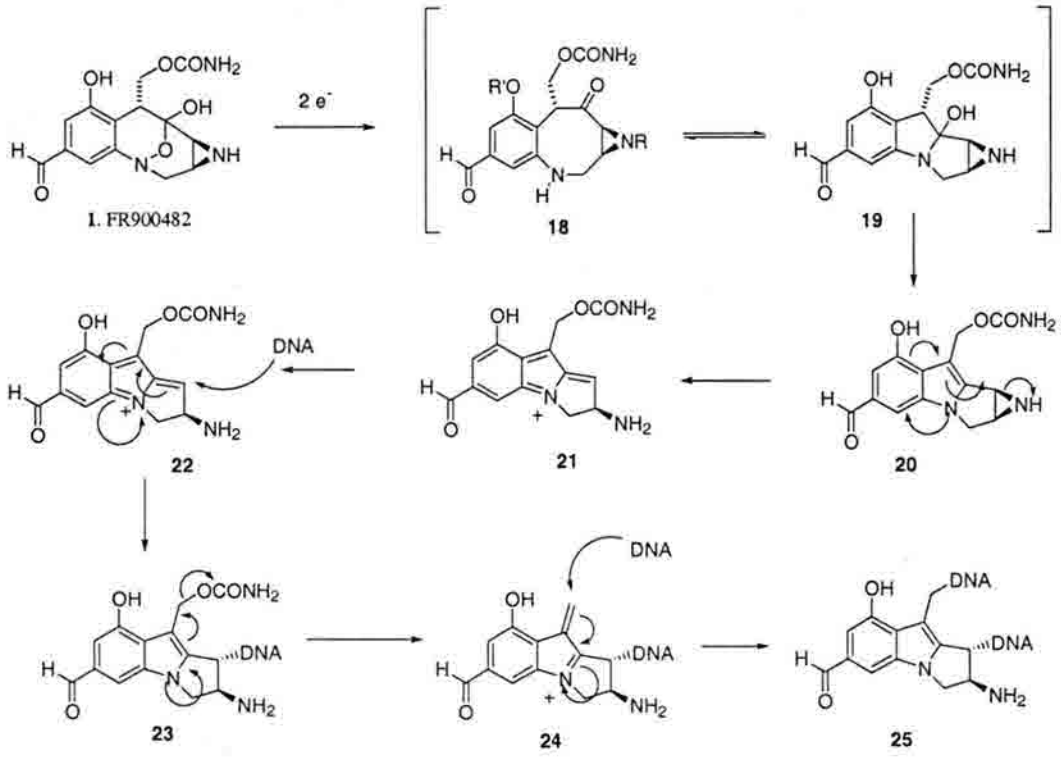


Nucleophilic attack by DNA at the C1 position leads to the first alkylation and regenerates the hydroquinone (**14**). Following expulsion of the carbamate, the second alkylation site is opened and subsequent Michael addition by the DNA affords the bis N2 guanosine cross-link (**17**). In contrast to the various monalkylation events seen with MMC, the interstrand cross-link is specific to only 5'-CG-3' steps in the minor grove.[8]

Likewise, studies by Rajski and Williams[9a-d] and by Hopkins and coworkers[10] have determined that FR compounds (**1**) and (**2**) cross-link duplex DNA with the 5'-CG-3' sequence specificity identical to MMC, in the minor groove. Both research groups demonstrated, moreover, that the cross-link depended on the N2 *exo*-cyclic amine groups on both guanidines. In addition, both families of compounds show the same preference for flanking base sequences in the order 5'-ACGT-3' >> 5'-TCGA-3' ~ 5'-CCGG-3'.[9b, 10a] MMC has additionally demonstrated a preference to cross-link 5'-mCG-3' sites with the cytosine methylated at C5 over the unmodified sequence.[11] Methylated cytosine 5'-CG-3' sequences represent a statistically rare but important sequence in gene expression, and whether this predilection for the methylated 5'-mCG-3' sequences translates to the FR compounds remains to be determined.

Based on their analogous dependence on an exogenous reductive agent and their comparable cross-linking ability, a mechanism of action for the FR series of compounds that is similar to that proposed for MMC (**6**) was put forth by Fukuyama and Goto in 1989 (Scheme **1.3**).[12]

Scheme 1.3. Proposed mechanism of DNA cross-linking by reductive activation of FR900482.



Initial two electron reduction of the hydroxylamine hemiketal bond results in formation of the mitosene intermediate **20**. In a manner similar to MMC, a cascade of events ensues, resulting in the opening of the aziridine ring and expulsion of the carbamate group. This allows for nucleophilic substitution by DNA at the N2 of guanosine. While the enzyme responsible for the *in vivo* reduction of MMC remains elusive, the reductase responsible for activation of the FR compounds has been shown to be DT diaphorase in studies with FK317.[6b] Additionally, it should be noted that the semi-

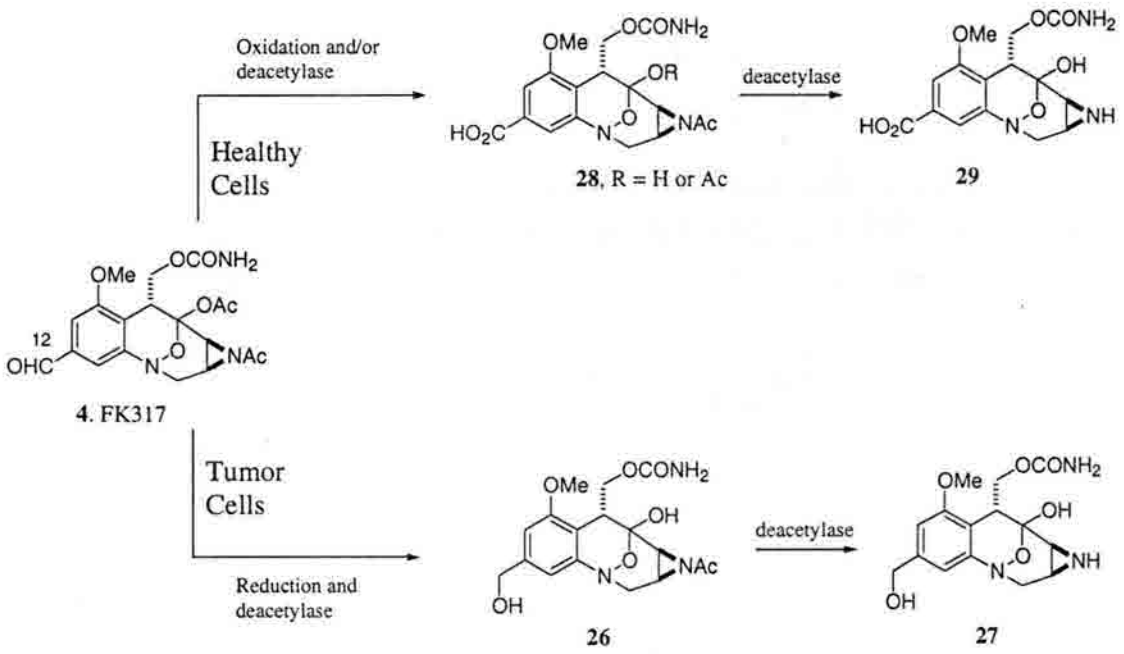
synthetic derivatives FK973 and FK317 require deacetylation of the hydroxylamine hemiketal before any reduction event can occur.[6b]

The proposed reductive pathway represents the generally accepted mechanism and was supported by work of Hopkins and coworkers.[10b,e] These *in vitro* experiments included characterization of various intermediates and derivatives in the reaction cascade,[10e] along with isolation and structural elucidation of the covalently cross-linked lesion by both FR900482 and FR66979 after reductive activation.[10b] The latter experiment followed precepts introduced by Tomasz *et al.* in the structure elucidation of MMC cross-linked DNA.[13] These protocols included enzymatic digestion of cross-linked DNA, HPLC purification of the guanosine lesion, acylation, and finally structure determination by means of spectroscopic analysis. By utilizing a palindromic sequence containing a single 5'-CG-3' site, Hopkins *et al.* were able to isolate the covalent cross-link establishing not only the site of alkylation, but also the existence and structure of the activated mitosene species.

Recent studies with the clinical candidate FK317 (**4**) have revealed an additional structural motif allowing for selective mitosene formation under hypoxic conditions.[6d] The oxidation state of the C12 position may play a key role in the reduction of the hydroxylamine hemi ketal functionality. Examination of the metabolites of FK317 *in vivo* revealed that the C12 aldehyde was reduced to the corresponding alcohols **26** and **27**. This oxidation state enhances the effectiveness of the cross-linking agent as demonstrated by the higher relative activity of FR66979 over that of FR900482 in *in vitro* studies. Moreover, in healthy cells, the majority of the drug was metabolized to the carboxylic

acid **28** and **29** at C12, derivatives which have been shown to be chemotherapeutically inactive.[6d]

Scheme 1.4. Metabolism of FK317 in both neoplastic and healthy cells.



The origin of the 5'-CpG-3' sequence specificity for both the MMC and FR series of molecules relies predominately on the ideal geometric fit of the resulting activated mitosene intermediate in the minor groove of DNA.[13,14] The basis for this ideal positioning is the distance between the *exo*-cyclic amine group (N2) of the guanosines in the 5'-CpG-3' sequence in B-DNA (3.1 Å) compared to the distance of the two sites of alkylation on the mitosene species (3.4 Å) (Figure **1.2**)

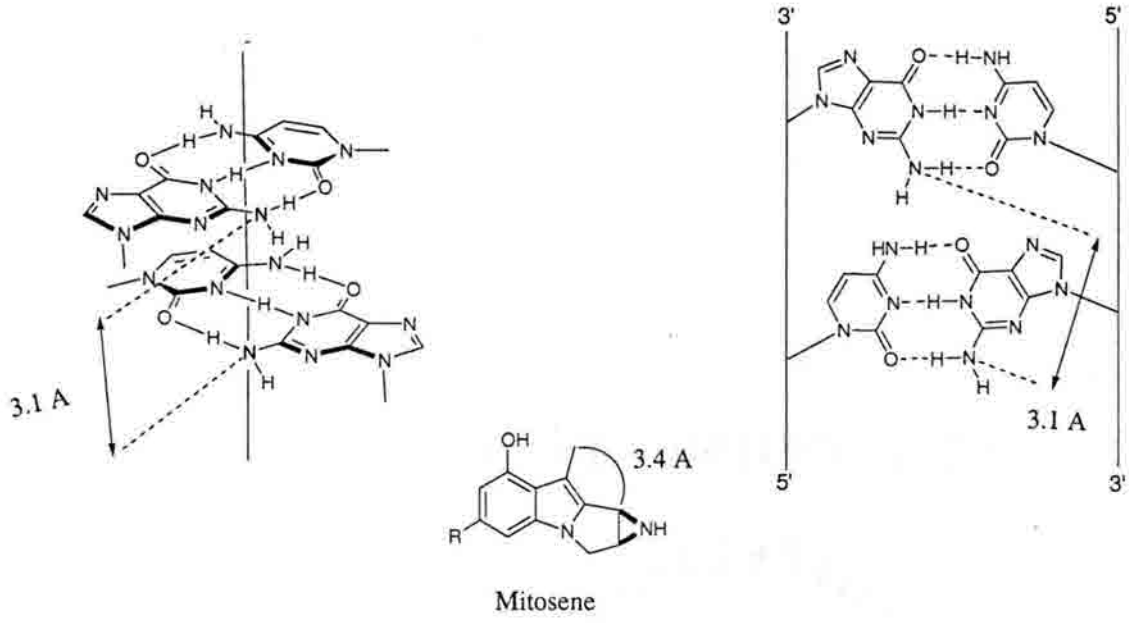


Figure 1.2. Ideal geometric fit of the mitosene in the 5'-CpG-3' sequence of B-DNA in the minor groove.

Notably, the consequence of geometric fit dictating both sequence selectivity and site of alkylation prevails over alternative factors, including greater base nucleophilicity and general accessibility, which in the case of CG base pairs resides at the N7 of guanosine in the major groove.[14, 9d]

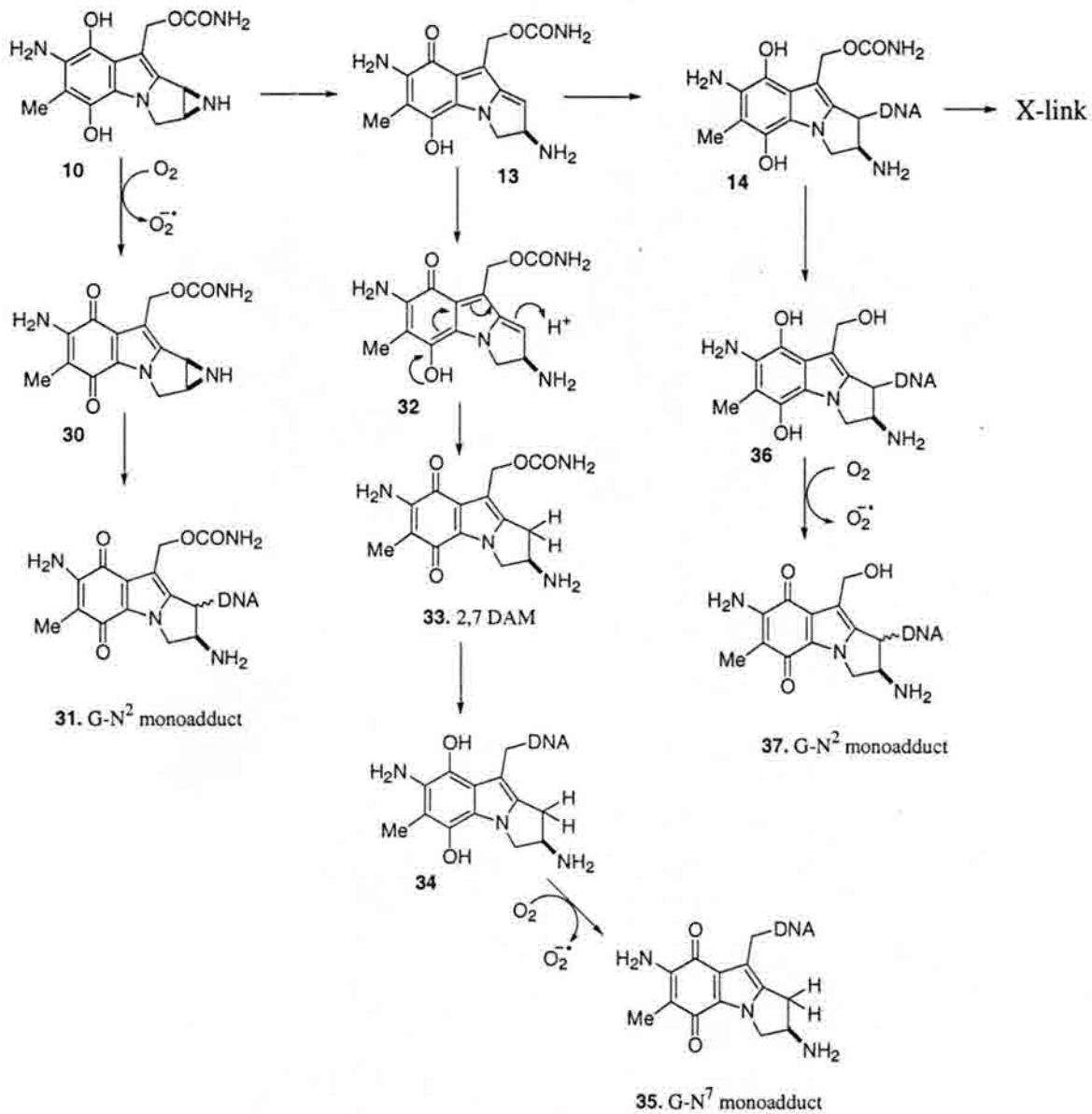
Although the initial evaluation of the natural products MMC and FR900482 demonstrated their capacity to induce protein-DNA cross-links in addition to DNA-DNA cross-links, the majority of biochemical studies for both compounds have solely focused on the latter event. In an effort to evaluate the FR compounds' ability to form covalent DNA-protein cross-links, Rajski and Williams studied the minor groove binding protein HMGA1 (formerly named HMG I/Y). High mobility group (HMG) proteins have been implicated in the regulation of genes associated with the immune system and cell growth as well as other processes. They are therefore preferentially expressed in rapidly

proliferating cells such as those found in tumor masses and represent a relevant drug target (ref. 15 and references therein). Rajski and Williams successfully used FR66979 to cross-link a synthetic peptide sequence of the binding domain of this protein to the corresponding synthetic oligonucleotide duplex containing the known HMG1A AT binding sequence.[9e,f] Following this initial biochemical study, Tepe and Williams, in collaboration with Reeves and coworkers, isolated both FR900482- and FK317-induced cross-links of HMGA1, HMGB1, and HMGB2 all minor groove-binding proteins, with DNA from human Jurkat cells *in vivo*.[15] This study demonstrated the viability of such an event. The clinical implications and significance of the DNA-protein cross-link, compared to DNA-DNA cross-linking, are currently being investigated

Although much discussion has detailed the similarities between the MMC and the FR series of compounds, a profound and clinically significant difference between the two families of compounds resides within the two separate structural motifs masking the active mitosene core. The redox activity of the quinone of MMC has two significant consequences, namely, superoxide production leading to nonspecific oxidative DNA damage and arbitrary arrest of the reductive pathway leading to monoalkylation.[8]

Following production of superoxide from both the initial one-electron reduction and other quinone redox reactions in the cascade, Haber-Weiss/Fenton cycling produces hydroxyl radicals and related reactive oxidants capable of mediating DNA single strand breaks and other indiscriminate cellular damage.[8] Additionally, reversion of the hydroquinone intermediates to the quinone species along the reaction cascade depicted in Scheme **1.5** allows for alternative reaction pathways to the therapeutically less significant monoalkylations.[8a]

Scheme 1.5. Mechanism of various monoalkylation events by mitomycin C.



Indeed, the majority of alkylation by MMC *in vivo* results in monoadducts and not the more lethal cross-links.[8a,16] Under aerobic conditions, these include both N2 adducts resulting from the exocyclic amine of guanosine. In tumors and other hypoxic areas of therapeutic interest, the majority of alkylation occurs via the innocuous monoalkylated

species **35**, resulting from S_N2 displacement of the C10 carbamate of 2,7 diaminomitomycin (2,7 DAM) by the N7 of guanidine.[8a]

In contrast to MMC, the reductive activation pathway of FR900482 (and by analogy FR66979, FK973, and FK317) circumvents the production of superoxide, and therefore obviating the detrimental non-specific oxidative damage, while maintaining a more efficient ability to cross-link DNA. These attributes distinguish the FR series of compounds as viable candidates to replace MMC as a chemotherapeutic agent.

Clinical testing by the Fujisawa Pharmaceutical Company began with the semi-synthetic triacetoxy derivative FK973 (**3**).[5] In addition to showing significantly less host toxicity, the compound showed nearly a three-fold increase in *anti*-tumor activity across a broad spectrum of murine and human carcinomas as compared to MMC. Additionally, FK973 showed strong *anti*-tumor activity against the drug-resistant (including MMC) P388 leukemia. Unfortunately, further clinical testing demonstrated the drug candidate caused vascular leak syndrome (VLS), necessitating its removal from Phase I clinical trials.[5e] VLS is a serious side effect characterized by an increase in vascular permeability, leading to increased leakage of fluids into interstitial space and ultimately organ failure.[5e,17]

Remarkably, the semi-synthetic derivative FK317 (**4**), differing from FK973 (**3**) only with a methoxyl group on the phenol in place of the acetate, was found not to induce VLS.[6] Furthermore, the activity of the compound was maintained in terms of the substantial decrease in host toxicity over MMC (**6**) along with enhanced *anti*-tumor activity equivalent to that seen with FK973.[6] Although the reason for such a drastic

difference remains unclear, work by Tepe and Williams, in collaboration with Reeves and coworkers, has led to some interesting observations when comparing the specific biological effects of FK317 and FR900482.[17] Treatment of human Jurkat cells with FR900482 induced cell death by necrosis only, independent of drug concentration. In contrast, human Jurkat cells treated with FK317 demonstrated a switch from necrosis- to apoptosis-induced cell death with increasing drug concentration. Programmed cell death through apoptosis is highly desirable in antitumor chemotherapeutic treatments; the alternative cell death by necrosis causes deleterious side effects including VLS. The necrosis to apoptosis switch may be related to the observed decrease in concentration of Blc-2 proteins with an increase in drug concentration in FK317 treated cells. This effect was not observed with cells treated with FR900482. The mode of cell death through either apoptosis or necrosis is directly dependent on the concentration of Blc-2 proteins.[17] An additional observation in the study demonstrated the ability of FR900482 to promote the expression of the cytokine IL-2, an agent directly related to inducing VLS, while FK317 did not. This phenomenon provides an alternative explanation for the differential induction of VLS by the two drugs.[17]

The dependence on VLS by the drug concentration of FK317 has been illustrated in a recent report by Fujisawa Pharmaceutical Company examining the survival time of mice bearing B16BL6 melanoma and Lewis lung carcinoma.[6f] Although FK317 showed an improved survival time of the mice at drug concentrations equal to MMC (3.2 mg/kg), an increase in drug concentration (10 mg/kg) resulted in a significant enhancement in survival time with both cancer types examined. Furthermore, 40% of the mice bearing

B16BL6 melanoma were totally cured; by comparison, MMC proved to be fatal at these drug concentrations.

The highly promising activity of FK317 necessitates further analysis including the elucidation of the entire spectrum of biological effects of these compounds along with synthetic work to devise analogs for biochemical analysis. Recent synthetic endeavors by Tersashima and coworkers[18c-h] and Fukuyama *et al.*[20j] have provided fully synthetic analogs providing additional insight into the interesting structure/activity relationships of the natural product **1**. Utilizing their synthetic route to the asymmetric total synthesis of FR900482 (**1**), Terashima *et al.* prepared the antipode of the natural product (**38**) and evaluated its *in vitro* activity against P388 murine leukemia cells.[18c-h]

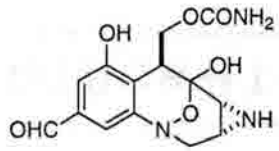
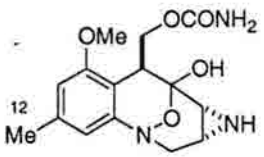


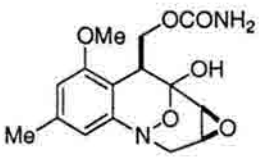
Figure 1.3. *ent*-FR900482 (**38**).

Significantly, **38** showed a 100 fold decrease in activity by a comparison of the IC_{50} values with **1**. The study demonstrated the importance of the stereochemistry of both the aziridine and C7 carbamoyl hydroxymethyl portion in the biological activity of these compounds.

Likewise, Fukuyama *et al.* investigated the biological activity of two intermediates **39** and **40** derived from a model system for an asymmetric total synthesis.[20j]



39



40

Figure 1.4. Fukuyama's synthetic analogs **39** and **40**.

In contrast to Terashima's findings, **39** showed activity against U937 human leukemia cells *in vitro* nearly identical to FR900482. Additionally, the epoxide **40** proved much less effective, with a 100 fold decrease in activity. It is not clear whether this decrease in activity was a result of the replacement of the aziridine ring by an epoxide or was a consequence of the *cis*- relationship between the C7 segment and the three-membered ring. Notably, although analog **39** demonstrated cytotoxicity comparable to **1**, circumventing the potential for oxidation state change activity at C12 by the replacement of the aldehyde with a methyl group, may have serious implications on the *in vivo* tumor selectivity.

Clearly, the results of these studies combined with the semi-synthetic studies from Fujisawa Pharmaceutical Company, demonstrate the importance of synthetic work on these natural products.

Section 1.2 Previous Total Syntheses of FR900482

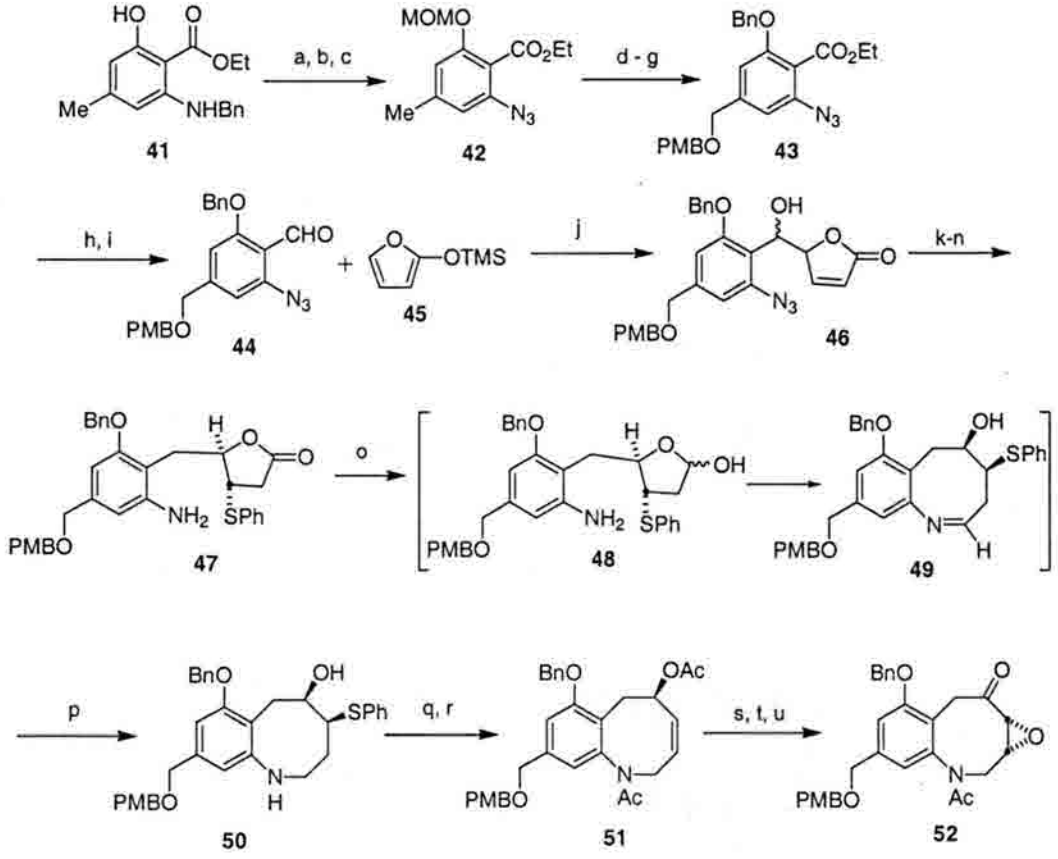
The significant biological activity of natural products FR66979 and FR900482, along with the clinical applications of various semi-synthetic derivatives, makes this family of compounds attractive for synthetic studies. Additionally, the dense functionality in such compact structures provides a formidable challenge for total synthesis. Prior to

our work, only three total syntheses[18] and one formal total synthesis of FR900482 had been reported,[19] two of which were racemic. In addition to these publications, several synthetic studies exploring alternative methods for installing the complex functionalities of the natural product have been disclosed.[20]

1.2.1 Fukuyama's Initial Total Synthesis of (±) FR900482

Fukuyama *et al.* reported the first total synthesis of (±) FR900482 just five years after its discovery in 1992.[18a] In addition to being racemic, the synthesis proved to be quite lengthy, requiring 43 synthetic steps. Despite this, many of the challenges in the construction of key functionalities, including the aziridine portion and the unique hydroxylamine hemiketal, were addressed. Indeed, much of the synthetic strategy employed is reminiscent of the total syntheses of the related mitomycins, namely Kishi's historic syntheses of Mitomycin A and C in the late 1970's and Fukuyama's improved total synthesis of Mitomycin C that followed a decade later.[21,22]

Scheme 1.6. Fukuyama's total synthesis of FR900482 Part I (43 steps).



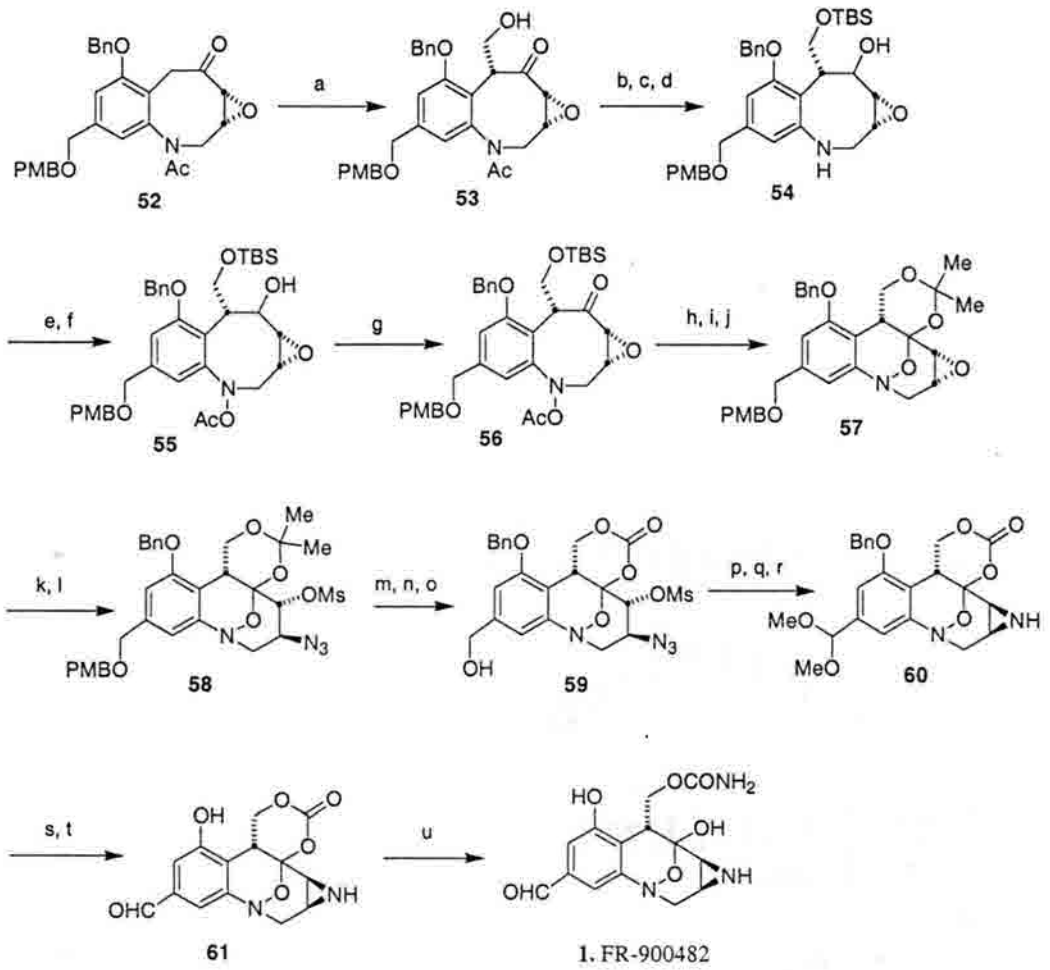
Key: (a) Pd/C, H_2 (1200 psi), HCO_2H, EtOH, 23 °C, 2 h; (b) $NaNO_2$, HCl, EtOH/H_2O, 0 °C, 20 min, then NaN_3, 0 °C, 40 min; (c) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, 23 °C, 98% for three steps; (d) NBS, $(BzO)_2$, PhH, reflux, 2 h; (e) *p*-$MeOC_6H_4OH$, K_2CO_3, DMF, 70 °C, 15 min, 47% for two steps; (f) TFA, CH_2Cl_2, 23 °C, 3 h; (g) BnCl, K_2CO_3, DMF, 80 °C, 98% for two steps; (h) DIBAL, CH_2Cl_2, -78 °C, 100%; (i) PCC, CH_2Cl_2, 23 °C, 98%; (j) 2-(trimethylsiloxy)furan, $SnCl_4$, CH_2Cl_2, -78 °C, 5 min, then HCl, THF/H_2O, 23 °C, 96%; (k) PhSH, Et_3N, CH_2Cl_2, 23 °C, 30 min; (l) Ac_2O, Py, 23 °C, 2 h; (m) Et_3SiH, BF_3•Et_2O, CH_2Cl_2, 23 °C; (n) Zn, AcOH, Et_2O/CH_2Cl_2, 23 °C, 47% for four steps; (o) DIBAL, CH_2Cl_2, -78 °C; (p) $NaBH_3CN$, TFA, CH_2Cl_2/MeOH, 23 °C, 10 min, 83% for two steps; (q) Ac_2O, Py, 60 °C; (r) *m*-CPBA, CH_2Cl_2, 0 °C, then PhMe, sealed tube, 170 °C, 7 h, 71% for two steps; (s) NaOH, MeOH, 23 °C; (t) *m*-CPBA, 23 °C, 4 h; (u) DMSO, $(ClCO)_2$, CH_2Cl_2, then Et_3N, 92% for three steps

The key steps in Fukuyama's FR900482 synthesis involve the formation of the eight-membered ring, a stereospecific aldol reaction to install the C9 hydroxymethyl

portion, construction of the hydroxylamine hemiketal, and a late stage installation of the aziridine portion. Lewis acid-mediated addition of 2-(trimethylsilyloxy)furan (**45**) to the azido benzaldehyde **46**, derived from ethyl benzoate **41**, provided the precursor for constructing the eight-membered ring. Thus, following Michael addition of thiophenol, reductive elimination of the secondary alcohol, and zinc-mediated reduction of the azide, substrate **47** was obtained over 5 synthetic steps. Reduction with DIBAL afforded the lactol, which in turn gave the intermediate imine (**48**), and subsequent *in situ* reduction ($NaCNBH_3$/TFA) produced the eight-membered cyclized intermediate **50**. Further elaboration gave the epoxy ketone **52**.

A stereospecific aldol reaction, utilizing LiOH and 38% aqueous formaldehyde, afforded the hydroxymethyl compound **53** possessing the desired *cis*-configuration relative to the epoxide (Scheme **1.7**). Presumably, the boat configuration of the eight-membered ring allowed hydrogen bonding between the hydroxyl group and the epoxide, dictating the stereochemical outcome of the reaction. The next challenge proved to be installing the hydroxylamine hemiketal portion of the molecule. The main difficulty encountered at this stage was the need to circumvent unmasking the secondary amine in the presence of the ketone. Such an event would lead to transannular cyclization and formation of the mitosene. Following reduction of the ketone **53** with $NaBH_4$ and selective protection of the primary hydroxyl group, the acetamide was reduced with DIBAL to afford the free secondary amine **54**. Subsequent oxidation of the secondary amine with mCPBA to the hydroxylamine and protection as the acetate permitted the

Scheme 1.7. Fukuyama's total synthesis, Part II.



Key: (a) CH_2O, LiOH, THF/H_2O, 0 °C, 2 h; (b) $NaBH_4$, EtOH, -78 to 23 °C, 71% for two steps; (c) TBSCl, Im, DMAP, CH_2Cl_2, 23 °C, 92%; (d) DIBAL, PhMe, -78 °C, 64%; (e) *m*-CPBA, CH_2Cl_2, 23 °C; (f) Ac_2O, 23 °C, 10 h, 83% for two steps; (g) DMSO, $(ClCO)_2$, CH_2Cl_2, then Et_3N, 83%; (h) NH_2NH_2, $MeOH/CH_2Cl_2$, 23 °C; (i) TBAF, THF, 23 °C, 96% for two steps; (j) dimethoxypropane, CSA, CH_2Cl_2, 23 °C, 100%; (k) NaN_3, DMF/H_2O, 125 °C, 6 h; (l) MsCl, Et_3N, CH_2Cl_2, 23 °C, 89% for two steps; (m) TFA, CH_2Cl_2, 23 °C, 10 min; (n) phosgene, Py, CH_2Cl_2, 23 °C; (o) CAN, CH_3CN/H_2O, 23 °C, 74% for three steps; (p) PCC, $MgSO_4$, CH_2Cl_2, 23 °C; (q) $CH(OMe)_3$, CSA, MeOH, 23 °C, 76% for two steps; (r) Ph_3P, *i*-Pr_2NEt, THF/H_2O, 60 °C, 30 min, 71%; (s) Pd/C, H_2 (1 atm), EtOH, 23 °C, 2 h, 100%; (t) $HClO_4$, THF/H_2O, 23 °C, 96%; (u) NH_3, CH_2Cl_2, 23 °C, 2 h, 95%.

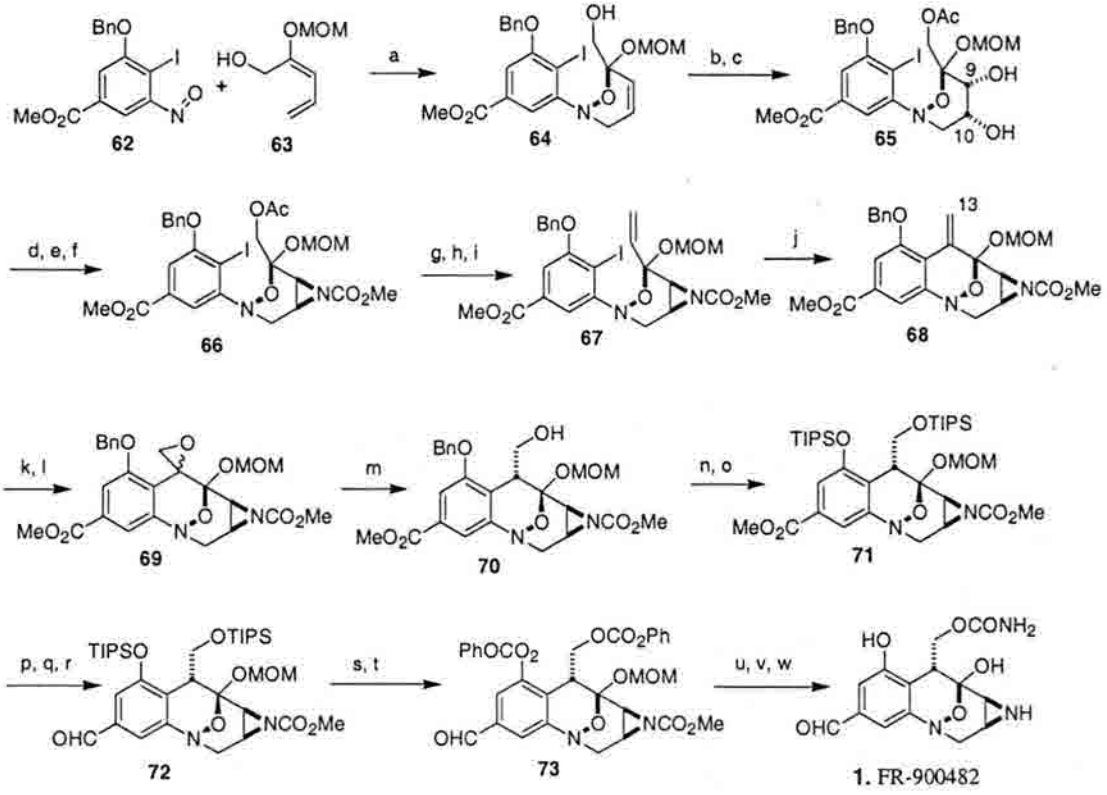
reinstallation of the ketone by Swern oxidation of the secondary alcohol (**56**). Finally, cleavage of the acetate group with hydrazine unmasked the hydroxylamine, which spontaneously closed to furnish the bicyclic hydroxylamine hemiketal.

In a manner similar to Kishi's mitomycin C synthesis, a Staudinger reduction was employed to install the aziridine ring in the final stage of the synthesis.[21] Due to the labiality of the aziridine functionality under acidic conditions, this strategy proved more convenient than having to carry this moiety through the majority of the synthetic route. After switching protecting groups on the hydroxylamine hemiketal and the hydroxymethyl portion of the molecule, the epoxide ring was opened with sodium azide. The secondary alcohol was treated with methanesulfonyl chloride and the resulting azido mesylate aziridine precursor was carried until the end of the synthesis. Following oxidation of C12 and several protecting group manipulations, a Staudinger reduction of the azide with triphenyl phosphine resulted in nucleophilic displacement of the mesylate to furnish aziridine **60** in 71% yield. Removal of the protecting groups and regioselective opening of the carbonate with ammonia furnished (±) FR900482, which shared identical spectroscopic properties and TLC behavior with an authentic sample of the natural product. Considering that Fukuyama's work represents the first successful synthesis of the natural product, the difficulties encountered and solutions developed in constructing the various complex functionalities for the first time should not be overshadowed by the lengthy sequence or racemic nature of the synthesis.

1.2.2 Danishefsky's Total Synthesis

Three years after Fukuyama's initial total synthesis, Danishefsky *et al.* completed the second racemic total synthesis of FR900482 in 1995.[18b] Several key steps in the synthesis allowed for a more concise construction of the molecule and provided the natural product in 34 steps, a noteworthy improvement over Fukuyama's synthesis.

Scheme 1.8. Danishefsky's total synthesis of FR900482 (34 steps)



Key: (a) PhH, 80 °C, 80%; (b) $(Ac)_2O$, Py, CH_2Cl_2, 22 °C, 92%; (c) OsO_4, $Me_3NO{\cdot}H_2O$, CH_2Cl_2/PhH, 22 °C, 71%; (d) $(Tf)_2O$, Py, CH_2Cl_2, 0 °C; (e) *n*-Bu_4NN_3, DMF, 22 °C, 74% for two steps; (f) i) $(Tf)_2O$, Py, CH_2Cl_2, 0 °C; ii) Ph_3P, THF, then NH_4OH; iii) $ClCO_2Me$, Py, CH_2Cl_2, 0 °C, 72%; (g) K_2CO_3, MeOH, 22 °C, 100%; (h) DMSO, $(ClCO)_2$, CH_2Cl_2, -78 °C then Et_3N; (i) Ph_3PCH_3Br, NaHMDS, THF, -20 °C, 75% for two steps; (j) $(Ph_3P)_4Pd$, Et_3N, CH_3CN, 90 °C, 18 h, 93%; (k) OsO_4, NMO, acetone/H_2O, 22 °C, 90%; (l) DIAD, Ph_3P, THF, 22 °C, 24 h, 86%; (m) SmI_2, *N,N*-dimethylethanolamine, THF, -78 °C, 86 to 92%; (n) Pd/C, H_2, EtOH, 30 min, 93%; (o) TIPSOTf, *i*-Pr_2NEt, CH_2Cl_2, 0 °C, 98%; (p) DIBAL, hexane/CH_2Cl_2, -78 °C, 93%; (q) *N*-((methoxycarbonyl)oxy)succinimide, Py, 22 °C, 2 h, 93%; (r) MnO_2, CH_2Cl_2, 22 °C, 1 h, 85%; (s) TBAF, THF, 22 °C, 12 h, 100%; (t) $ClCO_2Ph$, *i*-Pr_2NEt, CH_2Cl_2, 22 °C, 100%; (u) Ph_3CBF_4, di-*tert*-butylpyridine, CH_2Cl_2, 0 to 22 °C, 15 to 30 min, 75%; (v) NH_3, CH_2Cl_2, *i*-PrOH, 22 °C, 6 h, 80%; (w) K_2CO_3, MeOH/H_2O, 22 °C, 24 h, 76%.

The highlight of the synthesis proved to be a hetero Diels-Alder reaction between the nitroso iodobenzoate species **62**, prepared in eight steps from methyl vanillate (32%

overall yield), and the requisite diene **63**. The cycloaddition furnished the MOM-protected hydroxylamine hemiketal functionality in a single operation, avoiding the need for the lengthy manipulations employed in Fukuyama's synthesis. Following acetylation of the hydroxyl group, the aziridine ring was constructed in a manner similar to the initial FR900482 and mitomycin C syntheses. Reaction with OsO_4 produced the diol **65** in racemic fashion, which in turn was selectively converted to the C10 triflate. Displacement of the triflate by reaction with terabutylammonium azide, and conversion of the remaining alcohol to a triflate, set the stage for aziridine formation through a Staudinger reduction. Reduction of the azide with triphenyl phosphine furnished the corresponding free aziridine, which was protected as the methyl carbamate. Incorporation of the hydroxymethyl portion at C7 proved to be the next challenge of the synthesis. Following ring closure by Heck arylation, osmylation of the exocyclic methylene afforded the diol with high selectivity (10:1), presumably due to the approach from the less hindered β-face of the bicyclic ring system. Subsequent epoxide formation under Mitsonobu conditions and reductive opening of the oxirane with samarium iodide in the presence of *N,N*-dimethylethanolamine secured the hydroxymethyl group with the desired stereochemistry (**70**). In addition, the epoxide derived from the minor diastereomer of the osmylation also afforded the identical hydroxymethyl product. This observation led to a proposed sp^2-hybridized intermediate (at C7) in the reduction of both epoxides, which in turn undergoes kinetic protonation from the less-hindered β-face.

DIBAL reduction of both carbomethoxyl groups, reprotection of aziridine as the methyl carbamate, and oxidation furnished the aldehyde portion of target compound **72**. After exchanging the silyl protecting groups for phenyl carbonates the remainder of the

synthesis was completed through installation of the urethane segment by treatment of C14 phenyl carbonate with ammonia, and deprotection of the aziridine and both hydroxyl groups over three steps. The mild conditions employed for the hydrolysis of the methyl carbamate from the aziridine (K_2CO_3, $MeOH/H_2O$) warrants further comment. Under normal circumstances, liberation of a carbomethoxy-protected nitrogen requires strong reducing/hydrolytic conditions. In the case of an azirdine ring, however, the minimal overlap of the nitrogen lone pair with the carbonyl of a carbamoyl or acyl group allows for easier hydrolysis or reduction of such groups.

In addition to the reduction of synthetic steps and a key hetero Diels-Alder reaction, Danishefsky's synthesis demonstrates a successful protection strategy for the aziridine functionality, one that will be used in future synthetic studies, including our own.

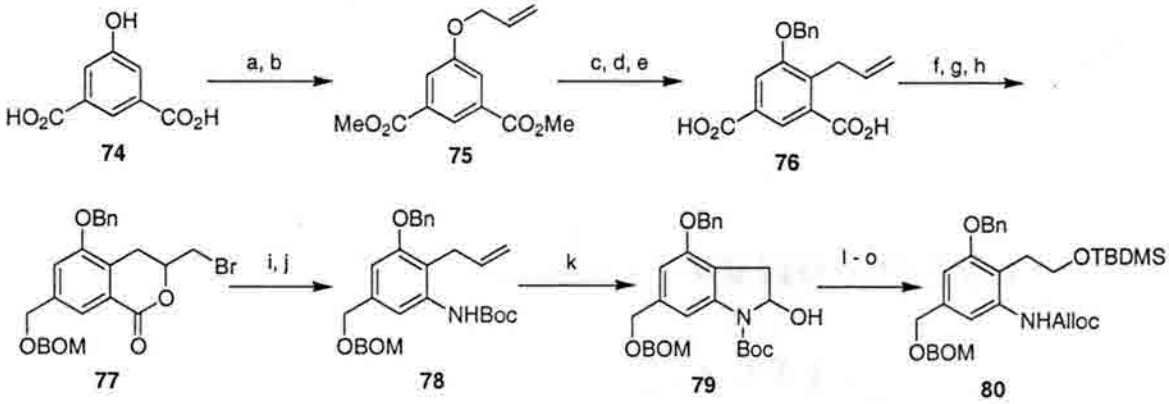
1.2.3 Terashima's Total Synthesis

Terashima *et al.* reported the first enantiospecific total synthesis of (+)-FR900482 in 1996 and 1997 over a series of seven publications.[18c-h] By starting with L-diethyl tartrate the template on which the enantiomerically pure aziridine ring could be constructed was present from the beginning of the synthesis. This strategy avoided the problem of constructing the aziridine during the late stages of the synthesis. Although the asymmetric nature of the synthesis underlies the importance of the work, the sheer number of synthetic steps (57 in total) detracts from its synthetic appeal.

The synthesis relies on the convergent coupling of an aromatic portion of the target compound with an aliphatic fragment 30 steps into the synthesis. The aromatic segment was prepared in 15 steps from 5-hydroxyisophtalic acid (**74**) following standard

synthetic operations. Noteworthy steps include differentiation of the carboxylic acids by formation of the bromolactone and the formation of the BOC-protected aniline **78** through a Curtius rearrangement.

Scheme 1.9. Terashima's total synthesis of (+) FR900482, Part I (57 steps total).

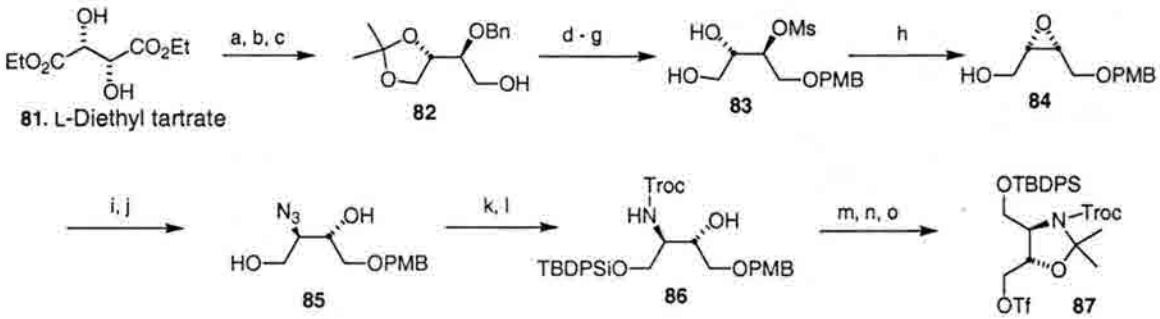


Key: (a) $SOCl_2$, MeOH, reflux, 100%; (b) allylbromide, K_2CO_3, acetone, reflux, 98%; (c) *N,N*-diethylaniline, reflux, 88%; (d) BnBr, K_2CO_3, acetone, reflux, 99%; (e) 2M NaOH, THF, reflux, 95%; (f) Br_2, aq $NaHCO_3$, $CHCl_3$, 0 °C, 72%; (g) $ClCO_2i$-Pr, Et_3N, THF, then $NaBH_4$, H_2O; 81%; (h) BOMCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 85%; (i) Zn, NH_4Cl, EtOH/H_2O, 81%; (j) DPPA, Et_3N, *t*-BuOH, rt to reflux, 76%; (k) OsO_4, $NaIO_4$, dioxane/H_2O, rt, 73%; (l) $NaBH_4$, EtOH, rt, 100%; (m) TBSCl, Et_3N, DMAP, CH_2Cl_2, rt, 97%; (n) TBSOTf, Py, CH_2Cl_2, rt, then TBAF, 92%; (o) AllocCl, aq $NaHCO_3$, CH_2Cl_2, rt, 98%.

The aliphatic portion of the molecule was derived from L-diethyl tartrate, which was selectively protected following a known sequence of reactions to give intermediate **82** and subsequently converted to the optically pure epoxide **84** (Scheme 1.10). Initially, it was realized that the epoxide could be more directly accessed through a Sharpless asymmetric epoxidation of the allylic alcohol in approximately 85% ee, but with an enantiomerically pure synthesis being one of the goals of the work, this alternative

strategy was not utilized. Opening of the epoxide with sodium azide, TBDPS protection of the primary alcohol, and Staudinger reduction, gave the intermediate amino alcohol. Not surprisingly, the ring opening gave a poor regioselectivity (3:2 favoring **85**) and isolation of the product from the undesired 1,2 diol was accomplished by chemoselective reaction of the mixture with sodium periodate allowing the resulting azido aldehyde byproduct to be readily separated. Further protection of the amino alcohol to the oxazoline and conversion to primary triflate **87**, furnished the aliphatic segment.

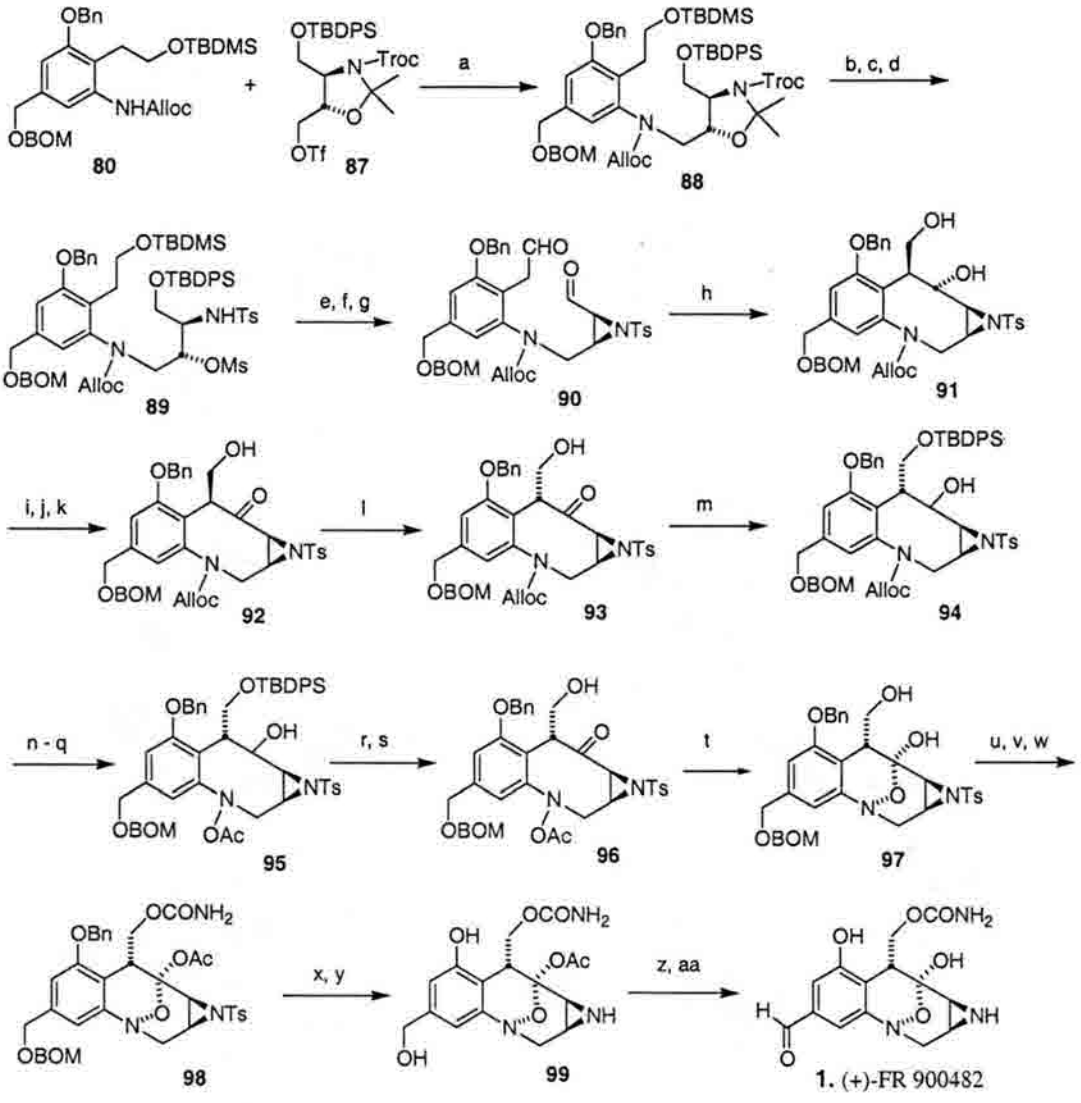
Scheme 1.10. Terashima's total synthesis of (+) FR900482, Part II.



81. L-Diethyl tartrate **82** **83** **84** **85** **86** **87**

Key: (a) PhCHO, *p*-TsOH, PhH; (b) $LiAlH_4$, $AlCl_3$, THF; (c) dimethoxypropane, acetone, 68% for three steps; (d) NaH, PMBCl, DMF, rt, 97%; (e) Raney Ni, H_2, EtOH, rt, 93%; (f) MsCl, Et_3N, CH_2Cl_2, 0 °C, 100%; (g) conc. HCl, MeOH, rt, 97%; (h) K_2CO_3, MeOH, rt, 88%; (i) NaN_3, NH_4Cl, EtOH, relux, 92%; (j) $NaIO_4$, THF/H_2O, rt, 55%; (k) TBSCl, Et_3N, DMAP, CH_2Cl_2, rt, 91%; (l) Ph_3P, THF/H_2O, rt, then TrocCl, aq $NaHCO_3$, rt, 98%; (m) TsOH, dimethoxypropane, acetone, rt, 97%; (n) DDQ, CH_2Cl_2/H_2O, rt, 98%; (o) Tf_2O, Et_3N, CH_2Cl_2, -78 °C, 94%.

Scheme 1.11. Terashima's total synthesis of (+) FR900482, Part III.



Key: (a) NaH, THF, -78 °C to rt, 100%; (b) Zn, AcOH, THF/H_2O, rt; (c) TsCl, Et_3N, DMF, 0 °C to rt, 77% for two steps; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 94%; (e) NaH, Im, THF, reflux, 92%; (f) HF•Py, Py, 0 °C, 99%; (g) DMPI, CH_2Cl_2, rt, 98%; (h) LiHMDS, THF, -78 to -5 °C, then $NaBH_4$, H_2O, -5 to 0 °C, 42%; (i) TBSCl, Et_3N, DMAP, CH_2Cl_2, rt, 79%; (j) DMPI, CH_2Cl_2, rt, 93%; (k) HF•Py, Py, 0 °C to rt, 93%; (l) DBU, THF, rt; separation (64% for desired isomer, 31% for undesired); (m) $NaBH_4$, THF/H_2O, 0 °C to rt, 87%; (n) TBDPSCl, Et_3N, DMAP, CH_2Cl_2, rt, 71%; (o) $(Ph_3P)_4Pd$, Ph_3P, THF, rt, 83%; (p) *m*-CPBA, CH_2Cl_2, -5 °C, 67%; (q) Ac_2O, $NaHCO_3$, rt, 69%; (r) DMPI, CH_2Cl_2, rt, 88%; (s) HF•Py, Py, 0 °C to rt, 88%; (t) K_2CO_3, MeOH, 0 °C to rt, 89%; (u) $ClCO_2CCl_3$, Py, 0 °C to rt, 81%; (v) NH_3, THF, 0 °C to rt, 94%; (w) Ac_2O, Py, DMAP, rt, 87%; (x) sodium naphthalenide, DME, -70 °C, 84%; (y) 10% Pd/C, H_2, EtOAc, rt, 81%; (z) DMSO, $(ClCO)_2$, Et_3N, CH_2Cl_2, -78 °C, 88%; (aa) NH_3, MeOH, rt, 73%.

Coupling of the two segments was accomplished by S_N2 displacement of the triflate **87** with the anion of the deprotonated Alloc-protected aniline (**80**). Further elaboration furnished the aziridine ring as the N-protected toluene sulfonamide. The key step in the synthesis proved to be formation of the eight-membered ring through an intramolecular aldol reaction. Thus, following desilylation and oxidation, dialdehyde **90** underwent the desired intramolecular addition upon treatment with LiHMDS. Following *in situ* reduction of the resulting hydroxy aldehyde ($NaBH_4$), diol **91** was isolated in 48% yield along with 33% unreacted substrate (isolated as the reduced uncyclized diol). Unfortunately, this stereoselective reaction resulted in a product possessing exclusively the C7 configuration opposite that of the natural product. Selective silylation, oxidation to the ketone, and deprotection with HF-pyridine furnished the hydroxyketone **92** affording a substrate that could be epimerized. Reaction with diazabicyclo[4,5,0]undec-7-ene (DBU) in THF over two hours gave a ~ 2:1 mixture of diastereomers favoring the product with the desired stereochemistry (**93**). Longer reaction times resulted in formation of the exocyclic methylene derivative resulting from elimination of the hydroxyl component. In nearly the identical manner as in Fukuyama's synthesis, the hydroxylamine hemiketal portion of the molecule was constructed from ketone **93** over 8 steps. The completion of the synthesis included formation of the carbamate at C13 through the cyclic carbonate and reaction with ammonia, deprotection of the aziridine and hydroxyl functionalities, and oxidation of the C12 hydroxymethyl group to the aldehyde. Notably, oxidation in the presence of the free aziridine was successful only when using Swern conditions; a variety of other oxidative methods explored led to decomposition.

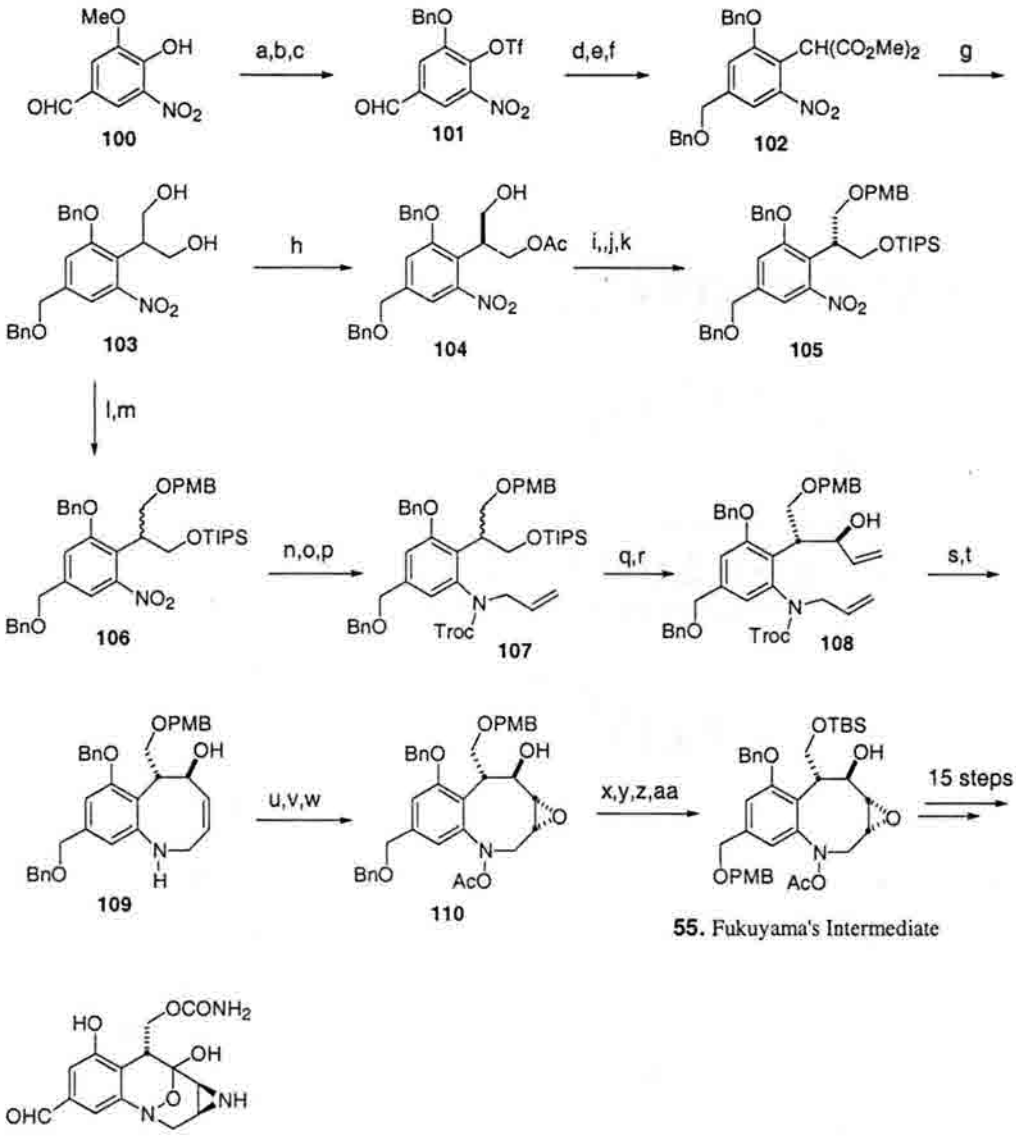
Terashima's synthesis proved important in providing the natural product in optically pure form, thereby confirming the absolute stereochemistry of the molecule. In addition, *epi*-FR900482, whose biological activity was previously discussed, was synthesized starting from the antipode of the diethyl tartrate. Finally, the work demonstrates the importance of a well-developed protecting group scheme for the total synthesis of this natural product, as evidenced by the numerous synthetic steps dedicated to protecting group manipulations.

1.2.4 Martin's Formal Total Synthesis

Recently, Martin *et al.* reported a formal total synthesis of (±) FR900482 that in principle could be applied to an asymmetric variant (Scheme **1.12**).[19] The key step utilized a ring-closing metathesis (RCM) reaction to construct an unsaturated eight-membered ring, which in turn could be elaborated to the target compound. The application of the RCM reaction followed preliminary reports on studies with functionally simpler model systems.

Starting from 5-nitrovanillian (**100**), three synthetic operations gave triflate **101**, which in turn was transformed into the malonate following displacement of the triflate with the malonate anion. Reduction with DIBAL afforded the 1,3-diol **103** in modest yield (38%). At this stage two separate routes were reported, one in which the ability to derive the protected diol in optically active form was demonstrated, with the alternative scheme producing the protected diol **106** in racemic fashion. Asymmetric desymmetrization of 1,3-diol **103** with *Pseudomonas* species lipase (PSL) afforded the optically active monoacetate (**104**) in 68% yield (94% ee).

Scheme 1.12. Martins Formal Total Synthesis of (±) FR900482 (38 steps).



Key: (a) HBr, HOAc, reflux, 82%; (b) NaH, BnBr, DMF, 0 °C; (c) Tf_2O, Py, CH_2Cl_2, 0 °C to rt, 78% for two steps; (d) $NaCH(CO_2Me)_2$, DMF 80%; (e) $NaBH_4$, THF, 0 °C; (f) NaH, BnBr, 0 °C to rt, 90% for two steps; (g) DIBAL, PhMe, 0 °C, 38%; (h) PSL, vinyl acetate, 4 Å sieves, 35 °C, 74%, 94% ee; (i) TIPSCl, Im, DMF, rt; (j) K_2CO_3, MeOH, rt, 50% for two steps; (k) $Cl_3CC(=NH)OMPM$, CH_3CN, rt, 62%; (l) NaH, MPMCl, DMF, rt; (m) TIPSOTf, 2,6 lutidine, CH_2Cl_2, 0 °C, 75% for two steps; (n) Raney Ni, H_2, THF/MeOH, rt, 99%; (o) TrocCl, K_2CO_3, CH_2Cl_2, 0 °C; (p) KH, allylbromide, DMF, 0 °C, 83% for two steps; (q) HF•Py, THF, 0 °C; (r) DMSO, $(ClCO)_2$, Et_3N, CH_2Cl_2/THF, -78 °C, then vinylmagnesium bromide, 0 °C, 65% for two steps; (s) $Cl_2(PCy_3)_2Ru{=}CH_2Ph$, Ph, 65 °C, 78%; (t) Zn, THF/HOAc, rt, 80%; (u) mCPBA, CH_2Cl_2, 0 °C; (v) Ac_2O, 0 °C to rt, 89% for two steps; (w) mCPBA, CH_2Cl_2, rt, 61%; (x) TFA, CH_2Cl_2, 0 °C; (y) TBSCl, DMAP, Im, CH_2Cl_2, rt, 61% for two steps; (z) 10% Pd/C, H_2, EtOAc, rt, 42%; (aa) *p*-$MeOC_6H_4OH$, PPh_3, DEAD, PhMe, rt, 80%.

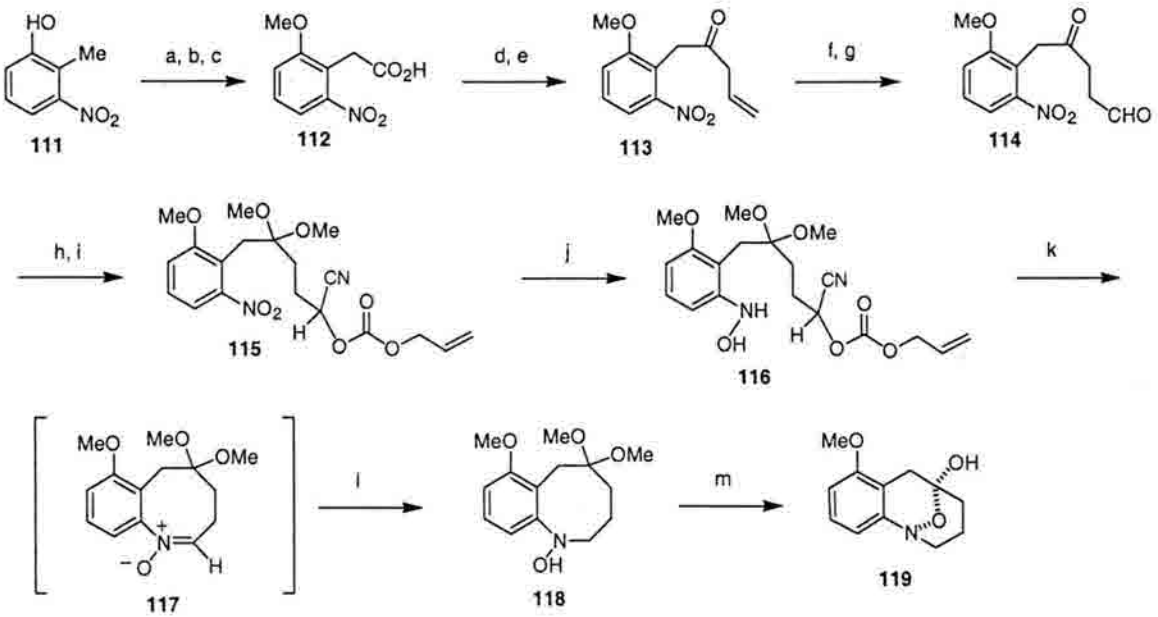
The resulting alcohol was protected as the triisopropylsilyl (TIPS) ether, which, after hydrolysis of the acetate, was converted to the PMB-protected diol (**105**). Additionally, the 1,3-diol **103** could be directly protected through similar conditions to give the achiral equivalent **106**. Due to the high cost of the enzymatic desymmetrization, and the low yield in the conversion to **105**, the racemic version of the protected diol was used for the completion of the formal synthesis. In the event, racemic **106** was elaborated to the N-Troc-protected allyl amine **107**. Desilylation, oxidation to the aldehyde, and addition of vinylmagnesium bromide afforded the diene with the resulting secondary alcohol *trans*- to the protected hydroxymethyl group. For the RCM reaction, the ruthenium-based Grubbs' catalyst was chosen, based on this catalyst's ability to tolerate free hydroxyl groups. Reaction of diene **108** under dilute conditions afforded the desired unsaturated eight-membered ring in 78% yield. Unable to install the aziridine ring in a stereocontrolled manner, a formal total synthesis was opted for by conversion of **110** into a late stage intermediate of Fukuyama's synthesis[18a] over eight steps.

The application of an RCM reaction for the construction of the natural product was successfully demonstrated, providing a slightly improved synthesis of a late stage intermediate in Fukuyama's racemic synthesis (38 total steps vs. 43 overall). The ability to derive the intermediate in optically active form with the insertion of the enzymatic desymmertization provides an alternative means of synthesizing (+)-FR900482 in 40 steps.

1.4 Model Studies

Since the isolation of FR900482 in 1987, several synthetic studies have been reported in addition to the total syntheses.[20] Initial studies involved methods for the construction of the unique hydroxylamine hemiketal functionality, the aziridine portion, and various eight-membered ring synthons. Indeed, Williams and Yasuda published the first synthetic study in 1989 describing the synthesis of the hydroxylamine hemiketal portion on a model substrate.[20a]

Scheme 1.15. Williams' and Yusuda's model study.



Key: a) MeI, K_2CO_3, acetone; (b) CH_2O, KOH, DMSO; (c) Jones oxidation, 74% for three steps; (d) $SOCl_2$; (e) $TiCl_4$, allyltrimethyl silane, 68% for two steps; (f) 9-BBN, H_2O_2, NaOH; (g) DMSO, $(ClCO)_2$, CH_2Cl_2, then Et_3N, 84%; (h) NaCN, allyl chloroformate, 94%; (i) $(MeO)_3CH$, H_2SO_4, MeOH, 89%; (j) Zn^0, NH_4Cl, THF/H_2O, 75%; (k) $Pd(Ph_3P)_4$, Ph_3P, THF, -20 °C; (l) 10 eq. $NaCNBH_3$, MeOH, rt, 51% for two steps; (m) 1N HCl, THF, rt, 64%.

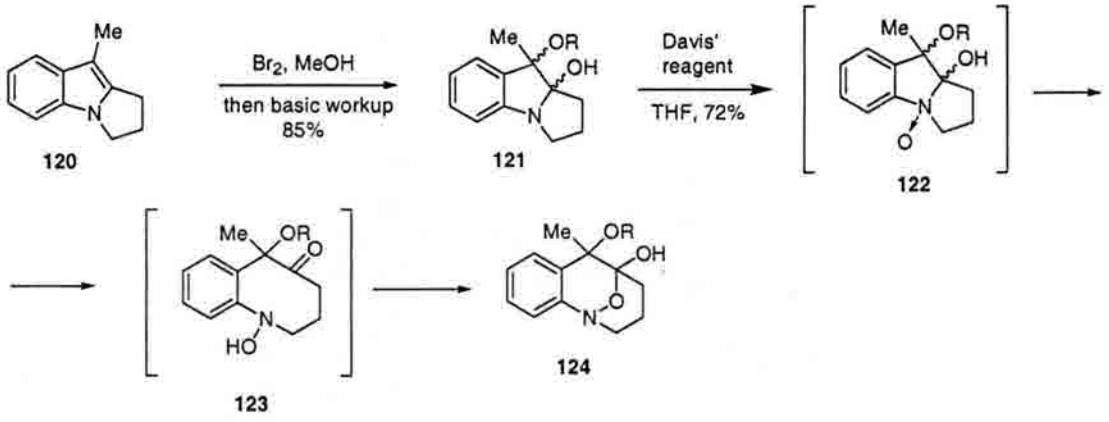
The synthetic strategy (Scheme **1.15**) relied on masking the carbonyls of intermediate **114** (derived from 2-methyl-3-nitroanisole in 7 steps) as the novel cyanohydrin allyl carbonate and dimethoxyacetal (**115**). Following Zn-mediated

reduction of the nitro function to the hydroxylamine; the cyanohydrin was selectively unmasked by cleavage of the allyl carbonate whereupon the cyclic nitrone **117** was spontaneously formed. Immediate reduction with sodium cyanoborohydride furnished the hydroxylamine. The ketone was unveiled through acid hydrolysis of the ketal, allowing ring closure to the hydroxylamine hemiketal **119** in 64% yield. Even with the work that soon followed, including the aforementioned total syntheses, the study still remains as a landmark for the first synthesis of this functionality.

Among other model studies,[20b,c] much attention has been focused on developing an efficient means to construct the hydroxylamine hemiketal functionality from an indole core. With the exception of Danishefsky's synthesis, this functionality has proven to be a hurdle in the total syntheses, requiring a multitude of tedious synthetic operations in the latter stages. Dmitrienko's pioneering work in this area has led to the development of an oxidative rearrangement from a dihydroindole core.

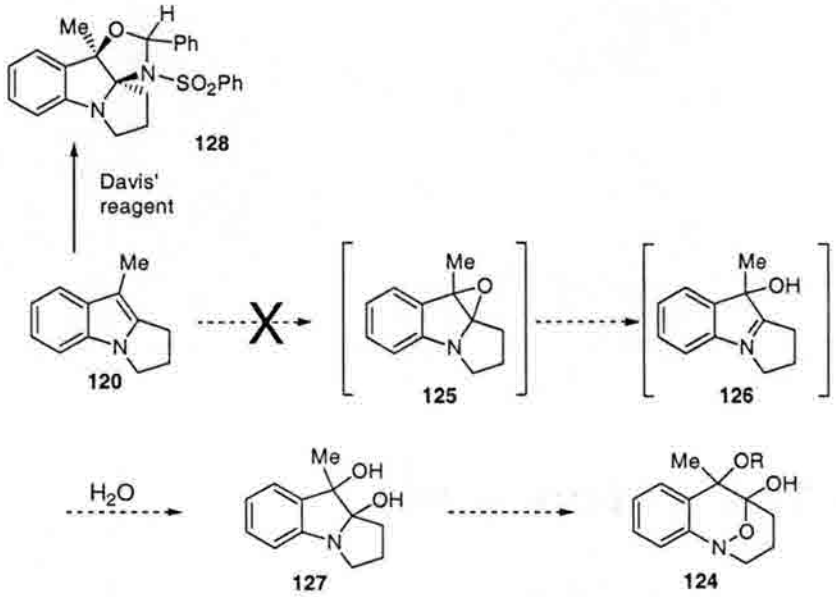
In a 1992 report, Dmitrienko *et al.* described how initial carbonolamine formation of a pyrrolo[1,2a]-indole (Br_2 and MeOH) (see Scheme **1.16**), provided a suitable precursor to the hydroxylamine hemiketal.[20d] Oxidation of the carbonolamine nitrogen of **121** with Davis' reagent or mCPBA furnished, presumably through the N-oxide, the hydroxylamine ketone intermediate **123**, which subsequently closed to give the desired bicyclic hemiketal bond.

Scheme 1.16. Dmitrienko's oxidative ring expansion model study.



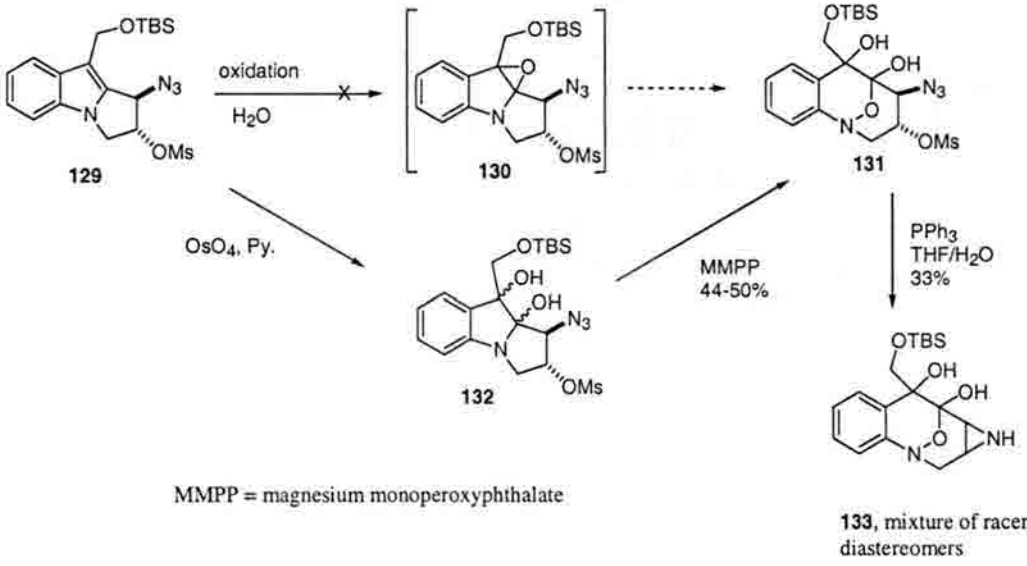
Ideally, a one-pot procedure was sought starting directly from the indole (Scheme **1.17**). Here, epoxidation of the 3-substituted pyrrolo[1,2a]-indole in the presence of water might lead directly to the diol following oxirane ring-opening by the indole nitrogen and addition of water. This intermediate could in turn undergo the so-called Dmitrienko rearrangement described above. Reaction of the starting pyrrolo[1,2a]-indole directly with Davis' reagent, however, resulted in only the formation of byproduct **128**.[20e]

Scheme 1.17. Unsuccessful oxidative ring expansion with Davis' reagent.



Recently Jimenez *et al.* explored this idea with a similar simple core structure.[20f] Substrate **129** (Scheme **1.18**) was subjected to reaction with Davis' reagent, dimethyldioxirane (DMDO), or 1,1,1-triflouromethyl, methyl dioxirane. All three oxidative conditions led to decomposition of the starting material with no desired product observed. Alternatively, a two-step procedure, as in Dmitrienko's system, afforded the desired hydroxylamine hemiketal (**131**).

Scheme 1.18. Jimenez's ring expansion model study



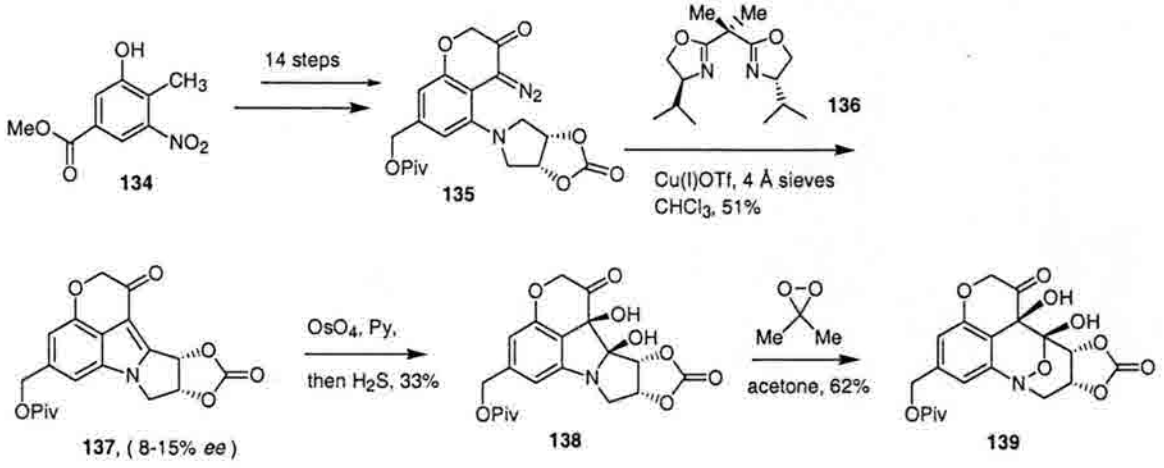
MMPP = magnesium monoperoxyphthalate

133, mixture of racemic diastereomers

Despite the inability to find a successful one-pot oxidative ring expansion, the two-step procedure has been employed on fully-functional core structures by two separate groups. One obstacle in applying this rearrangement to a total synthesis, however, seems to be the obligate formation of a quaternary substituted center at C7. Presumably, by formation of the quaternary C-7 center, elimination to the indole at the intermediate carbonolamine stage is precluded.

Sulikowski *et al.* reported the synthesis of diazoketone **135** from the known tri-substituted toluene derivative **134** over 14 steps (Scheme **1.19**).[20h] Based on results from a previous model study,[20g] an asymmetric oxidative insertion through a copper (I)-stabilized carbenoid with the chiral bis(oxazoline) ligand **136** was planned for constructing the mitosane core. Unfortunately, since higher temperatures were needed for the reaction to occur, the resulting enantioselectivity was minimal. Even more regrettably, the mitosene ring system was formed instead of the desired mitosane, the over-oxidation being attributed to the high copper (I) catalyst loading. Despite these disappointing results, mitosene **137** provided a suitable substrate for a Dmitrienko rearrangement. Thus, dihydroxylation with OsO_4 and DMDO oxidation of the resulting product furnished the hydroxylamine hemiketal **139**.

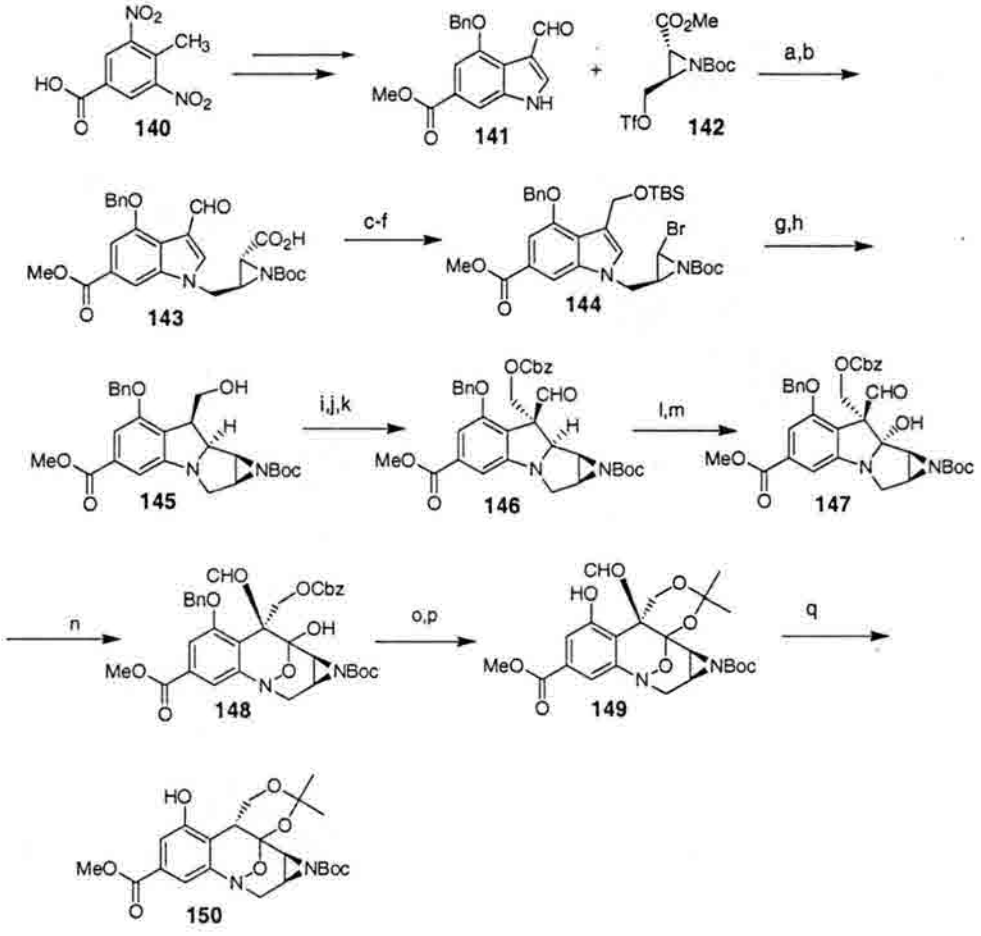
Scheme 1.19. Sulikowski's carbenoid insertion model study.



One year following Sulikowski's report in 1997, Ziegler *et al.* published a model study utilizing the Dmitrienko rearrangement to synthesis a late stage intermediate directed towards the total synthesis of FR900482.[20i] Significantly, the synthon **150** was

synthesized in asymmetric fashion and represents the most developed study using the oxidative ring expansion reported to date.

Scheme 1.20. Ziegler's radical cyclization and ring expansion model study.



Key: (a) NaHMDS, THF, -30 °C, 1 h, 66%;. (b) LiOH, 96%; (c) 2,2'-dithiobis(pyridine *N*-oxide), n-Bu_3P; (d) hν (visible), $BrCCl_3$, 69% for two steps; (e) $NaBH_4$, MeOH, 0 °C; (f) TBSCl, Im, CH_2Cl_2; (g) PhMe, ACCN, n-Bu_3SnH , 116 °C, 1 h; (h) TBAF, THF, 56% for four steps; (i) DMPI, CH_2Cl_2; (j) 37% CH_2O/H_2O, $NaHCO_3$, CH_2Cl_2/MeOH, 2 h, 86% (k) 1,1'-carbonyldiimidazole, CH_3CN, rt, 3.5 hr, then BnOH, DMAP, 63 °C, 3 h, 71%; (l) *m*-CPBA, CH_2Cl_2, 0 °C, 2 h, 98%; (m) Ac_2O, 24 h, 0 °C, then H_2O, 73%; (n) *m*-CPBA, 0 °C, 1.5 h, 81%; (o) 10% Pd/C, H_2, EtOH, 30 min, 92%; (p) dimethoxypropane, *p*-TsOH•H_2O, CH_2Cl_2, 20 min, 70%; (q) $(Ph_3P)_3RhCl$, Xy, 130 °C, 3.75 h, 77%

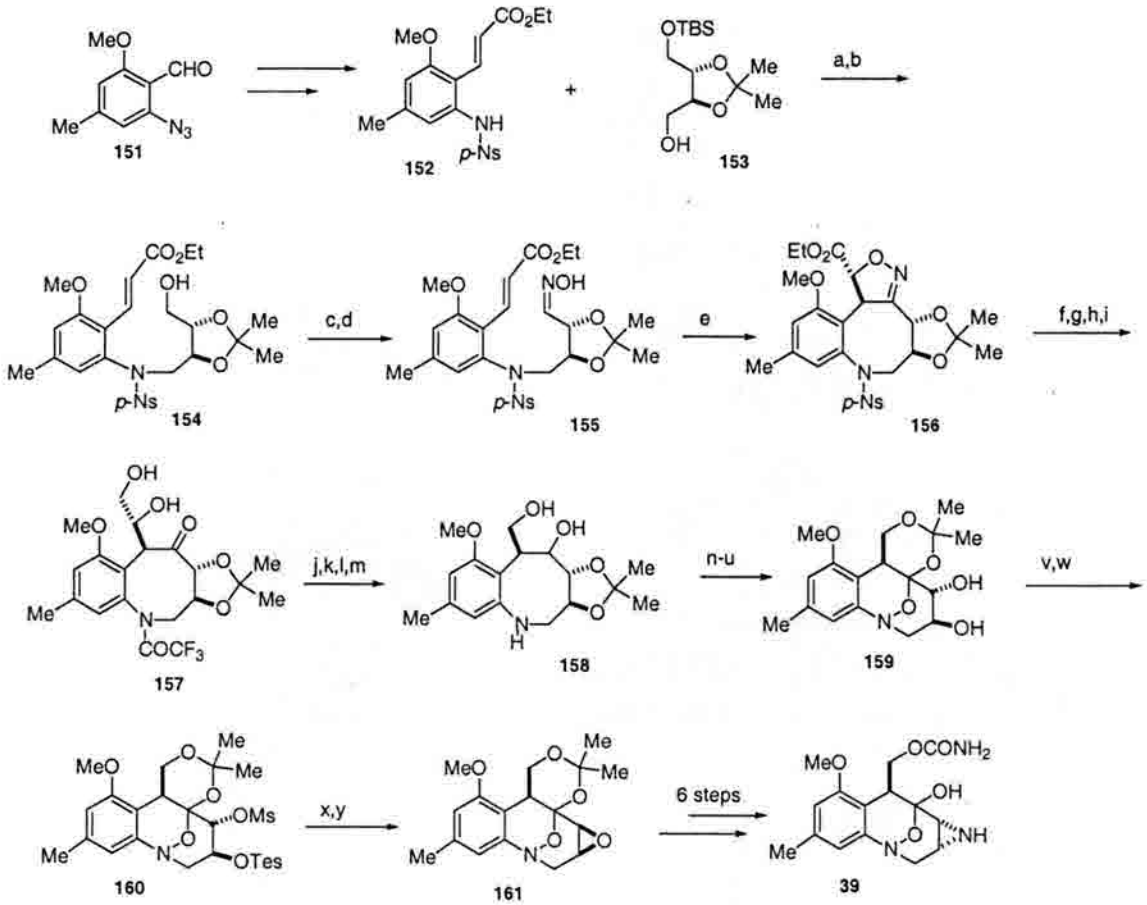
During the final stages of the model study, Ziegler *et al.* employed a series of interesting tactics to construct the core structure of the molecule. Indole **141**, synthesized from the corresponding tri-substituted benzoate, was coupled with the optically active aziridine triflate **142** derived from D-isoascorbic acid (Scheme **1.20**). The methyl ester was hydrolyzed, and subjected to a Barton-Samadi decarboxylation in $BrCCl_3$ to afford the bromoaziridine as 3.3:1 mixture of diastereomers. Following aldehyde reduction and protection, the mitosane bicyclic ring structure was installed by initial radical formation on the aziridine and subsequent reductive cyclization on the indole in the presence of n-Bu_3SnH. Desilylation of the crude mixture revealed nearly complete selectivity for the cyclized alcohol **145** possessing the stereochemistry shown. Oxidation to the aldehyde and aldol reaction with 38% aqueous formaldehyde furnished the hydroxymethyl group, which was protected as the benzyl carbonate over two steps. With a quaternary center at C7, the installation of the hydroxylamine hemiketal structure was carried out in two consecutive rearrangements. mCPBA oxidation to the N-oxide, acetylation, elimination to the imine, and addition of the acetate to the C8 imine carbon, effected a Polonovski rearrangement through the mitosane nitrogen furnishing the free carbonolamine **147** following aqueous workup. Dmitrienko rearrangement induced by mCPBA oxidation of the carbonolamine led directly to the hydroxylamine hemiketal **148** (58% yield over three steps). With the proper functionality installed at C8, the quaternary center at C7 was dismantled. Following deprotection of the primary alcohol and acetonide formation, decarbonylation with retention of configuration using Wilkinson's catalyst gave the desired product **150**, although the reaction reportedly was inconsistently reproducible. The capricious nature of the reaction combined with the possible incompatibility of the

Boc and acetonide groups with the acid-sensitive aziridine ring may provide reasons for why the total synthesis has not been completed. No further publications on this work have been reported to date.

Fukuyama *et al.* disclosed their progress towards an asymmetric synthesis of (+) FR900482 in 2001.[20j] Although much of the work relied on synthetic steps previously reported in their 1992 racemic synthesis, several strategies were explored to develop an asymmetric variant.

Starting from the known azidoaldehyde **151**, *p*-nitrobenzyl sulfonamide-protected aniline **152** was prepared over three steps. Compound **154** was obtained through a coupling of **152** to the known chiral acetonide alcohol (**153**) using Mitsunobu conditions and subsequent silyl deprotection. Swern oxidation followed by reaction with hydroxylamine furnished oxime **155**. The key nitrile oxide intramolecular [1,3] dipolar cycloaddition occurred following treatment of the oxime with sodium hypochlorite to afford the isoxazoline **156**; regrettably the product contained the incorrect stereochemistry at C7. Despite this setback, the synthetic endeavor was continued in an effort to construct biologically interesting analogs. Cleavage of the *p*-nitrobenzyl sulfonamide group and reprotection, ester reduction, and Raney nickel hydrogenation of the isoxazoline ring furnished diol **157**. Stereoselective ketone reduction, hydrolysis, sodium periodate cleavage of the diol, and subsequent reduction ($NaBH_4$), gave the free amino diol **158**. Utilizing nearly identical strategies as in the total synthesis, intermediate **158** was transformed to the acetonide-protected hydroxylamine hemiketal **159**. The diol functionality of **159** was converted to the epoxide by selective TES protection of the C10 hydroxyl group, mesylation at C9, desilylation, and sodium hydride-promoted ring

Scheme 1.21. Fukuyama's nitrile oxide dipolar cycloaddition model study for (+) FR900482



Key: (a) DEAD, PPh_3, benzene, 50 °C, 30 min, 93%; (b) TBAF, THF, rt, 45 min, 95%; (c) $(COCl)_2$, DMSO, CH_2Cl2, -78 °C; Et_3N; (d) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, rt, 30 min; (e) aq NaOCl, CH_2Cl_2, 0 °C, 4 h, 57% (3 steps); (f) PhSH, Cs_2CO_3, MeCN, 50 °C, 30 min, 74%. (g) $NaBH_4$, EtOH-THF (1:1), rt, 4 h, 99%; (h) TFAA, Et_3N, CH_2Cl_2; aq $NaHCO_3$, rt, 82%; (i) Raney-Ni, H_2, 5% aq H_3BO_3-EtOH (1:5), rt, 62%; (j) $NaBH(OAc)_3$, AcOH-THF (1:10), 0 °C, 30 min; (k) aq NaOH, MeOH, rt, 5 min, 85% (2 steps); (l) $NaIO_4$, MeCN-H_2O (3:2), 0 °C, 10 min; (m) $NaBH_4$, MeOH, rt, 10 min, 75% (2 steps); (n) TBSCl, Et_3N, DMAP, CH_2Cl_2, rt, 2 h, 70%; (o) MCPBA, CH_2Cl_2, 0 °C, 5 min; (p) Ac_2O, rt, 1 h, 65% (2 steps); (q) Dess-Martin periodinane, CH_2Cl_2, rt, 5 min, 95%. r) $NH_2NH_2{\cdot}H_2O$, CH_2Cl_2-MeOH (1:1), rt, 10 min, 78%; (s) TBAF, AcOH, THF, rt, 2 h, 98%; (t) Amberlist® 15E, MeOH, rt, 5 min; (u) $Me_2C(OMe)_2$, CH_2=C(OMe)Me, PPTS, DMF, 0 °C, 30 min, 78% (2 steps); (v) TESCl, Et_3N, DMAP, CH_2Cl_2, rt, 1 h, 75%; (w) MsCl, Et_3N, CH_2Cl_2, 0 °C, 20 min, 92%; (x) TBAF, THF, rt, 5 min, 95%; (y) NaH, DMF-THF (1:3), 60 °C, 5 min, 80%.

closure. The resulting oxirane **161** could then be elaborated to the *epi*-FR900482 analog **39** using previously reported procedures from the total synthesis. Alternatively, the

epoxide ring was maintained, and converted to the *cis*- analog **40**. The biological significance of both analogs **39** and **40** has previously been discussed.

1.5 Current Total Syntheses

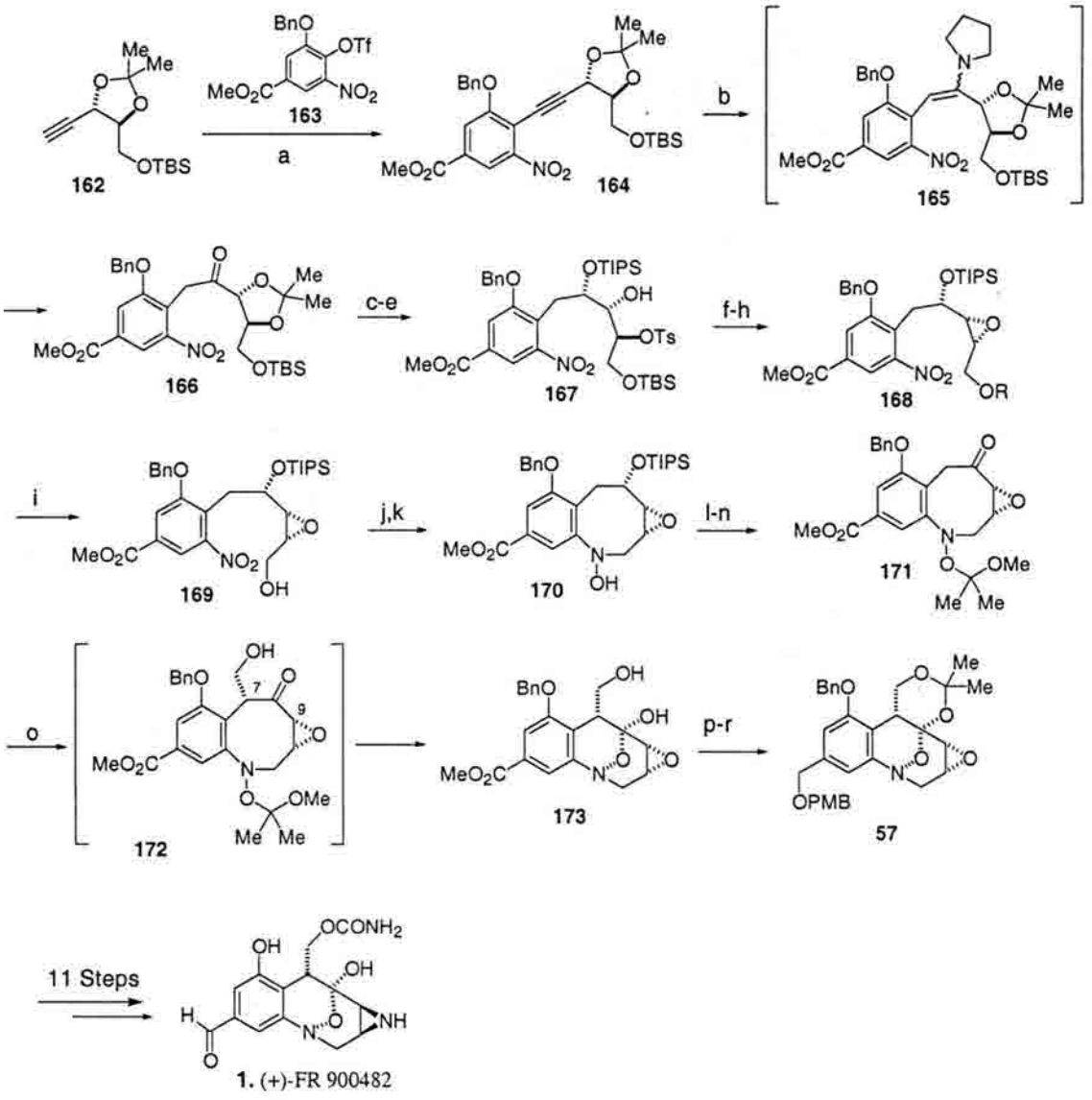
Following the completion of the total synthesis of (+) FR900482 and (+) FR66979 by Judd and Williams[23] as described in a later chapter (see Chapter 3) two additional total syntheses by both Fukuyama[24] and Ciufolini[25] were reported.

1.5.1 Fukuyama's Asymmetric Total Synthesis

In a second generation synthesis by Fukuyama *et al.*, optically pure acetonide **162** (derived from L-tartaric acid in 5 steps) provided a scaffold on which to complete an asymmetric synthesis (Scheme **1.22**).[24] Although much of the latter stages of the synthesis relied on nearly identical strategies as in the original racemic total synthesis, several key novel transformations early on allowed for an efficient construction of the core structure. Sonogashira-coupling of the optically pure acetylene **162** with the co-responding aromatic triflate **163** (6 steps from methyl vanillate) furnished alkyne **164** in good yield. Taking into account the conjugation of the alkyne functionality with the *O*-Nitro aromatic portion, selective addition of pyrrolidine followed by aqueous hydrolysis provided an efficient means of providing ketone **166**. Following several protecting group manipulations, construction of the eight-membered ring was accomplished by partial reduction of the nitro group to the hydroxyl amine and subsequent cyclization to the nitrone followed by *in-situ* reduction to the hydroxylamine. Interestingly, this identical strategy for ring-closure had failed during the total synthesis by Judd and Williams on a very similar substrate (see Chapter 2, Scheme **2.15**). The absence of the protected aziridine functionality along with the use of a sterically bulkier silyl protecting group

(TIPS in place of DEIPS) on the secondary hydroxyl group may have facilitated ring closure in this case.

Scheme 1.22. Fukuyama's asymmetric synthesis.



a) $[Pd(OAc)_2]$, PPh_3 (0.2 equiv), 2:1 THF/NEt_3, 65 °C, 1 h, then 25 °C, 75%; b) pyrrolidine, benzene, 25 °C, then aq. AcOH (50%), 25 °C; c) $Zn(BH4)_2$, Et_2O, -30 °C, 94% (2 steps), 9:1 diastereoselectivity; d) TIPSOTf, 2,6-lut., CH_2Cl_2, 25 °C; e) 5:1 AcOH/H_2O, 100 °C, 61% (2 steps); f) TBSCl, NEt_3, DMAP, CH_2Cl_2, 25 °C; g) TsCl, DABCO, CH_2Cl_2, 25 °C; h) NaH, DMF, 0 to 25 °C, 0.5 h, 76% (3 steps); i) CSA, MeOH, 25 °C; j) Dess-Martin periodinane, CH_2Cl_2, 0 to 25 °C; k) H_2 (1 atm), 5% Pt/C, MeOH, 25 °C, 89% (3 steps); l) 2-methoxypropene, TsOH\H_2O, CH_2Cl_2, 25 °C; m) TBAF, THF, 25 °C, 85% (2 steps); n) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C, then NEt_3, -78 to 25 °C, 82%; o) aq. HCHO (37%), LiOH, 20:3 THF/H_2O, 0 °C, then HCl, 0 to 25 °C; p) 2-methoxypropene, PPTS, 1:1 2,2-dimethoxypropane/acetone, 25 °C, (56%, two steps); q) DIBAL, CH_2Cl_2, -78 °C, 99%; r) 4-methoxyphenol, PPh_3, DEAD, benzene, 25 °C, 96%.

The resulting hydroxylamine was protected as the methoxy methylethyl ether **171**. Following aldol reaction with LiOH and aqueous formaldehyde as in the previous total synthesis, *in-situ* deprotection of the hydroxylamine with aqueous HCl gave the hydroxylamine hemiketal directly, circumventing the need for *in-situ* reduction of the ketone to the diol as in the previous racemic synthesis. Following several synthetic manipulations hydroxylamine hemiketal **173** was transformed to the identical intermediate **57** as in the original synthesis. With intermediate **57** in hand the total synthesis of (+) FR900482 was completed following essentially the same strategy as in the original synthesis.

In addition to being an asymmetric synthesis, Fukuyama's second generation synthesis demonstrated significant improvements over the original synthesis. An efficient coupling strategy through the acetylene substrate **162** followed by direct conversion to the ketone by conjugate addition of pyrolidine represents a novel approach to accessing the core structure. Additionally, the use of the methoxy methylethyl ether protecting group on the hydroxylamine allowed for *in-situ* production of the hydroxylamine hemi-ketal bond, precluding the need for a multi-step procedure to access this functionality as in the original synthesis.

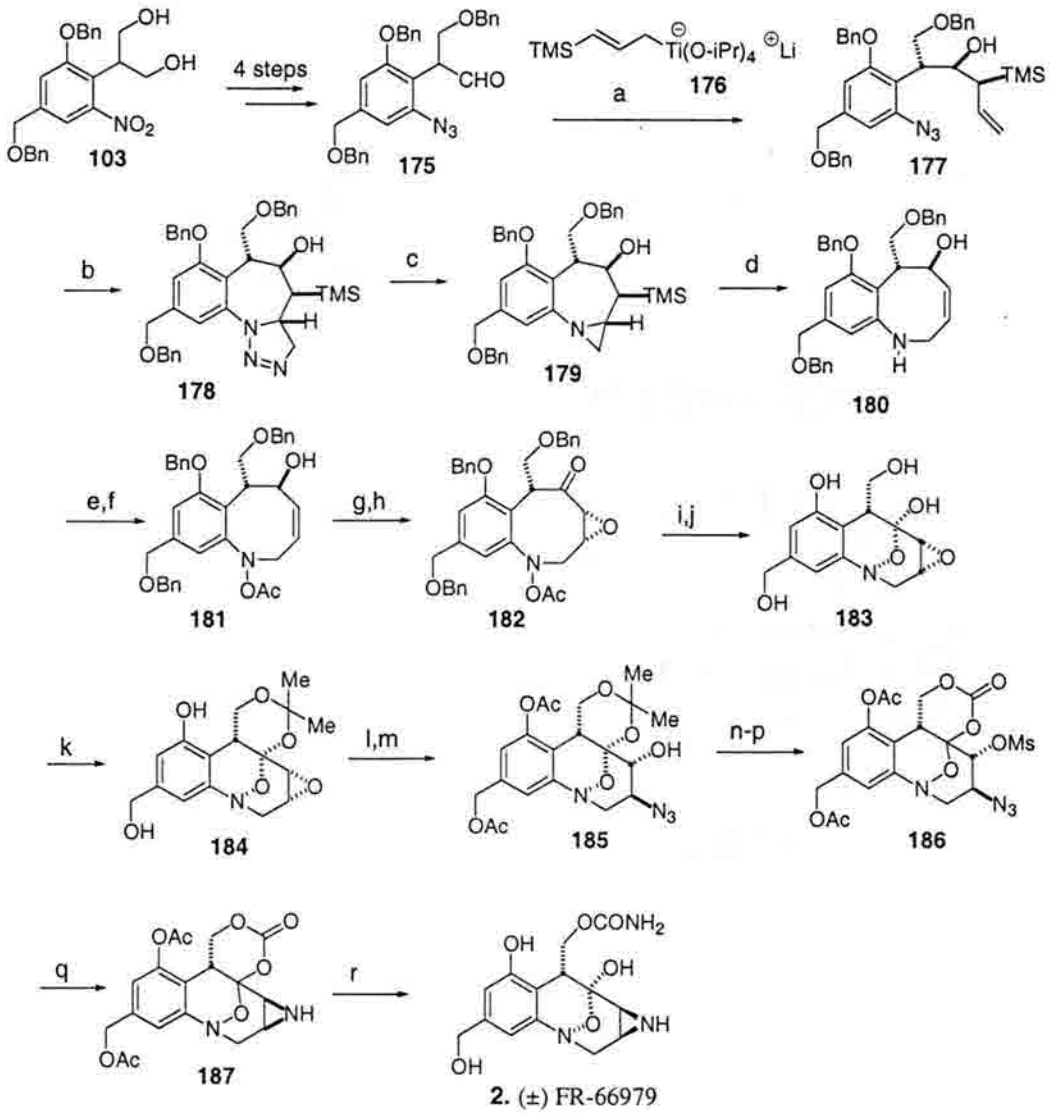
1.5.2 Ciufolini's Total Synthesis of FR66979

Following the completion of Fukuyama's second generation synthesis, Ciufolini and Ducray disclosed their synthesis of (±) FR66979.[25] Despite being racemic in nature, several key transformations in the middle of the synthetic sequence highlight a unique approach to this family of molecules. Utilizing an initial approach as in Martin's formal total synthesis (see Section **1.2.4**), aldehyde **175** was derived from the identical

intermediate **103** from Martin's formal total synthesis (11 steps total from 5-nitrovanillian (**100**)). Substrate **177** was constructed through allylation of aromatic aldehyde **175** *via* the lithiated allyltrimethylsilane species **176** and $Ti(O\text{-}iPr)_4$. Heating **177** in toluene induced an intramolecular 1,3-dipolar cycloaddition of the azido allylsilane intermediate affording triazoline **178** as a single diastereomer in good yield. Brief exposure to UV light resulted in direct conversion of the triazoline to the corresponding aziridine **179**. In the key synthetic transformation, hydroxide mediated homo-Brook rearrangement of the hydroxyl silyl intermediate **179** accompanied by fragmentation of the aziridine ring unveiled the eight-membered ring core structure of the nature product in a single step. This remarkable transformation along with the direct accessibility of the precursor through a 3-step sequence from aldehyde **175** demonstrated not only a unique synthetic approach to FR66979 and FR900482, but also to functionalized benzazocines in general. The completion of the total synthesis of FR66979 from the eight-membered ring (**80**) utilized nearly the identical overall strategy as in both Fukuyama's total syntheses including the synthesis of the aziridine and hydroxylamine hemi-ketal functionalities. One interesting feature however, involved using benzyl groups as the sole protecting groups of the various hydroxyl groups in the synthetic sequence. This strategy allowed for a global deprotection near the end of the synthesis to afford the tetraol hydroxylamine hemi-ketal **183**. Following selective protection of the primary hydroxyl and hydroxylamine hemi-ketal as the acetonide, the remainder of the synthetic intermediate could be acetylated allowing for deprotection during ammonolysis of the cyclic carbonate in the final synthetic step. Despite the moderate yield of this final step (40%), the protecting group strategy thus employed demonstrated the ability to

circumvent numerous selective protection-deprotection steps encountered during previous total syntheses.

Scheme 1.23. Ciufolini's racemic synthesis of FR66979.



Key: a) THF, -78 °C; b) toluene, 100 °C, 80% over two steps; c) *hv*, THF, 77%; d) nBu_4NOH, DMF, -20 °C, 49%; e) MCPBA, CH_2Cl_2, 0 °C; f) neat Ac_2O, 25 °C, 87% over two steps; g) MCPBA, CH_2Cl_2, $NaHCO_3$, 25 °C, 70%; h) cat. TPAP, NMO, CH_2Cl_2, 4 Å sieves, 25 °C, 83%; i) $N_2H_4\backslash H_2O$, MeOH, CH_2Cl_2, 100%; j) H_2 (1 atm), Pd/C, EtOAc, 97% crude k) 2-Methoxypropene, PPTS, DMF, 25 °C, 83%; l) LiN_3, DMF, 100 °C, 67%; m) Ac_2O, K_2CO_3, THF, 25 °C, 81%; n) MsCl, Et_3N, CH_2Cl_2, 25 °C, 71%; o) TFA, CH_2Cl_2, 25 °C; p) $COCl_2$, CH_2Cl_2, Et_3N, 0 °C, 28%; q) Ph_3P, iPr_2NEt, aqueous THF (90%), 60 °C, 78%; r) NH_3, MeOH, 25 °C, 40%.

Finally, although the natural product was synthesized in racemic form, the use of a nitro-benzyl propane diol intermediate as a precursor to aldehyde **175** should allow for an enantioselective version of the synthesis. By example of Martin's enzymatic resolution of the identical substrate **103** (see Section **1.2.4**) it should be possible to prepare aldehyde **175** in an optically pure form. Overall the total synthesis of (±) FR66979 by Ciufolini and Ducray details a unique approach to the eight-membered ring core structure of FR66979 and FR900482 and in combination with previous total syntheses represents a relatively quick access to both natural products.

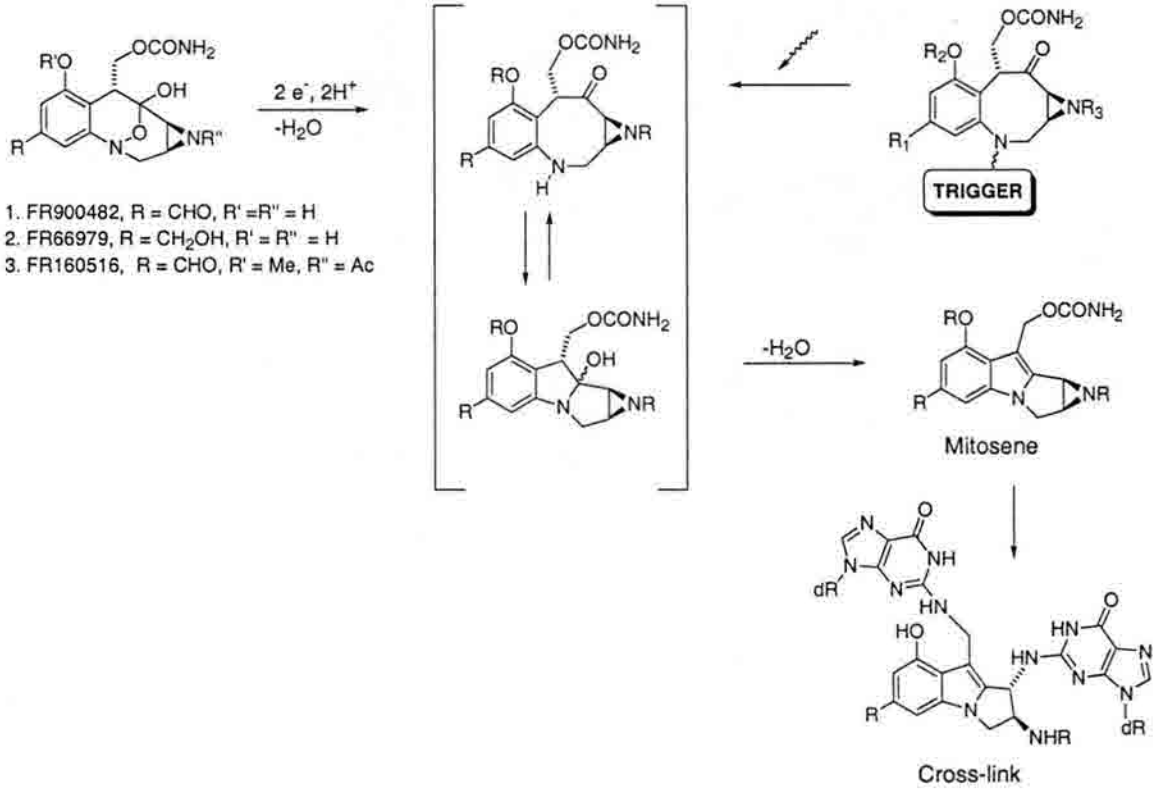
Chapter 2

Synthesis and Biochemical Studies of a Photo-Triggered Mitosene Progenitor

2.1 Introduction

The extensive biochemical studies investigating the DNA and DNA-protein interactions of the natural products FR900482 (**1**) and FR66979 (**2**) originating from this group[9,15,17] have inspired efforts towards synthesizing biologically interesting analogs. One such endeavor has focused on the design and synthesis of pro-mitosenes that are activated by alternative chemical signals. This strategy would abrogate the reductive activation pathway necessary for FR900482 and congeners (Scheme **2.1**).

Scheme **2.1.** Alternatively activated pro-mitosenes.



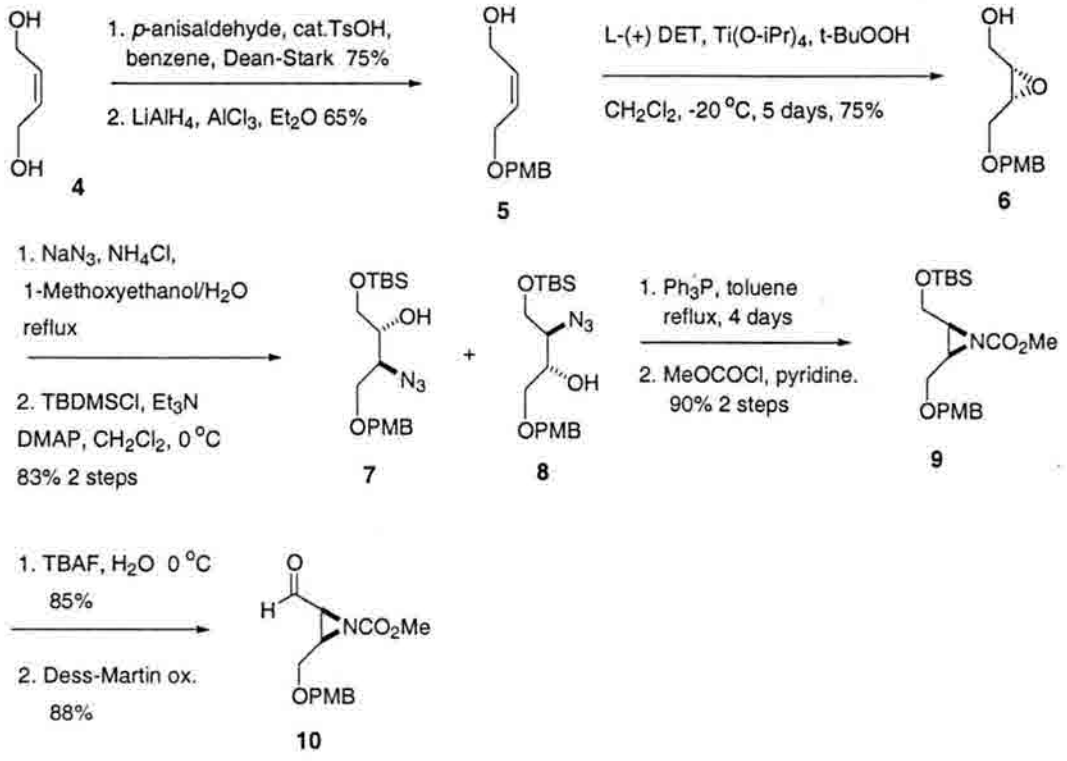
Central to this idea was the use of the 6-nitroveratryl carbamate (NVOC) group for the purpose of developing a photo-triggered analog that would enable activation at long UV wavelengths (350-410 nm). Indeed, since its introduction in 1970 by Patchornik and Woodward,[26] the NVOC group has found general use as a nitrogen protecting group in amino acids and nucleotide chemistry. The relatively long wavelength necessary for activation and cleavage is innocuous to both sensitive aromatic amino acids and oligonucleotides.[27]

2.2 Rollins' Model Study

The construction of the photo-triggered mitosene progenitor followed preliminary work by previous members of the group. Rollins and Williams published in 1997 the initial synthesis of a photo-triggered analog that furnished a mitosene core upon photo-activation.[28] The synthetic approach is outlined below depicting the synthesis of the analog over 25 steps (Schemes **2.2**-**2.4**).

Starting from *Z*-1,4-butene diol **4**, Rollins and Williams prepared the optically active aziridine aldehyde **10** over nine steps. The synthesis relied on previously published methods describing the preparation of azido silyl ethers **7** and **8** from *Z*-1,4-butene diol.[29] Hence, reaction of *p*-anisaldehyde with 1,4 butiene diol and reduction of the resulting acetal gave the mono protected PMB allylic alcohol **5**. Sharpless epoxidation[30] afforded the optically active epoxide (87% ee) whose enantiomeric ratio was determined by Mosher ester formation and ^{1}H and ^{19}F NMR analysis. Opening of the epoxide with sodium azide and selective TBS protection furnished the azido silyl ethers **7** and **8** as a mixture of regioisomers.[28]

Scheme 2.2. Synthesis of optically active aziridine aldehyde **10**

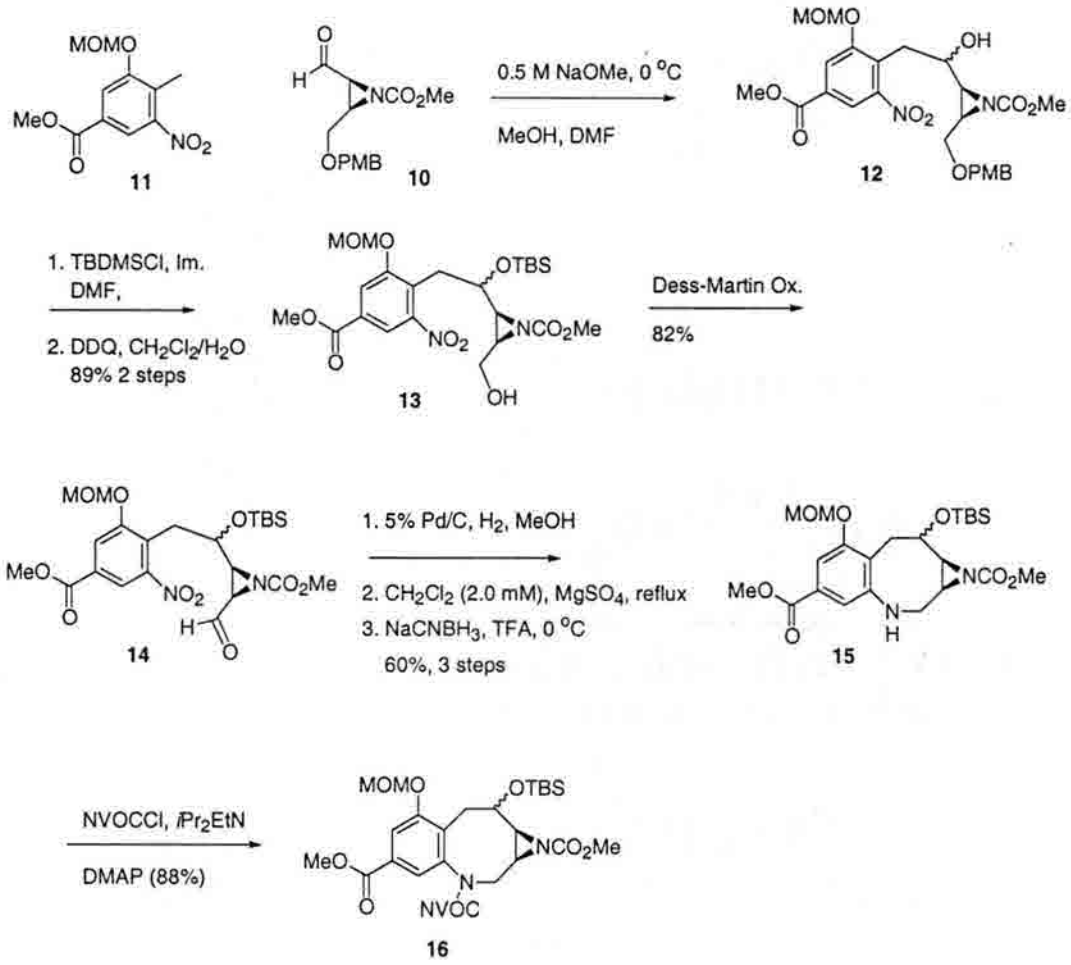


Accordingly, Rollins and Williams employed a Staudinger reaction to construct the aziridine ring. Refluxing both azido alcohols with triphenyl phosphine in toluene over 5 days gave the free aziridine, which was protected as the methyl carbamate in 90% yield. Finally, TBAF deprotection in THF/H_2O and Dess-Martin oxidation produced the aziridine aldehyde **10**.

Coupling of **10** with the known aromatic substrate **11** [20b,20i] by reaction with a catalytic amount 0.5 M sodium methoxide in DMF afforded the secondary alcohol **12**. TBS protection, oxidative PMB removal, and Dess-Martin oxidation furnished the aldehyde **14** in 73% yield over three steps. Reduction of the nitro group was accomplished by catalytic hydrogenation with 5% Pd/C and the resulting aniline was subjected to cyclization conditions with $MgSO_4$ and molecular sieves in CH_2Cl_2. With the tendency of the intermediate imine to dimerize, the reaction was found to require dilute

conditions (0.002 M). Reduction of the resulting imine with $NaCNBH_3$ and TFA gave the secondary amine **15**, which was acylated to the NVOC carbamate **16**.

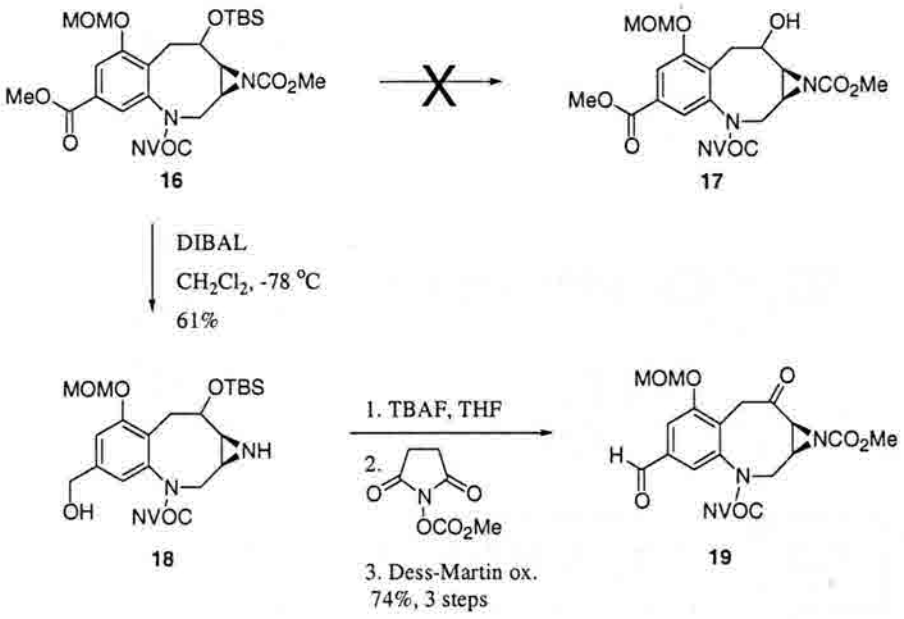
Scheme 2.3. Rollins' model study continued.



Attempts to remove the TBS group with substrate **16** were unsuccessful under a variety of conditions. Rather, the TBS ether could be removed only via prior deprotection of the aziridine nitrogen. Reduction of both carbomethoxy groups with DIBAL as precedence by Danishefsky's total synthesis, afforded **18**, which was deprotected by

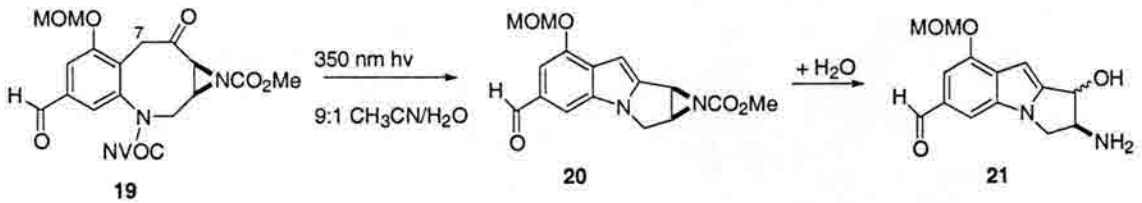
treatment with TBAF to give the diol. Re-protection of the aziridine and oxidation afforded the keto-aldehyde **19** as a model substrate for the mitosene progenitor (Scheme **2.4**).

Scheme 2.4. Completion of the model photo-triggered analog



Upon exposure to 350 nm light in 9:1 CH_3CN/H_2O, the model analog **19** successfully gave the mitosene core **21** as a mixture of diastereomers.

Scheme 2.5. Successful photo-activation to the mitosene core.



The model study by Rollins and Williams proved significant in demonstrating the viability of a photo-triggered progenitor to an active mitosene compound. However, the

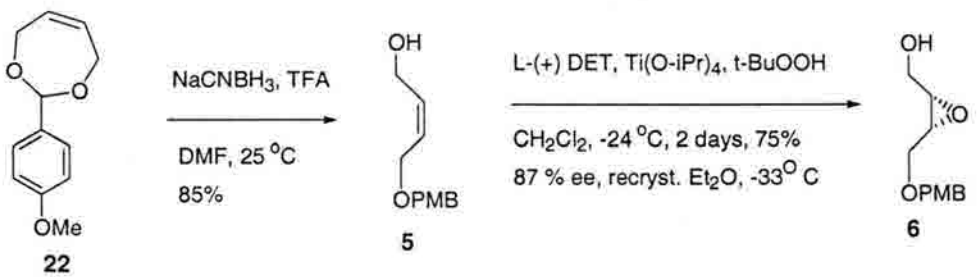
analog lacked the carbamoyl hydroxymethyl segment at C7 crucial for the ability to cross-link DNA. Despite this draw back, the preliminary study provided strategies for constructing the optically active aziridine, coupling of both the aromatic segment and the aziridine portion, and construction of the eight-membered ring. In developing a synthesis of a fully functional photo-triggered mitosene progenitor, it was hoped that much of these principles could still be utilized.

Section 2.3 Synthesis of a Fully Functional Photo-Triggered Mitosene Progenitor

The initial strategy for incorporating the C7 hydroxymethyl residue relied on an alternative coupling strategy between the aziridine aldehyde **10** and derivatives of the aromatic portion **4**. Aldehyde **10** was synthesized in much the same manner as the method reported by Rollins and Williams with a few modifications.

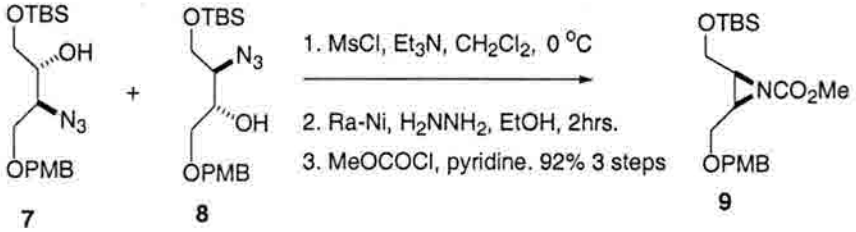
Reduction of acetal **22** with $NaCNBH_3$ and TFA was more amenable for large-scale production of the allylic alcohol **5**, which, in turn, was subjected to Sharpless asymmetric epoxidation as reported. Interestingly, the epoxide **6** could be recrystalized at low temperatures (-33° C in Et_2O), although this did not seem to enhance the optical activity but rather facilitated purification on scale up.

Scheme 2.6. Improved large scale preparation of optically active epoxide.



Difficulties in obtaining consistent results with the Staudinger reaction in large scale preparations required finding an alternative method for constructing the aziridine ring. Accordingly, mesylation of the secondary alcohol followed by azide reduction with hydrazine over Raney nickel afforded the free aziridine over 2 h. Reaction of the crude product with methyl chloroformate and pyridine furnished the protected aziridine **9** in 92% yield over three steps without the need for column chromatography. The convenience in reaction time and purification made this alternative procedure much more practical.

Scheme 2.7. Improved synthesis of the aziridine ring.

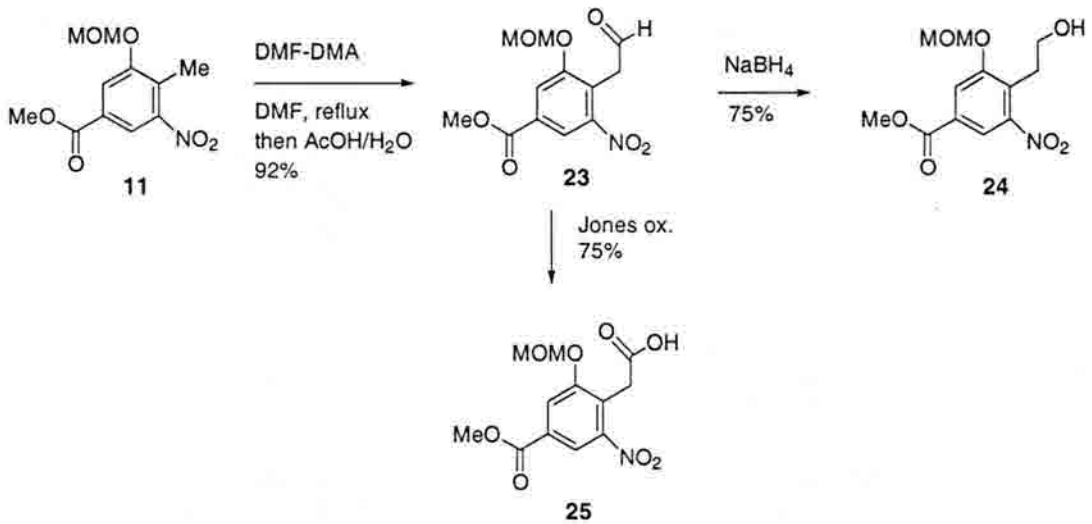


These minor modifications greatly facilitated large scale production of the aziridine aldehyde **10** with the overall preparation yielding between 5 and 7 grams from Z-1,4-butadiene diol **4**. The limitation in further scale-up resides with the Sharpless asymmetric epoxidation. In addition to difficulties encountered in maintaining the critical low reaction temperatures for this reaction at larger reaction scales, others have reported a significant decrease in optical purity with reactions performed on greater than 50 mmol of this substrate.[18d,f,h] Notably, Terashima and coworkers had used an enantiomerically pure form of the identical epoxide **6** in their total synthesis; [18d,f,h] use of their procedure would allow production of enantiomerically pure aziridine **10** with the addition of five synthetic steps. With the ability to synthesis the aziridine aldehyde in large quantities, the alternative coupling strategies could now be explored.

2.3.1 Alternative coupling strategies

Theoretically, having the hydroxymethyl segment or an equivalent synthon installed early in the synthesis would seem the most practical approach. Paralleling previous protocols from this group (Yasuda and Williams, unpublished results[31]) the trisubstituted toluene derivative **11**[20b,20i,28] was subjected to reaction with dimethylformamide dimethylacetal (DMF-DMA) in DMF at reflux to give aldehyde **23** following an aqueous acidic workup. The aldehyde, in turn, could be either reduced to the corresponding alcohol (**24**) with $NaBH_4$ or converted to the acid (**25**) through Jones' oxidation (Scheme **2.8**).

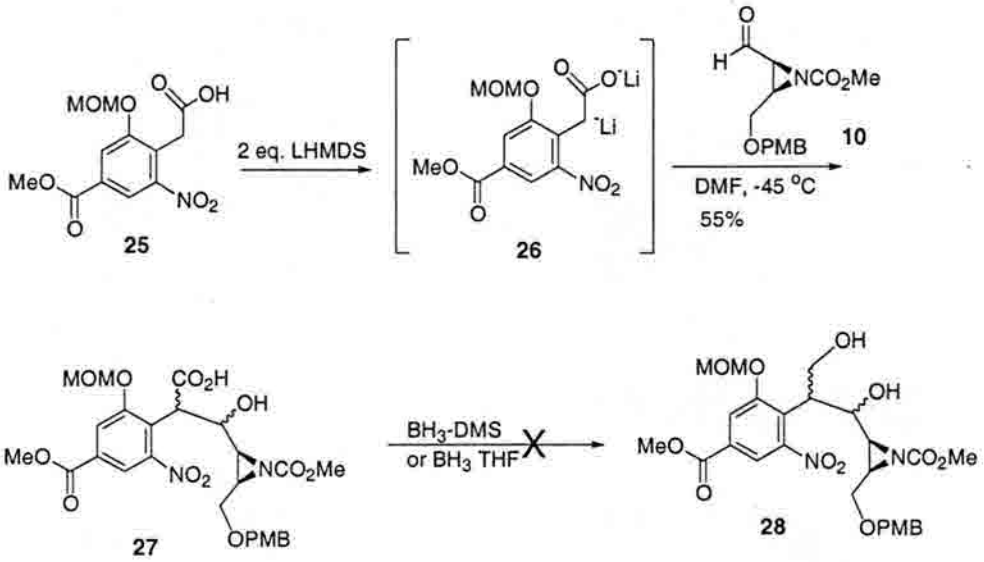
Scheme 2.8. Synthesis of alternative aromatic segments.



Attempts were made to couple each of these components with the aziridine aldehyde **10** following dianion formation with LHMDS. Although alcohol **24** failed to react, the dianion of the carboxylic acid **25** successfully gave the desired coupled product in 55% yield. The product was verified by mass spectrometry and ^1H NMR spectroscopy, which revealed that all four possible diastereomers had been produced. Separation of the diastereomers by chromatography was precluded by the carboxylic acid functionality and

necessitated carrying all four products to the next reaction. Attempts to selectively reduce the carboxylic acid with borane in THF or with BH_3-DMS complex failed to give the desired diol **28** (Scheme **2.9**) .

Scheme 2.9. Successful coupling of the dianion and the aziridine aldehyde.



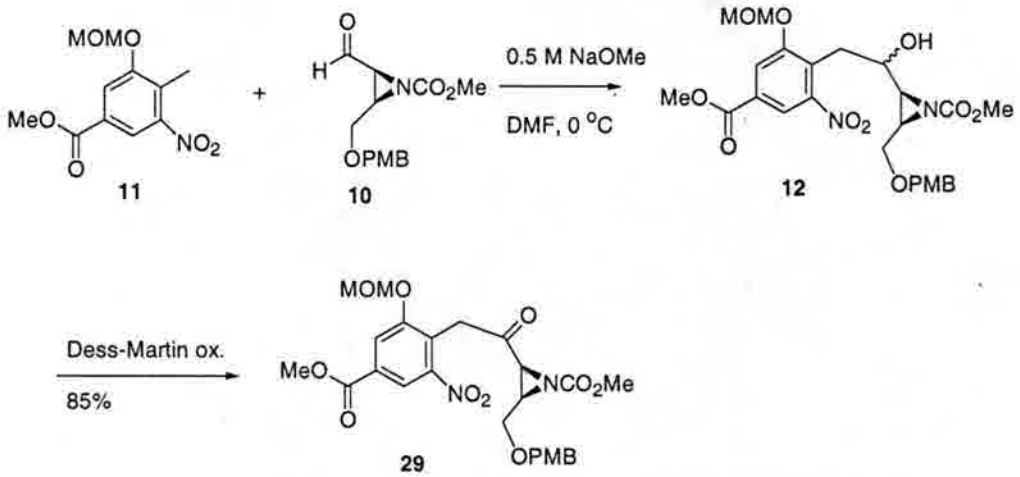
The inability to reduce the resulting carboxylic acid combined with the difficulty in dealing with the mixture of diastereomers led to the abandonment of this strategy. Furthermore, it should be noted that coupling reactions with aldehyde **23** or the dimethyl ester derivative from carboxylic acid **25** were not attempted since self-condensation with the former and the inability for selective reduction of the product of the latter would seem to impair both strategies.

2.3.2 Model Studies of the Aldol Reaction

Following the protocol by Rollins and Williams, coupling of the aziridine aldehyde with the aromatic segment **11** afforded the alcohol **12**.[28] Dess-Martin oxidation

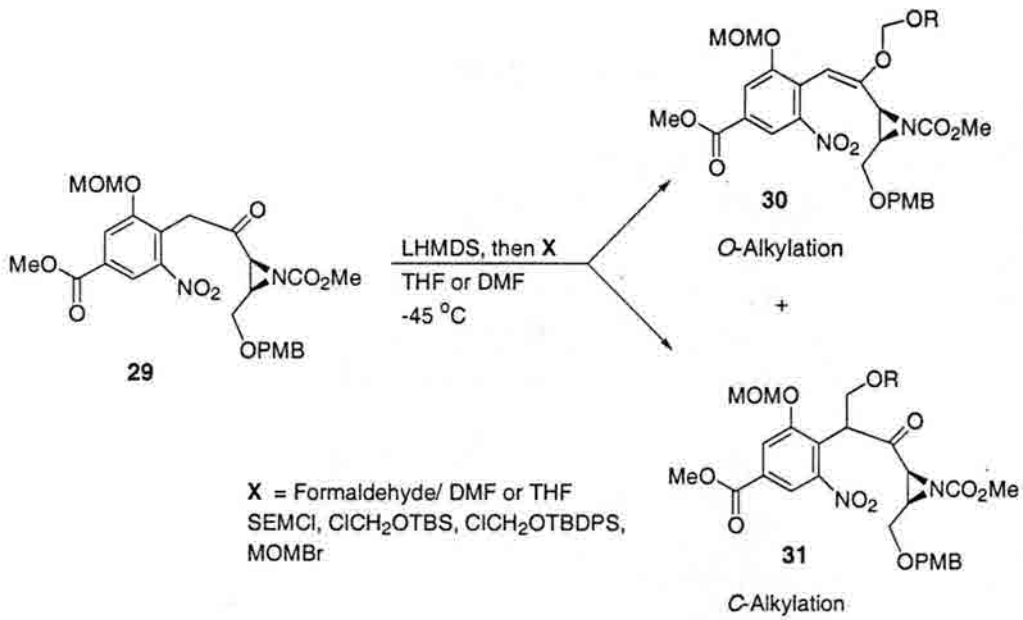
of the mixture of alcohols furnished ketone **29** (85% yield) to serve as a model system on which to study a late stage aldol reaction.

Scheme 2.10. Synthesis of the ketone substrate.



Initial attempts to perform an aldol reaction with formaldehyde in DMF or THF afforded no reaction. Not surprisingly, the enolate derived by reaction of **29** with LHMDS proved to be extremely unreactive owing to the stabilization by conjugation with the *para*-methyl benzoate and *ortho*-nitro functionalities. The anion's stability necessitated the use of highly reactive electrophiles in the form of α haloethers. Reaction with MOMCl, SEMCl and chloromethoxysilanes $ClCH_2OTBS$ and $ClCH_2OTBDPS$ (derived in three steps from ethanethiol according to Gunderen *et al.*[32]) resulted in selective O-alkylation only. By switching to a softer halogen with MOMBr, reaction in THF led to a 2:1 mixture of desired C-alkylation versus O-alkylation in 60% yield. The utility of this product would seem limited due to the difficulty of deprotecting the methyl ether in the presence of the remaining functionalities. The result remained important however, in demonstrating the possibility of an aldol reaction with a more advanced intermediate.

Scheme 2.11. Alkylation reactions on the model ketone substrate

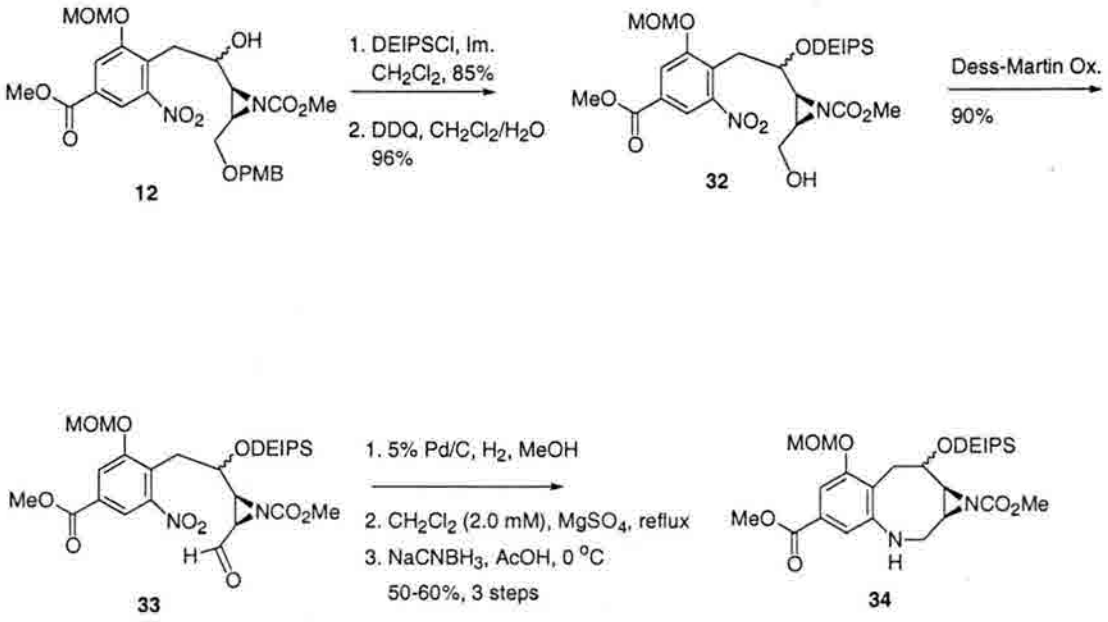


2.3.3 Completion of Photo-triggered Mitosene Progenitor

The approach for a late stage aldol reaction required the production of a ketone comparable to intermediate **19** (Scheme **2.4**), but with the methyl ester portion still intact. During the course of the synthetic study by Rollins and Williams, deprotection of the TBS-protected secondary alcohol following cyclization depended upon first removing the aziridine methyl carbamate. This observation suggested that the difficulty in desilylation was an issue of sterics, and could potentially be solved with an alternative protecting group. Hence, several less sterically-demanding protecting groups were evaluated and subsequently subjected to a similar synthetic sequence as in the previous study. Initial attempts with the triethylsilyl (TES) group and the TBS hydroxymethyl acetal, derived from the aforementioned chloromethoxysilane ($ClCH_2OTBS$), proved unsuccessful. The TES group in particular proved too labile during the cyclization with only a minimal yield of product isolated. This result did demonstrate however, the capacity to deprotect the secondary alcohol with the carbamate on the aziridine intact. Finally, the slightly

more bulky diethylisopropyl (DEIPS) protecting group demonstrated the desired traits of being stable during the synthetic manipulations while maintaining the ability to be cleaved in the presence of the protected aziridine.

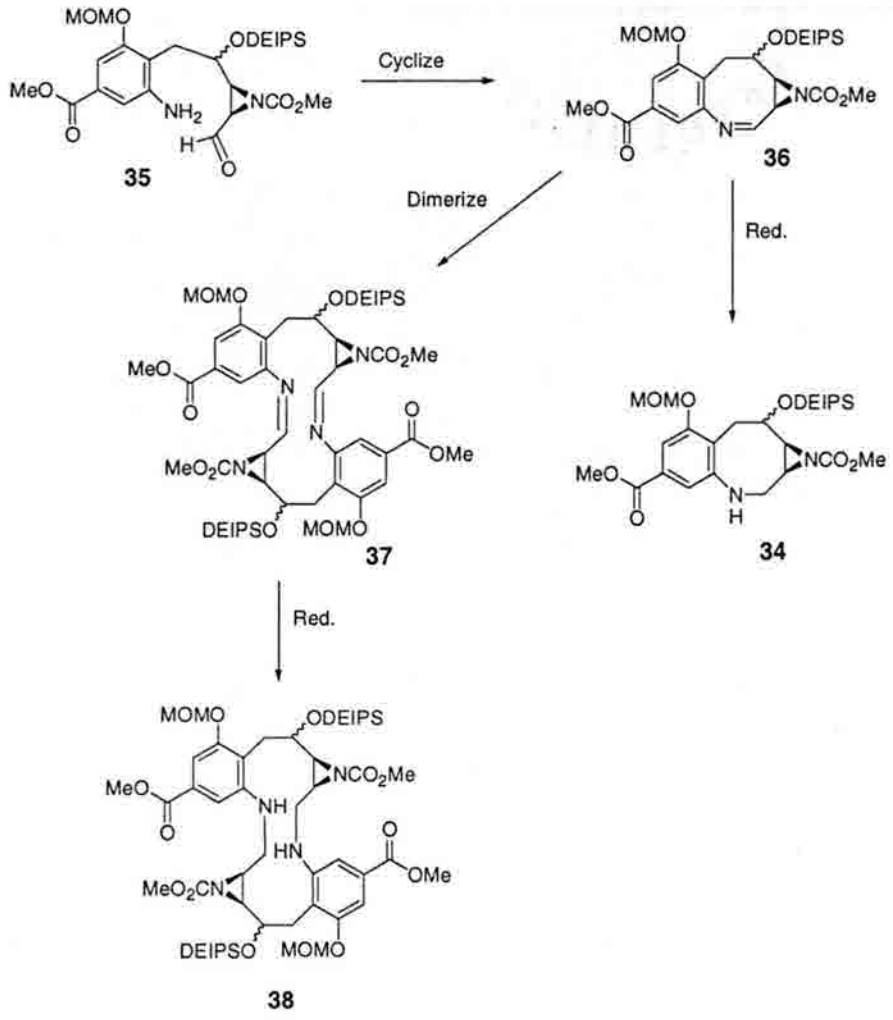
Scheme 2.12. Synthesis of eight-membered ring.



Accordingly, the coupling product **12** was silylated with DEIPSCl and imidazole in CH_2Cl_2 (85% yield). Oxidative removal of the *para*-methoxybenzyl group with DDQ and Dess-Martin oxidation of the primary alcohol afforded aldehyde **33** in 86% yield over two steps.[33] In analogy to the work by Rollins and Williams, a three step protocol was used to convert the aldehyde into the eight-membered ring **34**. Reduction of the nitro function by catalytic hydrogenation, and cyclization of the amino aldehyde to the eight-membered ring imine was effected with $MgSO_4$ and 4Å sieves under dilute conditions (~1.0 mM). Reduction of the imine with $NaCNBH_3$ and AcOH gave **34** in 55~75% yield.

As in the study by Rollins and Williams, high dilution conditions were necessary during the cyclization to circumvent dimerization of the intermediate imine (Scheme **2.13**).

Scheme 2.13. Dimerization of the intermediate imine.



This aspect of the synthesis precluded large-scale production of the eight-membered ring intermediate. Indeed, over forty different reaction conditions were examined in order to try to overcome this detrimental step, a few of which are tabulated below.

Scheme 2.14. Reductive amination of intermediate aniline.

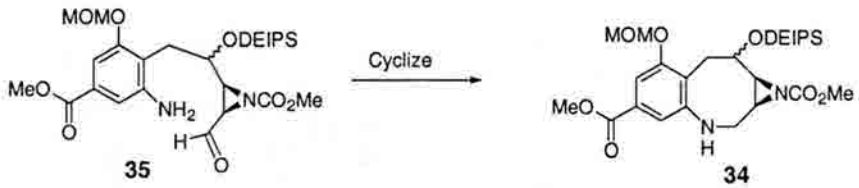


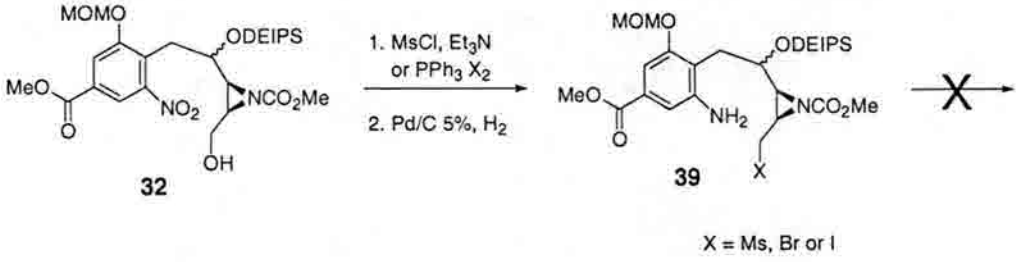
Table 1. Conditions for Reductive Amination of Aniline **35**.

Conditions	Result
$(Bu)_4NCNBH_3$, CH_2Cl_2, $MgSO_4$, 25 °C to Reflux	S.M.
$(Bu)_4NCNBH_3$, CH_2Cl_2, TFA, $MgSO_4$, Rt	Decomp.
DMF or EtOAc, $(Bu)_4NCNBH_3$, 4 A sieves	S.M.
$NaCNBH_3$, 4 A sieves, EtOH, Reflux	Decomp.
$NaCNBH_3$, 3 A sieves, MeOH, pH 5, Reflux	Decomp.
$ZnCl_2$, MeOH, Reflux, then $NaCNBH_3$	Dimer
1.5 eq. $ZnCl_2$ + $NaCNBH_3$, MeOH, reflux	1:1 Product/reduced Aldehyde
1 eq. $ZnCl_2$ + $NaCNBH_3$, MeOH, reflux	1:1 Product/S.M.
1.5 eq. $ZnCl_2$ + $NaCNBH_3$, MeOH/EtOH, Rt	Reduced Aldehyde
1.8 eq. $ZnCl_2$ + BH_3-pyridine, MeOH, reflux	Reduced Aldehyde
3 eq. $ZnCl_2$, $(nBu)_4NCNBH_3$, CH_2Cl_2, Rt	1:1 Product/reduced Aldehyde
$ZnCl_2$ + $NaCNBH_3$, THF, 25 °C to Reflux	Reduced Aldehyde

To overcome the dimerization of the intermediate imine at normal concentrations, conditions were needed which allowed for cyclization and immediate *in situ* reduction to the corresponding imine. More reactive reducing agents such as H_2/Raney Nickel and H_2/10% Pd/C resulted in decomposition through additional reduction of the aldehyde and/or aziridine ring. Further examination of the reaction revealed that the intermediate aniline aldehyde did not undergo cyclization in methanol, and only after switching solvents did such an event proceed. Cyclization attempts in the presence of tetrabutylammonium cyanoborohydride in various organic solvents were examined, but

to no avail. Likewise, mildly acidic conditions to promote the cyclization led only to decomposition. The sensitivity of the compound to protic acids and strong Lewis acids required that only mild Lewis acids be used. The use of $ZnCl_2$ with $NaCNBH_3$ has been shown to be an effective reagent for reductive aminations.[34] Unlike the related reaction with $NaBH_4$, $ZnCl_2$ and $NaCNBH_3$ have been reported to give a mixture of salts possibly including $Na[ZnCl(CNBH_3)_2]$, $Na_2[ZnCl_2\text{-}(CNBH_3)_2]$, $Na[Zn(CNBH_3)_3]$, in addition to just strictly $Zn(CNBH_3)_2$.[34] It was hoped however, that the reducing agent complexed to the Lewis acid would promote immediate reduction of the imine. Marginal success was seen in some examples, affording mixtures of product and starting material or reduced aldehyde, depending on the ratio of $ZnCl_2$ to $NaCNBH_3$. Unfortunately, the reaction was found to be capricious in nature and conditions to obtain high yields of the cyclic amine could not be found. Alternative approaches, such as converting the intermediate alcohol to the mesylate or other leaving group followed by displacement with the aniline failed to afford any reaction and it seemed the configuration of the aziridine ring precluded nucleophilic attack (Scheme **2.15**).

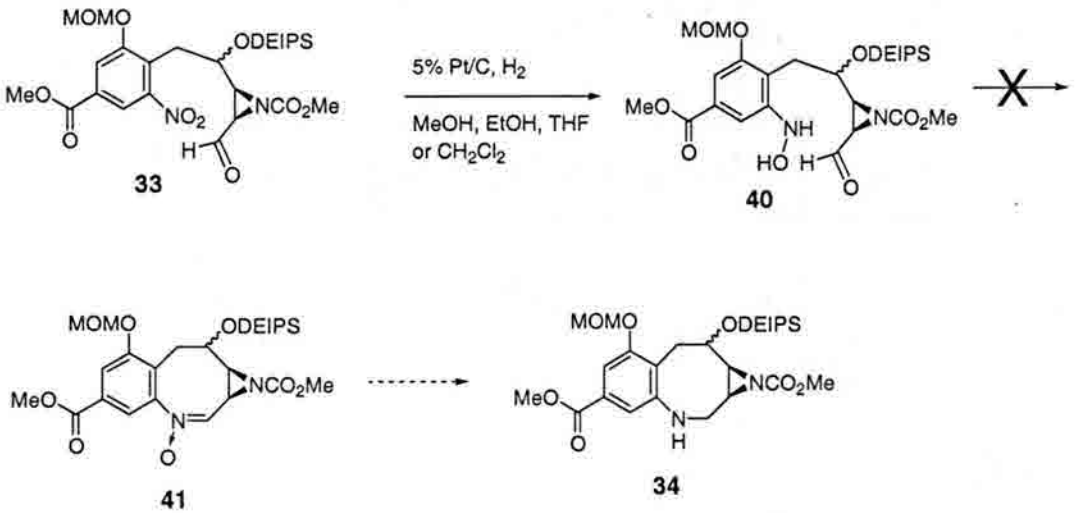
Scheme 2.15. Alternative cyclization attempts.



One final attempt involved using platinum on carbon to selectively reduce the nitro functionality to the hydroxylamine[35] **40** in the hope that the additional alpha-effect of the hydroxyl would enhance the nucleophilicity of the amine (Scheme **2.16**). Ideally this would result in the formation of the nitrone **41**, which could then be reduced to the

desired product. Although hydrogenation with 5% Pt/C successfully gave the hydroxylamine as the sole product, the intermediate was inert to further reaction in methanol or other solvents.

Scheme 2.16. Unsuccessful cyclizations of hydroxylamine **40** to nitrone **41.**

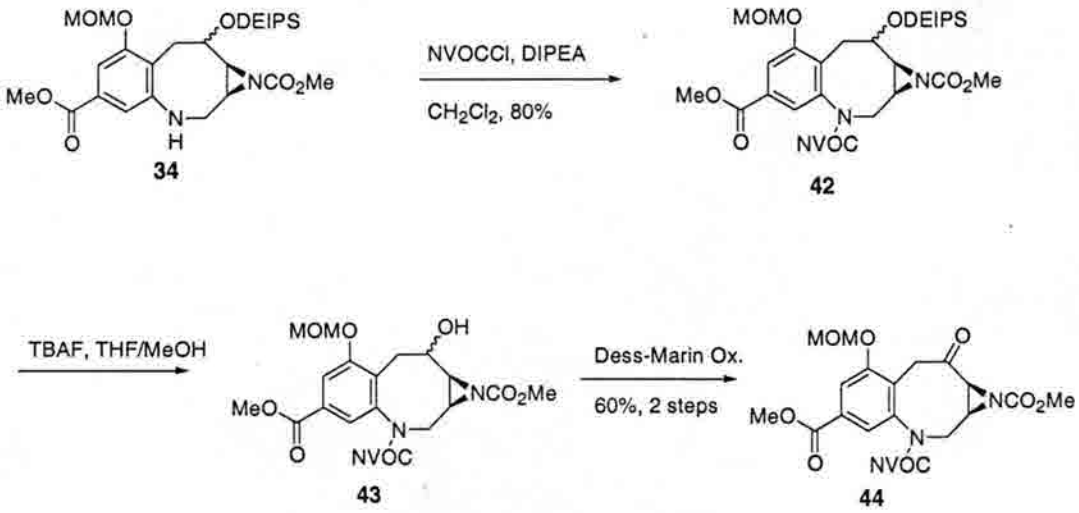


The failure to find improved conditions for this key transformation, mandated that the cyclizations be carried out at high dilution and subsequently reduced separately. One important result from these studies was discovering the stability of the intermediate aniline aldehyde in methanol even at high concentrations. This property allowed the hydrogenation to be scaled up and the intermediate aniline partitioned into separate flasks with methanol for simultaneous multiple cyclizations. Employing this strategy allowed for the reaction to at least be performed on a 1 gram scale.

With the eight-membered cyclic amine in hand, acetylation with 6-nitroveratryl chloroformate (75%) followed by removal of the DEIPS group with TBAF in THF/methanol afforded the free alcohol **43**. The use of methanol during the deprotection prevented any Payne-type rearrangement that would lead to the epoxide through opening

of the aziridine. Dess-Martin oxidation of the secondary alcohol provided the ketone **44** in 60% yield over two steps.

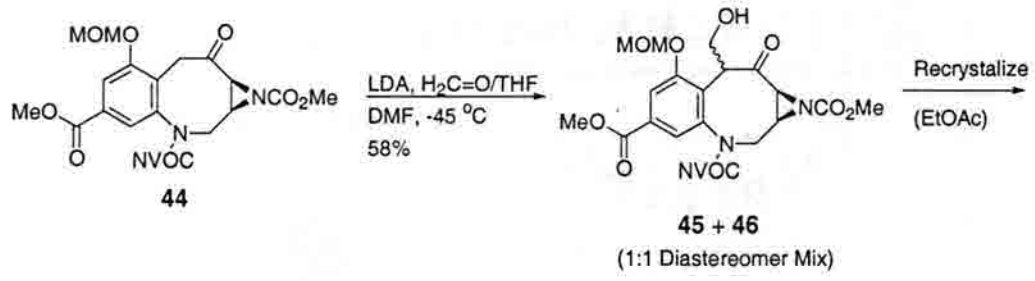
Scheme 2.17. Construction of ketone **44.**



It was now necessary to introduce the crucial hydroxymethyl group at C7. The susceptibility of both methoxycarbamoyl groups to aqueous base precluded the use of the commercially available 38 percent aqueous formaldehyde solutions for this reaction, and the preparation of an anhydrous alternative was sought. After trying several reported conditions for the generation of anhydrous formaldehyde solutions in ethereal solvents, a modified version of the procedure by Reetz *et al.* using α-polyoxymethylene polymer proved successful.[36] Thus reaction of ketone **44** with LDA in dry DMF at -45 °C, followed by addition of a freshly prepared anhydrous solution of formaldehyde in THF (ca. ~ 1.0 M) gave the desired aldol adduct in 58-70% yield as a 1:1 mixture of diastereomers. Fortunately, recrystalization from EtOAc was selective for the desired

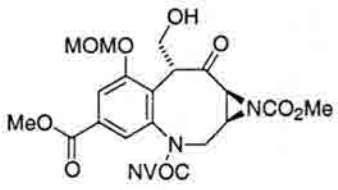
diastereomer **45** with the *anti*-configuration, whose identity was confirmed by X-ray analysis (Figure **2.1**) (See Appendix **1** for ORTEP drawing and related data).

Scheme 2.18. Key aldol reaction.



44

45 + 46
(1:1 Diastereomer Mix)



45

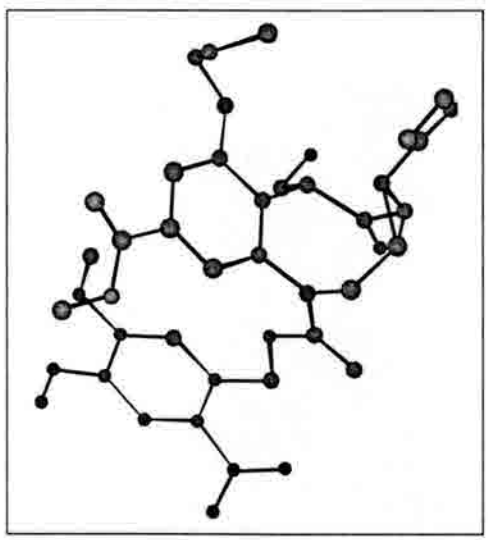
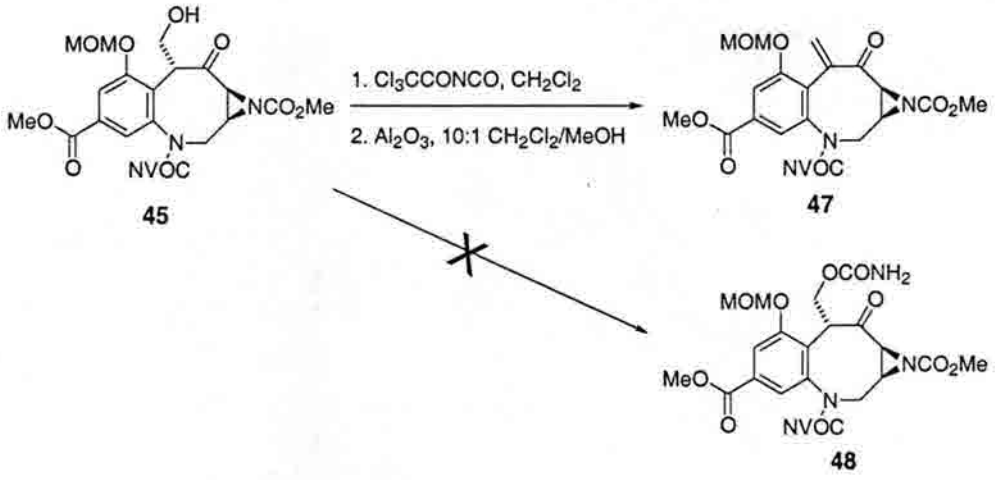


Figure 2.1. X-ray Structure of **45**.

Initial efforts to elaborate the aldol adduct into the carbamoyl adduct found in the FR compounds proved unsuccessful. Reaction of the primary hydroxyl group with trichloroacetyl isocyanate followed by cleavage of the trichloroacetamide intermediate with neutral alumina resulted in exclusive formation of the exocyclic methylene species **47** (Scheme **2.19**).

Scheme 2.19. Failed attempt to synthesis urethane **48.**



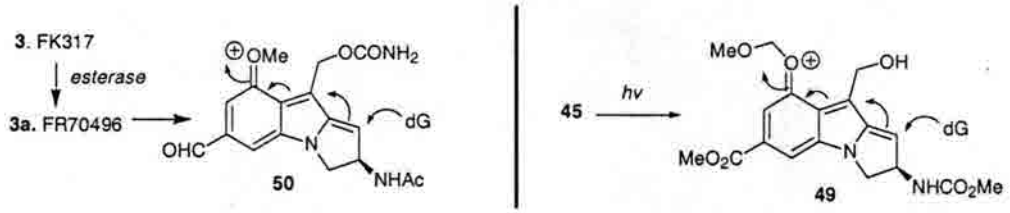
Alternative strategies involving ketone reduction, selective reaction of the primary alcohol, and re-oxidation to the ketone also failed to provide the desired product. It appeared that functionalization at this position rendered the compound too prone to elimination, and further efforts to synthesize the carbamate analog **48** were abandoned.

2.4 Biochemical Studies on Photo-Triggered Analog 45

Despite the synthetic difficulties encountered, further examination of the aldol product **45** revealed its viability as a DNA cross-linking agent. Although the related decarbamoyl mitomycin C derivative is known to only monoalkylate DNA and not produce cross-links, the minimal activity of that agent may be attributed to the quinone functionality, as previously discussed (Chapter **1**, Section **1.1**). Furthermore, in evaluating the remaining protecting groups, it is instructive to compare the proposed intermediate mitosene resulting from photo-activation of **45** with the clinical candidate FK317 (**3**). Theoretically, photolysis at 350 nm light would lead to the reactive mitosene

species **49**, capable of alkylating DNA. Likewise, following esterase deacetylation and reduction, FK317 would form the presumed mitosene intermediate **50**, as presented earlier. Both reactive intermediates share a strikingly similar core structure, and would be predicted to demonstrate the same ability to alkylate DNA.

Scheme 2.20. DNA alkylation of both FK317 and photo-triggered analog mitosene intermediates **49** and **50**.



The capacity of **45** to cross-link DNA was evaluated using linearized (*Eco*R1) pBR322 plasmid DNA by denaturing alkaline agarose gel electrophoresis according to Cech.[37] In order to compensate for the insolubility of compound **45** in H_2O, reactions with **45** were conducted in 1% or less DMSO/H_2O solutions. 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 was activated with 1 mM 2-mercaptoethanol in the presence of 0.5 μg of DNA duplex producing the interstrand cross-link (ICS) (lane 4).[9a-d] Interstrand cross-link formation was evident following 1 h irradiation of compound **45** at 10 μM and 1 μM concentrations with 0.5 μg of DNA duplex (lanes 6 and 7). Incubation of **45** at 10 μM concentration with 0.5 mg of DNA duplex for 12 h in the dark (lane 5) led to no detectable cross-link. The faster mobility of the cross-linked DNA at 10 μM concentration (lane 6) relative to that for the 1 μM concentration (lane 7) reaction is attributed to extensive (multiple) cross-links at the higher concentration.

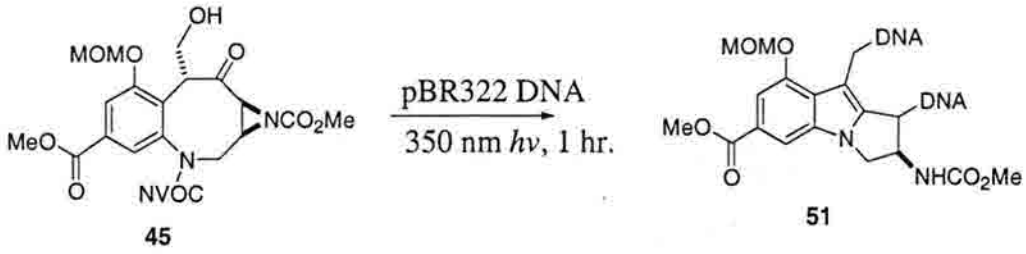


Figure 2.3. Photoactivated cross-link of pBR322 by analog 45. Reaction mixtures were prepared by addition of appropriate stock solutions to a total volume of 10 μL containing 0.5 μg of linearized DNA buffered to pH 8 with 10 mM Tris, 1 mM EDTA. Irradiations were performed in clear eppendorfs at 25 °C with a Rayonet® lamp equipped with four 350 nm bulbs. All samples were incubated 24 h. prior to analyses. The mixtures were analyzed on a 1.2% agarose gel at 80 volts for 3.5 h. Lane 1: Lambda Hind III. Lane 2: DNA, dark. Lane 3: DNA, *hv*, 60 min. Lane 4: 1.0 mM FR900482 + 1.0 mM 2-mercaptoethanol. Lane 5: 10 uM **45**, dark, 60 min. Lane 6: 10 uM **45**, *hv*, 60 min. Lane 7: 1 uM **45**, *hv*, 60 min. Lane 8: 100 nM **45**, *hv*, 60 min. Lane 9: 10 nM **45**, *hv*, 60 min.

Notably, the incubation period following photolysis was not necessary for interstrand cross-link production; ethanol precipitation and analysis by electrophoresis immediately following the photolysis revealed cross-link formation in much the same manner.

2.4.1 Determination of Cross-link Sequence Specificity

Having established the capacity of the mitosene progenitor analog **45** to efficiently cross-link DNA, further biochemical experiments were pursued in order to elucidate the sequence specificity of the interstrand lesion. The production of a mitosene

species structurally similar to that derived from the natural product and congeners would predict the identical preference for the 5'-CG-3' sequence. Following the precepts of extensive biochemical studies on FR900482 and FR66979 from this group,[9,38] synthetic oligonucleotides were used to further evaluate the cross-linking of analog **45**. Following 5' end-labeling of oligomer A with ^{32}P and annealing to the complimentary strand (oligomer B), the resulting duplex **1** provided a template containing two 5'-CG-3' sites for potential cross-links.

Figure 2.4. Duplex **1**.

*5' TTTATTAAC**G**TAATGCTTAATC**G**CAATGGGATT 3' Oligomer A
3' AAATAATT**G**CATTACGAATTA**G**CGTTACCCTAA 5' Oligomer B

Photolysis of compound **45** (10 mM in lane 5, and 2.5 mM in lane 6 [Figure **2.5**]) with duplex **1** (in 4:1 H_2O/CH_3CN lane 5, and 19:1 H_2O/CH_3CN lane 6) with 350 nm light in a Rayonet equipped with four bulbs for one hour led to cross-link formation. The use of acetonitrile in place of DMSO in experiments with synthetic duplexes was necessary as the latter solvent denatures duplexes of small oligomers. As with the plasmid studies, standard lanes were included demonstrating the integrity of the unreacted DNA (lane 1) and the stability of the duplex to the long wavelength UV light (lane 2). As a standard for cross-linking, reaction with the natural product FR900482 under reductive conditions was included (lane 3). Following ethanol precipitation, 20% DPAGE at 60 watts for 3.5 hrs produced the following image (Figure **2.5**).

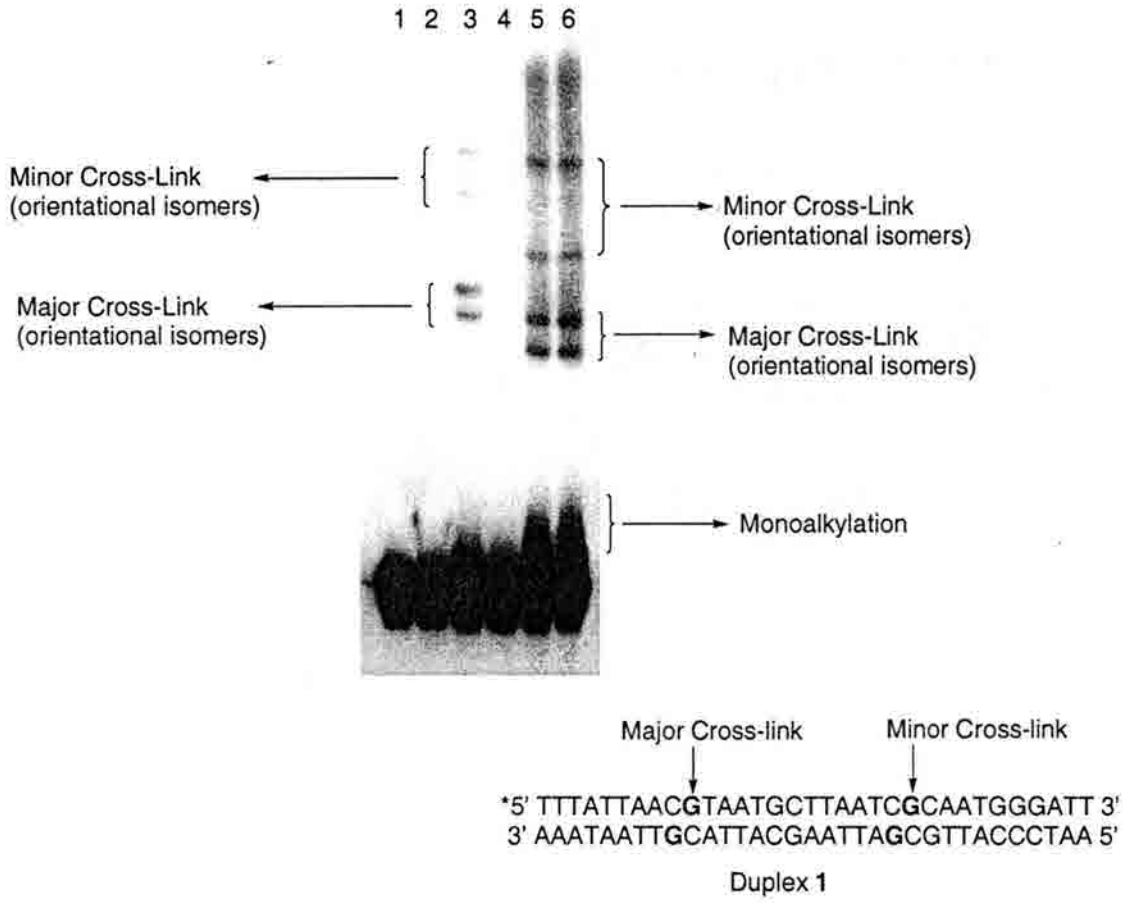


Figure 2.5. Autoradiogram of cross-linked duplex 1 (labeled at the 5' terminus of oligo A). Lane 1: DNA standard. Lane 2: DNA, *hv*, 60 min. Lane 3: DNA, 10 mM FR900482 + 10 mM 2-mercaptoethanol. Lane 4: 10 mM **45**, dark, 60 min. Lane 5: 10 mM **45**, *hv*, 60 min. Lane 6: 2.5 mM **45**, *hv*, 60 min.

As expected, the natural product FR900482 in lane 3 showed cross-links for each 5'-CG-3' site with a preference for the 5'-ACG-3' site.[9] Additionally, two bands appear for each cross-linkable site owing to the two different orientational isomers of the drug produced in this asymmetric (non-palindromic) DNA. This characteristic band shift of FR900482 and FR66979 resulting from orientational isomers has been rigorously characterized by Rajski and Williams.[9d]

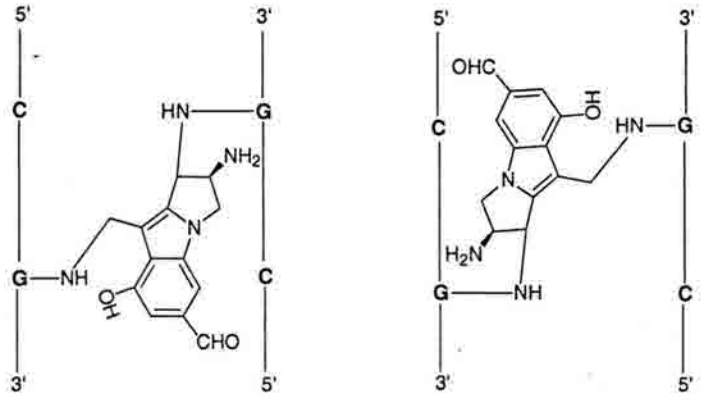


Figure 2.6. Orientational isomers of the natural product **1** cross-linked to DNA at 5'-CG-3'.

Photoactivation of compound **45** in lanes 5 and 6 afforded the same pattern of cross-links, producing orientational isomers for each cross-linkable site. These results suggested the analog adhered to the identical sequence specificity as the natural product, along with the complimentary preference for the 5'-ACG-3' site.

2.4.2 Isolation and Digestion of Cross-Linked Oligomers

In order to unambiguously establish both the sequence specificity and the identity of the cross-link lesion, efforts were undertaken to isolate and characterize by means of NMR spectroscopy the mitosene covalently bound to the guanosine nucleosides. As discussed previously, Tomasz *et al.* had developed this approach in the structure elucidation of the MMC cross-link,[13] and a similar protocol by Hopkins *et al.* had ultimately confirmed the structure of the cross-link lesion of the FR900482.[10b] The proposed strategy would employ a small palindrome sequence, whereupon following cross-link formation, subjection to enzymatic digestion with Snake Venom Diesterase (SVD) and Calf Intestinal Alkaline Phosphatase (AP) would enable isolation of the remaining covalently cross-linked guanosine bases.

Based on related work by Hopkins *et al.*, a self-complimentary 12-base-pair oligomer, whose small size would greatly facilitate the enzymatic digestion and isolation of the interstrand crosslink (ISC) lesion, was utilized.

5' AATTACGTAATT 3'

Figure 2.7. Self-complimentary Oligonucleotide C.

The size of the oligomer inherently resulted in a lower hybridization temperature (~ 40 °C), mandating a lower reaction temperature for production of the cross-link. Hence, it was found that the photolysis reactions had to be performed at 0° C with pre-cooled reaction samples. The instability of the duplex seemed to be more prevalent upon addition of the acetonitrile necessary for solvating the photo-triggered analog. The difficulties associated with the denaturing of the duplex, which precluded cross-link formation, seemed to be more problematic than precipitation of the NVOC analog. A series of reaction conditions were examined in order to maximize ISC formation.

Table 2. Cross-link experiments with oligomer C.

Entry	[DNA] duplex	[Compound **45**]	[NaCl]	% CH_3CN	Photolysis (hrs.)	ISC Yield[a]
1	0.2 mM	1.3 mM	-	3%	1.6	0%
2	0.5 mM	7.6 mM	-	15%	1	≤ 10%
3	0.5 mM	7.6 mM	-	15%	9	0%
4	0.4 mM	33.3 mM	-	67%	1.5	0%
5	0.4 mM	10.0 mM	14 mM	20%	1.5	≤ 10%
6	0.3 mM	20.0 mM	14 mM	40%	1.5	≤ 10%
7	0.4 mM	6.3 mM	500 mM	13%	2	≥ 10%
8	1.1 mM	2.0 mM	250 mM	4%	2.5	0%
9	0.4 mM	3.1 mM	400 mM	18%	1.5	≥ 10%
10	0.5 mM	3.6 mM	160 mM	22%	1.5	0%

[a] Yield based OD_{260} following cross-link isolation.

Initial results showed an ideal concentration of acetonitrile in water to be approximately 15%, with a ten fold excess of compound relative to DNA. Even under these conditions, the isolated yield of the crosslink was no better than 10%. Addition of a 2 M stock solution of NaCl helped stabilize the duplex, with concentrations of 400 to 500 mM giving the best results (10 to 20% cross-link yield). Addition of brine however, visibly increased the precipitation of the hydrophobic analog **45**, indicative of the dichotomy between duplex stability and the solubility of the analog.

Despite the low yield of cross-linked material, efforts focused on investigating the enzymatic digestion. As a standard reaction, the unmodified 12 base pair oligomer was initially digested with both SVD and AP and the progress of the reaction was monitored by reverse phase HPLC.

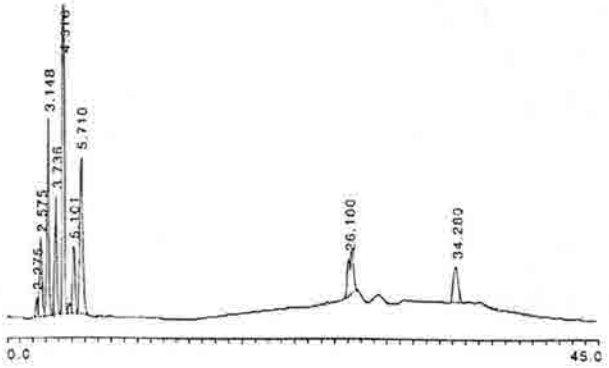


Figure 2.8. Reverse-Phase HPLC trace of the digestion of unmodified Oligomer C by SVD and AP after 2.0 hrs. C18 Column, 92:8 100 mM NH_4OAc/CH_3CN, 7 min; 70:30 100 mM NH_4OAc/CH_3CN, 13 min grad.; 60:40 100 mM NH_4OAc/CH_3CN, 10 min grad.; 92:8 100 mM NH_4OAc/CH_3CN, 10 min grad.

Indeed, HPLC analysis 2 hours into the digest showed several peaks with short retention times corresponding to various partially digested oligomers. Furthermore, 9 hours into the reaction, only four visible peaks were observed as a consequence of complete digestion of the individual nucleosides. This result was confirmed by individual

co-injection of the commercially available nucleosides, whose retention times matched each of the peaks from the completed digestion.

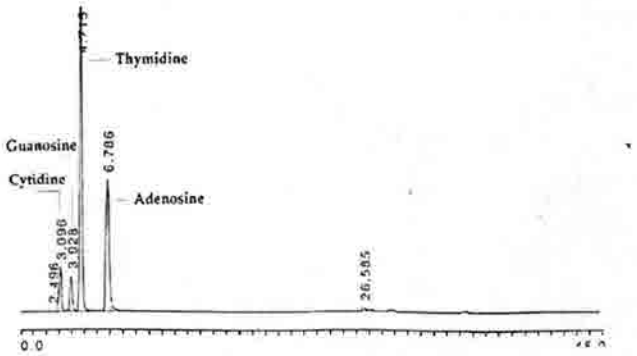


Figure 2.9. HPLC trace of digestion of unmodified oligomer C by SVD and AP following 9.0 hrs.

Following the successful digestion of the standard unmodified oligomer, the cross-linked material was subjected to the identical conditions. HPLC analysis of the digestion after 30 min. revealed a pattern much the same as the standard reaction with an additional major peak at a retention time of 31 minutes (Figure **2.10**).

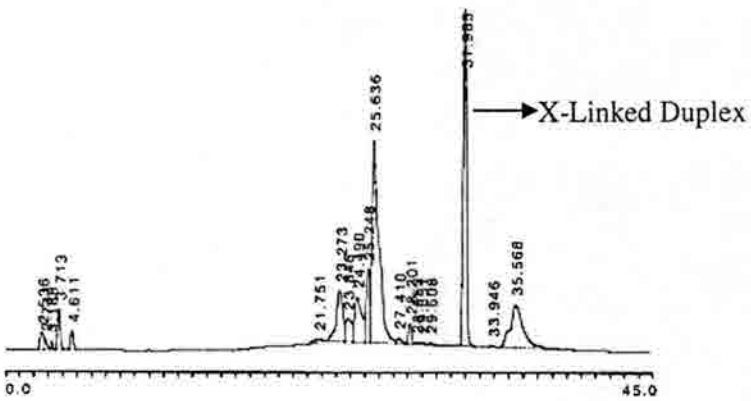


Figure 2.10. HPLC trace of digestion of cross-linked oligomer C by SVD and AP after 0.5 hrs.

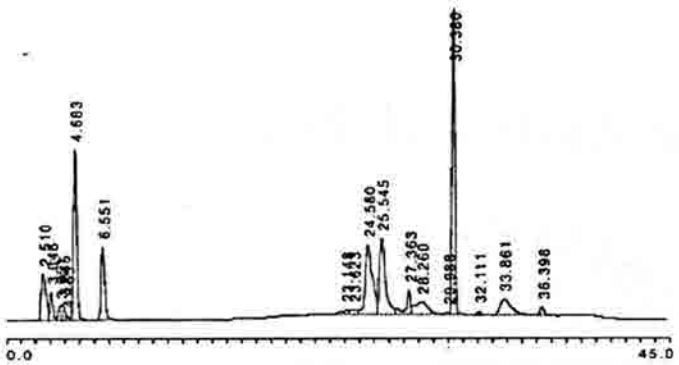


Figure 2.11. HPLC trace of digestion of cross-linked oligomer C by SVD and AP after 20 hrs.

The longer retention time relative to the unmodified sequence was indicative of the peak representing the cross-linked material. Indeed, comparison of HPLC traces of the cross-linked oligomers by MMC and FR900482 reported by Tomaz and Verdine[13,14a] and by Hopkins[10b] showed approximately the same retention time. Twenty hours into the enzymatic digestion, the reaction was far from complete as evidenced by the identical peak at 31 minutes with only a small increase in partially digested nucleotide sequences (represented by peaks having retention times between 2-6 minutes).

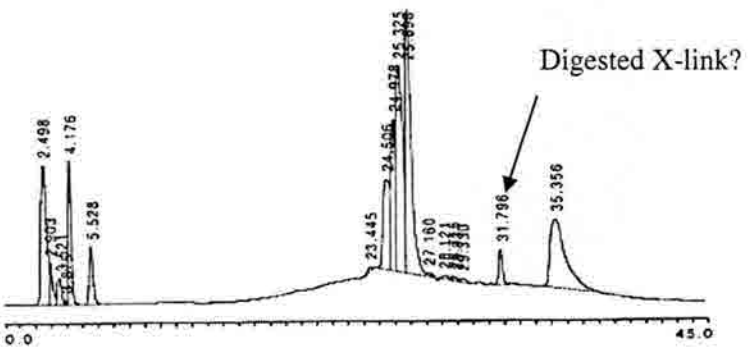


Figure 2.12. HPLC trace of digestion of cross-linked oligomer C by SVD and AP after 48 hrs.

With additional amounts of both enzymes added, the reaction was continued for an additional 28 hrs. HPLC analysis revealed the digestion had proceeded with a substantial decrease of the peak at 31 min. assigned to be the starting cross-linked oligomer. Furthermore, a proportional increase in the partially digested nucleotides was observed. After this point however, the enzymatic digestion ceased to proceed, and longer reaction times did not reveal the corresponding individual nucleosides. Of greater concern, a peak representing the cross-linked guanosines could not be identified. Although the reasons for the abrupt halt in the reaction are not clear, a similar incomplete digestion had been observed by Tomasz and Verdine when using the alternative P1 nucleases,[13,14a] suggesting the possibility that the enzyme efficacy is disrupted by the covalent cross-link. The inclusion of the enzyme DNAse I, a particularly useful endonuclease for pyrimidine sequences, may provide a means for completion of the digestion. Equally troubling though, was the low amount of material isolated from the initial cross-linking reactions. Efforts to improve this reaction utilizing longer sequences for added duplex stability did not succeed. The 18 base pair self-complimentary oligomer D failed to afford any cross-links when reacted at room temperature or without the high salt concentrations used with the 12 bp oligomer.

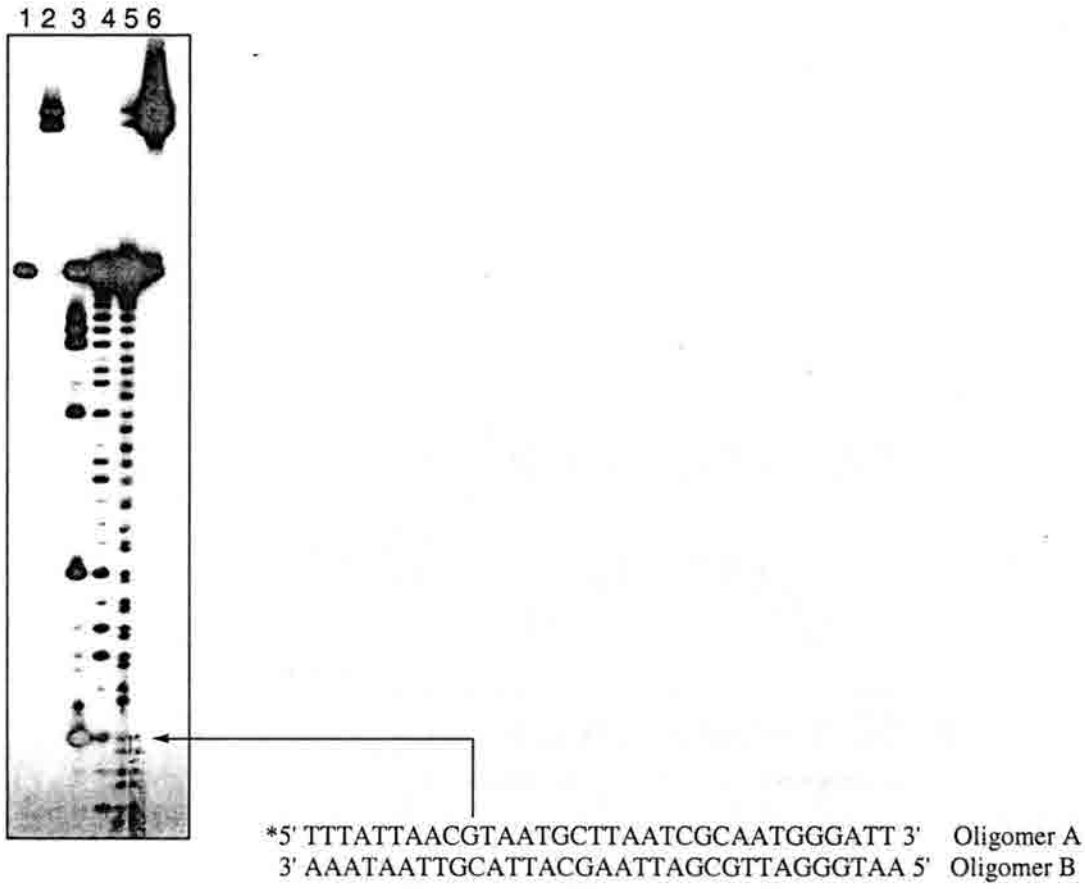
5' TATAATTACGTAATTATA 3'

Figure 2.13. Self-complimentary oligomer D.

The combination of low cross-link yields and incomplete enzymatic digestion required finding an alternative means of confirming the sequence specificity of the photo-triggered analog.

2.4.3 Footprinting Reactions

Footprinting reactions mediated by Fe(II)/EDTA cleavage of cross-linked duplexes offer an alternative means of determining sequence specificity. Developed by Tullius and Dombroski[39] and extended by Hopkins,[40] the technique involves initial isolation of the cross-linked lesion of an end-labeled duplex. Reaction of both the cross-linked duplex and unmodified labeled oligomer with Fe(II)/EDTA affords non-selective cleavage of the DNA backbone affording an equimolar assortment of all fragment sizes up to and including the full length of the strand. DPAGE analysis of the unmodified oligomer shows all possible fragment sizes whose difference in mobility changes as a consequence of the individual sequence length. Likewise, analysis of the digested cross-linked sequence produces a similar pattern for the small fragments up to and including the covalently modified residue. Fragment sizes following the site of alkylation have a retarded mobility as a consequence of being covalently attached to the complementary strand and appear near the origin of the gel. Hence, isolation of the major cross-linked band by reaction of duplex **1** with the photo-triggered analog **45** and subsequent foot-printing produced the image below following 20% DPAGE (Figure **2.14**).

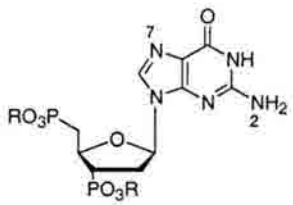


Duplex **1**

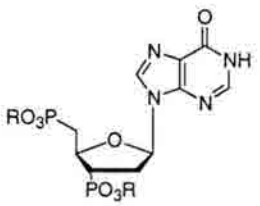
Figure 2.14. Autoradiogram of Fe(II)-EDTA footprinting of cross-linked 1 (labeled at the 5' terminus of oligo A). Lane 1: DNA standard (oligo A). Lane 2: cross-linked 1. Lane 3: Maxam-Gilbert G. Lane 4: Maxam-Gilbert G+A. Lane 5: 1 mM Fe(II)-EDTA digestion of oligo A (control). Lane 6: 1 mM Fe(II)-EDTA digestion of cross-linked 1.

The observed cleavage pattern of the major cross-linked lesion demonstrates the guanosine of the 5'-ACG-3' sequence as the site of alkylation. Consistently low yields of cross-link with further reactions using this duplex precluded additional foot-printing reactions with 5'-end-labeling on oligomer B and isolation of the minor cross-link lesion. Further experimentation with a synthetic duplex where the minor TCG cross-linking site was deleted also afforded low yields of cross-links. The capricious yield of interstrand

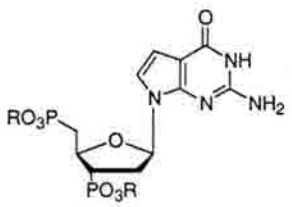
cross-link is attributed to the low solubility of the analog under aqueous conditions as evidenced by visible precipitation in the reaction mixture. Furthermore, the hydroxymethyl functionality results in a significant portion of the alkylation occurring as monoalkylation instead of cross-link. Corroboration of the footprinting data however, was secured by substitution of the 2'-deoxyguanosine base with 2'-deoxyinosine at the 5'-CpG- 3' steps of duplex **1** (Figure **2.15**).



52. 2'-deoxyguanosine



53. 2'-deoxyinosine



54. 2'-deoxy-7-deazaguanosine

Figure 2.15. Structures of modified 2'-deoxyguanosine residues

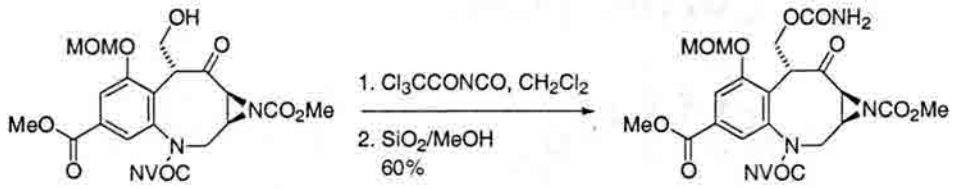
Reactions with the 2'deoxyinosine-modified duplex resulted in no observable cross-link, while the use of the 2'-deoxy-7-deazaguanosine showed cross-links in the same manner as the unmodified 2'-deoxyguanosine-containing duplex. These results strongly implicate the N2 exocyclic amine of 2'-deoxyguanosine as the site of alkylation in the same manner as the natural products **1** and **2**.

2.4.4 Synthesis of the Carbamoyl Photo-triggered Analog

Further efforts allowed for the successful construction of the carbamoyl version of the photo-triggered analog, which had previously eluded synthetic attempts. During the course of the total synthesis of the natural products FR900482 (**1**) and FR66979 (**2**), investigations into functionalization of a similar ketone substrate revealed that alumina was responsible for the production of the exocyclic methylene degradation product **47** (see Scheme **2.19**). Employing SiO_2 and MeOH as alternative conditions for the cleavage

of the trichloroacetate from the intermediate resulted in the successful production of **48** (Scheme **2.21**).[41]

Scheme 2.21. Successful installment of the carbamate functionality.



45 **48**

As expected, cross-linking experiments with linearized pBR322 revealed a capacity of **48** to cross-link DNA comparable to that of analog **45** following activation with 350 nm light. Furthermore, a comparison study was undertaken to evaluate if the additional carbamoyl functionalization at C13 enhanced the ability of **48** to produce interstrand cross-links relative to the hydroxymethyl precursor. Cross-linking experiments with duplex **1**, ^{32}P end-labeled at the 5'-end of oligomer A, demonstrated a significant increase of interstrand cross-link production compared to the hydroxymethyl analog **45** (Figure **2.15**).

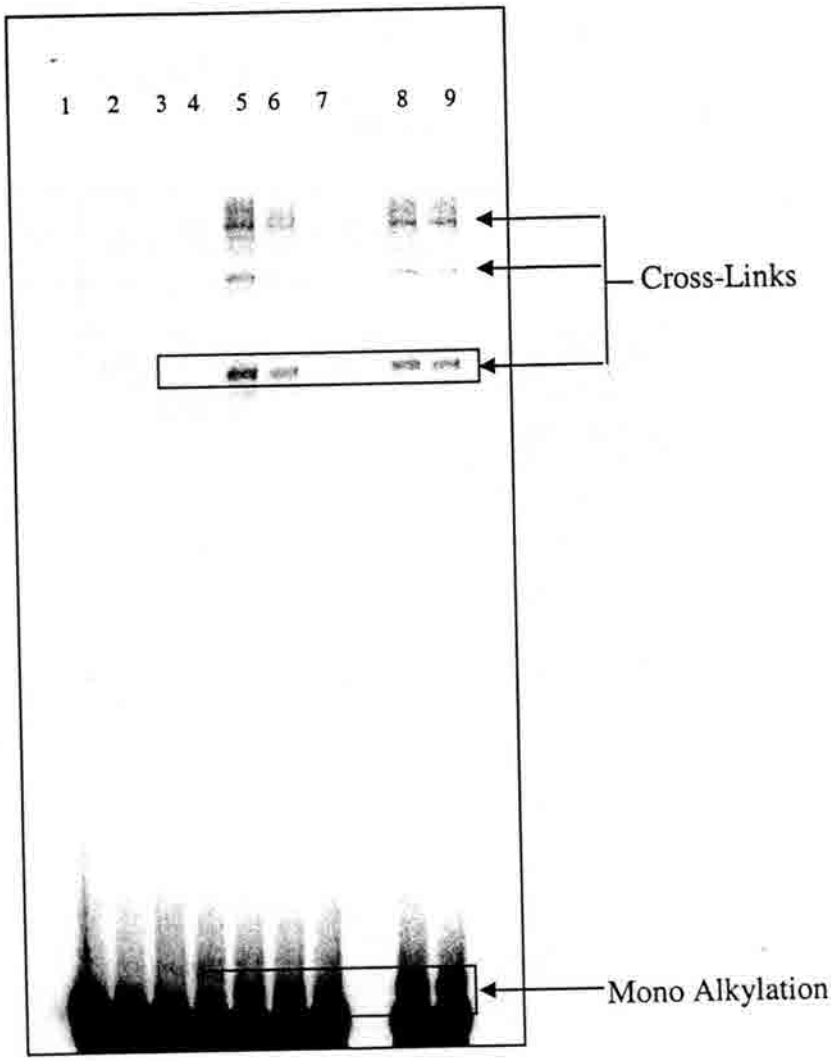
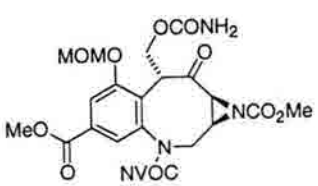
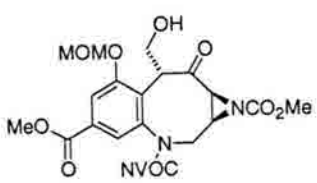


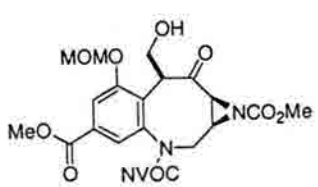
Figure 2.15. Autoradiogram of cross-linked duplex 1 (labeled at the 5' terminus of oligo A) with analogs 45, 46, and 48. Lane 1: DNA standard. Lane 2: DNA, *hv*, 60 min. Lane 3: DNA, 10 mM FR900482 + 10 mM 2-mercaptoethanol. Lane 4: 50 mM **48**, dark, 60 min. Lane 5: 50 mM **48**, *hv*, 60 min. Lane 6: 10 mM **48**, *hv*, 60 min. Lane 7: 1 mM **48**, *hv*, 60 min. Lane 8: 50 mM **45**, *hv*, 60 min. Lane 9: 50 mM **46**, *hv*, 60 min.



48. Carbamoyl analog



45. Hydroxymethyl analog



46. *epi*-Hydroxymethyl analog

Quantitation of the major cross-link bands by use of a phosphorimager reveals nearly twice the amount of cross-linked product (boxed area) for the carbamoyl analog compared to either of the decarbamoyl analogs.

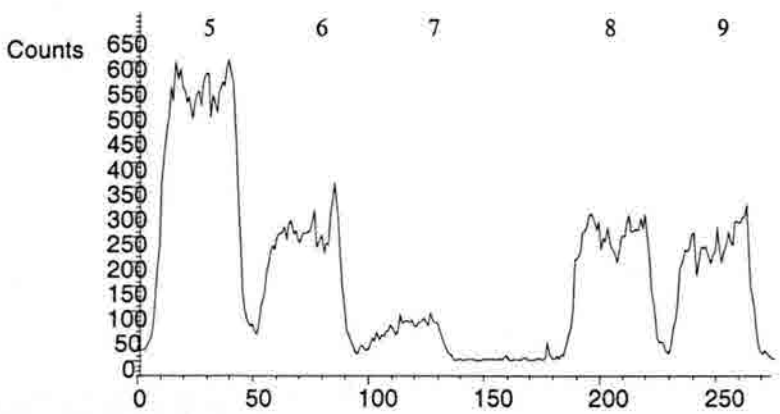


Figure 2.16. Quantitation of major cross-link by phosphorimagery.

Moreover, the enhanced ability of the C13 carbamoyl group as a leaving group led to only half the amount of monoalkylation (boxed area at the base of the gel in Figure **2.15**) observed with the analogs **45** and **46** as measured by phosphorimagery (Figure **2.17**). Notably, comparison of the decarbamoyl analogs **45** and **46** revealed approximately identical yields of cross-link and monoalkylation despite the difference of stereochemistry at C7.

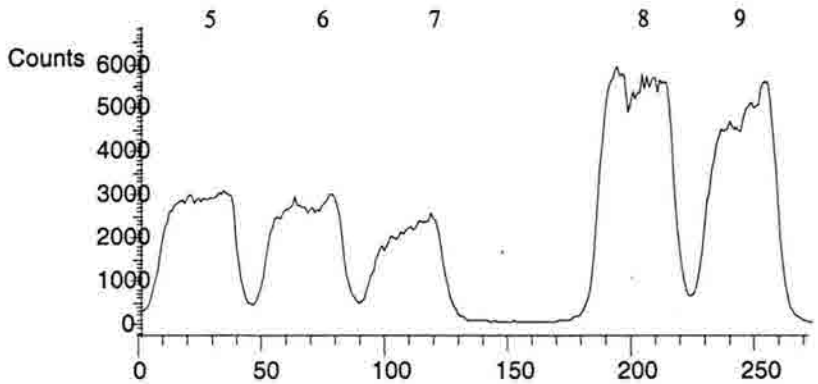
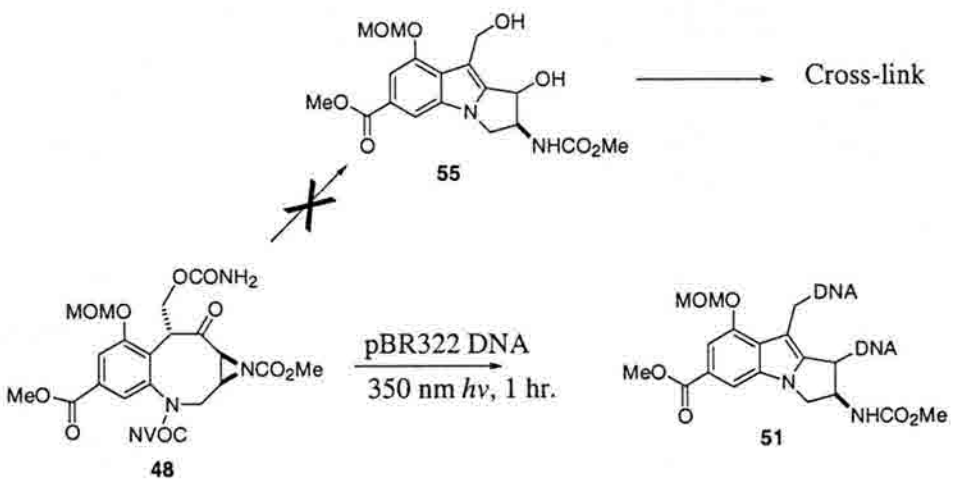


Figure 2.17. Quantitation of monoalkylation by phosphorimagery

The results of this study suggest a pre-covalent interaction between the analog and the DNA in a similar manner as the natural products. In the absence of such an interaction, photoactivation of analogs **45** and **48** in the aqueous media would be expected to generate the identical dihydromitosene core leading to indistinguishable yields of cross-link (Scheme **2.22**).

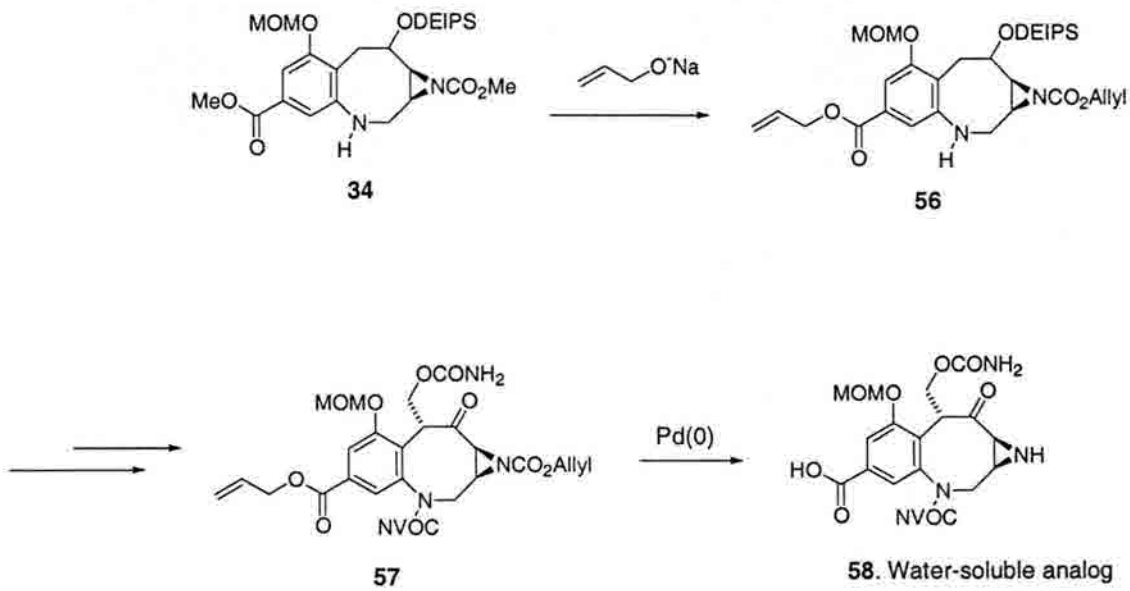
Scheme 2.22. Direct cross-linking of analog **48** in the absence of a dihydromitosene intermediate.



2.5 Future Directions

In general, the hydroxymethyl- and carbamoyl-photo-triggered analogs were successful in affording interstrand cross-links with DNA upon UV activation. However, their low solubility in aqueous media impedes their utility as biological probes or model compounds for future clinical applications. One possible solution would involve synthesizing a modified analog with both a free aziridine and carboxylic acid, providing a potentially water-soluble analog. This alternative compound could readily be synthesized by transesterification of both carbomethoxy groups at an earlier intermediate and cleaved following installation of the urethane functionality (Scheme **2.23**). As noted earlier, the minimal overlap of aziridine nitrogen lone pair of electrons with the carbamate carbonyl, as a consequence of being in the strained ring, makes the carbamate functionality much more susceptible to both hydrolysis and reduction in the same manner as an ester group.

Scheme 2.23. Proposed synthesis of a water-soluble photo-triggered analog (**58**).



2.6 Conclusion

A fully functional photo-triggered mitosene progenitor was synthesized in approximately 25 steps based on a previously reported synthetic model study. The capacity to cross-link duplex DNA was successfully demonstrated with linerized pBR322 and radiolabeled synthetic oligonucleotides. Furthermore, footprinting studies combined with cross-linking experiments with modified 2'-deoxyguanosine bases demonstrated the identical sequence specificity for 5'-CG-3' sites in the minor groove as the natural product FR900482. The ability to cross-link DNA gives rise to their utility as biological probes and templates for the design and synthesis of photo-triggered *anti*-neoplastic agents. Furthermore, the specificity for 5'-CG-3' sequences provides for a complementary biological tool to the naturally occurring and clinically used AT sequence-specific photo cross-linking psorelans.[42] The lengthy synthetic preparation and low solubility of these photo-triggered mitosene progenitors in aqueous media, however, requires future efforts to focus on structurally less complex and more hydrophilic analogs.

Chapter 3

Total Synthesis of (+)-FR66979 and (+)-FR900482

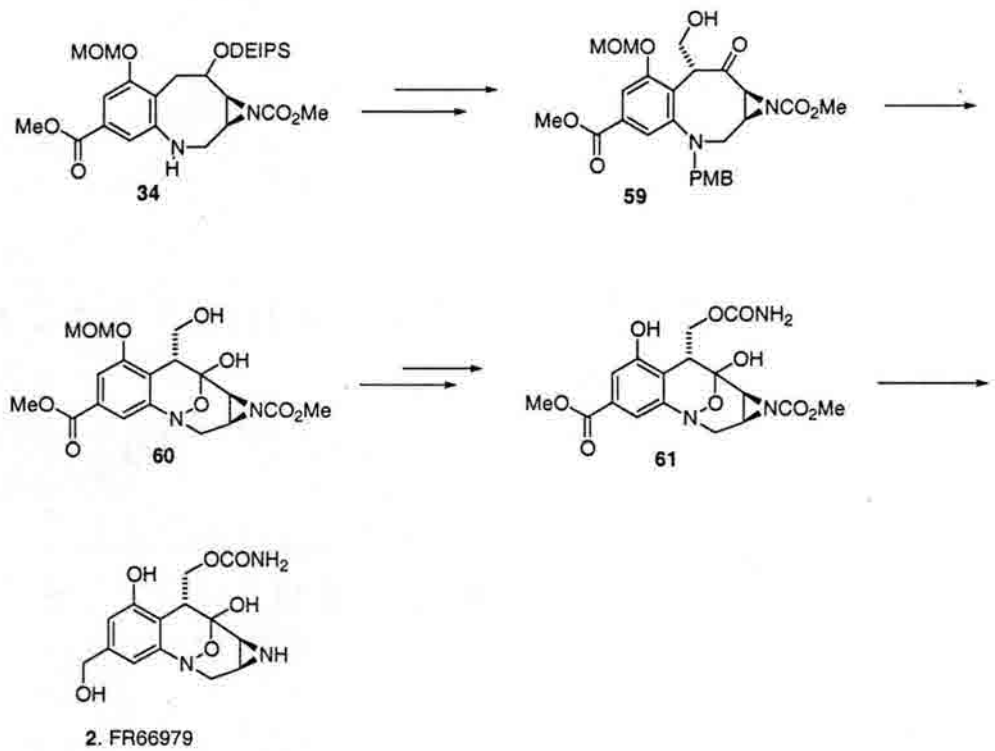
3.1 Introduction

In parallel with the synthetic and biochemical studies of a photo-triggered analog, the total synthesis of the natural products FR900482 (**1**) and FR66979 (**2**) was carried out. By example of the success and failures of the previous three total syntheses and formal total synthesis, a synthetic plan was devised that would culminate in an enantioselective synthesis requiring the fewest synthetic steps reported to date. Several elements of the strategy would allow this goal to be achieved. By utilizing the identical aziridine segment as in the photo-triggered analog study, the optical activity of the natural product would be secured from the start. Secondly, the overall number of synthetic transformations dedicated to protecting group manipulations would be kept to a minimum. Hence, both of the carbomethoxy groups and the phenolic MOM acetal would be maintained throughout the entire synthesis only to be removed at the very end. Lastly, a novel method of constructing the hydroxylamine hemiketal bond was planned that would allow a rapid assembly of the core structure of the natural product.

3.2 Proposed Synthetic Strategy and Model Studies

The following scheme depicts the general strategy envisioned to complete the total synthesis utilizing common precursors from the photo-triggered analog discussed in Chapter 2 (Scheme **3.1**).

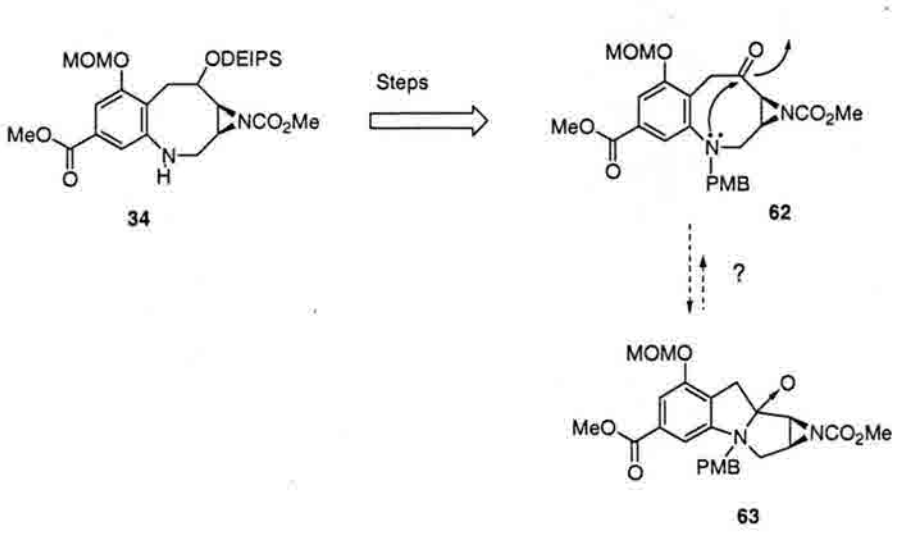
Scheme 3.1. Synthetic strategy for the synthesis of the natural product **1** and **2.**



In a manner similar to the previously discussed synthetic work (Chapter 2), the hydroxymethyl intermediate **59** was envisioned to be derived from eight-membered ring amine **34**. The significant difference would be the use of the *p*-methoxybenzyl (PMB) to protect the nitrogen in place of the NVOC group. The change in protecting groups, however, might give rise for potential problems. In the absence of the electron-withdrawing carbamate group, the proposed ketone intermediate might be in jeopardy of

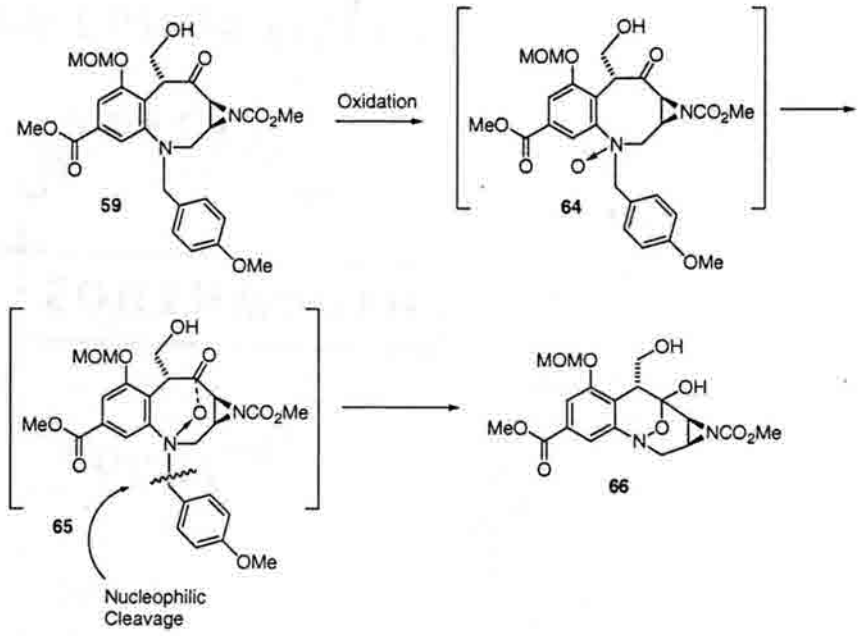
equilibrating to charged species **63**, consequently disrupting the aldol reaction and other proposed strategies (Scheme **3.2**).

Scheme 3.2. Potential equilibration of ketone intermediate **62**.



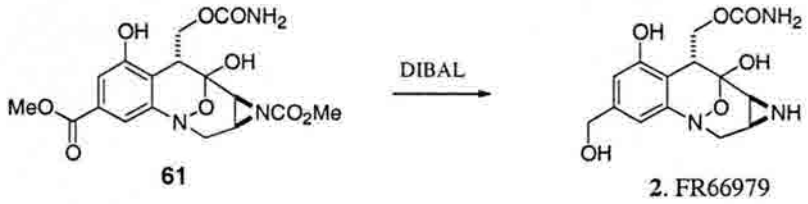
Despite this concern, and assuming that a successful synthesis of the aldol product **59** could be obtained, a novel strategy for installing the hydroxylamine hemiketal was devised. This approach involved oxidation of the tertiary amine to give N-oxide **64**. Relying on the reactive nature of the PMB methylene being enhanced by the formal charge of the adjacent nitrogen of the N-oxide, a mild nucleophile could potentially mediate the cleavage of the PMB resulting in closure to the desired hydroxylamine hemiketal **66** (Scheme **3.3**).

Scheme 3.3. Proposed strategy for incorporating the hydroxylamine hemiketal functionality.



Before embarking on the total synthesis of the natural products FR900482 (**1**) and FR66979 (**2**), an investigation was made into the feasibility of the proposed final deprotection. Following the proposed oxidative rearrangement (Scheme **3.3**), the hydroxymethyl intermediate would be elaborated to the final dicarbomethoxy compound **61**. Final DIBAL reduction of both carbomethoxy groups should allow for the completion of the synthesis of FR66979.

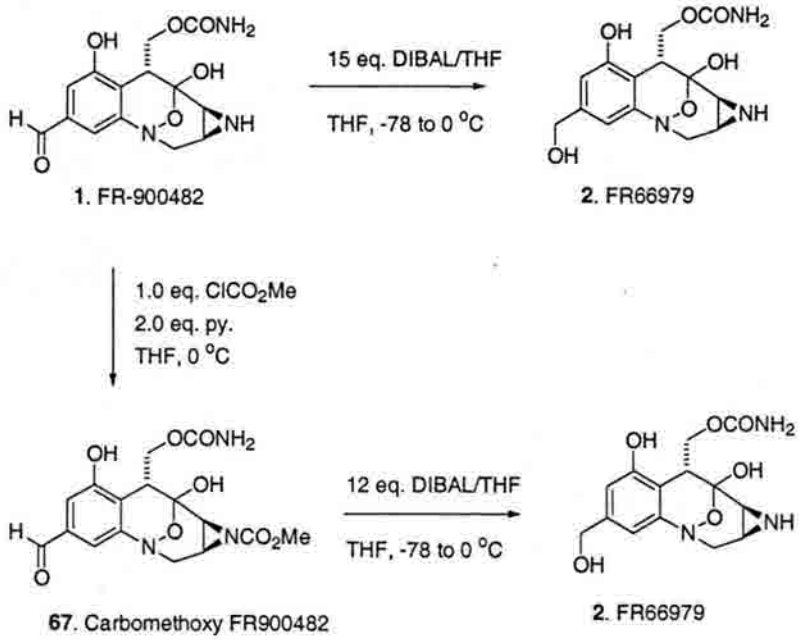
Scheme 3.4. Proposed final deprotection.



The capacity of diisobutylaluminium hydride (DIBAL) to reduce both methoxycarbonyl groups had been demonstrated in Danishefsky's total synthesis [18b] and later in the model study by Rollins and Williams.[28] Both substrates however, included protecting groups on the remaining functionalities of the molecule, allowing not only stability, but also solubility in most organic solvents. Indeed, both procedures used solutions of DIBAL/hexane, with methylene chloride as the reaction solvent. In the proposed reduction, the resulting natural product is water-soluble, making isolation from the crude product, which would adhere strongly to the aluminum, problematic. Compounding this problem is the presence of the unprotected phenol and free carbamate, which, would require having to cleave as many as 5 equivalents of aluminum "ate" salts from the reaction product. Finally, the stability of the unprotected hydroxylamine hemiketal in the presence of an excess amount of reducing agent was in question.

The availability of the natural product FR900482, by generous donation from the Fujisawa Pharmaceutical Company, allowed for a model study of the end-game strategy. After considerable experimentation, successful reduction conditions of FR900482 to FR66979 were established using DIBAL/THF solutions in THF followed by workup with sodium sulfate decahydrate (Scheme **3.5**). The fortuitous solubility of the natural product in THF allowed for the selective protection of the aziridine as the methyl carbamate **67**. This substrate provided the desired model compound for the reduction, confirming the ability to reduce this group. Reduction with DIBAL on both systems afforded the natural product FR66979 in moderate yield following workup with sodium sulfate decahydrate.

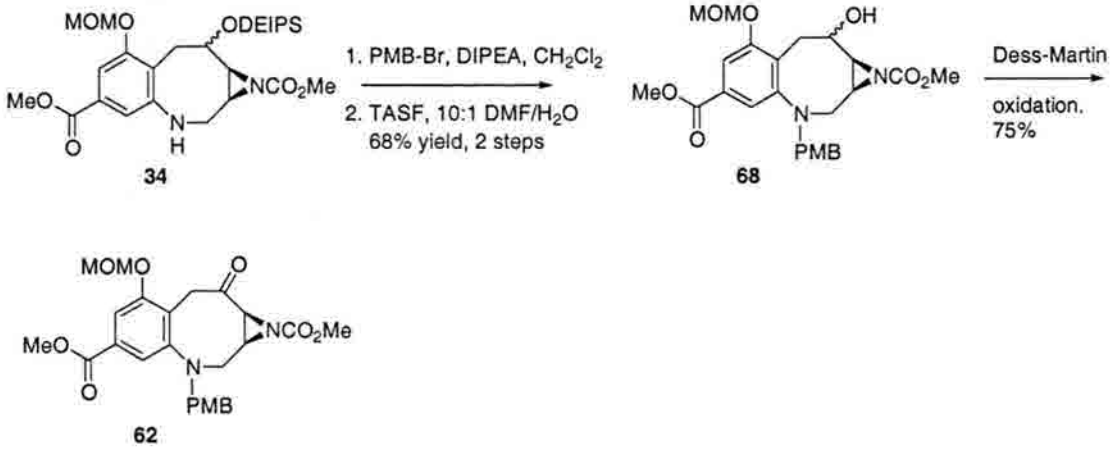
Scheme 3.5. Successful model studies for the final reduction.



3.3 Total Synthesis of (+) FR66979 and (+) FR900482

Having established a reasonable synthetic plan, efforts to carry out the total synthesis were initiated. Work toward FR900482 and FR66979 began with cyclic amine **34** whose preparation in twenty one steps was described in the previous chapter.

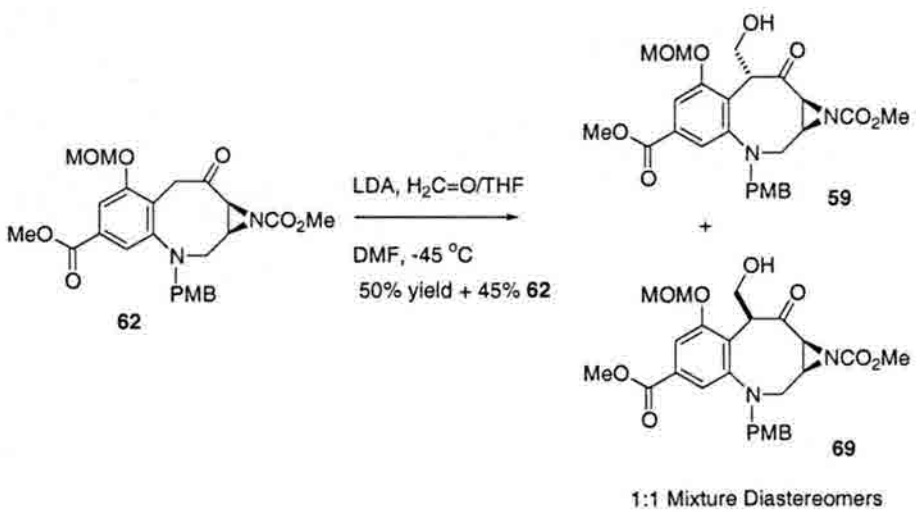
Scheme 3.6. Synthesis of ketone **62** from the common intermediate amine **34**.



Reaction of **34** with *p*-methoxybenzyl bromide and Hünig's base afforded the corresponding protected amine in high yield. Initial deprotection of the DEIPS group using TBAF in THF/MeOH proceeded in low yield in this case, with the Payne rearranged epoxide comprising most of the product. An alternative procedure used tris(dimethylamino)sulfonium diflourotrimethylsilicate (TASF) in 10:1 DMF/H_2O, which reportedly is effective in systems prone to side reactions of the resulting deprotected hydroxyls.[43] TASF-mediated deprotection afforded the secondary alcohol **68** (80% yield) which was oxidized the ketone **62** with Dess-Martin periodinane in good yield. Fortunately, the resulting ketone was stable, and no evidence of equilibration to the 5,5-bicylcic ring system was observed (Scheme **3.2**).

Utilizing aldol conditions identical to those used in the NVOC series, reaction with anhydrous formaldehyde produced the desired hydroxymethyl product, albeit in a lower yield (50%). As before, the reaction furnished approximately equal amounts of each diastereomer. The moderate yield of the reaction was accompanied by recovery of a large amount of starting ketone (45%), as was the case in the NVOC series.

Scheme 3.7. Key aldol reaction.



The exact reason for the low conversion in this reaction has not been identified. Competing O-alkylation could explain the high recovery of starting ketone **62**, which could come from hydrolysis of an enol hemiacetal on workup. Alternatively, a portion of the aldol product itself may undergo retro aldol reaction on workup due to the stability of the enolate. Extensive investigation into alternative work-up conditions did not result in any increase in yield. Furthermore, longer reaction times (over 2 hrs.) or higher reaction temperatures above (-45 °C) resulted in only production of the exocyclic methylene product **70** as a result of elimination.

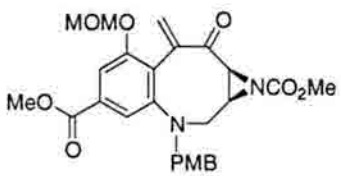


Figure 3.1. Elimination product **70.**

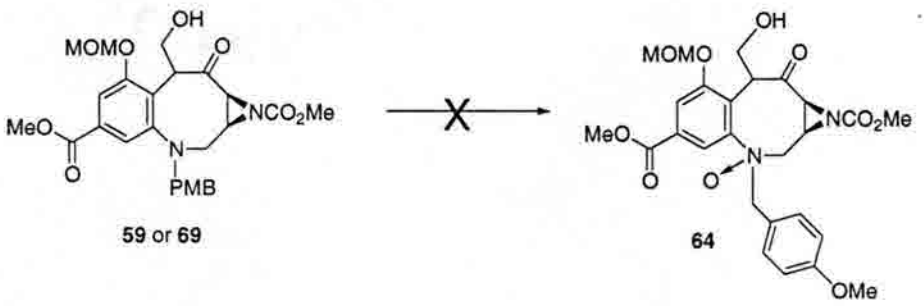
Moreover, conventional aldol reaction conditions such as using THF or THF/HMPA mixtures in place of DMF as the reaction solvent did not lead to any isolated product. Attempts to form the boron enolate also failed, since the compound decomposed when exposed to the dibutylboron triflate and triethylamine.[44] Despite the inability to improve the reaction, the moderate yield and recovery of starting material provided a workable means for continuing the synthesis.

Unfortunately, attempts to recrystalize the diastereomeric product mixture failed to afford any resolution. Preparative thin layer chromatography provided the only means of separating the diastereomers. Likewise, the stereochemistry of the hydroxymethyl group at C7 for each diastereomer could not be unambiguously assigned. Comparison of the ^{1}H NMR spectrum of either diastereomer with the aldol products from the NVOC series did not reveal any obvious correspondence. Ultimately, the diastereomer with the

correct stereochemistry would have to be determined by total synthesis and comparison to the natural product. Henceforth, both diastereomers were carried forward for the investigation of the key oxidative rearrangement.

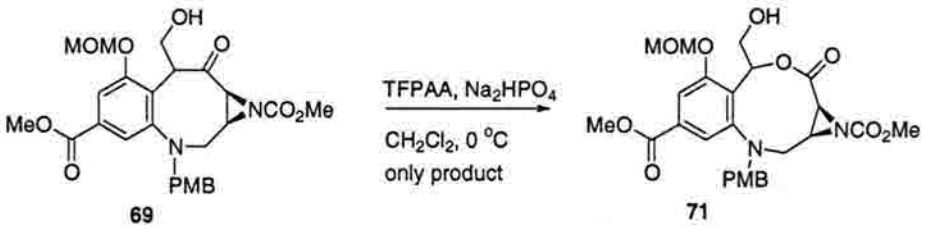
Initial attempts to oxidize the tertiary amine to the corresponding N-oxide met with a great deal of resistance. Surprisingly, reaction with mCPBA and Davis' reagent[45] at ambient temperature and dimethyldioxirane (DMDO)[46,47] at 0 °C led to no reaction.

Scheme 3.8. Failed oxidations to N-oxide **64**.



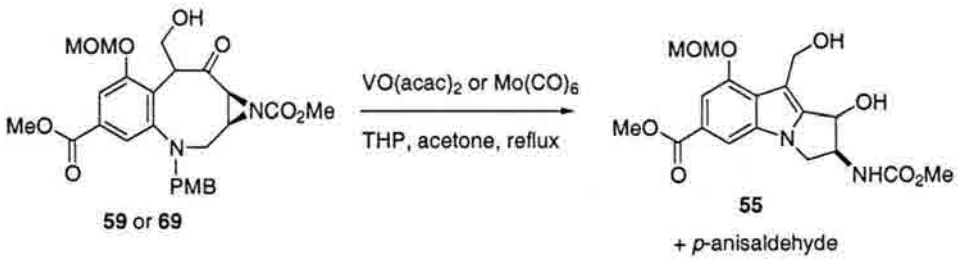
Apparently, the nucleophilicity of the nitrogen lone pair of electrons was attenuated by its proximity to the ketone carbonyl group, reducing reactivity. Treatment with excess DMDO at ambient temperature finally led to partial reaction, producing both *p*-anisaldehyde and an unidentified product in addition to starting material. The product from the oxidation proved difficult to identify since the ^{1}H NMR spectrum of the product consisted of a series of broad signals. Likewise, poor TLC mobility under various conditions hampered further attempts to purify the compound. Numerous repetitions of the reaction failed to provide any further ability to discern the structure of the product and alternative oxidation conditions were sought. Reaction with trifluoroperacetic acid (TFPAA)[48] led to Baeyer-Villiger reaction, furnishing the nine-membered lactone **71**, confirming the ineffectiveness of peracids for this reaction (Scheme 3.9).

Scheme 3.9. Undesired Baeyer-Villiger reaction.



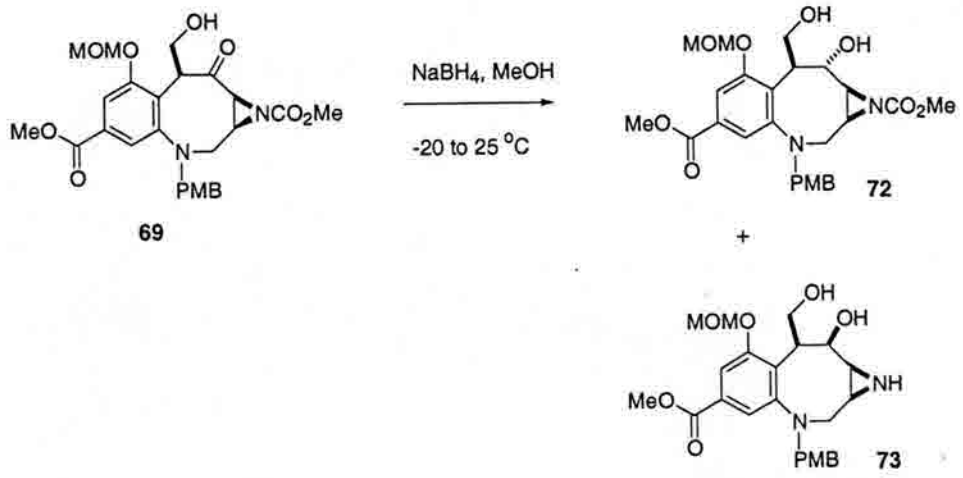
Attention was next focused on the use of the $VO(acac)_2$ and the related $Mo(CO)_6$ catalyst in conjunction with *t*-butyl hydrogen peroxide (THP).[49] Both catalysts resulted in the efficient production of the mitosene species **55** resulting from direct oxidative cleavage of the PMB group (Scheme **3.10**).

Scheme 3.10. Mitosene formation by direct oxidative PMB cleavage.



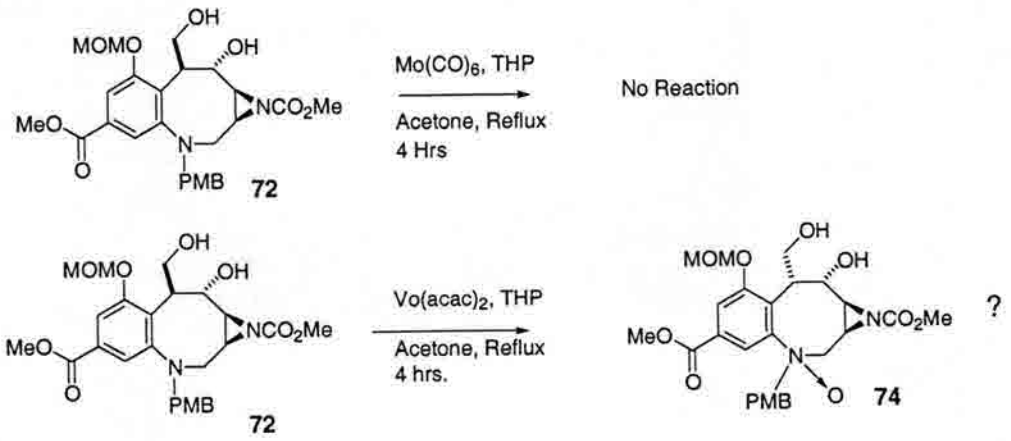
In order to further examine this result, the ketone of one diastereomer was reduced with $NaBH_4$ and, unexpectedly, the reaction required ambient temperature to proceed. The reduction afforded a mixture of diols with the methyl carbamate having additionally been cleaved on one diastereomer (the relative stereochemistry arbitrarily shown).

Scheme 3.11. Reduction to diols **72** and **73.**



Refluxing diol **72** with $Mo(CO)_6$ and *t*-butyl hydrogen peroxide in acetone however, lead to no reaction, while the use of $VO(acac)_2$ and *t*-butyl hydrogen peroxide (THP) slowly converted the starting material to a product having a baseline mobility by TLC analyses, presumed to be the N-oxide **74**. No PMB cleavage was observed with either of these reaction conditions.

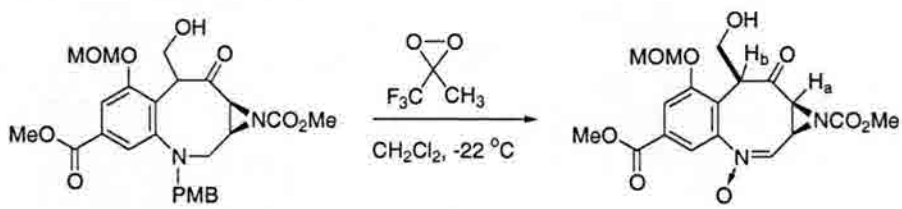
Scheme 3.12. Oxidation reactions with diol **72.**



The divergence in reactivity following the reduction of the ketone hinted that the interaction between the amine and carbonyl of the ketone had a major influence on the success of the oxidation reactions. The proposed strategy of generating the N-oxide in the presence of the ketone consequently seemed less promising.

The remaining options for effecting the proposed oxidation resided with DMDO. While it seemed probable that the unidentified product from the initial study with this reagent was some type of over-oxidized mitosene resulting from initial PMB cleavage, there remained the possibility of an alternate mode of reactivity. The more reactive trifluoromethyl methyl dioxirane (TFMD)[50] was used in combination with lower reaction temperatures in order to investigate this reaction further. Generation of a solution of TFMD (0.08 M) followed by rapid addition to a solution of **59** and **69** in methylene chloride at -22 °C resulted in the isolation of nitrone **75** along with *p*-anisaldehyde and *p*-anisic acid.

Scheme 3.13. Trifluoromethyl methyl dioxirane oxidation to nitrone **75**.

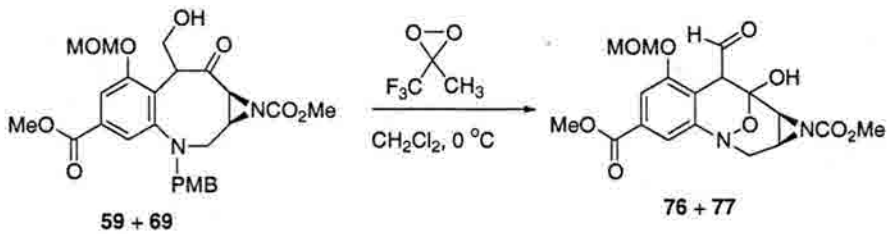


59 + 69 **75**

The stereochemical assignment was based on "W" coupling (J= 4.5 Hz) between the C7 proton and C9 proton of the aziridine ring (see Scheme **3.13**, H_a/H_b). The production of the nitrone represented a significant result, indicating the ability of the dioxiranes to oxidize the nitrogen prior to oxidative cleavage of the PMB group. The over-oxidation of the nitrogen before closing to the desired hydroxylamine hemiketal was

likely a consequence of the low reaction temperature. Performing the identical reaction at 0° C did not lead to the nitrone but rather afforded a new product, whose identity was initially thought to be the hydroxylamine hemiketal aldehyde (**76** and **77**) (Scheme **3.14**).

Scheme 3.14. Initial proposed reaction product from TFMD oxidation.



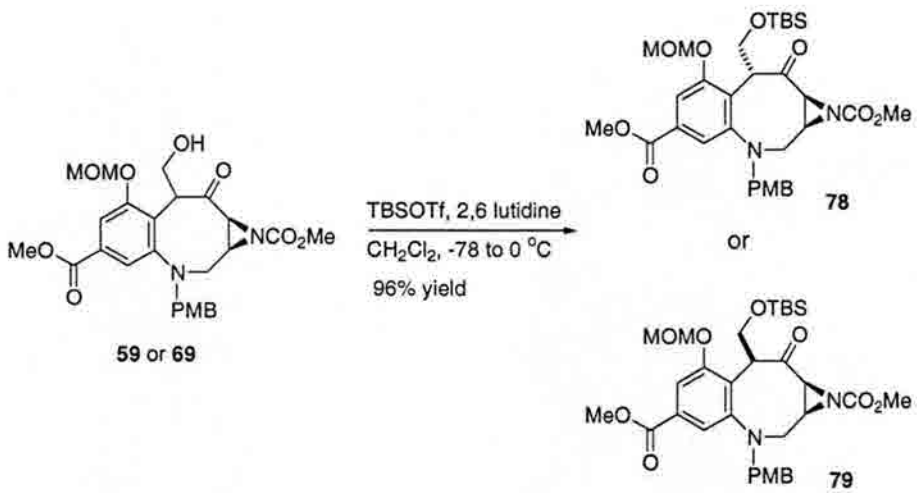
59 + 69 **76 + 77**

The ^{1}H NMR spectrum of the product proved interesting in that it showed an equilibrating mixture of two products. Additionally, reaction of each diastereomer led to the production of two separate sets of equilibrating products. The ^{1}H NMR spectrum showed a set of doublets, one from each product, appearing far downfield in the spectrum (9.7 and 10.1 ppm), corresponded to either the proton of an aldehyde or on the sp^2 carbon of a nitrone. Further analysis by COSY NMR spectrum revealed coupling of this proton to another at around 6.0 ppm and not the proton of the aziridine ring on C10. This suggested the aldehyde structure shown in Scheme **3.14**, although further structural elucidation was hampered by the appearance of two inseparable products in the spectrum. Additionally, mass spectral analysis did not have a mass corresponding to the proposed hydroxylamine hemiketal aldehyde product. Further attempts to convert this compound into the natural product through four additional synthetic steps failed. Finally, ^{19}F NMR spectroscopy revealed two fluorine peaks, presumably one from each equilibrating products, confirming suspicions that an equivalent of 1,1,1-triflouro-2-propanone had been incorporated into the product. The exact structure of either product and the

mechanism by which the 1,1,1-trifluoro-2-propanone was incorporated (presumably as a hydrate) remains to be determined.

The capacity of TFMD to oxidize the compound in the desired sequence led to the reinvestigation of the DMDO reaction. Presumably, both types of dioxirane reagents oxidized the molecule in a similar fashion, with additional over-oxidation by DMDO leading to an unidentifiable product. This event most likely occurred with oxidation of the hydroxymethyl group to the corresponding carboxylic acid. Protection of the alcohol of both diastereomers **59** and **69** with TBSOTf and lutidine at low temperatures furnished the silyl ethers **78** and **79** without production of eliminated side product.

Scheme 3.15. Silyl protection of aldol adducts.



For purposes of clarity, the stereochemistry of both diastereomers is included although their identity remained unknown at the time. Notably, diastereomer **78** demonstrated conformational exchange by ^{1}H NMR at ambient temperature. The results of a series of reactions on both diastereomers with dimethyldioxirane (0.1 M solution in acetone) are tabulated below (Table **3.1**).

Scheme 3.16. Dimethyldioxirane reactions on **78** and **79.**

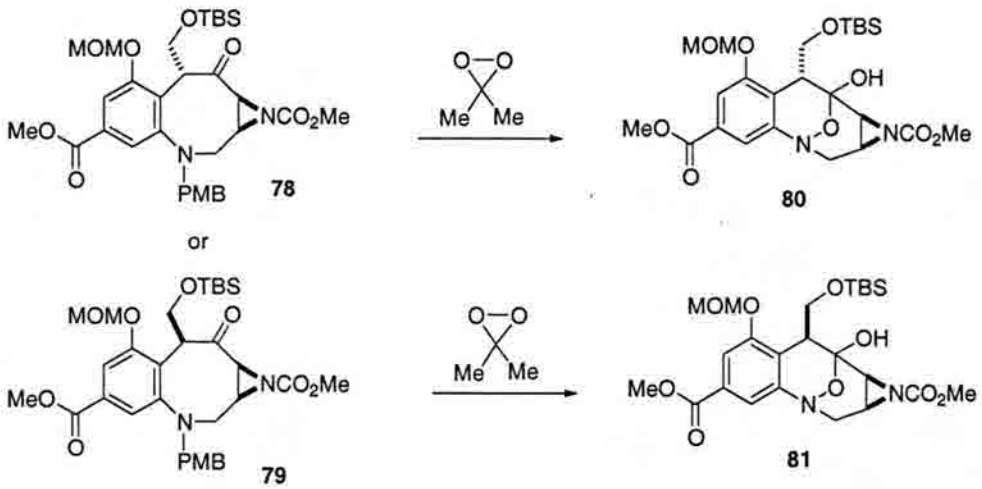
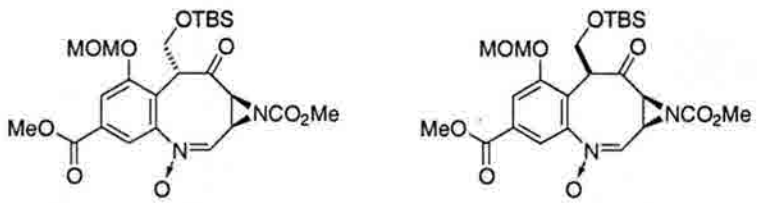


Table 3.1. Dimethyldioxirane reaction conditions.

Substrate	*Conditions*	*Result*
78	excess DMDO, CH_2Cl_2, K_2CO_3, 0-25 °C	Hydroxylamine hemiketal **80**+ **78** or Nitrone **82** + **78**
79	excess DMDO, CH_2Cl_2, K_2CO_3, 0-25 °C	Nitrone **83** + **79**
79	excess DMDO, CH_2Cl_2, Na_2HPO_4, 0-25 °C	Nitrone **83** + dec.
78	excess TFMD, CH_2Cl_2, -22 °C	Dec.
79	DMDO, 2 eq., CH_3CN/phosphate buffer (pH 6), 25 °C	No Reaction
79	excess DMDO, CH_3CN or DMF, Na_2HPO_4, 0-25 °C	**79** + dec.
78 or **79**	excess DMDO, CH_2Cl_2, Na_2HPO_4, 0-25 °C	Nitrone
78 or **79**	excess DMDO, CH_2Cl_2, K_2CO_3 (excess), 0-25 °C	*p*-Anisaldehyde
79	excess DMDO, CH_2Cl_2, K_2CO_3, 18-Crown-6, 0-25 °C	Elimination **70**

Dec. = decomposition

In general, reaction with excess DMDO in methylene chloride and K_2CO_3 resulted primarily in production of either nitrone **82** or **83** along with recovery of starting material.



82 **83**

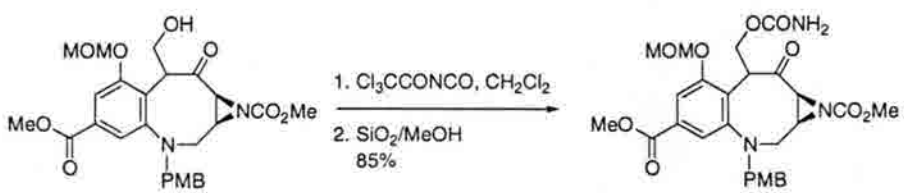
Figure 3.2. Nitrone byproducts **82** and **83**.

Potassium carbonate was employed based on literature reports describing the production of acetic acid from the degradation of DMDO.[51] In a single instance, the desired hydroxylamine hemiketal **80** was isolated by reaction with **78** in CH_2Cl_2 and K_2CO_3. Unfortunately, this result could not be repeated and only the production of the nitrone was observed in subsequent reactions. The isolation of both the nitrone product and starting material suggested that the amount of DMDO in the reaction was not a factor, the rate of over oxidation to the nitrone being faster then initial oxidation of the substrate. In the absence of a base or the use of the milder sodium phosphate, only an improved conversion to the nitrone was observed. Reactions in chlorinated solvents have been reported to facilitate the reactivity of DMDO,[50] and this proved accurate for this reaction, as use of acetonitrile or DMF in place of methylene chloride afforded no reaction. Remarkably, additional amounts of K_2CO_3 resulted in only *p*-anisaldehyde being isolated from the crude reaction mixture. This puzzling result suggested that some type of salt was produced with the K_2CO_3 and subsequently removed by filtration along with the carbonate prior to concentration of the reaction mixture. Unfortunately,

dissolving the isolated salts in aqueous media followed by extraction did not reveal any product.

The relative bulky size of the TBS group may be a possible reason for the inability for the oxidized intermediate to undergo the desired ring closure prior to nitrone formation. To investigate this possibility, efforts focused on constructing the primary carbamate function on the hydroxymethyl segment in place of the TBS group. Despite the failure to effect this transformation with the NVOC series, identical conditions were utilized (reaction with trichloroacetyl isocyanate followed by exposure to neutral alumina). Careful inspection of the reaction found the intermediate trichloroacetyl carbamate was stable; moreover, cleavage of the acetyl group with neutral alumina (Brockman I) did give the product by TLC analysis. However, continual exposure to the alumina rapidly degraded the product to the eliminated exocyclic methylene **70**. Having identified the alumina oxide as the source of the problem, milder conditions using SiO_2/MeOH provided a means of cleaving the intermediate trichloroacetyl portion without degrading the product.[41]

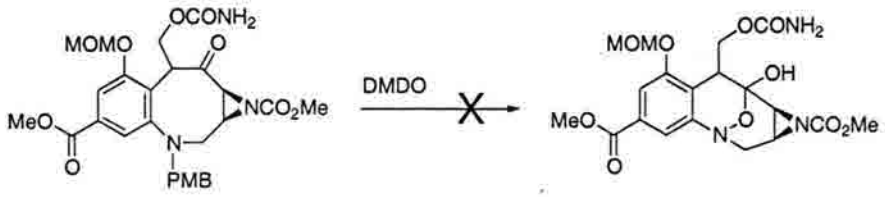
Scheme 3.17. Carbamate formation.



59 or **69** **84** or **85**

The resulting carbamate not only presented a much less bulky group, but also provided a means to a direct precursor of the natural product. Unfortunately, reactions with DMDO in CH_3CN afforded no reaction with only slow degradation, while use of DMDO in CH_2Cl_2 generated the nitrone as before along with significant decomposition.

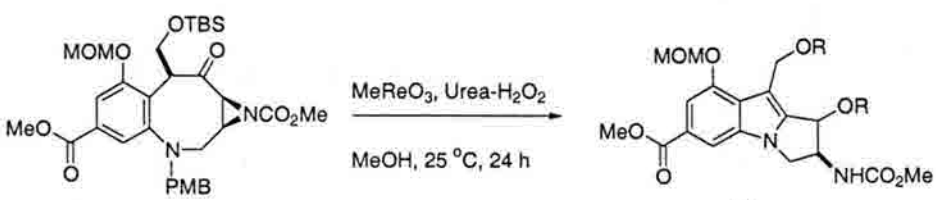
Scheme 3.18. Failed dimethyldioxirane oxidation with carbamates **84** and **85**.



84 or **85** **86** or **87**

As an alternative to DMDO, the methyltrioxorhenium-hydrogen peroxide system was explored with the *O*-TBS-protected intermediate **79**. Notably, this catalytic system has reportedly demonstrated reactivity very similar to the dioxiranes in the oxidation of secondary amines to nitrones, N-oxide production from tertiary amines, and oxidative C-H insertions.[52] Even with these apparent similarities, reaction of **79** with this reagent system led to clean conversion to mitosene species **55** by direct PMB cleavage. This result was reminiscent of the earlier reactions with THP oxidations catalyzed by $VO(acac)_2$ and $Mo(CO)_6$.

Scheme 3.19. Mitosene formation following oxidation by methyl rhenium trioxide.

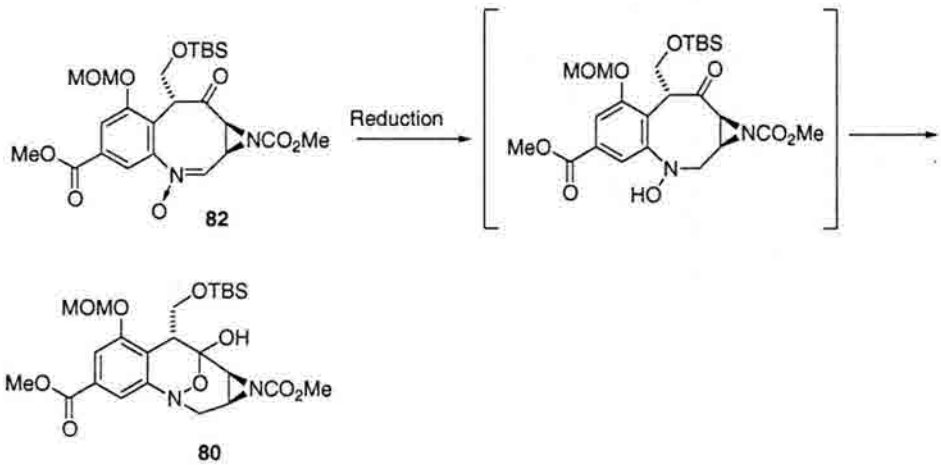


79 **55**
+ *p*-anisaldehyde
R = H or Me

Despite the difficulty in elucidating reaction conditions for the direct formation of the hydroxylamine hemiketal, the nitrone product did represent an alternative means to

introduce this functionality. Selective reduction of the nitrone to the hydroxylamine potentially could result in spontaneous closure to the desired [3.3.1] bicyclic ring system.

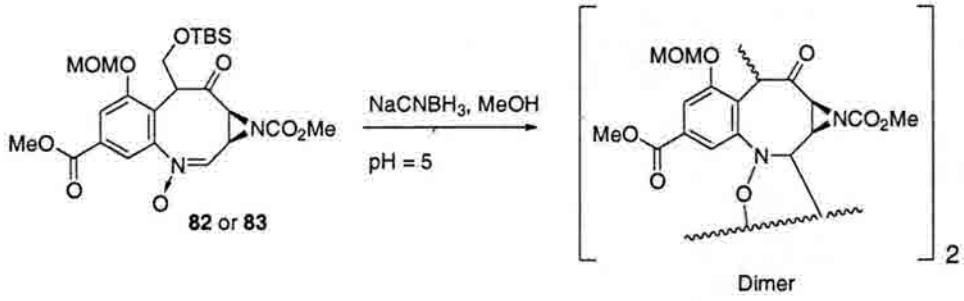
Scheme 3.20. Proposed hydroxylamine hemiketal formation by selective nitrone reduction.



Chemoselectively reducing the nitrone while maintaining the ketone functionality represented an intriguing synthetic challenge. Among the literature procedures for the partial reduction of nitrones, only a few procedures were found to be compatible with ketones. Those include the use of mild borohydride reductions such as those typically used for reductive aminations[53] as well as heterogeneous hydrogenations with platinum catalysts.[35] Additionally, one recent report described a homogeneous asymmetric hydrogenation of an aliphatic nitrone to the corresponding hydroxylamine using an iridium (I) catalyst.[54]

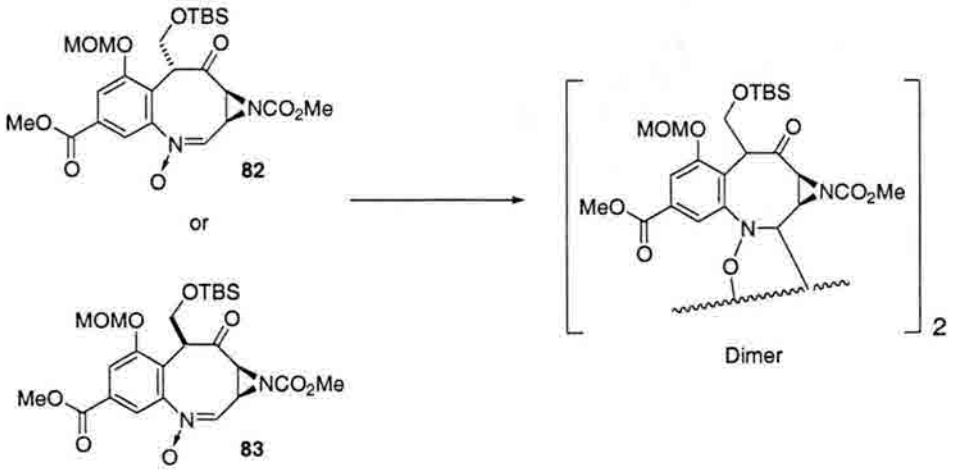
While use of $ZnCl_2$ with $NaCNBH_3$ afforded no reaction, $NaCNBH_3$ in MeOH at a pH of 5 led to dimerization of the nitrone, elimination, and finally 1,4 reduction of the resulting α, β unsaturated ketone.

Scheme 3.21. Reaction with sodium cyanoborohydride.



The tendency for these diastereomeric (at C7) nitrones to dimerize became apparent in certain solvents, including THF and EtOAc, with nitrone **82** being much more susceptible under all reaction conditions. In contrast, negligible dimerization of nitrone **83** was observed in methylene chloride and even **82** proved moderately stable in this solvent. The greater stability of **83**, therefore, dictated its use as the reduction substrate in the majority of the reactions.

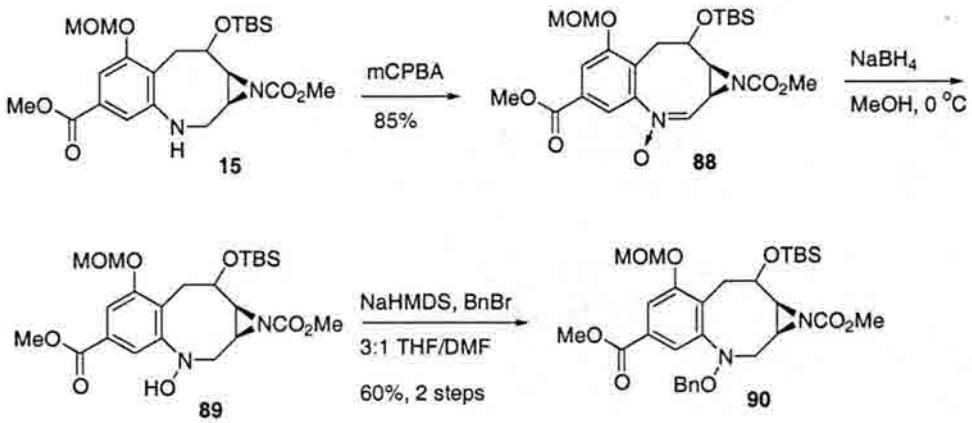
Scheme 3.22. Dimerization of nitrone diastereomers **82** and **83**.



Ketone reduction with $NaBH_4$ during a previous study had proven sluggish (Scheme **3.11**) requiring ambient reaction temperatures. This reactivity was hoped to

translate during attempts at a chemoselective reduction of the nitrone. However, reaction in MeOH at low temperatures (-20 to 0 °C) failed to afford any discernable product. In contrast, a model study employing nitrone **88** derived from direct mCPBA oxidation of amine **15**[55] successfully reduced to the hydroxylamine under identical reaction conditions with $NaBH_4$. Verification of the product included *O*-benzyl protection to **90** followed by analysis by ^1H NMR and mass spectrometry.

Scheme 3.23. Model study of sodium borohydride-mediated nitrone reduction.

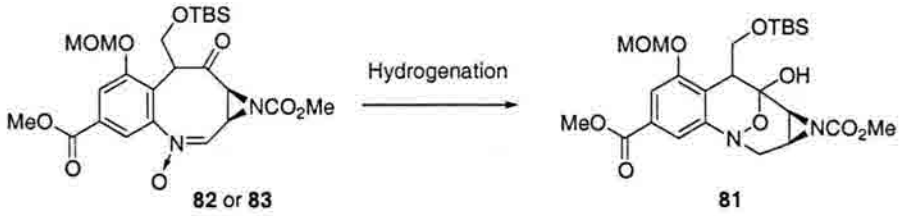


Although disappointing, the failure of the borohydride reductions was not entirely surprising. This method was limited in generating the free hydroxylamine only after the cleavage of the *N*-hydroxyborate following aqueous workup, leaving the ketone exposed throughout the reaction.

Catalytic hydrogenation represented a more viable approach by *in situ* production of the hydroxylamine leading to direct formation of the hydroxylamine hemiketal. Notably, the stability of the hydroxylamine hemiketal functionality to hydrogenation conditions was not in question, as one reported method for the semi-synthetic production of FR66979 from FR900482 includes hydrogenation of the aldehyde with 10% Pd/C.

Initial reactions utilized platinum catalysts because of their well-established selectivity in hydrogenations for C-N bonds over N-O bond cleavage in nitrones, particularly in acidic media.[35] Platinum oxide in MeOH only or in MeOH/AcOH led to no observable product. Additionally, the use of Pt/C (5%) under similar reaction conditions failed to furnish the desired product. The catalyst may have been too reactive, reducing the aziridine ring and/or the ketone. Attention was next focused on iridium (I) complexes, taking into consideration the aforementioned literature reference[54] and the related use of these homogenous catalysts for imine reductions.[56] The results of the hydrogenations with the iridium catalysts complexed with various phosphine ligands are collected below (Table 3.2).

Scheme 3.24. Proposed selective hydrogenation of nitrone **83.**



82 or 83 81

Entry	*Conditions*	*Result*
1	$[Ir(COD)Cl]_2$, BINAP (rac), $N(Bu)_4BH_4$, THF, H_2 (1200 PSI) 18 hrs.	Dec.
2	$[Ir(COD)Cl]_2$, BINAP (rac), K_2CO_3, THF, H_2 (1200 PSI), 18 hrs.	Dimer
3	$[Ir(COD)Cl]_2$, BPPM (S), CH_2Cl_2, H_2 (1200 PSI), 18 hrs.	Dimer
4	$[Ir(COD)Cl]_2$, BINAP (rac), CH_2Cl_2, H_2 (1200 PSI), 18 hrs.	Dimer
5	$[Ir(dppe)I_4]Li$, 3:1 THF/CH_2Cl_2, H_2 (400 PSI), 4 hrs.	Dec.

Predictably, following the reported methods, hydrogenation in THF resulted in dimerization of the nitrone.[54] The addition of $(Bu)_4NBH_4$ to increase reactivity as

reported, led to elimination and subsequent 1,4-reduction of the unsaturated ketone. Following the related literature on iridium reduction of imines, hydrogenation with catalysts derived from $[Ir(COD)Cl]_2$ and BPPM or BINAP in CH_2Cl_2 also led to dimerization.[56] These results proved unexpected, as the nitrone had shown moderate stability in this solvent. Ultimately the reason for the nitrone dimerization seemed to be dependent on trace amounts of unreacted $[Ir(COD)Cl]_2$. Although excess phosphine ligand prevented further dimerization, the desired product could not be isolated following hydrogenation. Notably, dimerization of the nitrone catalyzed by $[Ir(COD)Cl]_2$ may be related to recent literature describing use of this catalyst for additions of alkynes and other functionalities into imines.[57] As an alternative, the Ir(III) catalyst $[Ir(dppe)I_4]Li$ reported by Osborn *et al.* was synthesized from $[Ir(COD)Cl]_2$ in three steps.[58a,c] Although hydrogenation with this catalyst failed to afford any product, difficulties associated with the small reaction scale and problems in removing the excess LiI from the catalyst may have induced the degradation of the substrate. The $[Ir(BDPP)HI_2]_2$ catalyst reported by these authors is reported to be easier to purify and may provide a means of achieving this reaction.[58b,c]

The unanticipated complications during the hydrogenations and the instability of the substrate called for a re-evaluation of the DMDO reactions. Indeed, one instance had led to a successful oxidation reaction with at least one diastereomer, affording the desired hydroxylamine hemiketal (Scheme **3.16**). The single component of the reaction that could explain such inconsistencies resided with the production of the DMDO. The literature procedure for generating the *ca.* 0.1 M solution of dimethyldioxirane in acetone entailed mixing acetone, oxone, and an aqueous sodium bicarbonate solution, then distilling the

resulting dioxirane solution under reduced pressure. Consequently, varying amounts of water co-distill with the reagent and could explain the discrepancies observed. Furthermore, since K_2CO_3 acts as a desiccant as well as a base, an excessive amount in the reaction could effect the amount of water in the reaction.

To further examine this possibility, several reactions in aqueous solution were evaluated.

Scheme 3.25. Dimethyldioxirane oxidations in aqueous methylene chloride mixtures.

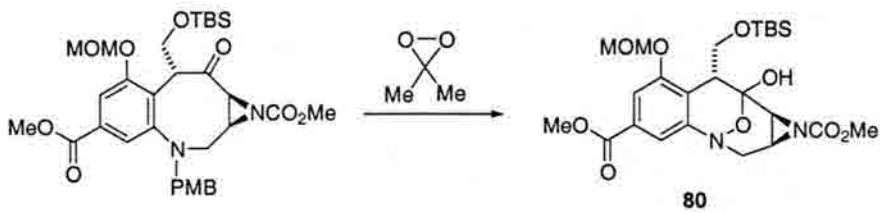


Table 3.3. Dimethyldioxirane reaction conditions

Entry	*Conditions*	*Result*
1	DMDO, 5:1 CH_2Cl_2/H_2O, K_2CO_3, 0-25 °C, 18 hrs.	Nitrone
2	DMDO, 1:1 CH_2Cl_2/ Sat. $NaHCO_{3\,(aq)}$, 0-25 °C, 3 hrs.	1:1:1.6 Product/Nitrone/**78**
3	DMDO, 1:1 CH_2Cl_2/ Sat. $Na_2CO_{3\,(aq)}$, 0-25 °C, 3.5 hrs.	Nitrone + **78**
4	DMDO, 1:1 CH_2Cl_2/ Sat. $K_2CO_{3\,(aq)}$, 0-25 °C, 6 hrs.	1:1 Product/**78**

Performing the reaction in a 5:1 CH_2Cl_2/H_2O mixture did not induce a change in the outcome of the reaction (Table **3.3**, entry 1). Employing a 1:1 mixture of CH_2Cl_2 and saturated $NaHCO_3$, however, resulted in an equal production of the nitrone and the desired hydroxylamine hemiketal **80** along with recovered starting material (**78**) (Table **3.3**, entry 2). This result suggested both the requirement for an aqueous phase and a

dependence on base to promote the bicyclic ring closure. Switching to a saturated Na_2CO_3 system resulted in nitrone (**82**) formation only and not the expected conversion to the desired product (Table **3.3**, entry 3). While the initial solvent mixture was biphasic, the addition of the DMDO solution results in a homogenous mixture with the precipitation of the Na_2CO_3. This is due to having added an equal or greater volume of acetone to the reaction as a consequence of the low molarity of the DMDO solution (0.1 M).

Finally, use of a saturated K_2CO_3 aqueous solution in conjunction with methylene chloride proved to be the ideal conditions, affording the desired hydroxylamine hemiketal **80** in 30-50% yield, with recovered starting material and *p*-anisaldehyde as the only observable by-products (Table **3.3**, entry 4). In contrast to the behavior observed with Na_2CO_3, the biphasic nature of the reaction was maintained after the addition of the acetone/DMDO solution. Moreover, neither hydrolysis of the carbomethoxy groups nor β-elimination of the sensitive *O*-TBS group was observed. Efforts to improve conversion using longer reaction times, a range of temperatures, or different reagent equivalents, did not succeed.

While the success of the reaction was certainly gratifying, the mechanism by which the DMDO-mediated formation of the hydroxylamine hemiketal remains unclear. In what manner does the dioxirane oxidize the tertiary amine in addition to cleaving the PMB group, rather than just oxidatively cleaving the PMB group as seen with all other reagents? One hypothesis is based on a literature report demonstrating the greater reactivity of vicinal diols than alcohols towards dioxiranes.[59] Oxidation of the secondary alcohol to the ketone of adamantane-1,2-diol **91** proved to be three times faster then

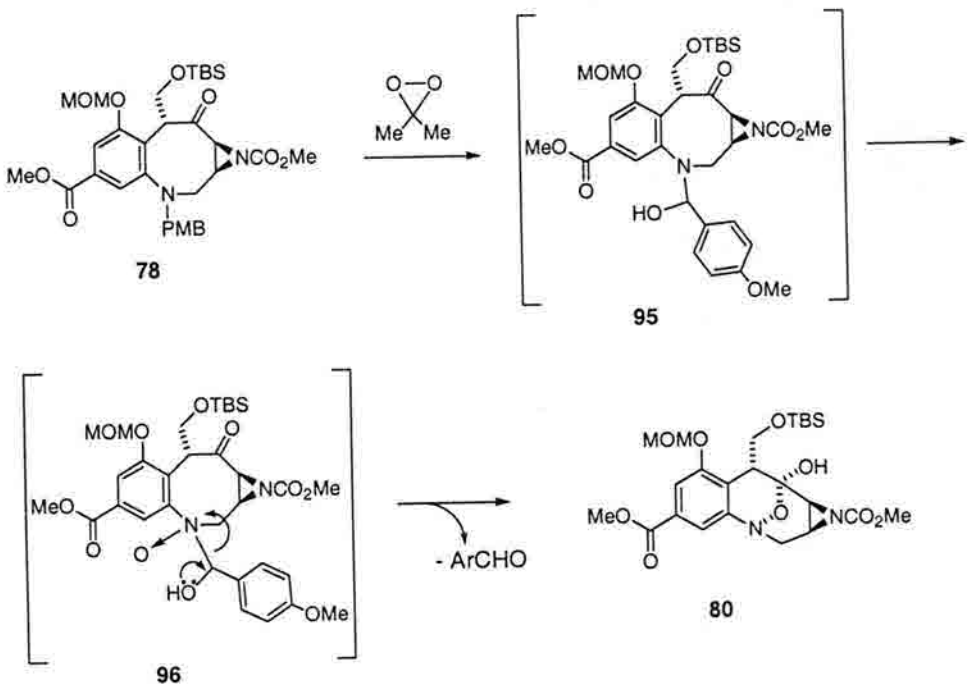
adamantane-2-ol **93** implying assistance of the proximal hydroxyl group in the oxidative insertion.

Scheme 2.36. DMDO oxidations of adamantane-1,2-diol **91** and adamantane-2-ol **93**.

92 **91** **93** **94**

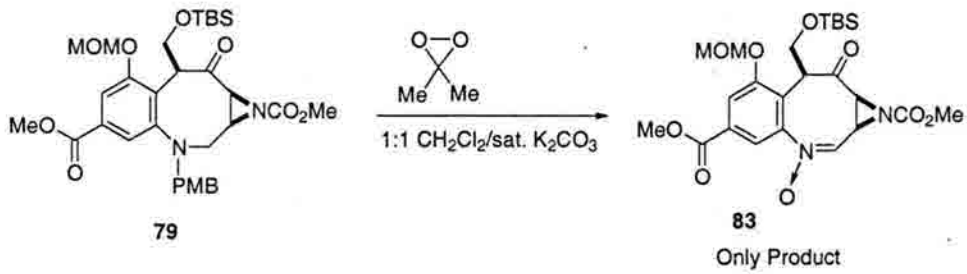
This effect, combined with the selective oxidation of the PMB methylene group with the other oxidizing reagents, suggests that the DMDO reaction may go through an initial oxidative C-H insertion to the carbonolamine **95** followed by a hydroxyl-assisted oxidation to the N-oxide **96**.

Scheme 3.27. Proposed mechanism of DMDO-mediated hydroxylamine hemiketal formation.



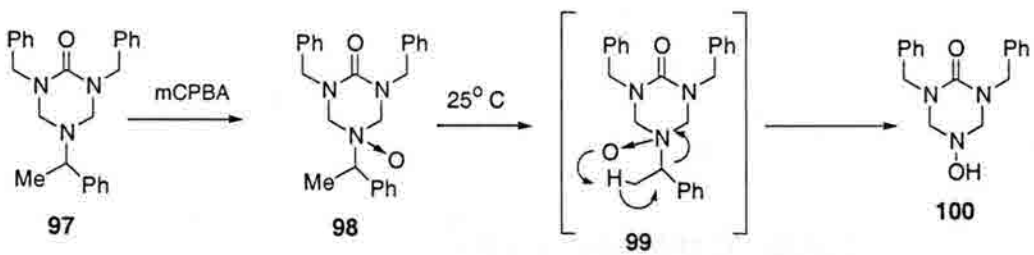
Although this proposal may seem reasonable, the failure of *epi*-substrate **79** to undergo the same conversion under identical conditions (Scheme **3.28**) and the requirement of a strongly basic aqueous phase are difficult to rationalize.

Scheme 3.28. DMDO oxidation of **79** to nitrone **83.**



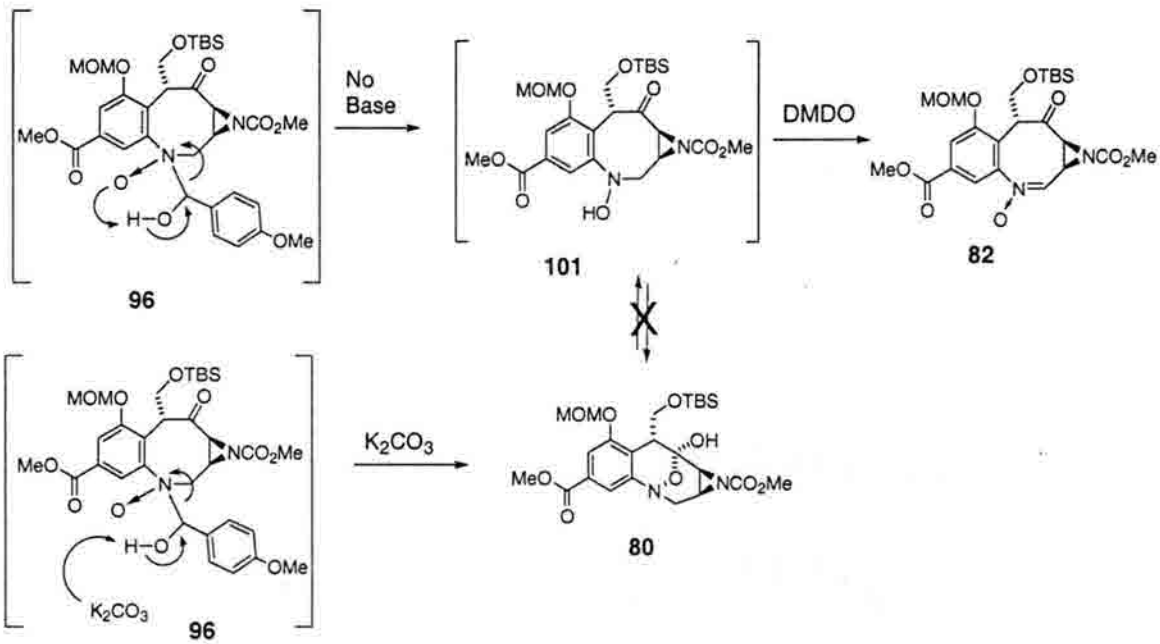
The role of the potassium carbonate in the formation of the hydroxylamine hemiketal with substrate **78** may be related to the known Cope elimination of α-substituted benzyl amine N-oxides. A related Cope elimination was reported by Knapp and coworkers, in which the α-methyl benzyl amine **97** was initially oxidized to the N-oxide and subsequently rearranged to give the hydroxylamine **100** (Scheme 3.29).[60a]

Scheme 3.29. Cope elimination of intermediate N-oxide.



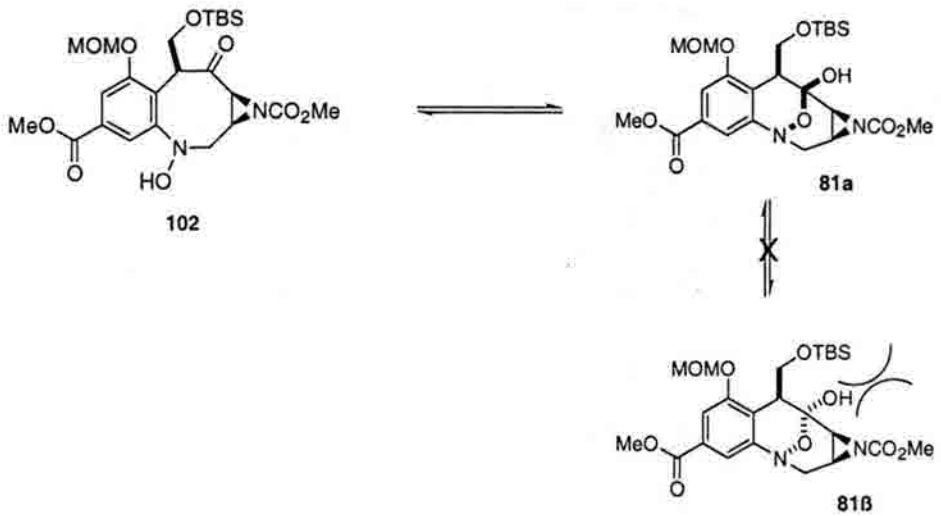
In the absence of a base, intermediate N-oxide **96** may undergo a similar transformation to give the hydroxylamine **101** which is subsequently oxidized to the nitrone. In contrast, the presence of strongly basic conditions may allow for an intermolecular deprotonation affording direct collapse to the hydroxylamine hemiketal **80** (or potassium salt, in the absence of water).

Scheme 3.30. Proposed role of potassium carbonate in DMDO reaction.



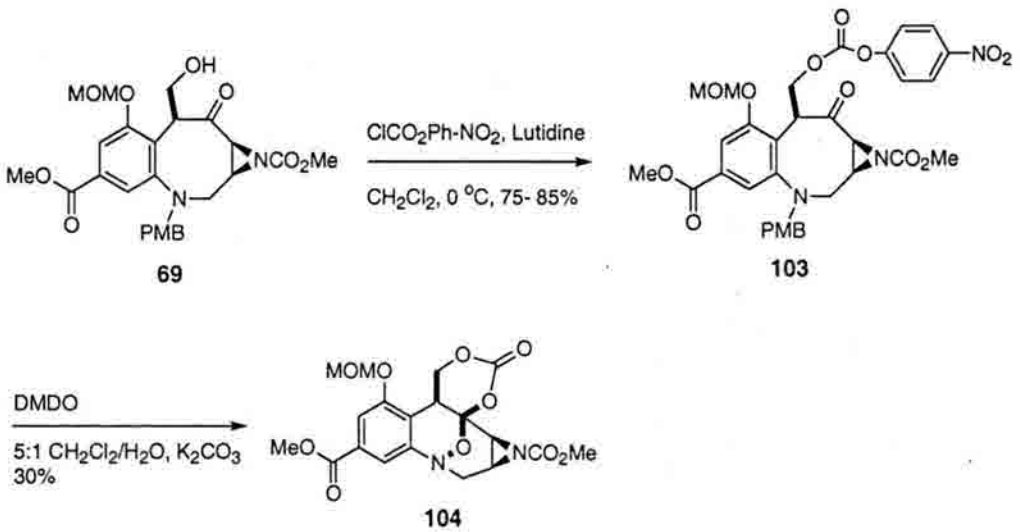
The hypothesis depicted above necessitates that hydroxylamine hemiketal **80** does not equilibrate to the hydroxylamine ketone **101,** allowing further oxidation. Notably, the ketone substrate **78** had shown conformational exchange between the *O*-TBS group and the aziridine protons by ^{1}H NMR spectroscopy while the product **80** did not. This suggests that unfavorable steric interactions may prevent such an equilibrium. Likewise, the *epi*-substrate **79** did not reveal the presence of any conformational isomers by ^{1}H NMR, perhaps indicating that equilibration and subsequent nitrone formation is allowed. Additionally, the *cis*-configuration of the silyoxymethyl segment and aziridine ring presumably favors the β-configuration of hydroxylamine **81** hemiketal to predominate.

Scheme 3.31. Equilibration of **102** with hydroxylamine hemiketal **81**.



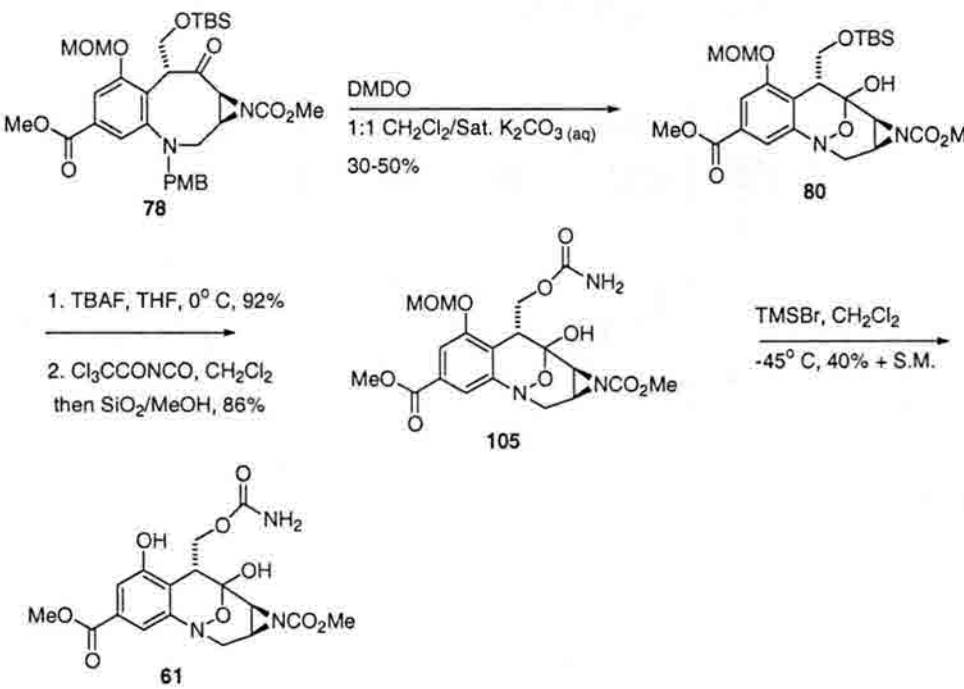
To investigate this idea, *p*-nitrophenyl carbonate derivative **103** was prepared from alcohol **69** by reaction with 1.1 equivalents of a pre-mixed solution of 2,6 lutidine and *p*-nitrophenyl chloroformate at 0 °C for 18 h (75-80% yield). While treatment with DMDO in the absence of a base afforded the nitrone, exposure to DMDO in a 5:1 mixture of CH_2Cl_2/H_2O with solid K_2CO_3 did give the cyclic carbonate **104** in ~30% yield (along with significant degradation). The successful production of the carbonate **104** would suggest that the equilibrium alluded to above had induced nitrone formation with the *O*-TBS substrate and lends credence to the proposed mechanism. By no means however, does this prove the existence of this mechanistic pathway, and further studies are needed.

Scheme 3.32. Formation of cyclic carbonate **104** from aldol adduct **69**.



In the event, the *O*-TBS protecting group on the resulting hydroxylamine hemiketal **80** was cleaved with TBAF (92% yield) and converted to the urethane **105** by reaction with trichloroacetyl isocyanate and workup with methanol and silica gel as before (86% yield) (Scheme **3.33**).

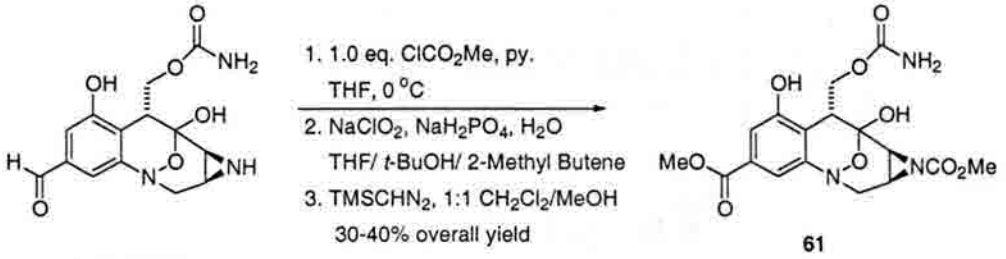
Scheme 3.33. Synthesis of phenol **61**.



Trimethylsilyl bromide (TMSBr) mediated removal of the methoxy methyl ether (MOM) in the presence of the acid-sensitive aziridine at -45 °C over 3 hrs. afforded phenol **61** in 60% yield.[61]

The correct stereochemistry of the product was unambiguously confirmed by comparison with an authentic, semi-synthetic sample prepared from the natural product. Thus, FR900482 was selectively protected as the methyl carbamate as before, oxidized to the carboxylic acid, and converted to the methyl ester **61** by careful addition of $TMSCHN_2$ (Scheme **3.34**).

Scheme 3.34. Semi-synthetic synthesis of intermediate phenol **61** from FR900482.



1. FR900482 (natural)

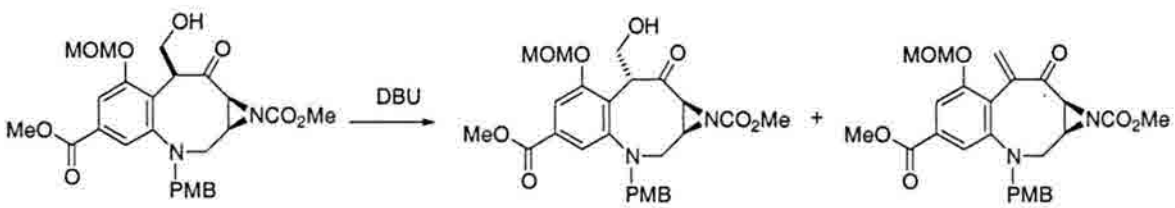
The resulting product exactly matched the fully synthetic intermediate **61** by ^1H NMR, ^{13}C NMR, IR, and mass spectrum. With the identity of the correct diastereomer secured, efforts focused on epimerization of the intermediate hydroxymethyl compound **69** possessing the *anti*-configuration.

Terashima *et al.* had employed a similar epimerization protocol in their total synthesis of FR900482 which gave complete selectivity for the undesired diastereomer during the intramolecular aldol reaction.[18c-h] Reaction of **92** (see Scheme **1.11**, Chapter 1) with 1,8-diazabicyclo[5.4.0]undeca-7-ene (DBU) in THF had resulted in a 2:1 mixture of

diastereomers after 3 hrs at room temperature. Longer reaction times had reportedly resulted in the generation of the eliminated exocyclic methylene product.

The additional electron-withdrawing methyl ester group in **69** suggested that a similar epimerization could be performed at a lower temperature, both increasing the selectivity and precluding degradation to the eliminated product.

Scheme 3.35. DBU-mediated epimerization of **69** to **59**.



69 **59** **70**

Table 3.3. Epimerization of **69**.

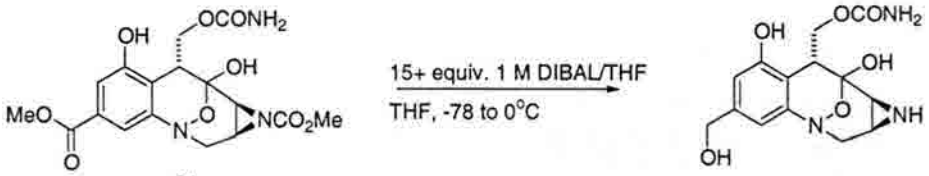
Entry	*Conditions*	*Result*
1	3.0 eq. DBU, THF, -45 to 0 °C, 3 hrs	No Reaction
2	3.0 eq. DBU, THF, 25 °C, 42 hrs	2:1:1 **59/ 69 /70**
3	3.0 eq. DBU, Toluene, 25 °C, 3 days	1.4:1:0.5 **59/ 69 /70**
4	8.0 eq. DBU, Toluene (diluted), 25 °C, 36 hrs	6.5:2.6:0.9 **59/ 69 /70**

Unexpectedly, DBU-mediated epimerization in THF led to no reaction from -45 to 0 °C. Moreover the reaction required 42 hours at room temperature and resulted in a 2:1 selectivity with an additional equivalent of the eliminated side product **70** also being produced. The solvent was switched to toluene with the expectation that providing a medium less amenable to elimination would significantly improved matters. Reaction with DBU in toluene afforded a 2.5:1 mixture of diastereomers in favor of the desired **59** after 36 hours with a minimal amount of elimination product **70** detected (< 10%). These

optimized conditions utilized excess DBU under dilute conditions. Presumably, dilution slows down the rate of elimination. With the ability to convert both diastereomers **59** and **69** to intermediate **61**, the final reduction step of the synthesis was initiated.

Despite the earlier success of the model study, the reduction of intermediate **61** by DIBAL in THF proved difficult. The resistance of the methyl ester functionality to the reduction required additional equivalents of the reducing reagent making the workup and isolation of the polar, water soluble natural product extremely complicated. Indeed, workup with sodium sulfate decahydrate provided the only means of extracting the natural product from the crude reaction, and at best yielded only 20 to 30% of FR66979.

Scheme 3.36. Low yielding DIBAL reduction to FR66979.



61

2. FR66979
(0-30% yield)

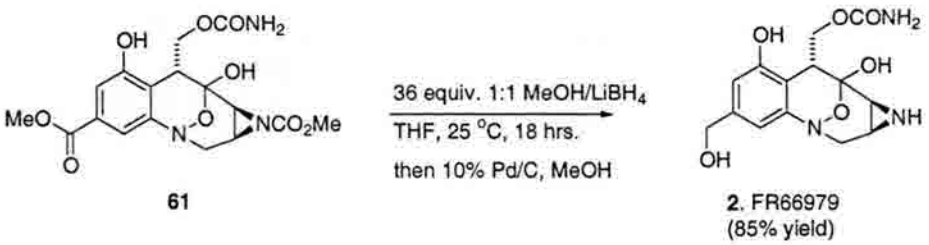
Despite the isolation of some natural product and successful completion of the total synthesis, the capricious and low yielding nature of the reaction required an alternative method to be devised.

The alternative reduction with excess $LiBH_4$ and methanol in THF proved to be a much more viable system.[62] Facile cleavage of the resulting borate complexes was accomplished with the addition of saturated ammonium chloride solution. The main difficulty encountered was the remaining nitrogen-boron ate complex.

Although the initial sigma nitrogen-boron bond is rapidly hydrolyzed with the aqueous workup, the resulting secondary amine of the aziridine ring forms a highly stabilized complex with the boron. Traditionally, cleavage of such complexes requires

harsh acidic or basic conditions, unsuitable for the sensitive functionalities of the natural product. A fortuitous report from a Pfizer group recently demonstrated that simple addition of a catalytic amount of 10% Pd/C rapidly cleaves these complexes.[63] Hence, following hydrolysis of the borate complexes and passage through a C18 sep pak (Waters), addition of a catalytic amount of 10% Pd/C in methanol liberated the natural product from the borate complex providing (+)-FR66979 (**2**) in 85% yield.

Scheme 3.37. Successful reduction to FR66979 with $LiBH_4$.



61

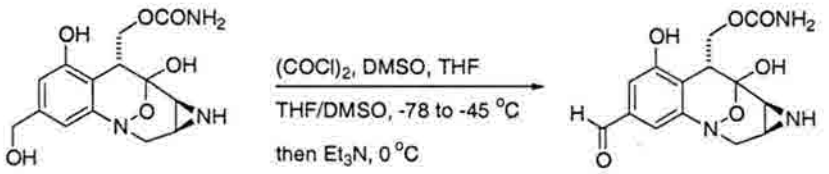
2. FR66979
(85% yield)

The fully synthetic compound matched a semi-synthetically derived sample of FR66979 by TLC mobility, ^1H NMR, IR, and mass spectrum. Additionally, the optical rotation of the semi-synthetic derivative was + 11.5 (c = 0.21, H_2O) (Lit. + 12.5, c = 0.87) while the synthetic compound showed a rotation of + 9.4 (c = 0.16, H_2O) which is attributed to the 87% ee of the Sharpless epoxidation.

The natural product FR900482 can be readily accessed by Swern oxidation of FR66979. The precedent for this conversion could be found in Terashima's total synthesis of FR900482 wherein the use of the Swern oxidation was found to be the only means capable of oxidizing a monoacetylated FR66979 derivative to the corresponding acetylated FR900482.[18f] The poor solubility of FR66979 in the reported solvent system (CH_2Cl_2/DMSO) required a slight modification in which THF/DMSO was used.[64] Even with the solvent change, the oxidation afforded FR900482 in only 30% yield along with

recovered starting material FR66979 (60%), presumably due to partial precipitation of the substrate during the reaction.

Scheme 3.38. Swern oxidation of FR66979 to FR900482.



2. FR66979

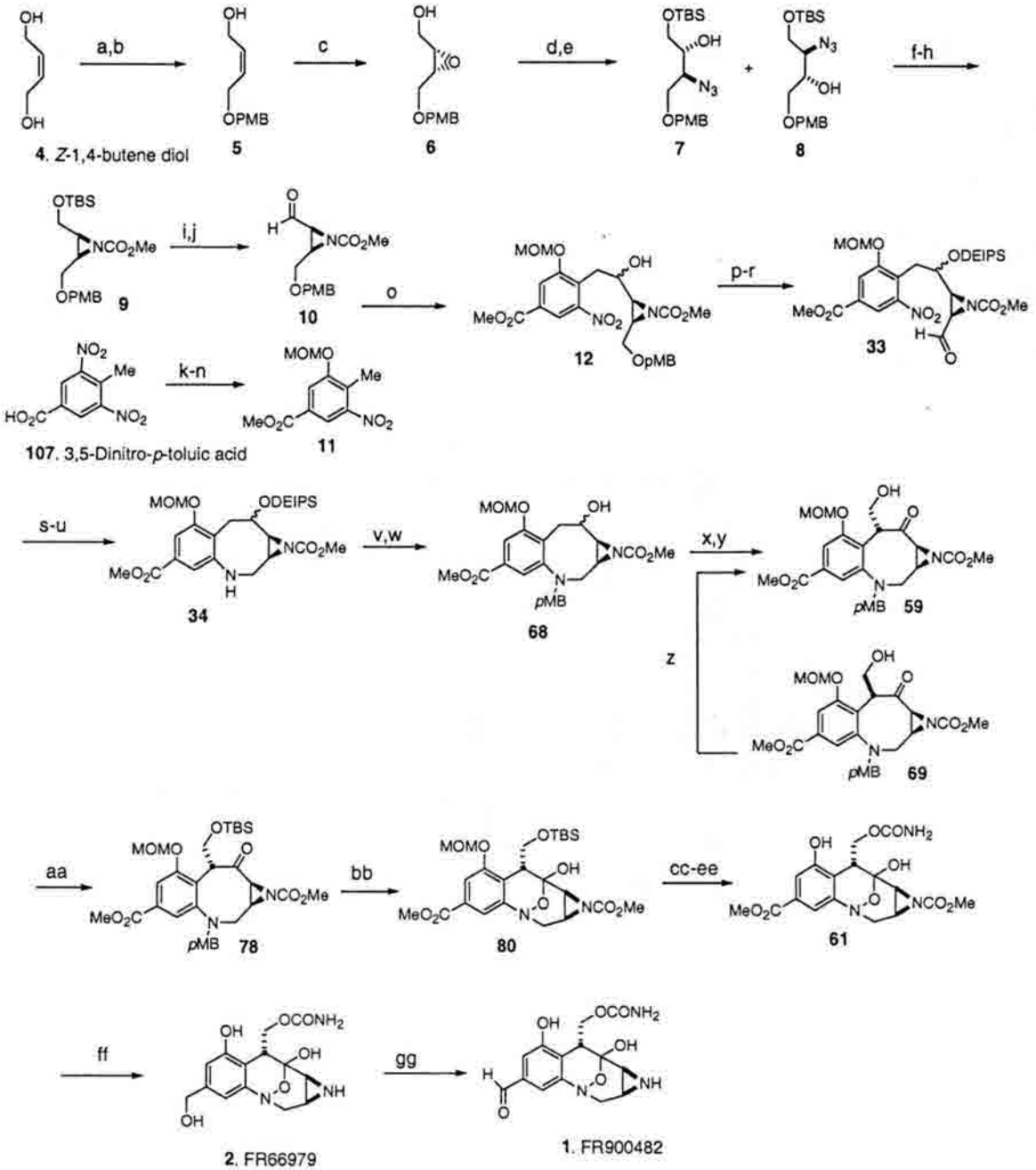
1. FR900482, 30 % + 60% FR66979

Despite the low conversion, the oxidation demonstrated the ability to synthesize both natural products from this synthetic route. A possible alternative would be to oxidize the intermediate nitrogen-borate complex. The borate protected nitrogen would perhaps allow the use of alternative oxidative conditions such as IBX in DMSO. The resulting FR900482 product could then be isolated after 10% Pd/C mediated cleavage of the borate as before.

3.4 Conclusion

The chemistry described herein represents the shortest total synthesis of (+) FR66979 and (+) FR900482 reported to date. Starting from 1,4-Z-butadiene and 3,5-dinitro-*p*-toluic acid, the natural product FR66979 can be obtained in an enantioselective manner over 32 synthetic steps (0.34 % yield). Likewise a single additional step provides the natural product FR900482 for a total of 33 steps overall. The synthesis includes an unprecedented method of constructing the hydroxylamine hemiketal through a DMDO-mediated oxidative rearrangement. The concise nature of the synthesis should allow the preparation of biologically significant analogs not readily available by semi-synthetic means from the natural product.

Scheme 3.39. Total Synthesis of (+) FR66979 and (+) FR900482.



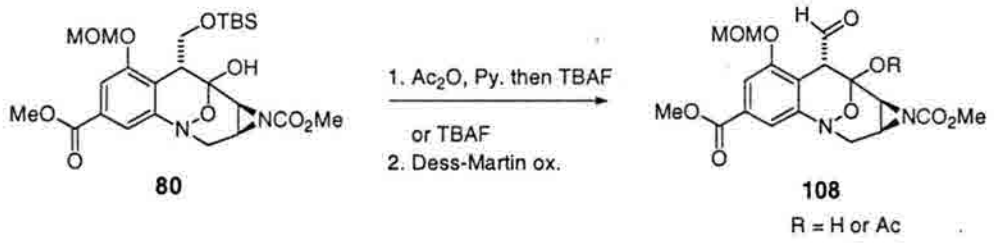
Key: a) *p*-Anisaldehyde, cat. TsOH, Ph, Δ (75%) b) $NaCNBH_3$, TFA, DMF, 25 °C (85%); c) L-(+) DET, $Ti(O\text{-}iPr)_4$, t-BuOOH, CH_2Cl_2, -24 °C (75%); d) NaN_3, NH_4Cl, 1-Methoxyethanol/H_2O, Δ; e) TBDMSCl, Et_3N, DMAP, CH_2Cl_2, 0 °C (83%, two steps); f) MsCl, Et_3N, CH_2Cl_2, 0 °C g) Ra-Ni, H_2NNH_2, EtOH; h) MeOCOCl, py. (92%, three steps); i) TBAF, THF/MeOH (85%); j) Dess-Martin oxidation (88%); k) $S(NH_4)_2$, EtOH, Δ (55%); l) $NaNO_2$, H_2SO_4/H_2O, 0 to 100 °C; m) MeOH, cat. H_2SO_4, Δ (85%, two steps); n) MOMCl, , *i*Pr_2NEt, CH_2Cl_2/THF, 0 to 25 °C (85%); o) NaOMe (0.5 M), DMF, 0° C (90%); p) DEIPSCl, Im., CH_2Cl_2 (86%); q) DDQ, CH_2Cl_2/H_2O (96%); r) Dess-Martin oxidation (90%) s) H_2, 5% Pd/C, MeOH; t) $MgSO_4$, 4Å sieves, CH_2Cl_2, Δ; u) $NaCNBH_3$, AcOH, CH_2Cl_2/MeOH, 0 °C (55-75%, three steps); v) *p*MBBr, *i*Pr_2NEt, CH_2Cl_2 (86%) w) TASF, DMF/H_2O, 25 °C; x) Dess-Martin oxidation (75%, 2 steps); y) LDA, DMF –45 °C; CH_2O/THF –45 °C (50%, 1:1 mixture **59** and **69**); z) DBU, toluene (70% + 30% starting material); aa) TBSOTf, 2,6 lutidine, CH_2Cl_2, -78 to 0 °C (96%); bb) dimethyldioxirane, aq. K_2CO_3/CH_2Cl_2, 0 °C to 25 °C (30-50%); cc) TBAF, THF, 0 °C (92%); dd) $Cl_3CCONCO$, CH_2Cl_2, 0 °C; MeOH, silica gel, 25 °C (86%); ee) TMSBr, CH_2Cl_2, -45 °C (60%); ff) $LiBH_4$, THF/MeOH, 25 °C, (85%); gg) $(COCl)_2$, DMSO, Et_3N, THF/DMSO, -78 to -45 °C then 0 °C (30%).

3.5 Future Directions

With the completion of a concise and efficient means of synthesizing the natural products FR900482 and FR66979, several possibilities aimed at developing a more efficient cross-linking and therapeutic agent become viable. One such area of interest lies in developing analogs capable of cross-linking DNA at alternative and/or multiple sequences in lieu of the 5'-CpG-3' sequence specificity of the natural product. It has been noted in the context of mitomycin C that the 5'-CpG-3' sequence in DNA would appear to represent an inefficient target for an antitumor agent as this sequence represents only 5% of all guanosines in the mammalian genome.[8b] Furthermore, it has been suggested that MMC (and by analogy FR900482) seemed to be more suited for their natural purpose of anti-microbial agents as contrary to the mammalian genome, the 5'-CpG-3' sequence in microorganisms can represent the majority of guanosines.[8b] Therefore it would seem advantageous to design analogs capable of cross-linking alternative sequences of DNA which are represented more frequently in the mammalian genome in order to provide for a more potent therapeutic agent. One such possible method to achieve this idea resides in modifying the proximal distance between the two sites of DNA alkylation. As previously discussed (see Chapter 1), the predilection of the FR-series of compounds and MMC for cross-linking the 5'-CpG-3' sequence resides with the ideal fit between the two sites of alkylation on the mitosene core with the distance between the N2 of both guanosines of the 5'-CpG-3' sequence. By extending the bond distance of the second site of alkylation at C13 it may be possible to change this ideal fit for an alternative DNA sequence. Such analogs could be readily accessed through the common intermediate **80** derived from the total synthesis as discussed previously. Direct

O-TBS deprotection of **80**, or alternatively following initial acylation of the hydroxylamine hemi-ketal group, would afford the corresponding primary alcohol along with aldehyde **108** following oxidation.

Scheme 3.40. Proposed synthesis of aldehyde **108**.

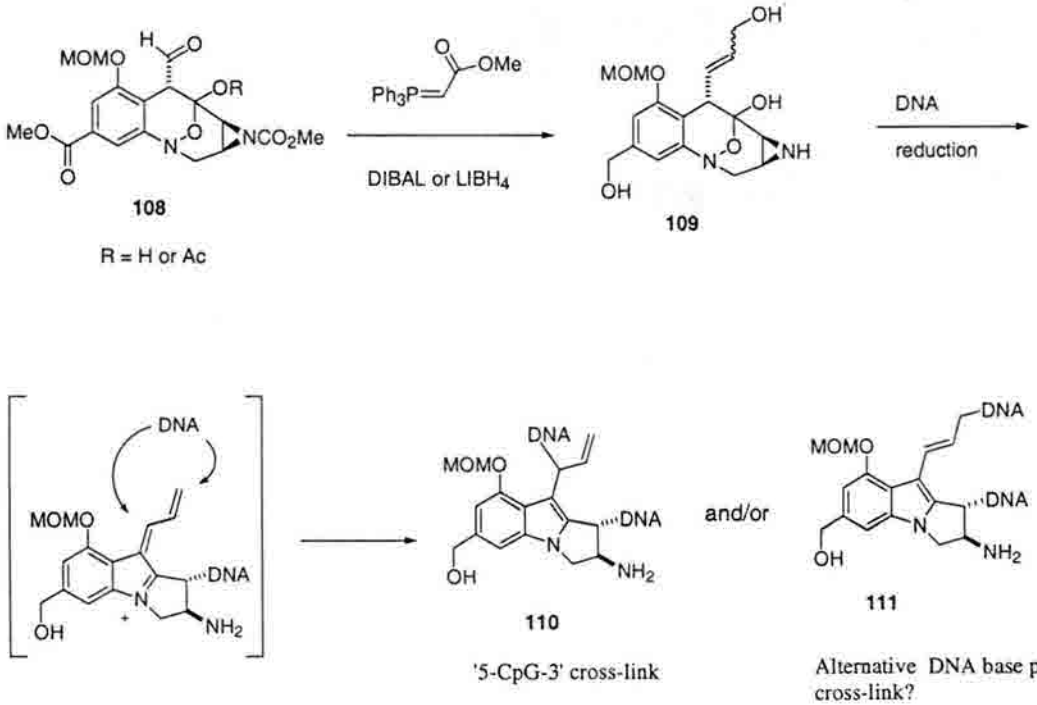


80 **108**

R = H or Ac

Wittig reaction of aldehyde **108** with the carbomethoxy ylide (Scheme **3.41**) followed by reduction to the allylic alcohol should give analog **109** representing a two-carbon extension to the cross-linking distance compared to the natural product **1**.

Scheme 3.41. Synthesis and alternative DNA sequence cross-linking of analog **109.**



108

R = H or Ac

109

110

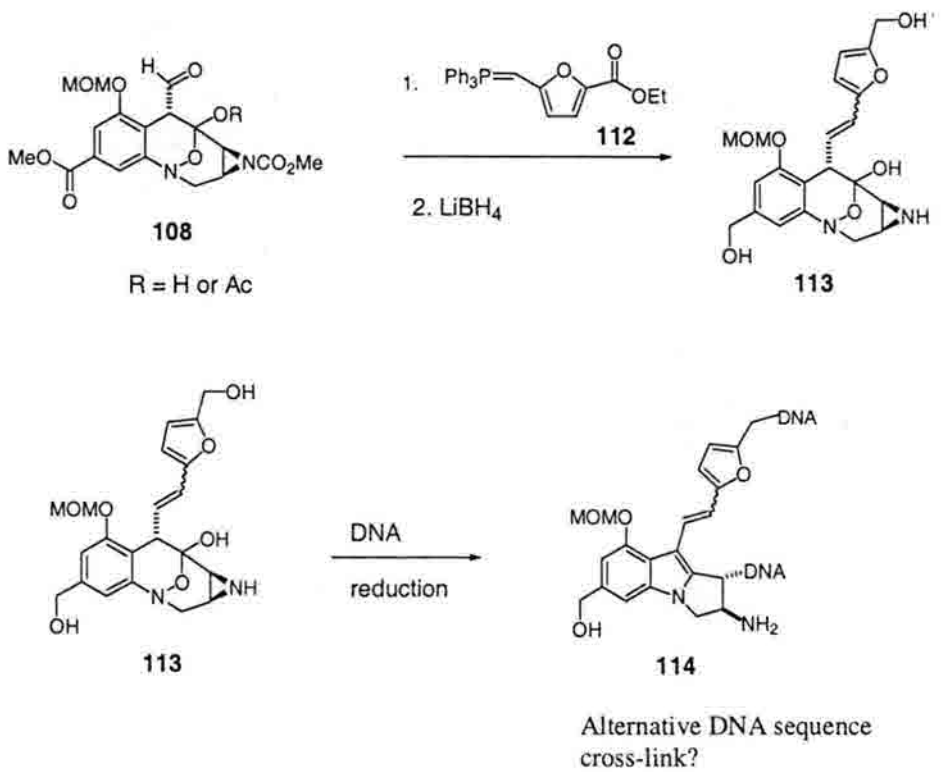
'5-CpG-3' cross-link

111

Alternative DNA base pair cross-link?

Alternatively, reaction with the known ylide **112**[65] and reduction would afford analog **113** designed to provide a greater proximal-distance between both sites of DNA alkylation while maintaining stabilization of the extensive conjugation through the furan ring.

Scheme 3.42. Synthesis and alternative DNA sequence cross-linking of furan analog **113.**



Notably, the capacity for decarbamoyl analogs to cross-link DNA with only the hydroxyl component in place of the carbamate functionality had been previously demonstrated through the photo-triggered analogs discussed in Chapter **2**. Additionally, modification of the second alkylation site in the FR900482 analogs may prove interesting

in probing additional protein-DNA cross-links in addition to alternative DNA-DNA cross-links.

3.6 Compounds Submitted for *In-vivo* Studies

The significant differences observed in both *anti*-tumor potency and toxicological properties among several semi-synthetic derivatives of FR900482 by the Fujisawa Pharmaceutical company prompted the submission of several synthetic derivatives obtained during this work.

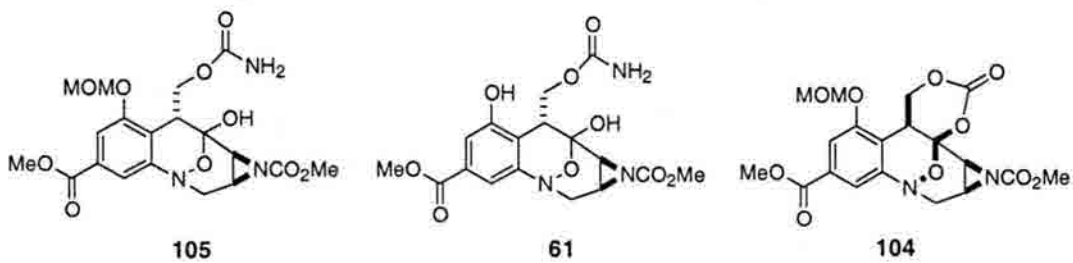


Figure 3.3. Compounds submitted for *in-vivo* testing.

Three compounds that were submitted to Fujisawa Pharmaceutical Company for further *in-vivo* studies include the methyl methoxy protected intermediate **105**, the dimethoxy carbonyl derivative **61**, and the *epi*-cyclic carbonate derivative **104** (Figure **3.3**). The evaluation of the *in-vivo* activity and *anti*-tumor properties of these analogs with respect to the clinical candidate FK317 is currently ongoing.

References

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[2] (a) *A New Antitumor Antibiotic, FR-900482. I. Taxonomic Studies on the Producing Strain: A New Species of the Genus Streptomyces* Iwami, M.; Kiyoto, S.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiotics*, (1987), *40*, 589; (b) *A New Antitumor Antibiotic, FR-900482. II. Production, Isolation, Characterization and Biological Activity,* Kiyoto, S.; Shibata, T.; Yamashita, M.; Komori, T.; Okuhara, M.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiotics*, (1987), *40*, 594; (c) *Structure of FR 900482, A Novel Antitumor Antibiotic From a Streptomyces,* Uchida, I.; Takase, S.; Kayakiri, H.; Kiyoto, S.; Hashimoto, M.; Tada, T.; Koda, S.; Morimoto, Y. *J. Am. Chem. Soc.*, (1987), *109*, 4108.

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[65] *Oxidation of 2,4-Alkadienoic Esters with Selenium Dioxide. A New Synthesis of Furans and Selenophenes*. Tsuboi, S.; Mimura, S.; Ono, S.; Watanabe, K.; Takeda, A., Bull. Chem. Soc. Japan, (1987), **60**, 1807.

Experimental Section

General Procedures

FR-900482 was generously supplied by the Fujisawa Pharmaceutical Co., Ltd., Japan. All drug stock solutions were made up to 50mM in sterile DDH_2O immediately prior to use unless otherwise noted. Oligodeoxynucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer using standard phosphoramidite chemistry (reagents and phosphoramidites from GLEN Research). Plasmid DNA substrate pBR322 was obtained from New England Biolabs or Roche Applied Science). Oligodeoxyribonucleotides (ODNs) were deprotected by heating 15 hrs. at 55° C in concentrated NH_4OH, followed by filtering of the CPG resin and concentration of supernatant *in vacuo*. All oligos were purified by 20% Denaturing Gel Electrophoresis (DPAGE). ODNs of interest were 5' end-labelled with [γ-^{32}P]ATP and T4 polynucleotide kinase (New England Biolabs). Labeled ODNs were then hybridized to their corresponding blunt ended complements in 200mM Tris (pH = 7.5) by heating the equimolar mixture of oligodeoxynucleotides to 75° C for 15 minutes then cooling to room temperature. $FeSO_4$ (from Mallinkrodt) solutions were made up to 4 mM using 4 mM EDTA 5. Mercaptoethanol (from Kodak) and dithiothreitol (from Gibco BRL) stock solutions were made using distilled deionized water immediately prior to use. Sodium acetate, tris, EDTA, and boric acid were also obtained from Gibco. DPAGE loading buffer contained .03% bromophenol blue, and .03% xylene cyanol in formamide. Alkaline agarose loading dye contained 50mM NaOH, 1mM EDTA, 2.5% (w/v) Ficoll, 0.25% (w/v) bromocresol blue. Dimethyl Sulfate and formic acid (88%) for Maxam-

Gilbert sequence reactions were obtained from Mallinkrodt. Centrex MF .45um cellulose acetate spin filters were obtained from Schleicher & Schuell. Samples were counted on a Packard 1500 Tri-Carb liquid scintillation analyzer. Phosphorimagery was performed on a Storm Phosphorimager (Amersham Biosciences).

Unless otherwise noted, materials were obtained from commercially available sources and used without purification. Toluene was freshly distilled from CaH_2. Diethyl ether and THF were freshly distilled from sodium benzophenone ketyl. 3Å molecular sieves were activated by heating for three minutes at the highest setting in a microwave followed by cooling under argon.

All reactions requiring anhydrous conditions were performed under a positive pressure of argon using oven-dried glassware (120°C) that was cooled under argon, unless stated otherwise.

Column chromatography was performed on Merck silica gel Kiesel 60 (230-400 mesh).

Mass spectra were obtained on Fisons VG Autospec.

^1H NMR, and ^{13}C NMR experiments were recorded on a Varian 300 or 400 MHz spectrometer. Spectra were recorded in $CDCl_3$ and chemical shifts (δ) were given in ppm and were relative to $CHCl_3$ unless otherwise noted. Proton ^1H NMR were tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant in hertz, and number of protons. When appropriate, the multiplicity of a signal is denoted as "br" to indicate the signal was broad.

IR spectra were recorded on a Perkin-elmer 1600 series FT-IR spectrometer.

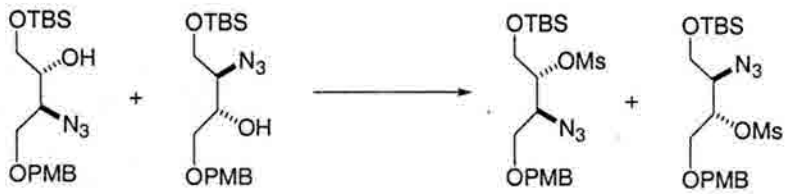
Optical rotations were determined with a Rudolph Research Autopol III automatic polarimeter referenced to the D-line of sodium.

(Z)-4-[(4-Methoxyphenyl)methoxy]-2-buten-1-ol.

22 5

The acetal **22** (4,7-dihydro-2-(4-methoxyphenyl)-1,3-dioxepin) (11.8 g, 57.2 mmol, 1 eq) and 180 mL of dry DMF were added to a 1 L flask. A solution of $NaCNBH_3$ (10.6 g, 168.7 mmol, 3 eq) and TFA (22.7 mL, 294.6 mmol, 5 eq) in 90 mL of dry DMF (prepared at 0 °C) was transfered via cannula into the flask under negative pressure at ambient temperature. The resulting solution was stirred 2.0 h until TLC analysis (1:1 EtOAc/Hex) showed the reaction to be complete. The reaction was quenched by dropwise addition of 1M $NaOH_{(aq)}$ until the solution reached a pH equal to 7. Following concentration *in vacuo*, the resulting oil was redissolved in 200 mL of CH_2Cl_2 and washed 2 x 100 mL H_2O and 1 x 150 mL sat $NaCl_{(aq)}$. The solution was dried over $MgSO_4$, filtered, concentrated, and dried under vacuum 12 h. The crude oil was purified by distillation using a kugelrohr apparatus (~1 mm Hg, 160 °C) to yield 10.0 g of the allylic alcohol **5** (85% yield) as a clear colorless oil (>95% pure). R_f = 0.40 1:1 Hex/EtOAc. ^1H NMR (300 MHz, $CDCl_3$) δ TMS: 2.04 (1H, br, D_2O exch.); 3.78 (3H, s); 4.04 (2H, d, J=6.0 Hz); 4.14 (2H, d, J=6.0 Hz); 4.44 (2H, s); 5.76 (2H, m); 6.86 (2H, d, J=8.6Hz); 7.23 (2H, d, J=8.6Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 55.2 (q), 58.7 (t), 65.3 (t), 72.1 (t), 113.8 (d), 128.4 (d), 129.5 (d), 129.9 (s), 132.3 (d), 159.3 (s)

[S-(R,S)]-3-Azido-4-[[(1,1-dimethlethyl)dimethylsilyl]oxy]-1-[(4-methoxyphenyl)methoxy]-methanesulfonate-2-butanol and **[R-(R,S)]-3-Azido-1-[[(1,1-dimethlethyl)dimethylsilyl]oxy]-4-[(4-methoxyphenyl)methoxy]-methanesulfonate-2-butanol.**



7 **8**

Alcohols **7** and **8** (15.8 g, 41.4 mmol, 1.0 eq) and 414 mL of CH_2Cl_2 were added to a 1 L round bottom flask. The stirred solution was placed on an ice bath for 15 min after which Et_3N (17.3 mL, 124.1 mmol, 3.0 eq) was added. The mixture was stirred for another 5 min after which methanesulfonyl chloride (4.8 mL, 62.0 mmol, 1.5 eq) was added to the flask dropwise over a minute. After 30 min, TLC analysis (2:1:2 CH_2Cl_2/Et_2O/Hex) of the reaction showed complete consumption of starting material. To the reaction mixture was added 200 mL of sat $NaHCO_{3(aq)}$, and the bilayer solution was stirred vigorously for 10 min. Following the addition of 100 mL of H_2O, the two layers were separated. The aqueous layer was extracted 2 x 150 mL EtOAc, and the combined organic layers were washed 1 x 200 mL sat $NaCl_{(aq)}$, and dried over Na_2SO_4. The reaction mixture was concentrated *in vacuo* and passed through a short plug of silica gel using 4:1 Hex/EtOAc as eluant to yield 19.0 g (96% yield) of product as a light yellow oil which was used without further purification. For analytical purposes, the mixture was further purified (>95% pure) by column chromatography (10:1:10 CH_2Cl_2/Et_2O/Hex). Mixture of isomers: R_f = 0.44 (2:1:2 CH_2Cl_2/Et_2O/Hex). ^1H NMR (300 MHz, $CDCl_3$) TMS: 0.03, 0.05, 0.06 (6H, s); 0.86, 0.87 (9H, s); 3.04, 3.05 (3H, s);

3.79 (3H, s); 3.64 - 3.88 (6H, m); 4.47, 4.48 (2H, ABq, J=11.4 Hz); 4.70, 4.80 (1H, q, J=5 Hz); 6.86, 6.87 (2H, d, J=8.6 Hz); 7.22, 7.25 (2H, d, J=8.6 Hz).

(2S-*cis*)-Methylester2-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-3-[[(4-methoxypheny)methoxylmethyl]-1-aziridinecarboxylic acid

9

The mesylate compound described above (19.0 g, 41.3 mmol, 1 eq.) and 440 ml of EtOH were added to a 1 L flask. To the resulting solution was added hydrazine monohydrate (34.3 ml, 707.1 mmol, 17.1 eq) followed by approximately 3 g of Raney Nickel. The reaction mixture was stirred 3 hrs. under an argon atmosphere until TLC analysis (1:1 EtOAc/Hex) showed the reaction to be complete. The reaction was filtered through a pad of Celite with EtOH and concentrated. The resulting oil was dissolved in 400 mL of EtOAc and washed 1 x $NaCl_{(aq)}$ and dried over Na_2SO_4. The mixture was filtered, concentrated, and placed under vacuum for 12 h. The clear yellow oil was dissolved in 300 mL of CH_2Cl_2 and cooled on an ice bath for ~15 min with stirring. Pyridine (10.0 mL, 20.8 mmol, 3.1 eq) was added to the mixture, and the mixture was stirred for another 5 min after which methyl chloroformate (6.4 mL, 82.8 mmol , 2.0 eq) was added dropwise over 2 min. The reaction was stirred for 20 min after which TLC analysis showed a complete loss of the unprotected aziridine (Rf = 0.73 10:1 CH_2Cl_2/MeOH). To the reaction mixture was added 300 mL sat $NaHCO_{3(aq)}$, and the

bilayer was stirred vigorously for 10 min. The two layers were separated. The aqueous layer was extracted 2 x 150 mL EtOAc, and the combined organic layers were washed 1 x 200 mL sat $NaCl_{(aq)}$. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by column chromatography (4:1 Hex/EtOAc) to yield 15.0 g (88% overall yield from **7** and **8**) of **9** as a light yellow oil (>95% pure).

^{1}H NMR (500 MHz, $CDCl_3$) TMS: 0.03 (3H, s); 0.05 (3H, s); 0.86 (9H, s); 2.69 (1H, dd, J=6.0,6.0 Hz); 2.77 (1H, dd, J=6.0,6.0 Hz); 3.55 (1H, dd, J=5.5, 11.2 Hz); 3.58 (1H, dd, J=6.3, 11.2 Hz); 3.60 (1H, dd, J=6.1, 11.4 Hz); 3.71 (3H, s); 3.77 (1H, dd, J=5.9, 11.4 Hz); 3.78 (3H, s); 4.46 (1H, d, J=11.5 Hz); 4.59 (1H, d, J=11.5 Hz); 6.85 (2H, d, J=8.6 Hz); 7.26 (2H, d, J=8.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) TMS: -5.4 (q); -5.3 (q); 18.2 (s); 25.7 (q); 40.4 (d); 41.8 (d); 53.5 (q); 55.1 (q); 61.2 (t); 67.1 (t); 72.4 (t); 113.7 (d); 129.4 (d); 129.9 (s); 159.2 (s); 163.5 (s).

(2S, 3R)-Methyl ester 2-[-1-[[(diethyl(1-methylethyl)silyl]oxy]-2-[4-(methoxycarbonyl)-2-(methoxymethoxy)-6-nitrophenyl]ethyl]-3-[[(4-methoxyphenyl)methoxy]methyl]-1-aziridinecarboxylic acid.

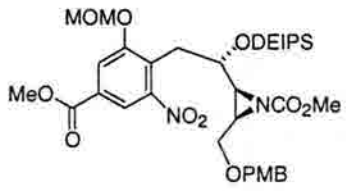
12

Alcohol **12** (2.65 g, 4.96 mmol, 1.0 equiv.) and imidazole (438 mg, 6.43 mmol, 1.3 equiv.) were dissolved in 50 mL of CH_2Cl_2. Following dropwise addition of DEIPSCl (1.10 mL, 5.96 mmol, 1.2 equiv.) the reaction mixture was stirred for 24 hrs after which TLC analysis (1:1 EtOAC/Hex) showed complete consumption of starting material. The reaction was quenched with sat $NaHCO_{3(aq)}$, diluted with CH_2Cl_2 and the layers were separated. The aqueous layer was extracted 2 X EtOAc, and the combined organic layers were washed with brine and dried over Na_2SO_4. Concentration followed by purification by column chromatography (1.5:1 Hex/EtOAc) yielded 2.82 g (86% yield) of the diethylisopropylsilyl ether as a light yellow oil.

Major Diastereomer: $[\alpha]_D^{25}$ = - 48.3 (c= 2.4, $CHCl_3$) ^{1}H N (300 MHz, $CDCl_3$) δ TMS: 0.37 (q, J = 8.1 Hz, 2 H), 0.43 (q, J = 8.1 Hz, 2 H), 0.67 - 0.80 (m, 13 H), 2.52 (t, J = 6.0 Hz, 1 H), 2.71 (dt, J = 4.8 Hz, 6.0 Hz, 1 H), 3.25 (dd, J = 4.8 Hz, 13.2 Hz, 1 H), 3.39 (dd, J = 6.0 Hz, 8.7 Hz, 1 H), 3.41 (s, 3 H), 3.57 (dd, J = 6.9 Hz, 11.1 Hz, 1 H), 3.63 (dd, J = 4.5 Hz, 10.8 Hz, 1 H), 3.65 (s, 3 H), 3.74 (s, 3 H), 3.88 (s, 3 H), 4.05 (m, 1 H), 4.49 (d, J = 12.0 Hz, 1H), 4.56 (d, J = 12.0 Hz, 1 H), 5.18 (s, 2 H), 6.83 (d, J = 9.0 Hz, 2 H), 7.25 (d, J = 9.0 Hz, 2 H), 7.88 (d, J = 1.8 Hz, 1 H), 8.04 (d, J = 1.8 Hz, 1 H); ^{13}C NMR (75

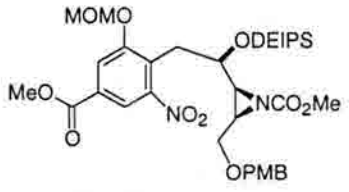
MHz)($CDCl_3$) δ TMS: 3.2, 3.3, 6.5, 6.6, 12.5, 16.8, 16.8, 32.2, 41.1, 45.0, 52.4, 53.4, 54.9, 56.3, 67.2, 67.8, 72.2, 94.7, 113.4, 113.4, 117.3, 118.1, 127.0, 129.3, 129.3, 129.6, 129.8, 151.6, 156.3, 159.0, 163.2, 164.6; IR (NaCl/neat) 2954, 1731, 1538, 1514, 1437 cm^{-1}; HRMS (FAB+) calcd for $C_{32}H_{47}N_2O_{11}Si_1$ (*m/z*) 663.2939, found (*m/z*) 663.2949.

Minor Diastereomer: $[\alpha]_D^{25}$ = + 0.70 (c=1.0, $CHCl_3$) ^1H NMR (300 MHz, $CDCl_3$) δ TMS: 0.63 (q, J = 7.8 Hz, 2 H), 0.75 (q, J = 7.8 Hz, 2H), 0.95 - 1.05 (m, 13 H), 2.70 (dt, J = 4.2 Hz, 7.5 Hz, 1 H), 2.82 (dd, J = 6.6 Hz, 9.0 Hz, 1 H), 2.96 (dd, J = 3.9 Hz, 11.4 Hz, 1 H), 3.20 (dd, J = 7.5 Hz, 10.8 Hz, 1 H), 3.32 (d, J = 6.9 Hz, 1 H), 3.52 (s, 3 H), 3.82 (s, 3 H), 3.89 (s, 3 H), 3.97 (dd, J = 7.5 Hz, 8.4 Hz, 1 H), 4.04 (s, 3 H), 4.50 (d, J = 11.7 Hz, 1 H), 4.63 (d, J = 11.7 Hz, 1 H), 5.30 (d, J = 6.9 Hz, 1 H), 5.33 (d, J = 6.9 Hz, 1 H), 6.95 (d, J = 8.4 Hz, 2 H), 7.30 (d, J = 8.7 Hz, 2H), 8.00 (d, J = 1.5 Hz, 1H), 8.12 (d, J = 1.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 3.4, 3.4, 6.7, 6.8, 12.7, 17.0, 17.0, 32.0, 41.0, 45.8, 52.5, 53.3, 54.9, 56.4, 66.7, 70.1, 71.9, 94.7, 113.4, 113.4, 117.3, 118.0, 125.6, 129.1, 129.1, 129.4, 129.9, 151.3, 156.3, 158.9, 163.1, 164.3; IR (NaCl/neat) 2954, 1731, 1538, 1514, 1437 cm^{-1}; HRMS (FAB+) calcd for $C_{32}H_{47}N_2O_{11}Si_1$ (*m/z*) 663.2949, found (*m/z*) 663.2951.



Major Diastereomer

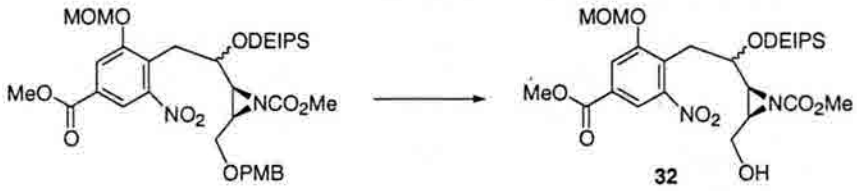
^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJI448II



Minor Diastereomer

^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJII234H

(2S,3R)-Methyl ester 2-[1-[[diethyl(1-methylethyl)silyl]oxy]-2-[4-(methoxycarbonyl)-2-(methoxymethoxy)-6-nitrophenyl]ethyl]-3-(hydroxymethyl)-1-Aziridinecarboxylic acid.

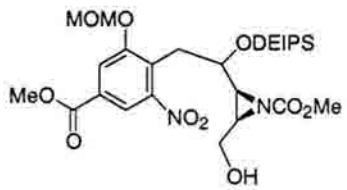


To a stirred mixture of the silyl ether described above (3.3 g, 4.98 mmol, 1.0 eq), 50 mL of CH_2Cl_2, and 2.41 mL of H_2O was added DDQ (1.47 g, 6.47 mmol, 1.3 eq) in one portion. The reaction mixture immediately turned dark green, and over the course of the next 1.5 h, the mixture slowly turned bright orange. After 1.5 h, the crude reaction mixture was passed through a short plug of activated alumina with 10:1 CH_2Cl_2/MeOH. After concentration *in vacuo*, the crude oil was purified by column chromatography (1.5:1 Hex/EtOAc) to give 2.6 g (96% yield) of product **32** as a clear orange oil (>95% pure).

Major Diastereomer: $[\alpha]_D^{25}$ = - 73.9 (c= 4.1, $CHCl_3$) ^1H NMR (300 MHz, $CDCl_3$) δ TMS: 0.34 (q, J = 7.8 Hz, 2 H), 0.44 (q, J = 8.1 Hz, 2 H), 0.67 - 0.80 (m, 13 H), 2.55 (t, J = 6.3 Hz, 1 H), 2.64 - 2.72 (m, 2 H), 3.25 (dd, J = 5.7 Hz, 13.2 Hz, 1 H), 3.41 (dd, J = 8.7 Hz, 13.2 Hz, 1 H), 3.43 (s, 3 H), 3.60 (s, 3 H), 3.66 - 3.84 (m, 2 H), 3.87 (s, 3 H), 4.15 (dt, J = 5.7 Hz, 8.7 Hz, 1 H), 5.24 (d, J = 6.9 Hz, 1 H), 5.25 (d, J = 6.9 Hz, 1 H), 7.86 (d, J = 1.5 Hz, 1 H), 8.01 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 3.4, 3.5, 6.5, 6.7, 12.7, 16.8, 16.8, 32.3, 43.0, 45.8, 52.4, 53.5, 56.4, 60.1, 68.1, 94.8, 117.3, 118.1, 126.7,

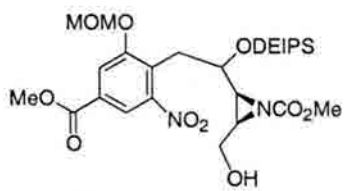
129.6, 151.4, 156.2, 163.2, 164.6; IR (NaCl/neat) 3498, 2955, 1731, 1538, 1438 cm^{-1}; HRMS (FAB+) calcd for $C_{24}H_{39}N_2O_{10}Si_1$ (*m/z*) 543.2374, found (*m/z*) 543.2372.

Minor Diastereomer: $[\alpha]_D^{25}$ = -14.5 (c=1.0, $CHCl_3$) ^1H NMR (300 MHz, $CDCl_3$) 0.51 (q, J = 7.8 Hz, 2 H), 0.61 (q, J = 7.8 Hz, 2 H), 0.80 - 0.98 (m, 13 H), 2.16 (t, J = 6.0 Hz), 2.52 (dd, J = 5.7 Hz, 6.6 Hz, 1 H), 2.73 (dd, J = 6.9 Hz, 8.7 Hz, 1 H), 3.02 (t, J = 5.7 Hz, 2 H), 3.19 (d, J = 7.5 Hz, 1 H), 3.20 (d, J = 6.6 Hz, 1 H), 3.46 (s, 3 H), 3.67 (s, 3 H), 3.92 (s, 3 H), 3.97 (m, 1 H), 5.28 (apparent singlet, 2 H), 7.92 (d, J = 1.5 Hz, 1 H), 8.04 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 3.5, 3.5, 6.8, 6.9, 12.8, 17.0, 17.0, 32.2, 43.1, 46.9, 52.7, 53.5, 56.6, 59.9, 69.9, 95.0, 117.5, 118.1, 125.6, 130.2, 151.4, 156.5, 163.3, 164.5; IR (NaCl/neat) 3519, 2954, 1731, 1538, 1437 cm^{-1}; HRMS (FAB+) calcd for $C_{24}H_{39}N_2O_{10}Si_1$ (*m/z*) 543.2374, found (*m/z*) 543.2372.

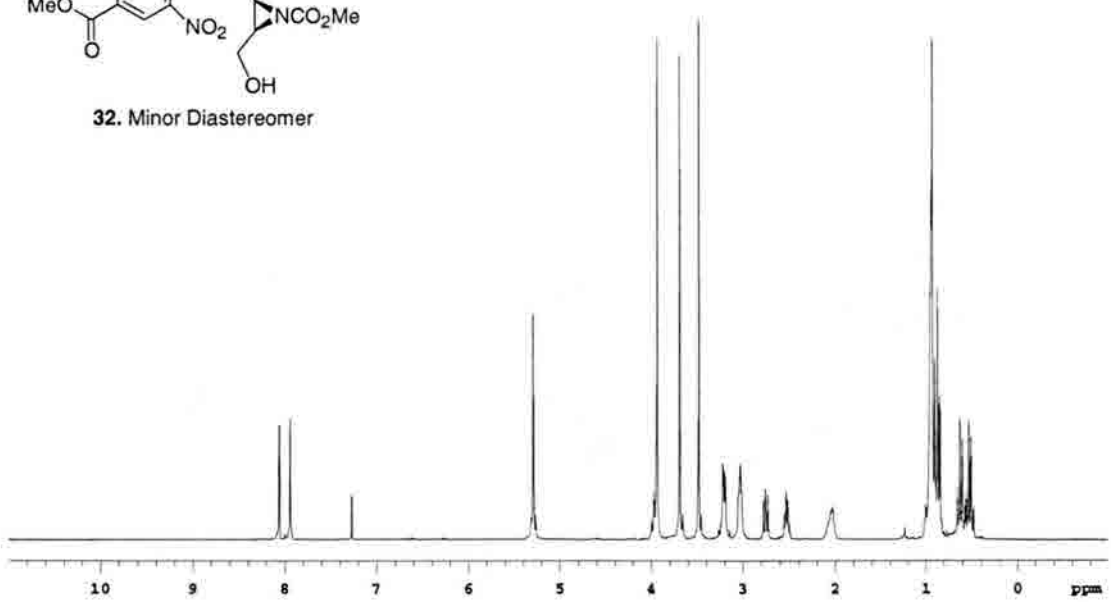


32. Major Diastereomer

^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJII194II

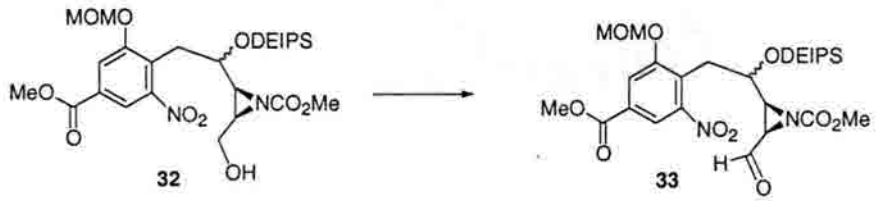


32. Minor Diastereomer



^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJII171II

(2S,3R)-Methyl ester 2-[1-[[diethyl(1-methylethyl)silyl]oxy]-2-[4-(methoxycarbonyl)-2-(methoxymethoxy)-6-nitrophenyl]ethyl]-3-formyl-1-Aziridinecarboxylic acid.

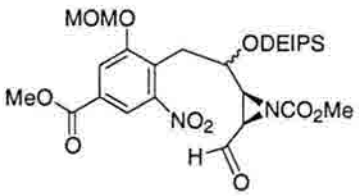


To alcohol **32** (2.5 g, 4.61 mmol, 1.0 eq) dissolved in 50 mL of reagent grade CH_2Cl_2 was added Dess-Martin reagent (3.0 g, 7.07 mmol, 1.5 eq) in one portion. After stirring for 2.5 h, the cloudy white mixture was diluted with Et_2O and poured into a solution of sat $NaHCO_{3(aq)}$ with excess $Na_2S_2O_3$. The milky biphasic mixture turned clear after 15 min of vigorous stirring. The two layers were separated, and the organic layer was washed with $NaHCO_{3(aq)}$, and H_2O. The combined aqueous layers were thoroughly extracted with Et_2O. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by flash chromatography (2:1 Hex/EtOAc) to give 2.2 g (90% yield) of **33** as a clear colorless oil (>95% pure).

Major Diastereomer: $[\alpha]_D^{25}$ = - 7.7 (c=1.0, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 0.49 (q, J = 7.8 Hz, 2H); 0.55 (q, J = 7.8 Hz, 2 H), 0.77 - 0.90 (m, 13 H), 2.75 (dd, J = 3.3 Hz, 6.6 Hz), 2.97 (dd, J = 4.5 Hz, 6.6 Hz, 1 H), 3.13 (dd, J = 7.2 Hz, 13.2 Hz, 1 H), 3.19 (dd, J = 7.2 Hz, 13.2 Hz, 1 H), 3.47 (s, 3 H), 3.65 (s, 3 H), 3.89 (s, 3 H), 4.50 (dt, J =3.3 Hz, 7.2 Hz, 1 H), 5.30 (s, 2 H), 7.92 (d, J = 2.1 Hz, 1 H), 8.07 (d, J = 1.2 Hz, 1 H), 9.44 (d, J = 4.5 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) d TMS: 3.4, 3.6, 6.7, 6.7, 12.7, 17.0, 17.0, 32.3, 45.1, 48.7, 52.6, 53.8, 56.6, 67.5, 94.8, 117.8, 118.3, 125.4, 130.2, 151.1,

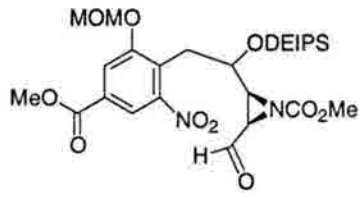
156.4, 161.8, 164.4, 196.6; IR (NaCl/neat) 2955, 1732, 1538, 1438 cm^{-1}; HRMS (FAB+) calcd for $C_{24}H_{37}N_2O_{10}Si_1$ (*m/z*) 541.2217, found (*m/z*) 541.2215.

Minor Diastereomer: $[\alpha]_D^{25}$ = + 103.5 (c=1.0, $CHCl_3$) ^1H NMR (300 MHz, $CDCl_3$) δ TMS: 0.56 (q, J = 7.8 Hz, 2 H), 0.67 (q, J = 8.1 Hz, 2 H), 0.83 - 1.00 (m, 13 H), 2.92 (dd, J = 5.2 Hz, 7.2 Hz, 1 H), 3.03 (dd, J = 6.9 Hz, 9.0 Hz, 1 H), 3.13 (dd, J = 7.2 Hz, 13.2 Hz, 1H), 3.20 (dd, J = 6.6 Hz, 13.2 Hz, 1 H), 3.47 (s, 3 H), 3.71 (s, 3 H), 3.93 (s, 3 H), 4.05 (dd, J = 6.9 Hz, 8.7 Hz, 1 H), 5.27 (d, J = 6.9 Hz, 1 H), 5.30 (d, J = 6.9 Hz, 1 H), 7.91 (d, J = 1.5 Hz, 1 H), 8.05 (d, J = 1.5 Hz, 1 H), 8.87 (d, J = 5.1 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 3.5, 3.5, 6.8, 6.9, 12.8, 17.1, 17.1, 32.1, 45.7, 48.7, 52.7, 53.9, 56.6, 69.6, 94.8, 117.7, 118.3, 124.8, 130.6, 151.3, 156.2, 161.4, 164.4, 194.7; IR (NaCl/neat) 2955, 1731, 1538, 1438 cm^{-1}; HRMS (FAB+) calcd for $C_{24}H_{37}N_2O_{10}Si_1$ (*m/z*) 541.2217, found (*m/z*) 541.2218.



33. Major Diastereomer

^1H NMR, 300 MHz, $CDCl_3$, filename: TCJII196



33. Minor Diastereomer

^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJII237H

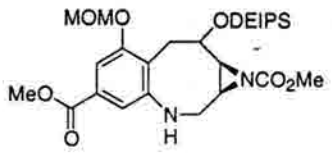
(1aS,9aS)-dimethyl ester 1,5-dicarboxylic acid, 9-[[diethyl(1-methylethyl)silyl]oxy]-1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-H-Azirino[2,3-c][1]benzazocine.

MeOH (40 mL) was saturated with H_2 for 10 min, flushed with argon, and 5% Pd/C (1.0 g, 0.48 mmol, 0.25 eq) was added in one portion. The mixture was saturated with H_2 for another 20 min, followed by addition of nitroaldehyde **33** (1.2 g, 2.22 mmol, 1.0 equiv) in 15 mL of MeOH. The H_2 gas was continually bubbled through the reaction and after 35 min, TLC analysis (10:1 CH_2Cl_2 /MeOH) of the reaction showed complete consumption of **33**. The reaction mixture was passed through a short pad of Celite with hot MeOH, and the filtrate was concentrated *in vacuo*. The reduced intermediate was dissolved in a minimum amount of MeOH and partitioned into four separate 1 L round bottom flasks and concentrated. The residue in each reaction flask was dissolved in 350 mL of CH_2Cl_2 with 20 g of activated 4Å molecular sieves and $MgSO_4$ (1 g) added to the solution. The stirred mixture was heated to reflux for 36 h and subsequently filtered and washed with 10:1 CH_2Cl_2/MeOH (400 mL). The filtrate was concentrated, and the residue was immediately dissolved in a mixture of 2:1 CH_2Cl_2/MeOH (15 mL). After the mixture was cooled in an ice bath for 10 min, $NaCNBH_3$ (170 mg, 2.70 mmol, 4.9 equiv.) was added in one portion followed by dropwise addition of AcOH (15 μL, 0.26 mmol, 0.5 equiv). After 5 min, TLC analysis of the reaction (1:1 Hex/EtOAc) showed no signs of starting material, and the reaction was quenched with sat $NaHCO_{3(aq)}$. Subsequently all

four individual reactions were combined, the layers were separated, and the aqueous layer was extracted 3 x CH_2Cl_2. The combined organic layers were washed 1 x sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (2:1 Hex/EtOAc) to give (55-75% yield from **33**) of **34** as a clear yellow oil (>95% pure).

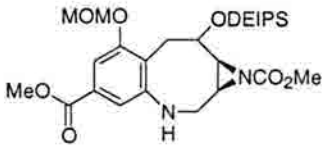
Major Diastereomer: $[\alpha]_D^{25}$ = + 60.6 (c=1.2, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 0.79 (m, 4 H), 0.90 - 1.11 (m, 13 H), 2.56 (m, 2 H), 2.87 (dd, J = 10.5 Hz, 13.8 Hz, 1 H), 3.24 (dd, J = 5.4, 13.8 Hz), 3.48 (s, 3 H), 3.68 (s, 3 H), 3.73 (m, 1 H), 3.87 (s, 3 H), 3.90 (m, 1 H), 4.56 (m, 1 H), 5.22 (d, J = 6.6 Hz, 1 H), 5.26 (d, J = 6.9 Hz, 1 H), 7.07 (d, J = 1.5 Hz, 1 H), 7.21 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 3.4, 3.5, 7.0, 12.9, 17.2, 17.2, 31.3, 41.3, 43.0, 47.3, 52.1, 53.4, 56.2, 68.7, 94.3, 105.3, 114.2, 118.3, 129.4, 148.3, 156.1, 163.9, 166.8; IR (NaCl/neat) 3380, 2953, 1724, 1587, 1437 cm^{-1}; HRMS (FAB+) calcd for $C_{24}H_{39}N_2O_7Si_1$ (*m/z*) 495.2527, found (*m/z*) 495.2524.

Minor Diastereomer: $[\alpha]_D^{25}$ = + 99.1 (c=1.25, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 0.64 - 0.73 (m, 4 H), 0.97 - 1.5 (m, 13 H), 2.56 (m, 1 H), 2.68 (dd, J = 5.0 Hz, 7.0 Hz, 1 H), 3.16 (m, 2 H), 3.48 (s, 3 H), 3.59 - 3.66 (m, 2 H), 3.68 (s, 3 H), 3.86 (s, 3 H), 4.29 (ddd, J = 4.5 Hz, 4.5 Hz, 4.5 Hz, 1 H), 5.17 (d, J = 7.0 Hz, 1 H), 5.21 (d, J = 7.0 Hz, 1 H), 7.09 (d, J = 1.5 Hz, 1 H), 7.32 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 3.5, 3.5, 7.0, 7.0,12.9, 17.2, 17.2, 30.7, 44.0, 48.8, 52.1, 52.8, 56.2, 67.5, 74.1, 94.2, 104.6, 109.4, 115.8, 129.5, 144.4, 154.6, 156.0, 167.0; IR (NaCl/neat) 3386, 2953, 1723, 1585, 1437 cm^{-1}; HRMS (FAB+) calcd for $C_{24}H_{39}N_2O_7Si_1$ (*m/z*) 494.2448, found (*m/z*) 494.2441.



34. Major Diastereomer

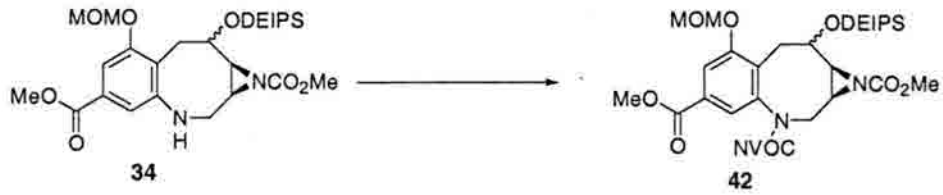
^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJII244II



34. Minor Diastereomer

^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJIII43

(1aS,9aS)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 1,5-dimethyl ester 9-[[diethyl(1-methylethyl)silyl]oxy]-1a,8,9,9a-tetrahydro-7-(methoxymethoxy)-1H-azirino[2,3-c][1]-benzazocine-1,3,5(2H)-tricarboxylic acid.

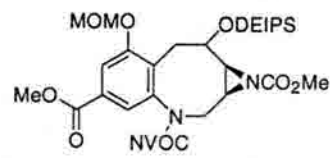


To a solution of **34** (130 mg, 0.26 mmol, 1.0 eq) in 3.2 mL of CH_2Cl_2 was added to a 25 *N,N*-diisopropylethylamine (152 μL, 0.77 mmol, 3.0 eq) and 6-nitroveratryl chloroformate (93 mg, 0.34 mmol, 1.3 eq). After 12 h, TLC analysis (1:1 Hex/EtOAc) of the reaction showed no starting material. The reaction was diluted with sat $NaHCO_{3(aq)}$ and extracted with 3 x EtOAc. The combined organic layers were washed 1 x sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (2:1 Hex/EtOAc) to give 143 mg (75% yield) of carbamate **42** as a clear yellow oil.

Major Diastereomer: $[\alpha]_D^{25}$ = + 45.2 (c=1.3, $CHCl_3$) ^{1}H NMR (300 MHz, DMSO, 393.1 K): 0.65 - 0.75 (m, 4 H), 1.00 (m, 13 H), 2.67 (br, 1 H), 2.86 (br, 4 H), 2.98 (br, 1 H), 3.47 (s, 3 H), 3.62 (s, 3 H), 3.80 (s, 3 H), 3.86 (s, 3 H), 3.87 (s, 3 H), 3.92 (t, J = 4.2 Hz, 1 H), 4.35 (br, 1 H), 5.28 (d, J = 6.9 Hz, 1 H), 5.32 (d, J = 6.3 Hz, 1 H), 5.41 (s, 2 H), 6.93 (br, 1 H), 7.46 (d, J = 1.8 Hz, 1 H), 7.63 (d, J = 1.5 Hz, 1 H), 7.65 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) (Note: at room temperature, this compound exists as an equilibrating mixture of conformational isomers of the NVOC-urethane on the nmr time scale): 3.5, 3.6, 7.0, 7.0, 12.7, 17.2, 17.2, 29.3, 29.5, 40.4, 47.6, 48.5, 52.2, 52.4, 53.5, 53.6, 53.6,

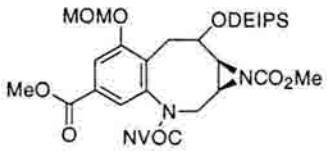
56.2, 56.4, 64.5, 64.8, 70.4, 94.3, 107.8, 108.2, 113.6, 122.1, 128.5, 130.6, 131.3, 138.6, 142.1, 147.4, 153.4, 153.9, 155.7, 164.1, 165.6; IR (NaCl/neat) 2951, 1725, 1581, 1522, 1438 cm^{-1}; HRMS (FAB+) calcd for $C_{34}H_{48}N_3O_{13}Si_1$ (*m/z*) 734.2956, found (*m/z*). 734.2941

Minor Diastereomer: $[\alpha]_D^{25}$ = + 19.6 (c=1.2, $CHCl_3$) ^1H NMR (300 MHz, DMSO, 393.1 K): 0.62 - 0.69 (m, 4 H), 0.94 - 1.02 (m, 13 H), 3.04 – 3.20 (br, 4 H), 3.47 (s, 3 H), 3.62 (s, 3 H), 3.77 (s, 3 H), 3.87 (s, 6 H), 3.92 (m, 1 H), 4.10 (br, 1 H), 5.26 (d, J = 6.6 Hz, 1 H), 5.31 (d, J = 6.6 Hz, 1 H), 5.40 (d, J = 14.4 Hz, 2 H), 5.46 (d, J = 14.4 Hz, 2 H), 6.86 (br, 1 H), 7.50 (s, 1 H), 7.64 (s, 1 H), 7.65 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) (Note: at room temperature, this compound exists as an equilibrating mixture of conformational isomers of the NVOC-urethane on the nmr time scale): 3.5, 3.6, 7.0, 7.0, 12.8, 13.0, 17.2, 17.2, 33.2, 34.3, 37.5, 38.2, 46.5, 49.1, 50.3, 52.4, 53.2, 53.6, 56.1, 56.3, 56.6, 56.6, 64.7, 64.9, 69.7, 74.7, 76.6, 94.8, 107.9, 108.5, 108.8, 114.1, 122.8, 123.0, 127.6, 127.7, 129.8, 130.1, 132.5, 133.1, 139.0, 139.8, 143.1, 147.6, 153.3, 153.5, 154.1, 155.5, 155.9, 162.6, 162.9, 165.7; IR (NaCl/neat) 2953, 1718, 1577, 1522, cm^{-1}; HRMS (FAB+) calcd for $C_{34}H_{48}N_3O_{13}Si_1$ (*m/z*) 734.2956, found (*m/z*). 734.2962



42. Major Diastereomer

^{1}H NMR, 300 MHz, 393 K, DMSO, filename: TCJII246HT



42. Minor Diastereomer

^{1}H NMR, 300 MHz, 393 K, DMSO, filename: TCJIII337IIHT

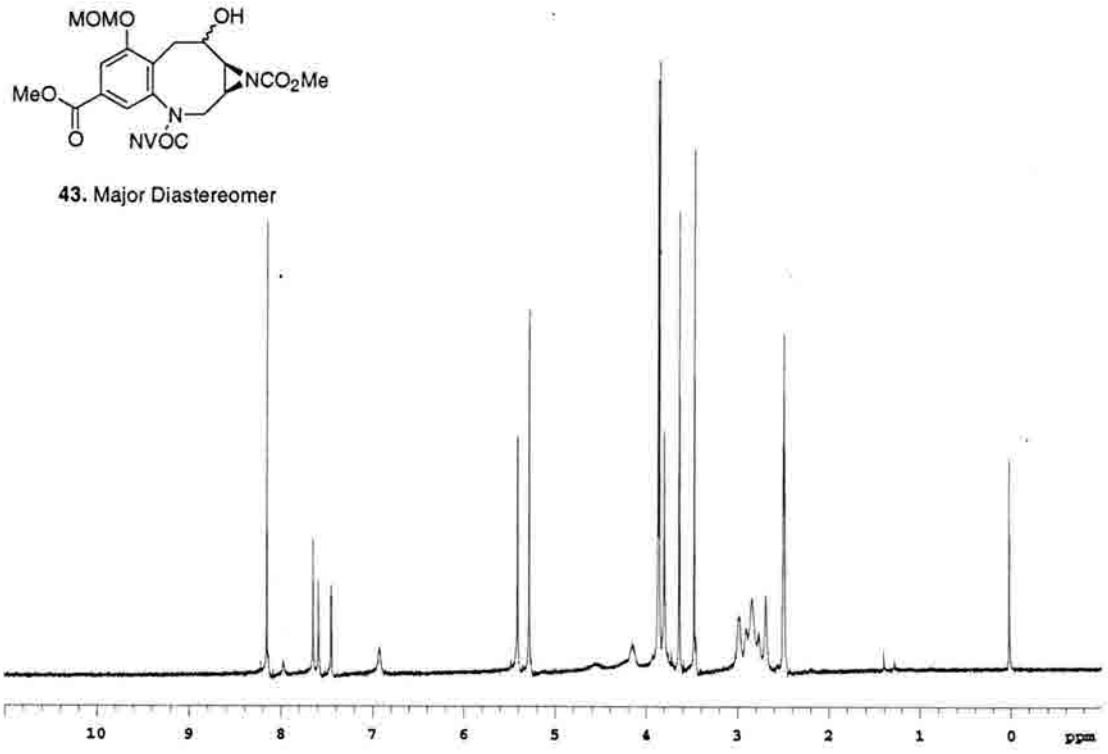
(1aS,9aS)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 1,5-dimethyl ester-1a,8,9,9a-tetrahydro-7-(methoxymethoxy)-9-hydroxy-1H-azirino[2,3-c][1]-benzazocine-1,3,5(2H)-tricarboxylic acid.

Compound **42** (110 mg, 0.15 mmol, 1.0 equiv.) was dissolved in 1.5 mL THF and cooled to 0 °C. Next, 1 M TBAF in THF (225 μL, 0.23 mmol, 1.5 equiv.) was added dropwise and the reaction was strirred for 1 h at 0 °C until TLC analysis (EtOAc) showed the complete consumption of **42**. The reaction was quenched with sat $NH_4Cl_{(aq)}$ and the layers were separated. The aqueous layer was diluted with H_2O and subsequently extracted 3 X EtOAc. The combined organic layers were washed with brine and dried over Na_2SO_4. Following concentration, the crude product was purified by column chromatography (EtOAc) to yield 60 mg (65% yield) of alcohol **43** as an amorphous solid.

Major Diastereomer: mp = 96-98 °C; $[\alpha]_D^{25}$ = + 21.1 (c=1.4, $CHCl_3$); ^1H NMR (300 MHz, DMSO, 393.1 K): 2.69 (br, 1 H), 2.85 (br, 2 H), 2.99 (br, 1 H), 3.45-3.51 (m, 1H), 3.48 (s, 3 H), 3.64 (s, 3 H), 3.72-3.82 (m, 1H), 3.81 (s, 3 H), 3.86 (s, 3 H), 3.87 (s, 3 H), 4.15 (br, 2 H), 4.59 (br, 1 H), 5.29 (s, 2 H), 5.41 (s, 2 H), 6.93 (br, 1 H), 7.46 (d, J = 1.5 Hz, 1 H), 7.60 (d, J = 1.5 Hz, 1 H), 7.66 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) (Note: at room temperature, this compound exists as an equilibrating mixture of conformational isomers

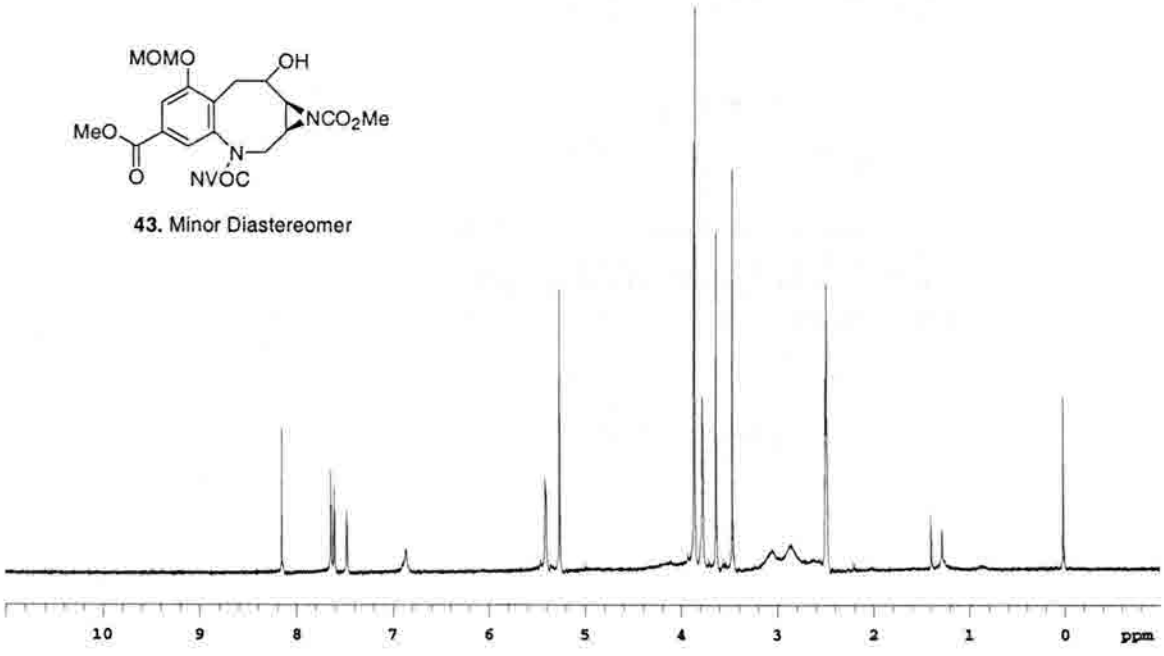
of the NVOC-urethane on the nmr time scale): 27.8, 29.0, 39.7, 41.4, 45.9, 46.3, 48.6, 51.0, 52.3, 54.2, 56.0, 56.5, 63.1, 64.8, 65.8, 69.4, 94.4, 94.8, 107.9, 108.4, 113.7, 114.3, 122.3, 122.8, 128.0, 138.8, 141.6, 147.5, 153.4, 153.9, 154.2, 155.6, 156.3, 162.8, 163.6, 165.6; IR ($NaCl/CHCl_3$) 3429, 2951, 1720, 1582, 1523, 1438 cm^{-1}; HRMS (FAB+) calcd for $C_{27}H_{32}N_3O_{13}$ (*m/z*) 606.1935, found (*m/z*) 606.1940.

Minor Diastereomer: $[\alpha]_D^{25}$ = + 17.7 (c=1.2, $CHCl_3$); ^1H NMR (300 MHz, DMSO, 393.1 K): 2.70 – 3.20 (br, 4 H), 3.45-3.51 (m, 1H), 3.47 (s, 3 H), 3.64 (s, 3 H), 3.78 (s, 3 H), 3.86 (s, 3 H), 3.87 (s, 3 H), 3.90 – 3.96 (br, 2 H), 5.27 (s, 2 H), 5.41 (d, J = 14.4 Hz, 1 H), 5.42 (d, J = 14.4 Hz, 1 H), 6.87 (br, 1 H), 7.48 (d, J = 1.5 Hz, 1 H), 7.61 (d, J = 1.5 Hz, 1 H), 7.65 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) (Note: at room temperature, this compound exists as an equilibrating mixture of conformational isomers of the NVOC-urethane on the nmr time scale): 29.7, 30.4, 32.1, 38.2, 38.3, 46.3, 48.7, 49.2, 51.0, 52.5, 53.0, 53.9, 56.1, 56.1, 56.4, 56.5, 56.7, 64.9, 69.5, 74.5, 94.9, 107.9, 108.5, 108.7, 114.1, 114.8, 122.9, 123.2, 127.5, 129.9, 130.4, 132.4, 133.2, 138.9, 140.2, 143.0, 147.6, 153.3, 153.7, 154.1, 155.1, 155.9, 156.3, 162.6, 162.9, 165.6; IR ($NaCl/CHCl_3$) 3480, 2954, 1716, 1580, 1523, 1437 cm^{-1}; HRMS (FAB+) calcd for $C_{27}H_{32}N_3O_{13}$ (*m/z*) 606.1935, found (*m/z*)



43. Major Diastereomer

^{1}H NMR, 300 MHz, 393 K, DMSO, filename: TCJII30HT



43. Minor Diastereomer

^{1}H NMR, 300 MHz, 393 K, DMSO, filename: TCJIII338HT

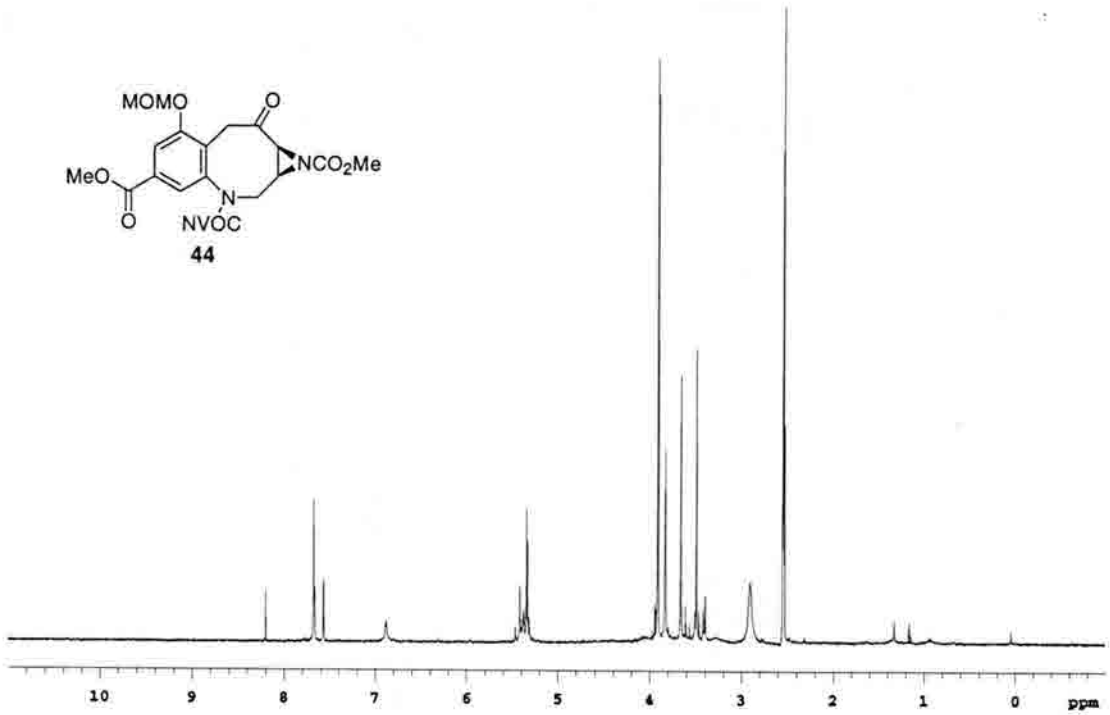
(1aS,9aS)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 1,5-dimethyl ester-1a,8,9,9a-tetrahydro-7-(methoxymethoxy)-9-oxo-1H-azirino[2,3-c][1]-benzazocine-1,3,5(2H)-tricarboxylic acid.

43 **44**

Alcohol **43** (106 mg, 0.175 mmol, 1.0 eq.) was dissolved in 1.8 ml of reagent grade CH_2Cl_2. Dess-Martin reagent (111 mg, 0.263 mmol, 1.5 eq.) was added in one portion and the reaction was allowed to stir 1 h. Dilution with Et_2O followed by the addition of a solution of $Na_2S_2O_3$ in sat. $NaHCO_{3\,(aq)}$ gave a cloudy biphasic mixture that was vigorously stirred until clear. Following separation, the aqueous layer was extracted 3 X EtOAC. The combined organic layers were washed 1 X $NaCl_{(aq)}$, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (3:2 CH_2Cl_2/Et_2O) to give 96 mg (91% yield) of ketone **44** as a white amorphous solid.

Compound **19**: mp = 89-93 °C; $[\alpha]_D^{25}$ = -66.4 (c=1.1, $CHCl_3$); ^1H NMR (300 MHz, DMSO, 393 K): 3.20 (br, 21 H), 3.38 (d, J = 6.6 Hz, 1 H) 3.45 (s, 3 H), 3.47 (d, J = 13.2 Hz, 1 H), 3.57 (d, J = 13.2 Hz, 1 H), 3.62 (s, 3 H), 3.79 (s, 3 H), 3.87 (s, 3 H), 3.88 (s, 3 H), 3.89 (m, 1 H), 3.93 (d, J = 9.0 Hz, 1 H), 5.29 (d, J = 6.6 Hz, 1 H), 5.33 (d, J = 6.6 Hz, 1 H), 5.38 (s, 2 H), 6.84 (br, 1 H), 7.53 (d, J = 1.5 Hz, 1 H), 7.63 (d, J = 1.5 Hz, 1 H), 7.64 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) (Note: at room temperature, this compound exists as an equilibrating mixture of conformational isomers of the NVOC-urethane on the nmr time scale): 40.6, 42.3, 45.3, 46.3, 46.7, 52.4, 53.9, 56.1, 56.3, 56.4, 65.0, 65.4,

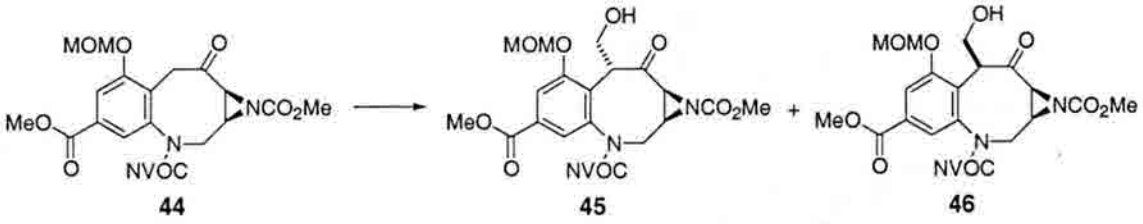
94.3, 94.7, 107.9, 108.7, 113.8, 121.5, 127.5, 129.7, 130.3, 132.2, 139.1, 140.3, 147.7, 153.5, 153.8, 155.5, 161.5, 165.7, 203.6; IR ($NaCl/CHCl_3$) 2956, 1724, 1581, 1524, 1437 cm^{-1}; HRMS (FAB+) calcd for $C_{27}H_{30}N_3O_{13}$ (*m/z*) 604.1779, found 604.1783 (*m/z*).



^{1}H NMR, 300 MHz, 393 K, DMSO, filename: TCJII205HT

(1aS,8R,9aS)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 1,5-dimethyl ester-1a,8,9,9a-tetrahydro-8-(hydroxymethyl)-7-(methoxymethoxy)-9-oxo-1H-azirino[2,3-c][1]-benzazocine-1,3,5(2H)-tricarboxylic acid. (45)

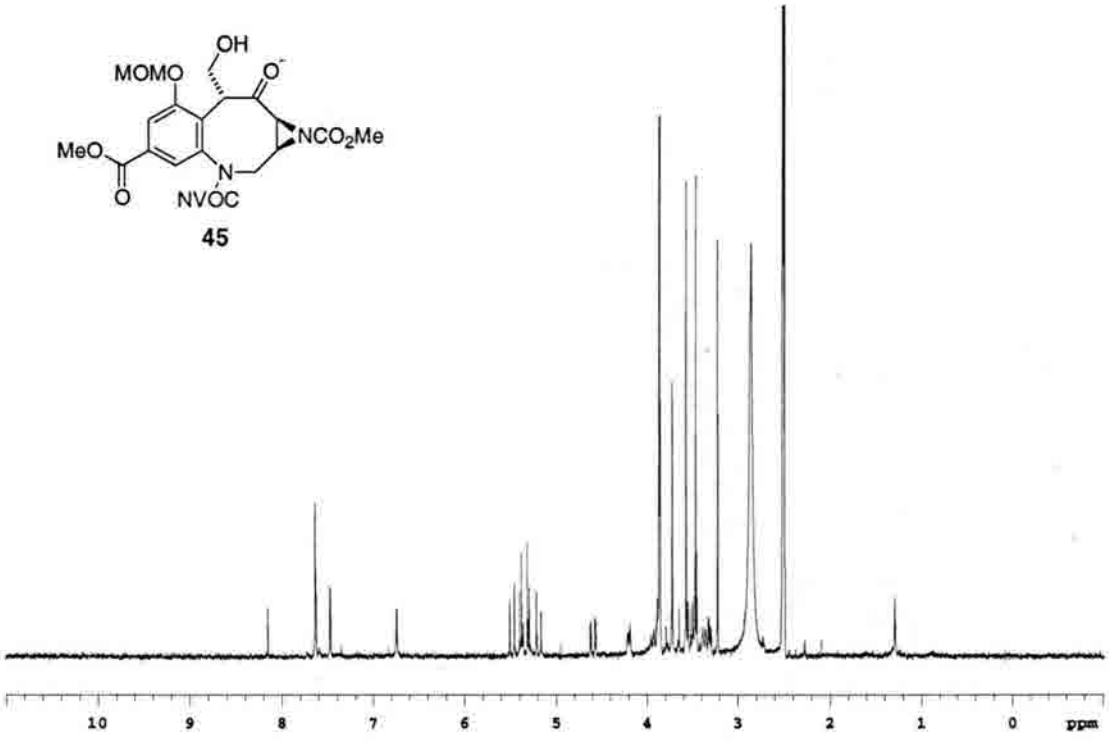
(1aS,8S,9aS)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 1,5-dimethyl ester-1a,8,9,9a-tetrahydro-8-(hydroxymethyl)-7-(methoxymethoxy)-9-oxo-1H-azirino[2,3-c][1]-benzazocine-1,3,5(2H)-tricarboxylic acid. (46)



Ketone **44** (96 mg, 159 μmol, 1.0 equiv.) was dissolved in 900 μL of dry DMF and cooled to -45 °C. A freshly prepared solution of LDA 1.0 M (166 μL, 166 μmol, 1.05 equiv.) was added dropwise and the resulting solution was allowed to warm to 0 °C for 15 min and subsequently cooled back to -45 °C. To the bright yellow solution, 1.4 mL of a 0.15 M anhydrous formaldehyde solution in THF was added dropwise. The reaction was allowed to stir 1.5 h whereupon the color slowly faded to a light yellow solution. Sat $NH_4Cl_{(aq)}$ was added to quench the reaction followed by dilution with EtOAc. Upon reaching ambient temperature the resulting layers were separated and the aqueous layer was thoroughly extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. Drying overnight under vacuum followed by purification of the crude oil by column chromatography (EtOAc) afforded 57 mg (58 % yield) of **45** and **46** as a mixture of diastereomers. Selective recrystalization of **45** from EtOAc afforded crystals suitable for X-ray analysis.

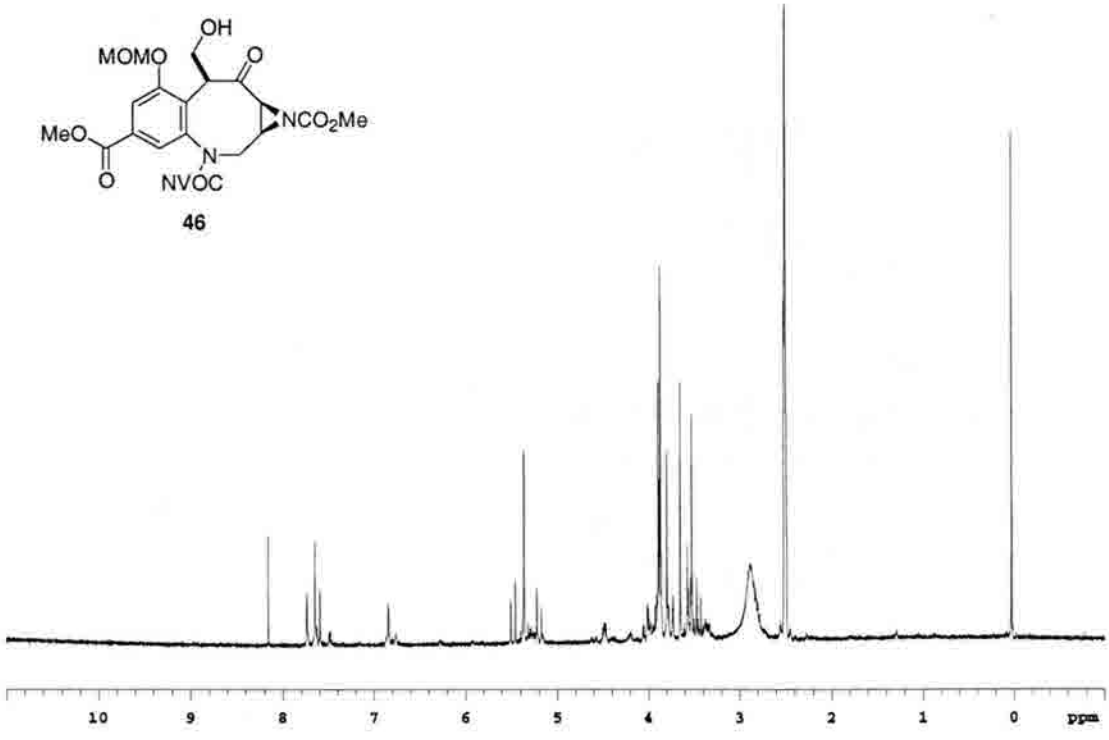
Compound **45**: mp = 200-203 °C; $[\alpha]_D^{25}$ = -20.3 (c=0.8, $CHCl_3$); ^1H NMR (300 MHz, DMSO, 393 K): 3.29 (dd, J = 7.0 Hz, 2.5 Hz, 1 H), 3.36 (m, 1 H), 3.46 (s, 3 H), 3.47 (d, J = 7.0 Hz, 1 H), 3.52 (m, 1 H), 3.57 (s, 3 H), 3.72 (s, 3 H), 3.86 (s, 3 H), 3.87 (s, 3 H), 3.89 (m, 1 H), 4.19 (dd, J = 2.7 Hz, 8.1 Hz, 1 H), 4.61 (dd, J = 3.3 Hz, 15.9 Hz, 1 H), 5.22 (d, J = 15.0 Hz, 1 H), 5.32 (d, J = 6.3 Hz, 1 H), 5.38 (d, J = 6.6 Hz, 1 H), 5.51 (d, J = 15.0 Hz, 1 H), 6.74 (s, 1 H), 7.48 (d, J = 1.8 Hz, 1 H), 7.63 (d, J = 1.5 Hz, 1 H), 7.64 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) (Note: at room temperature, this compound exists as an equilibrating mixture of conformational isomers of the NVOC-urethane on the nmr time scale): 45.4, 46.0, 46.2, 52.6, 53.3, 54.0, 56.1, 56.4, 56.5, 61.5, 65.3, 94.1, 108.0, 114.4, 121.3, 126.9, 131.1, 133.2, 140.5, 147.4, 153.4, 154.0, 161.3, 165.4, 205.5; IR ($NaCl/CHCl_3$) 3544, 2954, 1723, 1581, 1523, 1436 cm^{-1}; UV λ_{max} (CH_3CN) nm (ε): 345 (6766), 295 (7813), 243 (18101); MS (ES+) (*m/z*) 634 (M+H) (100%).

Compound **46**: mp = 98-104 °C; ^1H NMR (300 MHz, DMSO, 393 K): 3.3-3.4 (m, 1 H), 3.47 (d, J = 13.2 Hz, 1 H), 3.53 (s, 3 H), 3.56 (d, J = 7.5 Hz, 1 H), 3.65 (s, 3 H), 3.73 (d, J = 14.7 Hz, 1 H), 3.80 (s, 3 H), 3.87 (s, 3 H), 3.89 (s, 3 H), 3.8-4.1 (m, 2 H), 4.48 (m, 1 H), 5.22 (d, J = 14.7 Hz, 1 H), 5.36 (s, 2 H), 5.46 (d, J = 14.7 Hz, 1 H), 6.84 (s, 1 H), 7.59 (d, J = 1.5 Hz, 1 H), 7.65 (s, 1 H), 7.74 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) (Note: at room temperature, this compound exists as an equilibrating mixture of conformational isomers of the NVOC-urethane on the nmr time scale): 45.3, 45.4, 46.0, 48.6, 51.7, 52.6, 54.1, 56.3, 56.3, 56.8, 61.5, 65.1, 94.8, 108.0, 114.7, 123.0, 127.1, 130.7, 132.5, 139.4, 140.0, 148.0, 153.5, 156.0, 162.0, 165.3, 201.8; IR ($NaCl/CHCl_3$) 3583, 2954, 1723, 1580, 1523, 1438 cm^{-1}.



45

^{1}H NMR, 300 MHz, 393 K, DMSO, filename: TCJII206SDHT



46

^{1}H NMR, 300 MHz, 393 K, DMSO, filename: TCJIIIEpiAldIIHT

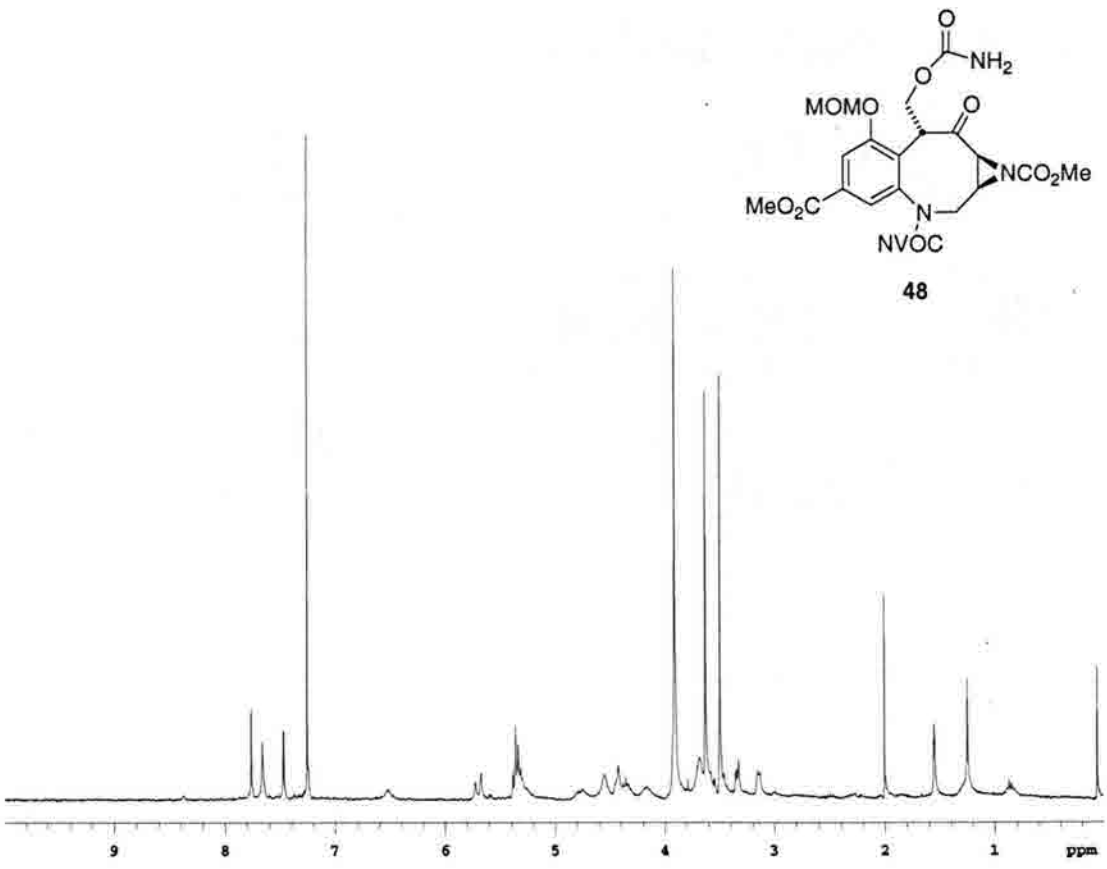
(1aS,8R,9aS)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 1,5-dimethyl ester-1a,8,9,9a-tetrahydro-8-[[(aminocarbonyl)oxy]methyl]-7-(methoxymethoxy)-9-oxo-1H-azirino[2,3-c][1]-benzazocine-1,3,5(2H)-tricarboxylic acid.

45 48

To a solution of alcohol **45** (4.5 mg, 7.1 μmol, 1.0 equiv.) in 800 μL CH_2Cl_2 at 0 °C was added trichloroacetyl isocyanate (5 μL, 42.0 μmol, 6.0 equiv.). The reaction was stirred 10 min until TLC analysis (EtOAc) showed complete reaction of the starting material. The reaction was quenched with the addition of 5 drops of MeOH, concentrated *in vacou*, and the crude reaction mixture was dissolved in 3 mL of MeOH. Silica gel was added and the mixture was stirred approximately 2.5 h at ambient temperature until TLC analysis (EtOAc) showed mostly product (the trichloroacetyl intermediate and the elimination by-product co-spot by TLC analysis). The reaction was filtered with 10:1 CH_2Cl_2/MeOH and concentrated. Purification of the crude mixture by PTLC (20:1 CH_2Cl_2/MeOH) furnished 2.9 mg (60% yield) of carbamate **48** as a yellow/white crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$) (Note: at room temperature, this compound exists as an equilibrating mixture of conformational isomers of the NVOC-urethane on the nmr time scale): 3.15 (m, 1 H), 3.33 (d, J = 6.6 Hz, 1 H), 3.50 (s, 3 H), 3.63 (s, 3 H), 3.68 (br s, 3 H), 3.92 (s, 6 H), 4.1-4.8 (br m, 5 H), 5.31 (br d, 1 H), 5.34 (d, J = 6.9 Hz, 1 H), 5.38 (d, J = 6.9 Hz, 1 H), 5.68 (d, J = 15.0 Hz, 1 H), 6.53 (br s, 1 H), 7.49 (d, J = 1.5 Hz, 1 H), 7.68

(s, 1 H), 7.78 (d, J = 1.5 Hz, 1 H); IR ($NaCl/CHCl_3$) 3542, 3373, 2922, 1725, 1581, 1524, cm^{-1}; MS (*m/z*) dec.



^1H NMR, 300 MHz, $CDCl_3$, filename: TCJIII12-21-NVOC

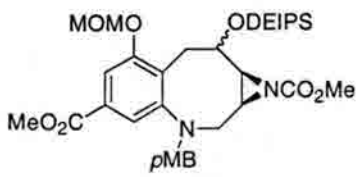
(1aS,9aS)-Dimethyl ester -1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-3-[(4-methoxyphenyl)methyl]-9-[[diethyl(1-methylethyl)silyl]oxy]-1H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid.

Aniline **34** (200 mg, 0.404 mmol, 1.0 equiv.) was dissolved in 6 mL of dry CH_2Cl_2. DIPEA (211 uL, 1.21 mmol, 3.0 equiv.) was added followed by dropwise addition of PMB-Br (106 uL, 0.525 mmol, 1.3 eq.). The solution was allowed to stir 48 h until TLC analyses (2:1 Hex/EtOAc) showed loss of starting material. The reaction was quenched by the addition of sat $NaHCO_{3\,(aq)}$. The layers were separated, and the aqueous layer was extracted 3 X CH_2Cl_2. The combined organic layers were concentrated, and the crude material was taken up in a 2:1 Hex/EtOAc solution, washed 2 X H_2O, 1 X $NaCl_{(aq)}$ and dried over Na_2SO_4. Following concentration, the crude oil was purified by column chromatography (3:1 Hex/EtOAc) to afford 215 mg (86 % yield) of the PMB protected aniline as a colorless oil.

Major Diastereomerer: $[\alpha]_D^{25}$ = + 77.1 (c=1.0, $CHCl_3$) ^1H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 0.70 – 0.77 (m, 4 H), 1.00 – 1.04 (m, 13 H), 2.23 (dd, J = 6.0 Hz, 12.0 Hz, 1 H), 2.49 (dd, J = 4.5 Hz, 6.0 Hz, 1 H), 3.07 (dd, J = 10.5 Hz, 13.5 Hz, 1 H), 3.18 (dd, J = 4.5 Hz, 13.5 Hz, 1 H), 3.46 (m, 2 H), 3.50 (s, 3 H), 3.65 (s, 3 H), 3.80 (s, 3 H), 3.89 (s, 3 H), 4.33 (1/2 ABq, J = 13.5 Hz, 1 H), 4.41 (1/2 ABq, J = 13.5 Hz, 1 H), 4.55 (m, 1 H), 5.21 (1/2 ABq, J = 7.5 Hz, 1 H), 5.26 (1/2 ABq, J = 7.5 Hz, 1 H), 6.85 (d, J = 9.0 Hz, 2 H),

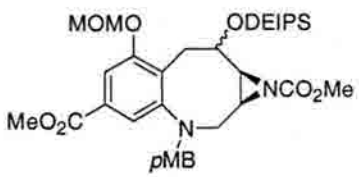
7.27 (d, J = 9.0 Hz, 2 H), 7.38 (d, J = 1.5 Hz, 1 H), 7.47 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 3.5, 3.5, 7.0, 12.9,17.2, 17.2, 31.2, 40.8, 44.9, 52.0, 53.3, 53.3, 55.1, 56.1, 58.7, 68.2, 94.3, 107.7, 113.7, 113.7, 115.0, 125.3, 129.0, 129.6, 129.6, 130.3, 151.7, 155.4, 158.7, 163.6, 166.8; IR (NaCl/neat) 2952, 1733, 1718, 1653, 1576, 1559 cm^{-1}; HRMS (FAB+) calcd for $C_{32}H_{47}N_2O_8Si_1$ (*m/z*) 615.3102, found (*m/z*) 615.3094.

Minor Diastereomer: $[\alpha]_D^{25}$ = + 83.9 (c=0.87, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 0.69 – 0.74 (m, 4 H), 0.99 – 1.07 (m, 13 H), 2.22 (dd, J = 6.0 Hz, 13.5 Hz, 1 H), 2.51 (dd, J = 7.5 Hz, 7.5 Hz, 1 H), 2.96 (dd, J = 6.0 Hz, 12.0 Hz, 1 H), 3.04 (dd, J = 6.0 Hz, 15.0 Hz, 1 H), 3.27 (m, 2H), 3.50 (s, 3H), 3.62 (s, 3H), 3.79 (s, 3H), 3.91 (s, 3H), 4.06 (1/2 ABq, J = 12 Hz, 2 H), 4.14 (1/2 ABq, J = 12 Hz, 2 H), 4.41 (m, 1 H), 5.20 (1/2 ABq, J = 7.5 Hz, 1 H), 5.25 (1/2 ABq, J = 7.5 Hz, 1 H), 6.80 (d, J = 9.0 Hz, 2 H), 7.23 (d, J = 9.0 Hz, 2 H), 7.53 (d, J = 1.5 Hz, 1 H), 7.62 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 3.7, 3.7, 7.0, 7.1,13.0, 17.3, 17.3, 34.3, 38.6, 47.7, 52.2, 53.3, 55.2, 56.3, 56.6, 59.5, 71.6, 94.7, 111.4, 113.6, 113.6, 117.5, 129.2, 129.9, 129.9, 130.3, 131.8, 152.1, 155.4, 158.6, 163.2, 166.6; IR (NaCl/neat) 2952, 1726, 1512 cm^{-1}; HRMS (FAB+) calcd for $C_{32}H_{47}N_2O_8Si_1$ (*m/z*) 615.3102, found (*m/z*) 615.3080.



Major Diastereomer

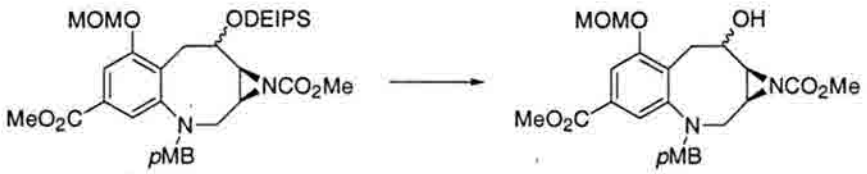
^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJII475



Minor Diastereomer

^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJIII47

(1aS,9aS)-Dimethyl ester -1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-3-[(4-methoxyphenyl)methyl]-9-hydroxy -1H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid.



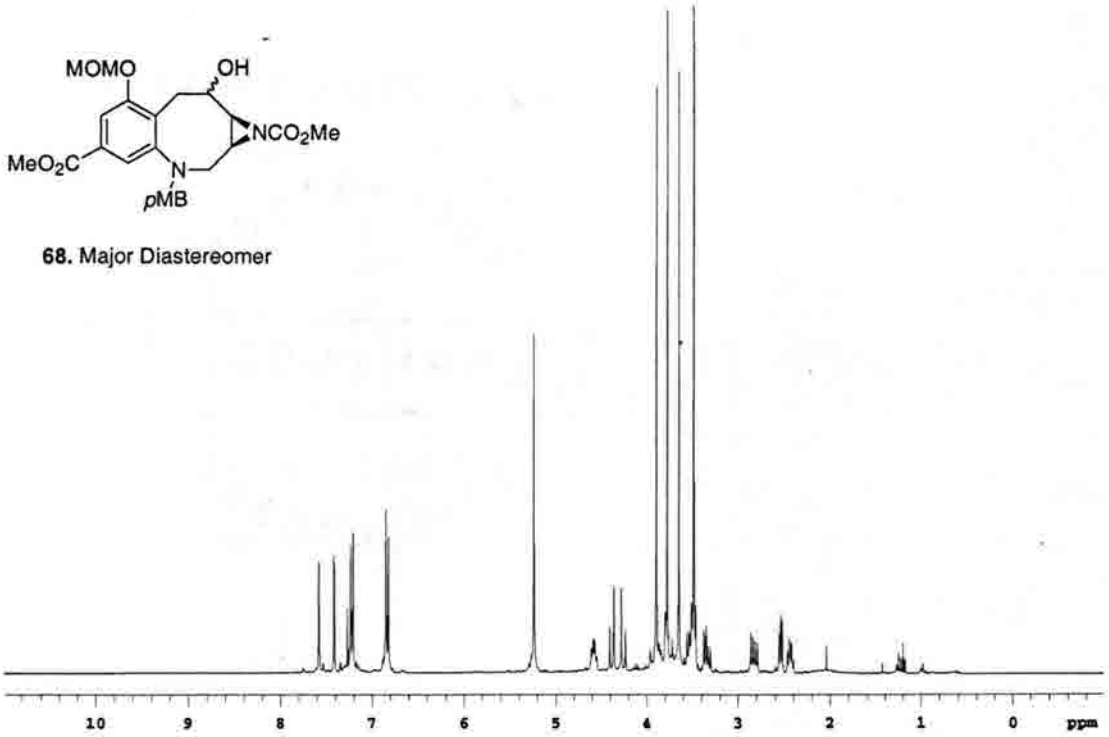
68

To a solution of the silyl ether from the previous reaction (760 mg, 1.23 mmol, 1.0 equiv.) in 16 mL of a 10:1 solution of DMF/H_2O was added Tris(dimethylamino)sulfur (trimethylsilyl)difluoride (TASF) (700 mg, 2.54 mmol, 2.1 equiv.). After stirring at ambient temperature for 4 h, an additional portion of TASF (350 mg, 1.27 mmol, 1.0 equiv.) was added and the reaction was continued for 2 h until TLC analyses (2:1 CH_2Cl_2/Et_2O) showed complete consumption of the starting material. Addition of saturated $NH_4Cl_{(aq)}$ followed by dilution with EtOAC and H_2O afforded a biphasic mixture that was subsequently separated. Extraction of the aqueous phase with 3 X EtOAc followed by washing the combined organic extracts 1 X brine and drying over Na_2SO_4 afforded a crude oil that was dried under vacuum overnight. Purification of the crude material by column chromatography (2:1 CH_2Cl_2/Et_2O) furnished 515 mg of alcohol **68** (86% yield) as a white crystalline solid following concentration in Et_2O.

Major Diastereomer: $[\alpha]_D^{25}$ = + 105.1 (c=1.0, $CHCl_3$) m.p. 48 – 50 °C ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 2.40 (m, 1 H), 2.52 (dd, J = 4.5 Hz, 6.0 Hz, 1 H), 2.79 (dd, J = 7.5 Hz, 15.0 Hz, 1 H), 3.30 (dd, J = 7.5 Hz, 13.5 Hz, 1 H), 3.49 (s, 3 H), 3.51 (m, 2 H), 3.65 (s, 3 H), 3.78 (s, 3 H), 3.89 (s, 3 H), 4.26 (1/2 ABq, J = 13.5 Hz, 2 H), 4.39 (1/2 ABq, J = 13.5

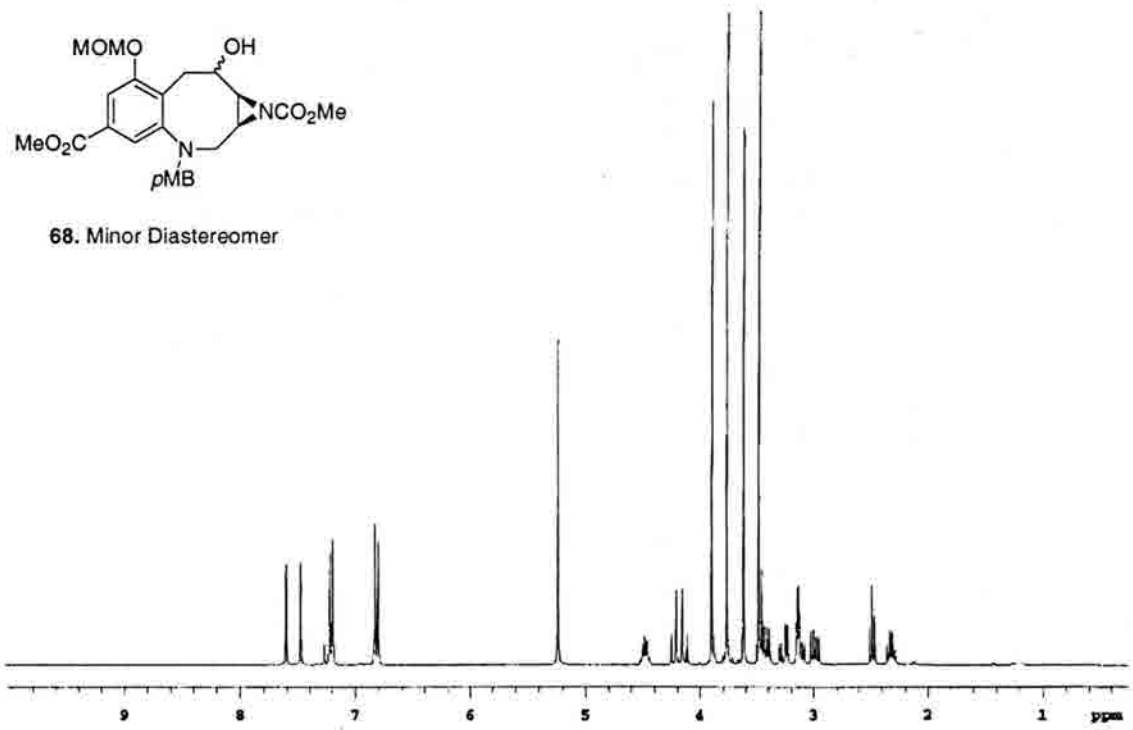
Hz, 2 H), 4.55 (m, 1 H), 5.24 (ap. s, 2 H), 6.82 (d, J = 9.0 Hz, 2 H), 7.21 (d, J = 9.0 Hz, 2 H), 7.41 (d, J = 1.5 Hz, 1 H), 7.58 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 31.6, 39.7, 44.0, 52.2, 53.7, 54.1, 55.2, 56.3, 59.3, 64.7, 94.4, 109.1, 113.8, 113.8,116.5, 126.8, 129.4, 129.6, 129.6, 150.8, 155.2, 158.8, 163.2, 166.5; IR (NaCl/neat) 3462, 2953, 1720, 1576, 1512 cm^{-1}; HRMS (FAB+) calcd for $C_{25}H_{31}N_2O_8$ (*m/z*) 487.2080, found (*m/z*) 487.2068.

Minor Diastereomer: $[\alpha]_D^{25}$ = + 103.6 (c=0.87, $CHCl_3$) m.p. 57 – 59 °C ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 2.29 (m, 1 H), 2.95 (dd, J = 7.5 Hz, 13.5 Hz 1 H), 3.08 (dd, J = 6.0 Hz, J = 15.0 Hz, 1 H), 3.13 (d, J = 4.5 Hz, 1 H), 3.23 (dd, J = 6.0 Hz, J = 15.0 Hz, 1 H), 3.39 (m, 1 H), 3.49 (s, 3 H), 3.62 (s, 3 H), 3.77 (s, 3 H), 3.90 (s, 3 H), 4.13 (1/2 ABq, J = 13.5, 1 H), 4.23 (1/2 ABq, J = 13.5, 1 H), 4.44 (m, 1 H), 5.25 (ap. s, 2 H), 6.81 (d, J = 9.0 Hz, 2 H), 7.20 (d, J = 9.0 Hz, 2 H), 7.48 (d, J = 1.5 Hz, 1 H), 7.61 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 31.8, 38.8, 47.4, 52.2, 53.6, 55.1, 55.4, 56.4, 59.4, 71.2, 94.7, 110.4, 113.7, 113.7, 117.4, 129.4, 129.7, 129.7, 130.0, 151.8, 155.1, 158.6, 163.0, 166.4; IR (NaCl/neat) 2953, 1718, 1701, 1684, 1653 cm^{-1}; HRMS (FAB+) calcd for $C_{25}H_{31}N_2O_8$ (*m/z*) 487.2080, found (*m/z*) 487.2083.



68. Major Diastereomer

^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJIII491



68. Minor Diastereomer

^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJIII48

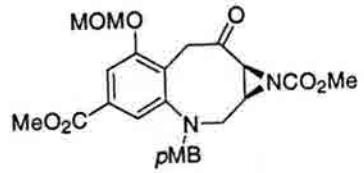
(1aS,9aS)-Dimethyl ester 1a,2,3,8,9,9a-hexahydro-4-(methoxymethoxy)-7-[(4-methoxyphenyl)methyl]-9-oxo-1H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid.

68 62

Alcohol **68** (65 mg, 0.134 mmol, 1.0 eq.) was dissolved in 4 mL of reagent grade CH_2Cl_2. Dess-Martin reagent (85 mg, 0.200 mmol, 1.5 eq.) was added in one portion and the reaction slowly turned a light purple color. The reaction was allowed to stir 1.5 hr. and was diluted with Et_2O followed by a solution of $Na_2S_2O_3$ in sat. $NaHCO_{3\,(aq)}$. The layers were vigorously stirred until clear. Following separation, the aqueous layer was extracted 4 X EtOAC. The combined organic layers were washed 1 X $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (2:1 CH_2Cl_2/Et_2O) to give 48 mg (75% yield) of ketone **62** as a white crystalline solid following concentration with Et_2O.

$[\alpha]_D^{25}$ = - 45.6 (c=1.0, $CHCl_3$) m.p. 58 – 62 °C ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 2.77 (m, 1 H), 3.06 (d, J = 6.9 Hz, 1 H), 3.35 (d, J = 3.0 Hz, 2 H), 3.44 (s, 3 H), 3.46 (m, 2 H), 3.61 (s, 3 H), 3.76 (s, 3 H), 3.91 (s, 3 H), 3.94 (m, 2 H), 5.20 (1/2 ABq, J = 6.6 Hz, 1 H), 5.25 (1/2 ABq, J = 6.6 Hz, 1 H), 6.78 (d, J = 7.5 Hz, 2 H), 7.08 (d, J = 2.5 Hz, 2 H), 7.53 (d, J = 1.5 Hz, 1 H), 7.56 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 39.1, 45.5, 46.3, 52.3, 52.4, 53.7, 55.1, 56.2, 56.3, 60.6, 94.2, 112.0, 113.5, 118.4, 128.2, 129.7,

130.9, 131.1, 131.1, 149.9, 153.8, 158.9, 161.7, 166.3, 200.0; IR (NaCl/neat) 2953, 1722, 1611, 1583 cm^{-1}; HRMS (FAB+) calcd for $C_{25}H_{29}N_2O_8$ (*m/z*) 485.1924, found (*m/z*) 485.1931.

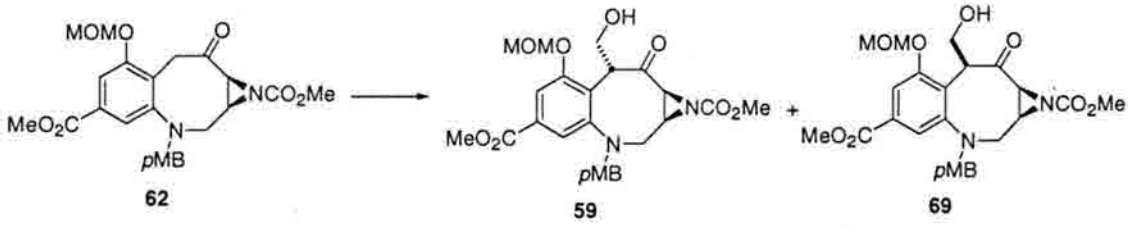


62

^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJIII496

(1aS,8R,9aS)-Dimethyl ester 1a,2,3,8,9,9a-hexahydro-8-(hydroxymethyl)-7-(methoxymethoxy)-3-[(4-methoxyphenyl)methyl]-9-oxo-1H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid. (59)

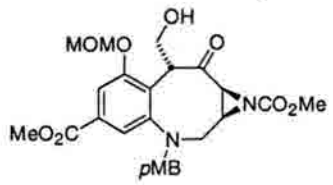
(1aS,8S,9aS)-Dimethyl ester 1a,2,3,8,9,9a-hexahydro-8-(hydroxymethyl)-7-(methoxymethoxy)-3-[(4-methoxyphenyl)methyl]-9-oxo-1H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid. (69)



Ketone **62** (28 mg, 57.8 μmol, 1.0 equiv.) was dissolved in 600 μL of dry DMF and cooled to -45 °C over 20 min. Next, a 0.86 M solution of LDA (67.2 μL, 57.8 μmol, 1.0 equiv.) was added dropwise and the solution turned a deep orange. After stirring 15 min., a 0.2 M solution of anhydrous formaldehyde in THF (491 μL, 98.2 μmol, 1.7 equiv.) was added dropwise. The color of the solution faded to a light yellow over 15 min. and the reaction was allowed to stir an additional 30 min. at - 45 °C before being quenched with a solution of sat. $NH_4Cl_{(aq)}$. The reaction was diluted with EtOAc and allowed to come to room temperature. The layers are separated and the aqueous layer was extracted 4 X EtOAc. The combined organic layers are washed 1 X brine, dried over Na_2SO_4, concentrated and dried overnight under vacuum. The product was purified by column chromatography (3:3:1 $CHCl_3$/benzene/acetone) to give 15 mg (54% yield) of alcohols **59** and **69** as a 1:1 mixture of diastereomers along with 10 mg of recovered **62**. The diastereomers were further separated by PTLC (2:2:1 $CHCl_3$/benzene/acetone) to furnish both **59** and **69** as clear colorless oils.

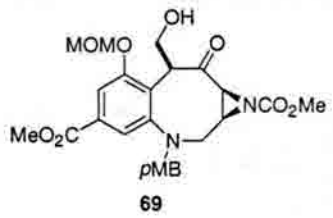
Compound **59**: $[\alpha]_D^{25}$ = - 19.3 (c=1.8, $CHCl_3$) ^1H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 3.02 (dd, J = 3.0 Hz, 6.6 Hz, 1 H), 3.17 (d, J = 6.6 Hz,1 H), 3.21 (m, 2 H), 3.48 (s, 3 H), 3.56 (s, 3 H), 3.80 (m, 2 H) 3.81 (s, 3 H), 3.86 (br d, J = 4.5 Hz, 2 H), 3.92 (s, 3 H), 4.14 (dd, J = 4.5 Hz, 7.5 Hz 1 H), 5.27 (1/2 ABq, J = 7.5, 1 H), 5.37 (1/2 ABq, J = 7.5, 1 H), 6.86 (d, J = 9.0, 2 H), 7.09 (d, J = 9.0, 2 H), 7.56 (d, J = 1.5 Hz, 1 H), 7.64 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 44.8, 47.7, 49.5, 51.3, 52.3, 53.6, 55.3, 56.2, 62.2, 62.8, 93.8, 112.9, 113.8, 113.8, 119.2, 127.7, 130.3, 131.3, 131.3, 133.2, 150.4, 153.3, 159.1, 161.4, 166.1, 201.1; IR (NaCl/neat) 3530, 2954, 1724, 1678, 1583 cm^{-1}; HRMS (FAB+) calcd for $C_{26}H_{31}N_2O_9$ (*m/z*) 515.2030 found (*m/z*) 515.2026.

Compound **69**: $[\alpha]_D^{25}$ = - 41.3 (c=1.4, $CHCl_3$) ^1H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 2.66 (m, 1 H), 3.08 (dd, J = 3.0 Hz, 12.0 Hz, 1 H), 3.11 (d, J = 6.0 Hz, 1 H), 3.16 (dd, J = 6.0 Hz, 12.0 Hz, 1 H), 3.52 (s, 3 H), 3.66 (s, 3 H), 3.79 (s, 3 H), 3.56 – 3.83 (m, 2H), 3.87 (m, 2 H), 3.95 (s, 3 H), 4.21 (dd, J = 3.0 Hz, 7.5 Hz, 1 H), 5.30 (1/2 ABq, J = 7.5, 1 H), 5.35 (1/2 ABq, J = 7.5, 1 H), 6.84 (d, J = 9.0 Hz, 2 H), 7.07 (d, J = 9.0 Hz, 2 H), 7.73 (d, J = 1.5 Hz, 1 Hz), 7.77 (d, J = 1.5 Hz, 1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 43.2, 46.0, 50.0, 52.5, 53.4, 53.9, 55.2, 56.7, 61.3, 62.9, 94.4, 113.0, 113.9, 113.9, 119.9, 127.8, 130.3, 130.3, 130.4, 131.7, 150.3, 155.1, 159.0, 162.4, 165.8, 199.8; IR (NaCl/neat) 3525, 2954, 1724, 1514 cm^{-1}; HRMS (FAB+) calcd for $C_{26}H_{31}N_2O_9$ (*m/z*) 515.2030 found (*m/z*) 515.2034.



59

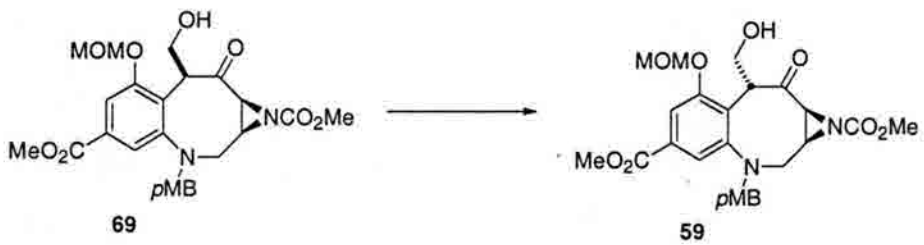
^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJIII293



69

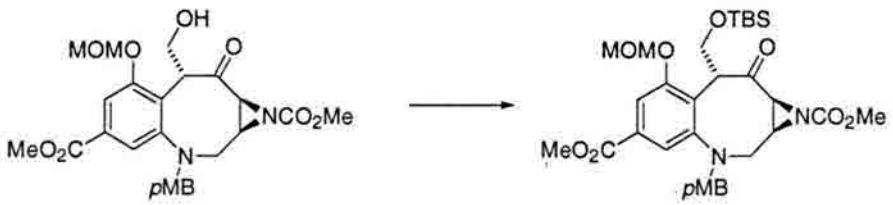
^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJIII261tdHnmr

(1aS,8R,9aS)-Dimethyl ester 1a,2,3,8,9,9a-hexahydro-8-(hydroxymethyl)-7-(methoxymethoxy)-3-[(4-methoxyphenyl)methyl]-9-oxo-1H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid. (59)



To a solution of alcohol **69** (9.0 mg, 17.5 μmol, 1.0 equiv.) in 4 mL of dry toluene was added 1,8-diazabicyclo[5.4.0]unde-7-ene (DBU) (16 μL, 107.0 μmol, 6.0 equiv.) and the reaction was stirred at ambient temperature. After 48 h, no further progress of the reaction was observed by TLC analysis (2:2:1 CH_3Cl/benzene/acetone) and sat. $NH_4Cl_{(aq)}$ was added to the mixture followed by dilution with CH_2Cl_2. The layers were separated and following dilution with H_2O the aqueous layer was extracted 3 X CH_2Cl_2. The combined organic layers were washed 1 X brine, dried over Na_2SO_4, filtered, and concentrated. Purification by PTLC (2:2:1 CH_3Cl/benzene/acetone) afforded 5.9 mg of alcohol **59** (65% yield) along with 2.3 mg of recovered starting material **69**.

(1aS,8R,9aS)-Dimethyl ester 8-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-3-[(4-methoxyphenyl)methyl]-8-oxo-1H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid.

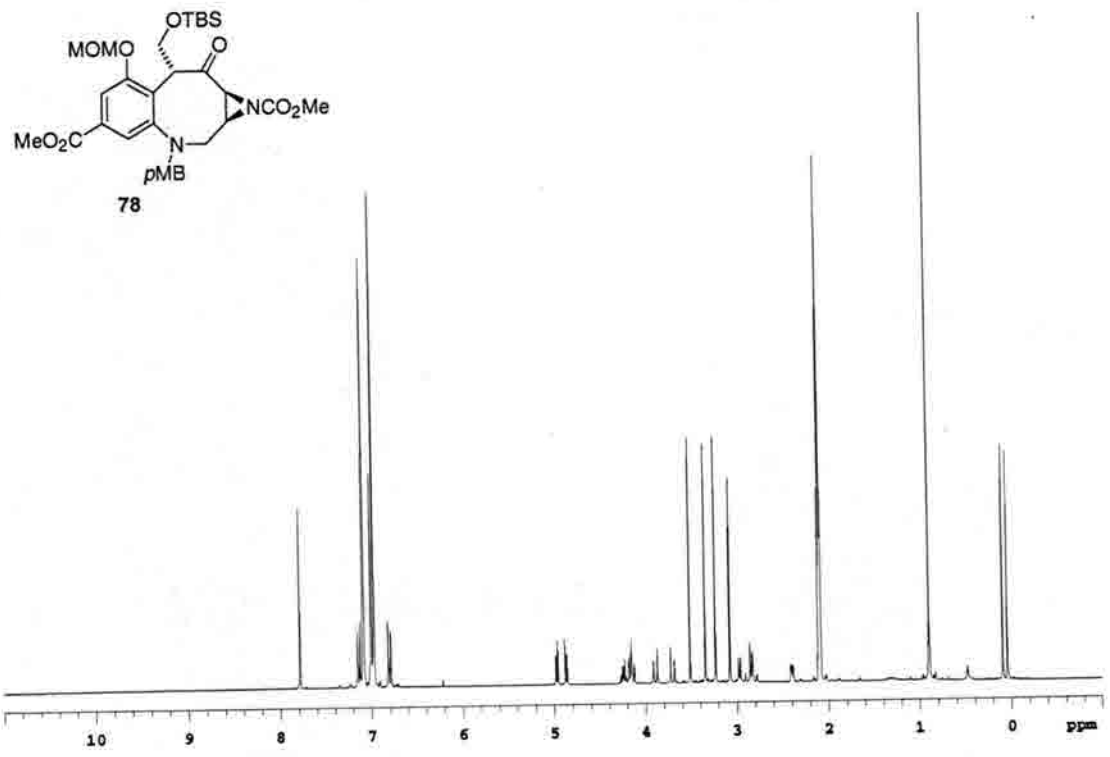


59 **78**

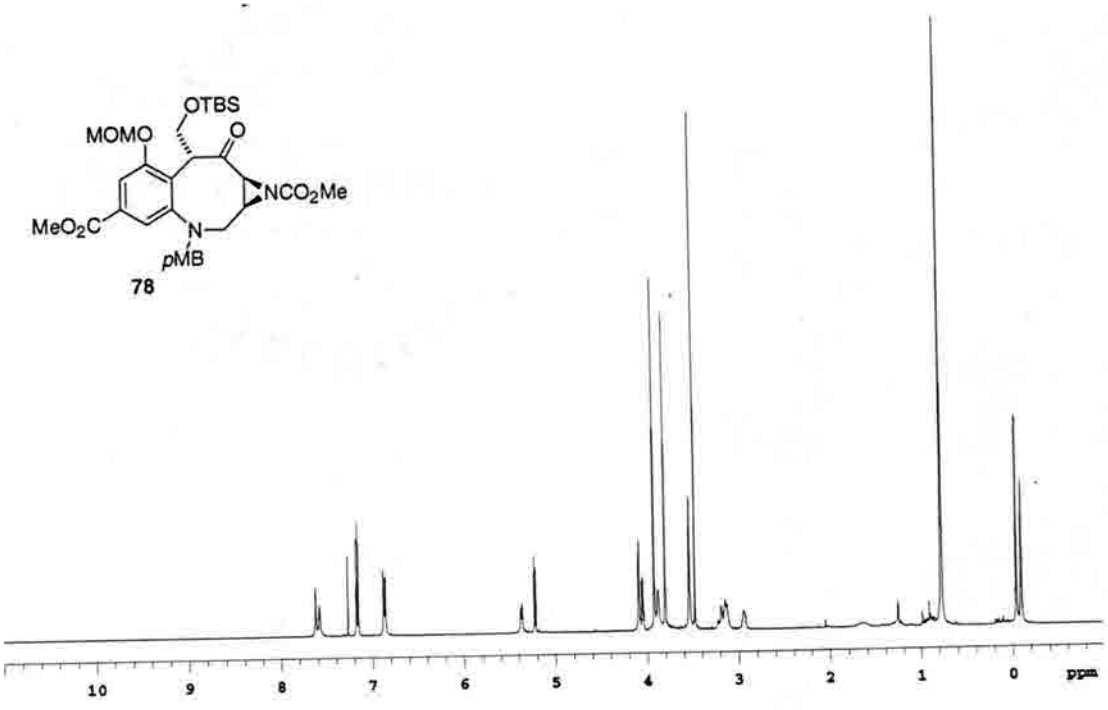
Alcohol **59** (34 mg, 66.1 μmol, 1.0 equiv.) was dissolved in 1 mL of CH_2Cl_2 and cooled to -78 °C over 20 min. Next, 213 μL of a premixed solution of 1:1 TBSOTf (43 μL, 187 μmol, 2.8 equiv.) and 2,6 lutidine (24 μL, 206 μmol, 3.1 equiv.) in CH_2Cl_2 was added dropwise. The solution was allowed to warm to 0 °C and stirred at this temperature for 1.5 h after which TLC analysis (2:1 CH_2Cl_2/Et_2O) showed complete consumption of the starting material. The reaction was quenched with $NaHCO_{3(aq)}$ followed by dilution with CH_2Cl_2 and H_2O and the layers were separated. The aqueous phase was extracted 3 X CH_2Cl_2 and the combined organic layers washed 1 X H_2O, 1 X brine, and dried over Na_2SO_4. Concentration and purification by PTLC (2:1 CH_2Cl_2/Et_2O) afforded 40 mg (96% yield) of product **78** as a clear colorless oil.

$[\alpha]_D^{25}$ = - 18.8 (c=1.7, $CHCl_3$) ^1H NMR (300 MHz, toluene-d6, 323 K) δ : 0.04 (s, 3 H), 0.09 (s, 3 H), 0.89 (s, 9 H), 2.38 (dd, J = 2.5 Hz, 6.5 Hz, 1 H), 2.77 (dd, J = 3.0 Hz, 14.0 Hz, 1 H), 2.85 (d, J = 14.0 Hz, 1 H), 2.95 (d, J = 6.5 Hz, 1 H), 3.07 (s, 3 H), 3.23 (s, 3 H), 3.34 (s, 3 H), 3.51 (s, 3 H), 3.68 (d, J = 13.0 Hz, 1 H), 3.87 (d, J = 13.0 Hz, 1 H), 4.11 (dd, J = 2.5 Hz, 8.5 Hz, 1 H), 4.15 (dd, J = 2.5 Hz, 7.5 Hz, 1 H), 4.22, (dd, J = 5.0 Hz, 8.0 Hz, 1 H), 4.86 (d, J = 6.5 Hz, 1 H), 4.96 (d, J = 6.5 Hz, 1 H), 6.79 (d, J = 8.5 Hz, 2 H),

7.12 (d, J = 8.5 Hz, 2 H), 7.78 (s, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: -5.2 (x2), 18.8, 26.2 (x3), 45.3, 47.2, 49.4, 51.5, 52.4, 53.7, 55.4, 56.3, 61.6, 61.8, 94.3, 113.0, 113.8, 113.8, 119.4, 128.7, 130.1, 131.9, 131.9, 134.7, 151.7, 153.9, 159.3, 162.0, 166.7, 197.8; IR (NaCl/neat) 2953, 1726, 1692, 1514 cm^{-1}; HRMS (FAB+) calcd for $C_{32}H_{45}N_2O_9Si_1$ (*m/z*) 629.2894 found (*m/z*) 629.2896.

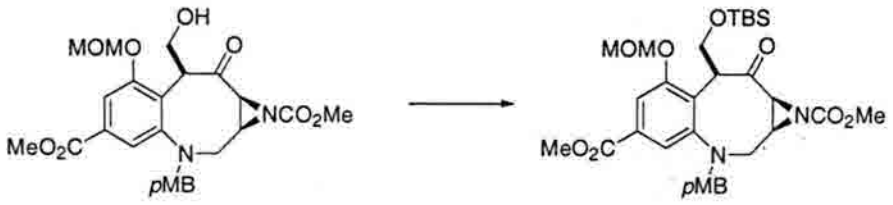


^1H NMR, 300 MHz, 323 K, toluene-d6, filename: TCJIIITBS50C-6-9-02



^{1}H NMR, 300 MHz, $CDCl_3$, filename: TCJIIITBS50C-6-9-02

(1aS,8S,9aS)-Dimethyl ester 8-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-3-[(4-methoxyphenyl)methyl]-8-oxo-1H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid.

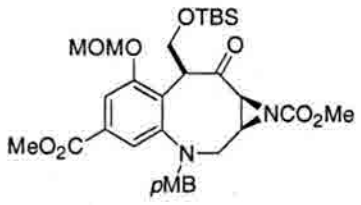


69 **79**

Alcohol **69** (10 mg, 19.2 μmol, 1.0 equiv.) was dissolved in 1 mL of CH_2Cl_2 and cooled to -78 °C over 20 min. Next, 62 μL of a premixed solution of 1:1 TBSOTf (12 μL, 54 μmol, 2.8 equiv.) and 2,6 lutidine (7.0 μL, 60 μmol, 3.1 equiv.) in CH_2Cl_2 was added dropwise. The solution was allowed to warm to 0 °C and stirred at this temperature for 1.5 h after which TLC analysis (2:1 CH_2Cl_2/Et_2O) showed complete consumption of the starting material. The reaction was quenched with $NaHCO_{3(aq)}$ followed by dilution with CH_2Cl_2 and H_2O and the layers were separated. The aqueous phase was extracted 3 X CH_2Cl_2 and the combined organic layers washed 1 X H_2O, 1 X brine, and dried over Na_2SO_4. Concentration and purification by PTLC (2:1 CH_2Cl_2/Et_2O) afforded 12 mg (96% yield) of product **79** as a clear colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ : -0.05 (s, 3 H), -0.11 (s, 3 H), 0.77 (s, 9 H), 2.54 (m, 1 H), 2.99 (dd, J = 3.6 Hz, 12.5 Hz, 1 H), 3.07 (dd, J = 6.0 Hz, 12.5 Hz, 1 H), 3.10 (d, J = 7.0 Hz, 1 H), 3.54 (s, 3 H), 3.63 (s, 3 H), 3.80 (s, 3 H), 3.81 (m, 1 H), 3.88 (d, J = 14.0 Hz, 1 H), 3.93 (d, J = 14.0 Hz, 1 H), 3.96 (s, 3 H), 4.26 (dd, J = 5.5 Hz, 5.5 Hz, 1 H), 4.33 (dd, J = 5.5 Hz, 9.5 Hz, 1 H), 5.25 (d, J = 6.5 Hz, 1 H), 5.34 (d, J = 6.5 Hz, 1 H), 6.85 (d, J = 8.5 Hz, 2 H), 7.11 (d, J = 8.5 Hz, 2 H), 7.72 (d, J = 1.2 Hz, 1 H), 7.80 (d, J = 1.2 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: -5.5, -5.3, 25.9 (x3), 42.5, 46.0, 50.4, 52.4, 53.2, 53.7,

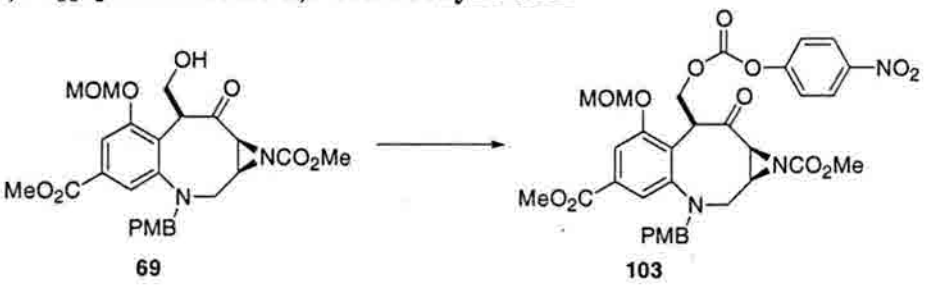
55.1, 56.6, 60.7, 62.8, 94.9, 98.3, 113.1, 113.9, 113.9, 119.8, 128.5, 130.2, 130.2, 131.4, 132.7, 150.9, 156.1, 159.0, 162.7, 166.2, 196.0; IR (NaCl/neat) 2953, 1724, 1513 cm^{-1}; HRMS (FAB+) calcd for $C_{32}H_{45}N_2O_9Si_1$ (*m/z*) 629.2894 found (*m/z*)



79

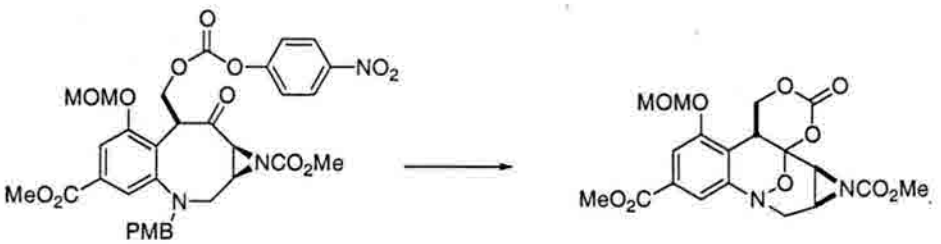
^1H NMR, 300 MHz, $CDCl_3$, filename: TCJIII56

(1aS,8S,9aS)-Dimethyl ester 8-[[4-nitro-phenoxycarbonyloxy]methyl]-1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-3-[(4-methoxyphenyl)methyl]-8-oxo-1H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid.



A stock solution of *p*-nitrophenyl chloroformate (21 mg, 104 μmol, 4.1 equiv.) and 2,6 lutidine (12.5 μL, 107 μmol, 4.2 equiv.) was stirred in 800 μL of CH_2Cl_2 for 1.5 h at ambient temperature to give a light red solution. Next, 230 μL of the mixture was added to a solution of alcohol **69** (13mg, 25.3 μmol, 1.0 equiv.) in 500 μL of CH_2Cl_2 at 0 °C and the reaction was stirred at this temperature for 18 h. Following the addition of sat. $NaHCO_{3\,(aq)}$, the layers were partitioned, and the aqueous layer was extracted 3 X CH_2Cl_2. The combined organic extracts were washed with 2 X sat. $NaHCO_{3\,(aq)}$, 2 X H_2O, 1 X brine, and dried over K_2CO_3. Purification by PTLC (1:1 CH_2Cl_2/Et_2O) affords 8 mg of carbonate **103** (47% yield) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) 2.68 (m, 1H); 3.14 (d, J = 7.2 Hz, 1H); 3.20 (m, 2H); 3.55 (s, 3H); 3.66 (s, 3H); 3.78 (s, 3H); 3.90 (d, J = 12.8 Hz, 1H); 3.98 (s, 3H); 4.07 (d, 12.8, 1H); 4.47 (d, J = 8.4, 10.5 Hz, 1H); 4.51 (m, 1H); 4.74 (dd, J = 5.0, 10.51, 1 H); 4.34 (s, 2H); 6.87 (d, J = 8.8 Hz, 2H); 7.12 (d, J = 8.8 Hz, 2H); 7.19 (d, J = 9.3 Hz, 2H); 7.78 (d, J = 1.3 Hz, 1H); 7.87 (d, J = 1.3, 1H); 8.22 (d, J = 9.3, 2H); IR (NaCl/neat) 2953, 1724, 1513 cm^{-1}; Mass Spec (ES^+) 680 (M+H).

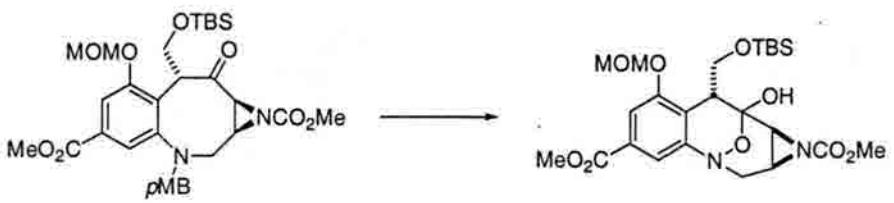


Nitrophenyl carbonate **103** was dissolved in 1.5 ml CH_2Cl_2 and cooled to 0° C. Solid K_2CO_3 followed by 200 μL of H_2O was added to the reaction with stirring. Freshly prepared DMDO solution (-20 °C) was added via a cold syringe (1.5 ml), and the reaction was allowed to come to ambient temperature over 5 hrs. Following addition of sat. $NaHCO_{3(aq)}$ and dilution with CH_2Cl_2, the reaction was separated. Extraction of the aqueous layer 2 X CH_2Cl_2 followed by 1 X brine wash and drying over Na_2SO_4 afforded a crude solid. Purification by PTLC (2:1 CH_2Cl_2/Et_2O) afforded ~2 mg of **4** as a white solid.

Mass Spec (ES^+) 437 (M+H, 25), 413 (M-24, 100)

(MALDI-TOF) 475 (M+K), 459 (M+Na), 437 (M+H), 413 (M-24)

(1aS,3R,8R,9R,9aS)-Dimethyl ester 8-[[[(1,1 dimethylethyl)dimethylsilyl]oxy]methyl]-1,1a,2,8,9,9a-hexahydro-9-hydroxy-7-(methoxymethoxy)-3,9-epoxy-3H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid.

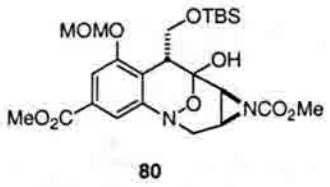


78 80

Compound **78** (25 mg, 39.7 μmol, 1.0 equiv.) was dissolved in 2 mL of CH_2Cl_2 and cooled to 0 °C over 20 min. Next, 2 mL of sat. $K_2CO_{3(aq)}$ was added and the mixture was stirred for 5 min after which 3.5 mL of a freshly prepared solution of DMDO at -20 °C (0.8-0.9 M, determined by titration with PPh_3 according to Murray *et al.*[46b]) was added via a cold syringe. The resulting biphasic mixture was protected from light and allowed to slowly come to room temperature over 6.5 h with vigorous stirring. The reaction was poured into a separatory funnel and the layers were separated. Dilution of the aqueous layer with H_2O was followed by extraction with 3 X CH_2Cl_2. The combined organic extracts were washed 3 X sodium phosphate buffer (pH=6), 1 X brine and dried over Na_2SO_4. Concentration afforded a crude yellow oil that was purified by PTLC (2:1 CH_2Cl_2/Et_2O) to give 9.0 mg of hydroxylamine hemi-ketal **80** (43% yield) as a light yellow oil along with recovery of 12 mg of unreacted ketone **78**.

$[\alpha]_D^{25}$ = + 8.0 (c=0.48, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ $CHCl_3$: 0.16 (s, 3 H), 0.21 (s, 3 H), 0.97 (s, 9 H), 2.76 (dd, J = 2.0 Hz, 6.0 Hz, 1 H), 3.21 (d, J = 6.0 Hz, 1 H), 3.48 (s, 3 H), 3.47 - 3.51 (m, 1 H), 3.58 (s, 3 H), 3.68 (d, J = 14.0 Hz, 1 H), 3.89 (s, 3 H), 3.93 (dd, J = 2.0 Hz, 14.0 Hz, 1 H), 4.00 (dd, J = 10.4, J = 10.4, 1 H), 4.11 (dd, J = 4.0 Hz, 10.4 Hz, 1 H), 5.24 (1/2 ABq, J = 6.0 Hz, 1 H), 5.34 (1/2 ABq, J = 6.0 Hz, 1 H), 7.12 (d,

J = 1.2 Hz, 1 H), 7.36 (d, J = 1.6 Hz, 1 H), 7.84 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: -5.63, -5.41, 25.7 (x3), 29.7, 32.7, 40.6, 44.1, 52.2, 52.4, 53.5, 56.2, 63.3, 93.4, 94.0, 108.8, 113.3, 119.3, 129.8, 149.3, 153.9, 161.8, 166.5; IR (NaCl/neat) 3339, 2926, 1726, 1584 cm^{-1}; HRMS (FAB+) calcd for $C_{24}H_{37}N_2O_9Si_1$ (*m/z*) 525.2268 found (*m/z*) 525.2261.



80

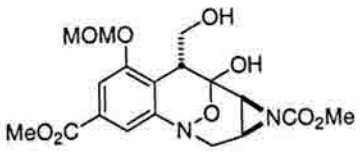
^{1}H NMR, 400 MHz, $CDCl_3$, filename: TCJIII295

(1aS,3R,8R,9R,9aS)-Dimethyl ester 1,1a,2,8,9,9a-hexahydro-9-hydroxy-8-(hydroxymethyl)-7-(methoxymethoxy)-3,9-epoxy-3H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid.

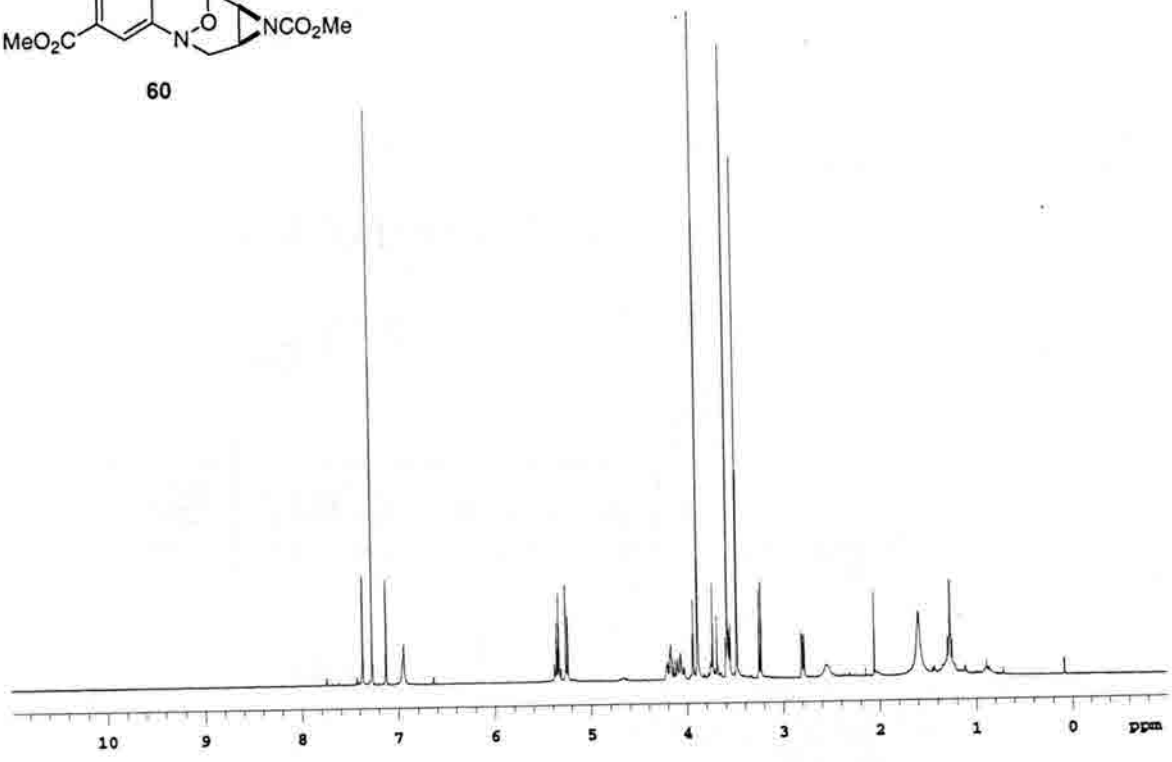
Compound **80** (7 mg, 13.3 μmol, 1.0 equiv.) was dissolved in 500 μL of THF and cooled to 0 °C over 20 min. To the resulting solution was added 1 M TBAF in THF (23 μL, 23.0 μmol. 1.7 equiv.) and the solution was stirred for 1 h after which TLC analysis (20:1 CH_2Cl_2/MeOH) revealed complete consumption of the starting material. The reaction was quenched with sat. $NH_4Cl_{(aq)}$, stirred vigorously for 10 min., and diluted with EtOAc and H_2O. The layers were partitioned and the aqueous layer was extracted with 3 X CH_2Cl_2. The combined organic layers were washed 1 X brine and dried over Na_2SO_4. Purification by PTLC (20:1 CH_2Cl_2/MeOH) afforded 5 mg (92% yield) of alcohol **60** as a colorless oil.

$[\alpha]_D^{25}$ = + 10.5 (c = 0.40, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 2.55 (s, br, 1 H), 2.78 (dd, J = 2.1 Hz, 6.9 Hz, 1 H), 3.22 (d, J = 6.9, Hz, 1 H), 3.48 (s, 3 H), 3.55 (m, 1 H), 3.58 (s, 3 H), 3.68 (d, J = 15.0 Hz, 1 H), 3.89 (s, 3 H), 3.94 (d, J = 2.1 Hz, 15.0 Hz), 4.03 (dd, J = 12.0 Hz, 12.0 Hz, 1 H), 4.17, (dd, J = 3.0 Hz, 12.0 Hz, 1 H), 5.25 (1/2 ABq, J = 6.0, 1 H), 5.35 (1/2 ABq, J = 6.0, 1 H), 6.95 (s, br, 1 H), 7.13 (d, J = 1.5 Hz, 1 H), 7.37 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.7, 32.7, 40.8, 43.9, 52.2, 53.6, 56.2, 62.8, 93.4, 94.0, 109.0, 113.2, 119.4, 129.9, 149.1, 153.9, 161.7, 166.4; IR

(NaCl/neat) 3331, 2924, 1717, 1684 cm^{-1}; HRMS (FAB+) calcd for $C_{18}H_{22}N_2O_9$ (*m/z*) 410.1325 found (*m/z*) 410.1314.

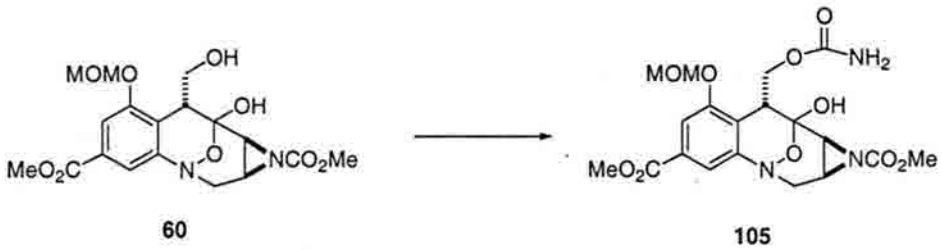


60



^1H NMR, 300 MHz, $CDCl_3$, filename: TCJIII303II

(1aS,3R,8R,9R,9aS)-Dimethyl ester 8-[[(aminocarbonyl)oxy]methyl]-1,1a,2,8,9,9a-hexahydro-9-hydroxy-7-(methoxymethoxy)-3,9-epoxy-3H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid.

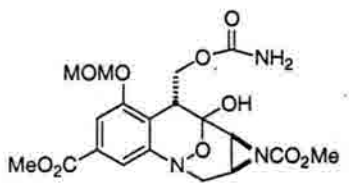


60 **105**

To a solution of alcohol **60** (6.5 mg, 15.8 μmol, 1.0 equiv.) in 600 μL CH_2Cl_2 at 0 °C was added trichloroacetyl isocyanate (5 μL, 42.0 μmol, 2.7 equiv.). The reaction was stirred 5 min and quenched with the addition of 5 drops of MeOH. The reaction was concentrated *in vacou* and the crude intermediate was dissolved in 3 mL of MeOH. Silica gel was added to give a slurry and the mixture was stirred for 8 h at ambient temperature. The reaction was filtered with 10:1 CH_2Cl_2/MeOH and concentrated. Purification of the crude mixture by PTLC (20:1 CH_2Cl_2/MeOH) furnished 6.2 mg (86% yield) of carbamate **105** as a white amorphous solid.

$[\alpha]_D^{25}$ = -29.7 (c=0.70, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 2.77 (dd, J = 2.0 Hz, 6.5 Hz, 1 H), 3.19 (d, J = 6.5, Hz, 1 H), 3.33 (d, J = 3.5 Hz, 1 H), 3.48 (s, 3 H), 3.57 (s, 3 H), 3.71 (d, J = 6.5 Hz, 1 H), 3.86 (m, 1 H), 3.90 (s, 3 H), 4.59 (dd, J = 4.0 Hz, 12.5 Hz, 1 H), 4.78 (s, br, 2 H), 5.09 (d, J = 12.5 Hz, 1 H), 5.29 (d, J = 6.5 Hz, 1 H), 5.35 (d, J = 6.5 Hz, 1 H), 6.92 (s, br, 1 H), 7.15 (d, J = 1.5 Hz, 1 H), 7.41 (d, J = 1.5 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.4, 41.5, 43.2, 52.1, 53.6, 56.2, 61.3, 92.1, 93.9, 109.0, 113.4,

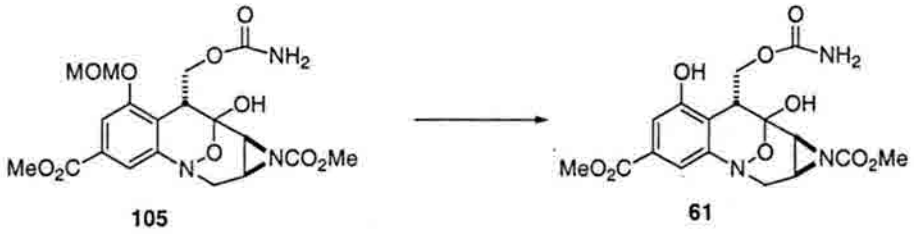
120.3, 129.8, 149.7, 153.8, 158,6, 161.7, 166.5; IR (NaCl/neat) 3359, 2954, 1722, 1584 cm^{-1}; HRMS (FAB+) calcd for $C_{18}H_{23}N_3O_9$ (*m/z*) 453.1383 found (*m/z*) 453.1314.



105

^1H NMR, 300 MHz, $CDCl_3$, filename: TCJIII320IV

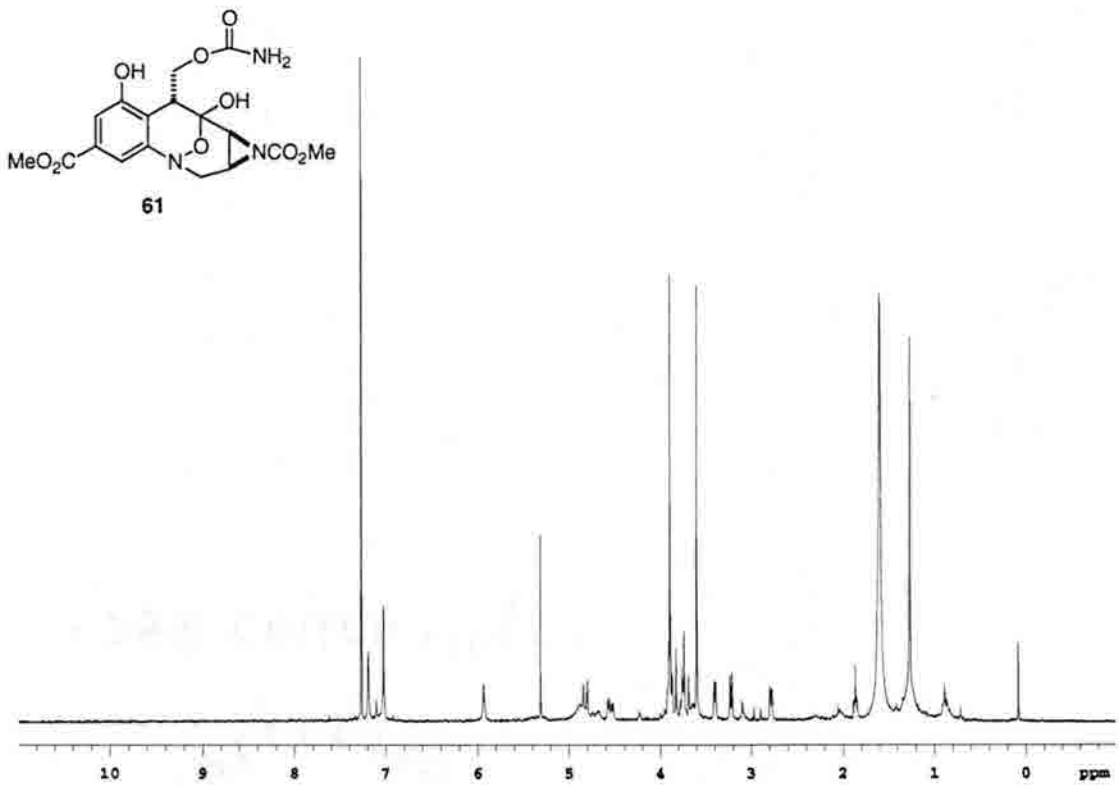
(1aS,3R,8R,9R,9aS)- dimethyl ester 8-[[(aminocarbonyl)oxy]methyl]-1,1a,2,8,9,9a-hexahydro-7,9-dihydroxy-3,9-epoxy-3H-azirino[2,3-c][1]benzazocine-1,5-dicarboxylic acid.



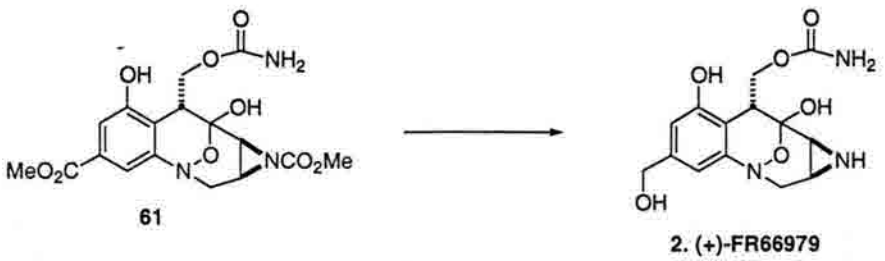
Compound **105** (8.5 mg, 18.8 μmol, 1.0 equiv.) was dissolved in 600 μL of CH_2Cl_2 and cooled to -45 °C over 20 min. TMSBr (15 μL, 114 μmol, 6.0 equiv.) was added dropwise and the solution was stirred 1.5 h while maintaining a reaction temperature between -45 and -40 °C. An additional portion of TMSBr (15 μL, 114 μmol, 6.0 equiv.) was added and the reaction was continued for 2 h. The reaction was quenched with $NaHCO_{3(aq)}$ and allowed to come to ambient temperature. The mixture was diluted with CH_2Cl_2 and the layers were separated. Sat. $NH_4Cl_{(aq)}$ was added to the aqueous portion until a pH < 9 was obtained and the resulting solution was extracted 1 X CH_2Cl_2 and 3 X THF. The combined organic layers were washed 1 X brine, dried over Na_2SO_4, and concentrated. Purification by PTLC (10:1 CH_2Cl_2/MeOH) afforded 4.6 mg (60% yield) of phenol **61** as a white solid.

$[\alpha]_D^{25}$ = +10.5 (c=0.42, $CHCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ $CHCl_3$: 2.77 (dd, J = 2.0 Hz, 6.5 Hz, 1 H), 3.20 (d, J = 6.5 Hz, 1 H), 3.39 (d, J = 5.5 Hz, 1 H), 3.60 (s, 3 H), 3.69 (d, J = 14.5 Hz, 1 H), 3.87 (m, 1 H), 3.89 (s, 3 H), 4.50 (dd, J = 5.5 Hz, 13.0 Hz, 1 H), 4.79 (d, J = 13.0 Hz, 1 H), 4.7 – 4.9 (s, br, 2 H), 5.79 (s, br, 1 H), 6.74 (s, br, 1 H), 7.03 (d, J = 2.0

Hz, 1 H), 7.19 (d, J = 2.0 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.6, 41.0, 43.2, 52.2, 53.9, 62.2, 92.4, 111.4, 111.6, 117.5, 129.6, 149.1, 154.1, 158.8, 162.0, 166.9; IR (NaCl/neat) 3363, 2917, 2849, 1701, 1539 cm^{-1}; HRMS (FAB+) calcd for $C_{16}H_{23}N_3O_8$ (*m/z*) 410.1199 found (*m/z*) 410.1199.

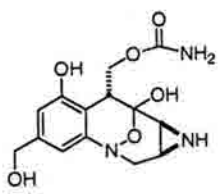


^1H NMR, 300 MHz, $CDCl_3$, filename: TCJIII345



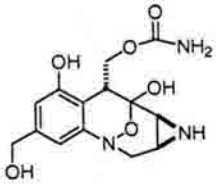
To a solution of compound **61** (4.0 mg, 9.8 μmol, 1.0 equiv.) in 400 μL THF were added a 2 M $LiBH_4$ solution in THF (30 μL, 60 μmol, 6.1 equiv.) and dry MeOH (2.4 μL, 59 μmol, 6.0 equiv.). The reaction was stirred at ambient temperature and following 1 h an identical aliquot of $LiBH_4$ solution and MeOH were added. The additions were continued with two more aliquots every hour followed by two additional portions every three hours for a total of nine hours. Following the additions, the reaction was continued for nine more hours. The reaction was quenched with sat. $NH_4Cl_{(aq)}$ and stirred for 30 min. The THF was concentrated off under reduced pressure and the remaining aqueous mixture was passed through a C_8 Sep Pak (Waters) pre-washed with 1 X MeOH, 3 X H_2O. The Sep Pak was washed 3 X H_2O and the product was eluded off with MeOH. Next, 10% Pd/C (~15 mg) was added to the MeOH filtrate and the mixture was stirred for 12 hrs. Filtration through a celite pad with hot MeOH and concentration gave crude FR66979 which was purified by PTLC (9:4 CH_2Cl_2/MeOH, eluted off plate with 2.5:1 CH_2Cl_2/MeOH) to furnish 2.7 mg of (+)-FR66979 as a white solid.

$[\alpha]_D^{25}$ = + 9.4 (c = 0.16, H_2O) ^1H NMR (400 MHz, CD_3OD) δ CD_3OH: 2.45 – 2.80 (br, 2 H), 3.66 (br, 2 H), 3.69 (m, 1 H), 4.47 (s, 2 H), 4.68 (d, J = 11.2 Hz, 1 H), 5.06 (dd, J = 6.4 Hz, 11.2 Hz, 1 H), 6.34 (s, 1 H), 6.51 (s, 1 H), 8.57 (br, 1 H); IR (NaCl/neat) 3302, 2924, 1709, 1587 cm^{-1}; MS (ES) (% Total ion count) 346 (M+Na, 38), 324 (M+H, 40).



2. (+)-FR66979

Full synthetic



2. (+)-FR66979

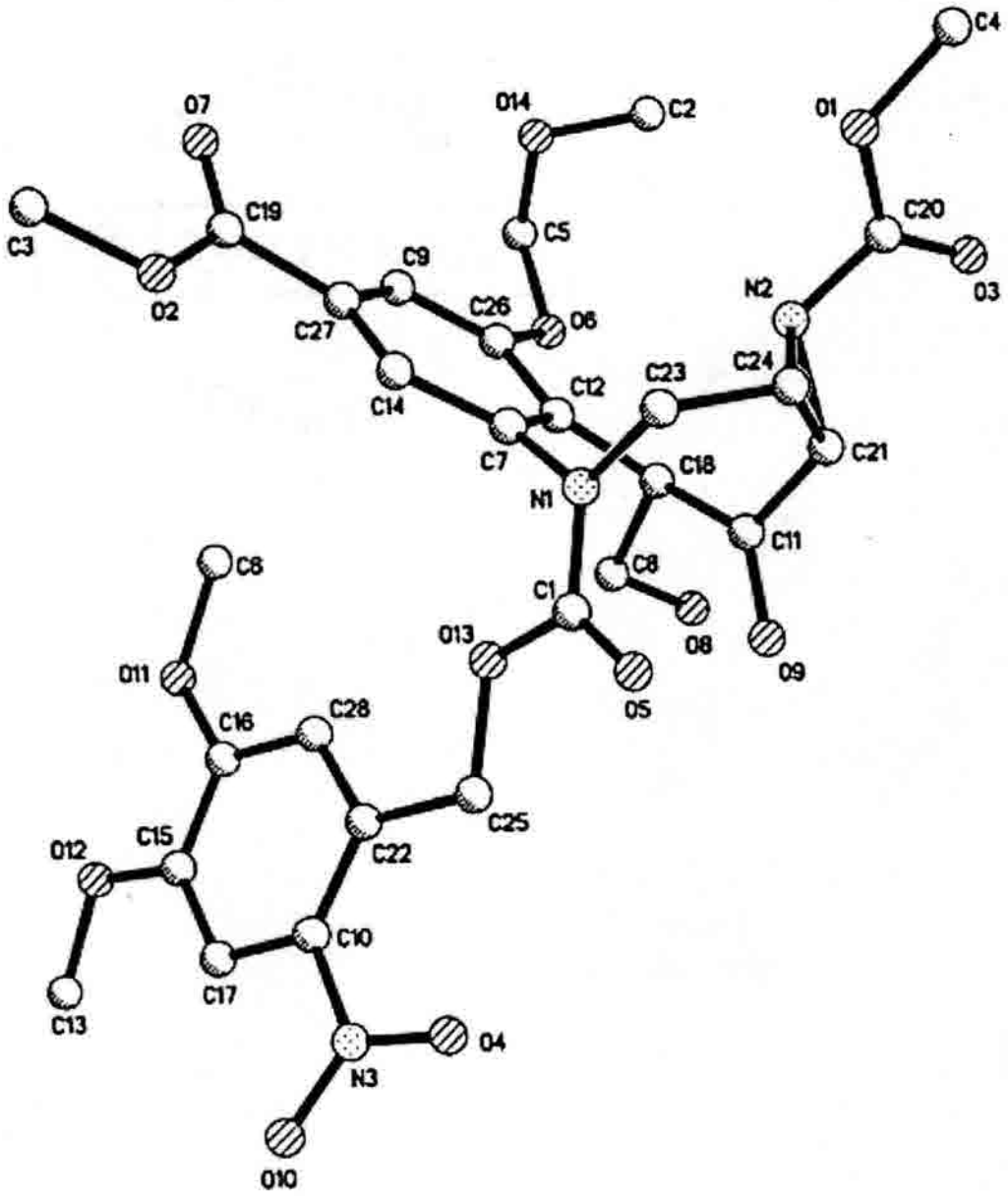
semi-synthetic

2. (+)-FR66979 1. (+)-FR900482

Oxalyl chloride (10.4 uL, 119.2 umol, 4.5 eq.) was added to 417 uL of THF in a 5 mL flask cooled to -78 °C. Following the addition, 122 uL of a stock solution consisting of 190 uL of DMSO in 1.2 mL of THF was added dropwise and the resulting solution was warmed to -35 °C for approximately 3 min. and then cooled back to -78 °C. A solution of FR66979 (**2**) (8.5 mg, 26.3 umol, 1.0 eq.) in 418 uL of a 4:1 solution of DMSO/THF was added dropwise and the solution was allowed to warm to -35 °C for 15 min. Next, Et_3N (48 uL, 344.4 mmol, 13.1 eq.) was added and the reaction was stirred at 0 °C for 20 min. before being quenched by the addition of brine. Following dilution with THF, the reaction was stirred vigorously and separated. The aqueous layer was extracted 3 x THF and the combined organic layers were washed 1 x brine, dried over Na_2SO_4, and concentrated. After drying under vacuum 24 hrs., the crude material was purified by PTLC using 9:4 CH_2Cl_2/MeOH to give 2.5 mg of FR900482 (**1**) in 30% yield along with 60% recovered FR66979 (**2**).

Appendix 1

Crystal Structure of Compound **45**



ORTEP of compound 45

Table 1. Crystal data and structure refinement for 1.

Identification code	rwccd12(TCJ-II-29)
Empirical formula	$C_{28}H_{31}N_3O_{14}$
Formula weight	633.56
Temperature	170(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 10.824(2) Å alpha = 90° b = 15.469(2) Å beta = 90° c = 18.085(3) Å gamma = 90°
Volume, Z	3028.1(8) Å^3, 4
Density (calculated)	1.390 Mg/m^3
Absorption coefficient	0.113 mm^{-1}
F(000)	1328
Crystal size	0.20 x 0.20 x 0.36 mm, clear colorless cubes
θ range for data collection	1.73 to 28.31°
Limiting indices	$-14 \le h \le 14$, $-20 \le k \le 12$, $-23 \le l \le 23$
Reflections collected	19745
Independent reflections	7271 (R_{int} = 0.1198)
Absorption correction	SADABS
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	7271 / 0 / 407
Goodness-of-fit on F^2	1.018
Final R indices [I>2σ(I)]	R1 = 0.1120, wR2 = 0.2059
R indices (all data)	R1 = 0.2089, wR2 = 0.2597
Extinction coefficient	0.0015(11)
Largest diff. peak and hole	0.313 and -0.248 eÅ^{-3}

Comment:highly mosaic quality of XTLS resulted in higher R values.

Table 2. Atomic coordinates [x 10^4] and equivalent isotropic displacement parameters [$Å^2$ x 10^3] for 1. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U(eq)
C(1)	7987(5)	8722(4)	8092(3)	24(1)
C(2)	8576(6)	8341(4)	7489(4)	31(2)
C(3)	8264(7)	8620(4)	6782(4)	33(2)
C(4)	7387(6)	9264(4)	6674(4)	32(2)
C(5)	6846(6)	9649(4)	7277(4)	30(2)
C(6)	7152(6)	9405(4)	8004(4)	25(2)
C(7)	6618(6)	9934(4)	8637(3)	26(1)
C(8)	6620(6)	9500(4)	9400(3)	25(2)
C(9)	5783(6)	8740(4)	9531(3)	27(2)
C(10)	6167(6)	7834(4)	9340(3)	25(2)
C(11)	7299(6)	7605(4)	9013(4)	28(2)
C(12)	8794(8)	8187(5)	6115(4)	39(2)
C(13)	7286(7)	10799(4)	8666(4)	36(2)
C(14)	10410(10)	7363(7)	5594(5)	74(3)
C(15)	5310(8)	10337(6)	6531(4)	49(2)
C(16)	3761(9)	9339(7)	6828(5)	67(3)
C(17)	3994(7)	8093(5)	8945(4)	33(2)
C(18)	2511(7)	7091(6)	8547(5)	53(2)
C(19)	9043(6)	8613(4)	9296(3)	22(1)
C(20)	10721(6)	9563(5)	9478(4)	30(2)
C(21)	11496(6)	10159(4)	9011(3)	26(2)
C(22)	11118(6)	10380(4)	8298(4)	28(2)
C(23)	11782(6)	10933(4)	7847(4)	29(2)
C(24)	12906(6)	11287(4)	8103(4)	27(2)
C(25)	13317(6)	11066(4)	8796(4)	31(2)
C(26)	12622(6)	10503(4)	9236(3)	27(2)
C(27)	10410(6)	10735(6)	6839(4)	44(2)
C(28)	14469(6)	12352(5)	7931(4)	38(2)
N(1)	8150(5)	8353(4)	8814(3)	27(1)
N(2)	5255(5)	8319(4)	8889(3)	28(1)
N(3)	13166(5)	10271(4)	9954(3)	33(1)
O(1)	8283(6)	8192(4)	5525(3)	54(2)
O(2)	9854(5)	7788(4)	6242(3)	57(2)
O(3)	5969(5)	10295(3)	7214(2)	42(1)
O(4)	4752(5)	9563(4)	6349(3)	50(2)
O(5)	6598(5)	11450(3)	9032(3)	41(1)
O(6)	7172(4)	9819(3)	9915(2)	34(1)
O(7)	3227(4)	8499(3)	9270(3)	45(1)
O(8)	3780(4)	7387(3)	8537(3)	40(1)
O(9)	9211(4)	8306(3)	9901(3)	34(1)
O(10)	9732(4)	9255(3)	9001(2)	30(1)
O(11)	11461(4)	11148(3)	7148(2)	36(1)
O(12)	13476(4)	11845(3)	7636(2)	34(1)
O(13)	14258(4)	10446(4)	10065(3)	43(1)
O(14)	12515(5)	9903(4)	10407(3)	45(1)

Table 3. Bond lengths [Å] and angles [°] for 1.

C(1)-C(6)	1.398(9)	C(1)-C(2)	1.394(9)
C(1)-N(1)	1.437(8)	C(2)-C(3)	1.390(9)
C(3)-C(4)	1.390(10)	C(3)-C(12)	1.494(10)
C(4)-C(5)	1.374(9)	C(5)-O(3)	1.383(8)
C(5)-C(6)	1.408(9)	C(6)-C(7)	1.521(9)
C(7)-C(13)	1.522(9)	C(7)-C(8)	1.535(9)
C(8)-O(6)	1.212(7)	C(8)-C(9)	1.503(9)
C(9)-N(2)	1.449(8)	C(9)-C(10)	1.502(9)
C(10)-N(2)	1.485(8)	C(10)-C(11)	1.501(9)
C(11)-N(1)	1.459(8)	C(12)-O(1)	1.202(9)
C(12)-O(2)	1.322(9)	C(13)-O(5)	1.416(8)
C(14)-O(2)	1.473(10)	C(15)-O(4)	1.381(10)
C(15)-O(3)	1.427(9)	C(16)-O(4)	1.423(10)
C(17)-O(7)	1.196(8)	C(17)-O(8)	1.338(9)
C(17)-N(2)	1.412(9)	C(18)-O(8)	1.448(8)
C(19)-O(9)	1.206(7)	C(19)-O(10)	1.351(7)
C(19)-N(1)	1.362(8)	C(20)-O(10)	1.456(7)
C(20)-C(21)	1.505(9)	C(21)-C(26)	1.391(9)
C(21)-C(22)	1.395(9)	C(22)-C(23)	1.383(9)
C(23)-O(11)	1.351(7)	C(23)-C(24)	1.413(9)
C(24)-O(12)	1.356(8)	C(24)-C(25)	1.374(9)
C(25)-C(26)	1.399(9)	C(26)-N(3)	1.469(8)
C(27)-O(11)	1.418(8)	C(28)-O(12)	1.434(8)
N(3)-O(14)	1.222(7)	N(3)-O(13)	1.229(7)

C(6)-C(1)-C(2)	121.7(6)	C(6)-C(1)-N(1)	118.8(5)
C(2)-C(1)-N(1)	119.2(6)	C(3)-C(2)-C(1)	118.5(6)
C(2)-C(3)-C(4)	121.2(6)	C(2)-C(3)-C(12)	120.6(7)
C(4)-C(3)-C(12)	118.0(6)	C(5)-C(4)-C(3)	119.4(6)
C(4)-C(5)-O(3)	122.7(6)	C(4)-C(5)-C(6)	121.7(6)
O(3)-C(5)-C(6)	115.7(6)	C(1)-C(6)-C(5)	117.4(6)
C(1)-C(6)-C(7)	124.5(6)	C(5)-C(6)-C(7)	117.9(6)
C(6)-C(7)-C(13)	108.6(5)	C(6)-C(7)-C(8)	116.1(5)
C(13)-C(7)-C(8)	110.7(5)	O(6)-C(8)-C(9)	119.6(6)
O(6)-C(8)-C(7)	120.9(6)	C(9)-C(8)-C(7)	118.9(5)
N(2)-C(9)-C(8)	117.6(5)	N(2)-C(9)-C(10)	60.4(4)
C(8)-C(9)-C(10)	121.8(5)	N(2)-C(10)-C(9)	58.0(4)
N(2)-C(10)-C(11)	119.5(5)	C(9)-C(10)-C(11)	123.8(5)
N(1)-C(11)-C(10)	113.9(5)	O(1)-C(12)-O(2)	123.7(7)
O(1)-C(12)-C(3)	122.5(7)	O(2)-C(12)-C(3)	113.8(6)
O(5)-C(13)-C(7)	113.1(6)	O(4)-C(15)-O(3)	112.6(6)
O(7)-C(17)-O(8)	125.5(7)	O(7)-C(17)-N(2)	125.1(7)
O(8)-C(17)-N(2)	109.3(6)	O(9)-C(19)-O(10)	124.4(6)
O(9)-C(19)-N(1)	124.7(6)	O(10)-C(19)-N(1)	110.8(5)
O(10)-C(20)-C(21)	106.1(5)	C(26)-C(21)-C(22)	115.7(6)
C(26)-C(21)-C(20)	123.9(6)	C(22)-C(21)-C(20)	120.3(6)
C(23)-C(22)-C(21)	123.0(6)	O(11)-C(23)-C(22)	124.8(6)
O(11)-C(23)-C(24)	115.6(6)	C(22)-C(23)-C(24)	119.6(6)
O(12)-C(24)-C(25)	125.5(6)	O(12)-C(24)-C(23)	115.8(6)
C(25)-C(24)-C(23)	118.7(6)	C(24)-C(25)-C(26)	120.0(6)
C(21)-C(26)-C(25)	122.9(6)	C(21)-C(26)-N(3)	121.1(6)
C(25)-C(26)-N(3)	116.0(6)	C(19)-N(1)-C(1)	123.4(5)
C(19)-N(1)-C(11)	118.2(5)	C(1)-N(1)-C(11)	118.0(5)
C(17)-N(2)-C(9)	115.9(5)	C(17)-N(2)-C(10)	118.6(5)
C(9)-N(2)-C(10)	61.6(4)	O(14)-N(3)-O(13)	123.1(6)
O(14)-N(3)-C(26)	118.4(5)	O(13)-N(3)-C(26)	118.5(6)
C(12)-O(2)-C(14)	115.2(7)	C(5)-O(3)-C(15)	116.6(5)
C(15)-O(4)-C(16)	113.3(7)	C(17)-O(8)-C(18)	114.5(6)
C(19)-O(10)-C(20)	114.3(5)	C(23)-O(11)-C(27)	117.6(5)
C(24)-O(12)-C(28)	117.2(5)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters [$Å^2 \times 10^3$] for 1.

The anisotropic displacement factor exponent takes the form:

$-2\pi^2 [(ha^*)^2 U_{11} + \ldots + 2hka^*b^* U_{12}]$

	U11	U22	U33	U23	U13	U12
N(1)	21(2)	26(2)	29(2)	3(2)	-2(2)	-4(2)
O(1)	24(2)	24(2)	34(2)	6(1)	-2(1)	0(1)
O(2)	37(2)	25(2)	33(2)	6(1)	4(1)	-3(1)
O(3)	22(2)	35(2)	45(2)	-2(2)	-1(1)	-4(1)
O(4)	34(2)	45(2)	31(2)	-3(2)	6(1)	2(2)
C(1)	34(3)	35(3)	29(2)	6(2)	-10(2)	-3(2)
C(2)	23(2)	27(2)	34(2)	-3(2)	-4(2)	-1(2)
C(3)	24(2)	22(2)	31(2)	-1(2)	-2(2)	-1(2)
C(4)	25(2)	21(2)	21(2)	-1(2)	-2(2)	1(2)
C(5)	35(3)	24(2)	22(2)	-2(2)	4(2)	2(2)
C(6)	28(2)	23(2)	35(2)	6(2)	2(2)	-1(2)
C(7)	24(2)	20(2)	22(2)	1(2)	1(2)	-2(2)
C(8)	26(2)	23(2)	21(2)	-3(2)	-2(2)	-4(2)
C(9)	33(2)	25(2)	31(2)	-2(2)	4(2)	-1(2)
C(10)	45(3)	19(2)	46(3)	3(2)	5(2)	-3(2)
C(11)	49(3)	31(3)	46(3)	2(2)	8(3)	-10(2)
C(12)	31(3)	39(3)	41(3)	-4(2)	12(2)	-4(2)
C(13)	33(3)	29(2)	36(2)	0(2)	1(2)	0(2)
C(14)	22(2)	34(2)	23(2)	2(2)	-6(2)	5(2)
C(15)	37(3)	42(3)	26(2)	1(2)	-4(2)	-12(2)
C(16)	53(3)	32(3)	32(3)	3(2)	-9(2)	-12(2)
C(17)	42(3)	37(3)	34(3)	-7(2)	-6(2)	0(2)
C(18)	35(3)	46(3)	27(2)	-1(2)	-4(2)	1(2)
C(19)	30(2)	31(2)	28(2)	5(2)	-3(2)	-3(2)
C(20)	25(2)	27(2)	29(2)	1(2)	-3(2)	7(2)
C(21)	30(3)	66(4)	39(3)	6(3)	14(2)	-8(3)
C(22)	77(4)	70(4)	38(3)	0(3)	21(3)	12(3)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for 1.

	x	y	z	U(eq)
H(2A)	9176(6)	7901(4)	7559(4)	37
H(4A)	7164(6)	9436(4)	6188(4)	38
H(7A)	5736(6)	10058(4)	8509(3)	31
H(9A)	5219(6)	8793(4)	9967(3)	32
H(10A)	5801(6)	7376(4)	9664(3)	30
H(11A)	7862(6)	7247(4)	9372(4)	33
H(11B)	7263(6)	7249(4)	8565(4)	33
H(13A)	8085(7)	10721(4)	8924(4)	43
H(13B)	7465(7)	10991(4)	8155(4)	43
H(14A)	11194(10)	7094(7)	5737(5)	111
H(14B)	10561(10)	7793(7)	5206(5)	111
H(14C)	9846(10)	6919(7)	5406(5)	111
H(15A)	4670(8)	10793(6)	6566(4)	59
H(15B)	5889(8)	10503(6)	6132(4)	59
H(16A)	3404(9)	8786(7)	6671(5)	101
H(16B)	3125(9)	9789(7)	6808(5)	101
H(16C)	4070(9)	9286(7)	7336(5)	101
H(18A)	2431(7)	6577(6)	8234(5)	79
H(18B)	1971(7)	7549(6)	8358(5)	79
H(18C)	2272(7)	6948(6)	9055(5)	79
H(20A)	11224(6)	9073(5)	9660(4)	36
H(20B)	10378(6)	9877(5)	9908(4)	36
H(22A)	10370(6)	10141(4)	8115(4)	33
H(25A)	14072(6)	11296(4)	8978(4)	37
H(27A)	10282(6)	10944(6)	6333(4)	66
H(27B)	9681(6)	10869(6)	7139(4)	66
H(27C)	10542(6)	10109(6)	6832(4)	66
H(28A)	14806(6)	12725(5)	7543(4)	57
H(28B)	15119(6)	11967(5)	8115(4)	57
H(28C)	14160(6)	12710(5)	8339(4)	57
H(5A)	6453(5)	11294(3)	9468(3)	62

Appendix 2

Publications

1. *Synthesis of the First Photo-Triggered Pro-mitosene Based on FR-900482,* Williams, R. M.; Rollins, S. B.; Judd, T. C., *Tetrahedron* (2000), **56**, 521.

2. *Synthesis and DNA Cross-Linking of a Phototriggered FR900482 Mitosene Progenitor,* Judd, T. C.; Williams, R. M., *Org. Lett.* (2002), **4**, 3711.

3. *Concise Enantioselective Synthesis of (+)-FR66979 and (+)-FR900482: Dimethyldioxirane-Mediated Construction of the Hydroxylamine Hemiketal*, Judd, T. C.; Williams, R. M., *Angew. Chem. Int. Ed.* (2002), **41**, 4683.

Pergamon

Tetrahedron 56 (2000) 521–532

TETRAHEDRON

Synthesis of the First Photo-Triggered Pro-mitosene Based on FR-900482

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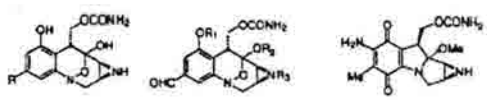
Abstract—A stereocontrolled synthesis of an eight-membered ring precursor to a photo-triggered mitosene is described.

Introduction

FR-900482 (**1**), and FR-66979 (**2**) are anti-tumor antibiotics that were obtained from the fermentation harvest of *Streptomyces sandaensis* No. 6897 at the Fujisawa Pharmaceutical Co. in Japan.[1–3] The triacetate derivative FK-973 (**3**)[4] and the recently disclosed drug candidate FK-317 (**4**),[5] both semi-synthetically derived from FR-900482, have shown highly promising anti-tumor activity in human clinical trials. FK-317, now in phase II clinical trials holds considerable promise to replace the structurally related and widely used anti-tumor drug mitomycin C (MMC, **6**).[2] In initial phase I clinical trials, patients treated with FK-973 exhibited vascular leak syndrome and this substance was subsequently withdrawn from clinical development. FK-317, on the other hand, was found not to induce vascular leak syndrome in patients and recently passed phase I clinical trials.[5]



1, FR-900482, R = CHO
2, FR-66979, R = CH_2OH

3, FK-973, $R_1 = R_2 = R_3 = Ac$
4, FK-317, $R_1 = Me$, $R_2 = R_3 = Ac$
5, FR-70496, $R_1 = Me$, $R_2 = OH$, $R_3 = Ac$

6, mitomycin C (MMC)

Previous reports from the Fujisawa group have shown that FK-973 forms DNA–DNA interstrand cross-links and DNA–protein cross links in L1210 cells.[6] Similarly, FK-317 was shown to lead to the formation of DNA–DNA and DNA–protein cross-links in human non-small cell lung cancer cells (A549) and human colon cancer cells (SW-480 and SW-620) but requires deacetylation in vivo and in vitro to FR-70496 (**5**), for the expression of biochemical and cytotoxic activity.[5] In contrast to mitomycin C, FK-973 and FK-317 do not cause oxidative single strand scission of DNA.[6–9] The Fujisawa drugs have been shown to be a significantly less host toxic than mitomycin C and are approximately three-fold more potent. In addition, FK-317 has recently been shown to be more cytotoxic than adriamycin and *cis*-platin, drugs that are frequently used in the clinic.

It is well established that MMC is reductively activated to provide an electrophilic mitosene via the in situ bioreductive formation of a semi-quinone radical anion.[9] Non-specific oxidative damage to DNA and other cellular macromolecules mediated by MMC is a manifestation of superoxide production resulting from the reduction of molecular oxygen by the semi-quinone radical anion intermediates: subsequent Haber–Weiss/Fenton cycling produces hydroxyl radical and related highly reactive and diffusable oxidants capable of causing non-selective tissue damage.[10] The relatively low host toxicity of FK-973 and FK-317 in clinical trials relative to MMC may be correlated to the incapacity of these agents to cause indiscriminate oxidative damage to DNA and other healthy cellular targets. Since FK-973 and MMC both share the ability to cross-link DNA, it is very clear that the lack of oxidation chemistry inherent in FK-973 has not at all compromised its efficacy as an anti-tumor drug relative to MMC.

It has been demonstrated that FR-900482 (and by analogy, FR-66979, FK-973 and FK-317) undergo reductive activation in vitro to form the reactive mitosene derivative **9** (Scheme 1) which preferentially cross-links duplex DNA at ^{5}CpG^3 steps.[7,8] The mechanism of reductive activation involves the thiol-mediated two-electron reduction of the N–O bond[11] in the presence of trace Fe(II) salts[8c] generating the transient ketone **7** which rapidly cyclizes to the

Keywords: mitosene; photochemical trigger; aziridine; FR-900482; anti-tumor antibiotic.

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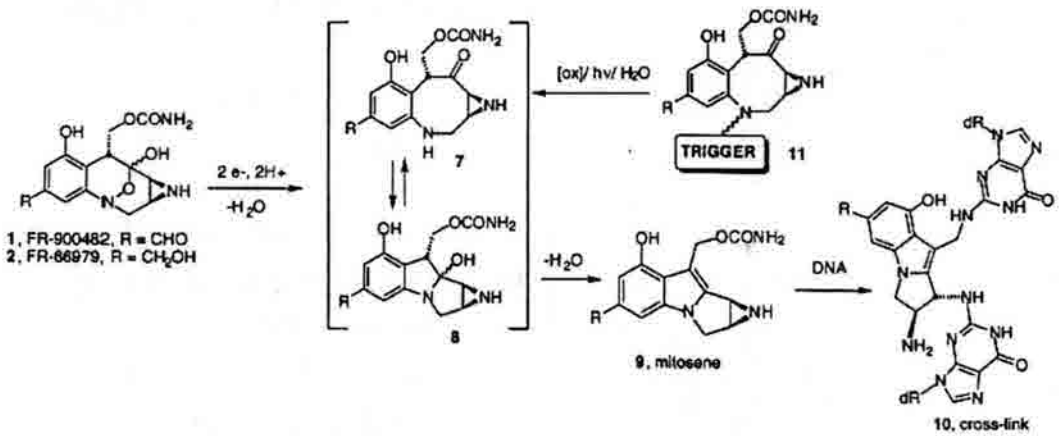


Scheme 1.

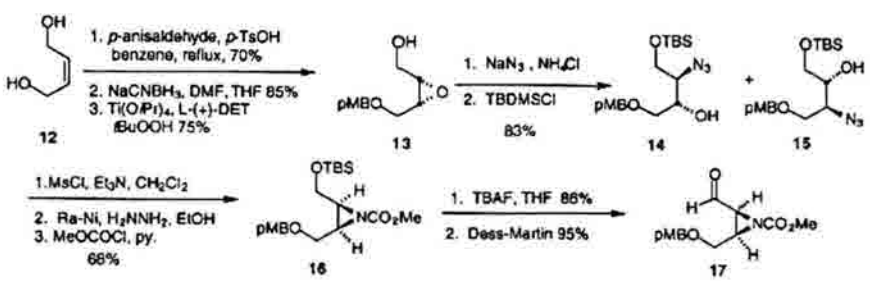
carbinolamines **8**. Expulsion of water has been inferred as the rate-determining step *enroute* to the electrophilic mitosene.[8d]

It can thus be seen that MMC, FR-900482 and congeners are actually naturally occurring clever 'pro-drugs' that must be reductively activated in vivo to expose the highly reactive electrophilic mitosene derivatives that are responsible for the biological activity displayed by these substances.[10] Our objectives in this area are aimed at exploiting the concept of latent triggering of pro-mitosenes by designing and synthesizing new masked mitosenes of the general structure **11** that can be triggered by alternative chemical and/or biochemical means. The ultimate goals of these strategies are to improve the tumor selectivity of such agents. Since many anti-tumor drugs display multiple modes of action such as, intercalation, oxidative scission of double- and single-stranded DNA, cross-linking, alkylation and membrane effects amongst others, the issue of chemical and biological selectivity is of paramount, fundamental importance to provide the next generation of highly effective and selective anti-tumor drugs.

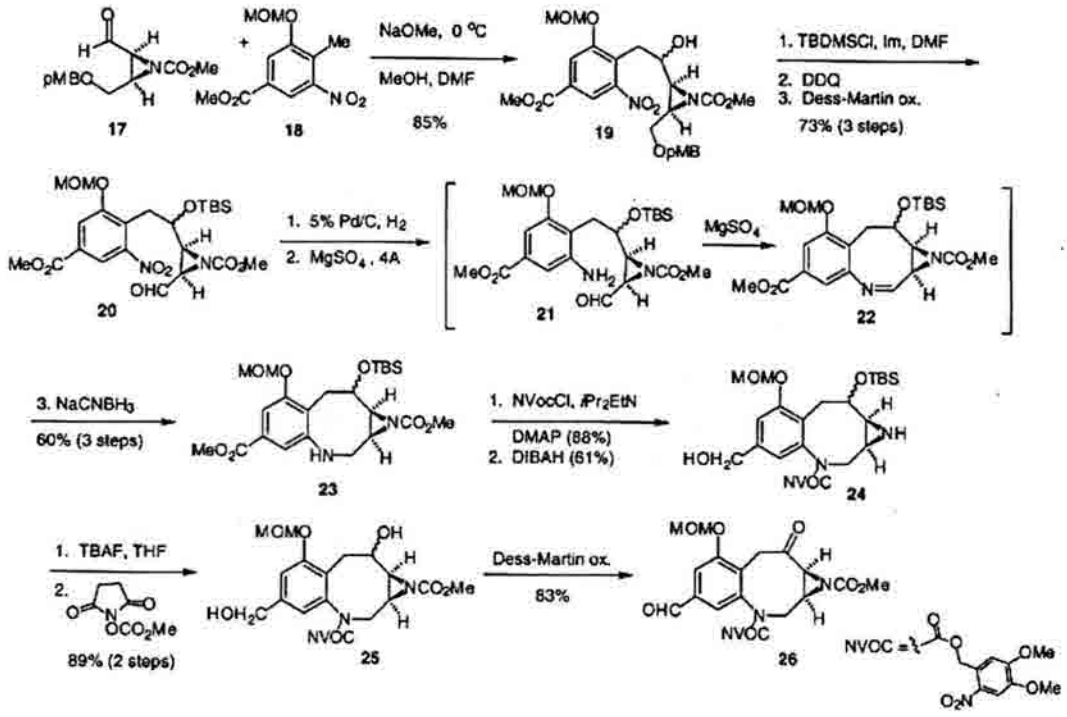
The unique structure of the Fujisawa drugs and their extraordinary anti-tumor activities have made these substances attractive synthetic targets. Several different approaches to the core nucleus of **1** have been published,[12] and three groups have successfully completed the syntheses of FR-900482.[13] 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 suitably masked activation cascade intermediate corresponding to **11** (Scheme 1) that is not reductively activated but that could, in principle, be triggered photochemically, oxidatively, or hydrolytically to form a reactive mitosene. As a first step toward testing this hypothesis, the synthesis of the first light-activated pro-mitosene is described herein.[14]

Results and Discussion

The aliphatic portion of the pro-drug model system was prepared from commercially available *cis*-2-butene-1,4-diol (**12**) (Scheme 2). Formation of the cyclic acetal with *p*-anisaldehyde and sodium cyanoborohydride reduction of the acetal gave the mono protected *cis*-diol in 60% yield over two steps. Sharpless epoxidation of the allylic alcohol gave epoxide **13** (75%) in approximately 94:6 *er*. Non-selective ring opening of **13** with sodium azide gave a mixture of the corresponding azido alcohols in a ~3:2 ratio (the mixture was not purified except for characterization purposes). Selective protection of the primary alcohols of gave a mixture of the corresponding O-TBS ethers **14** and **15** (83%, for the two steps).[15] Reduction of the azides with Raney Nickel and carbomethoxylation of the resulting



Scheme 2.



Scheme 3.

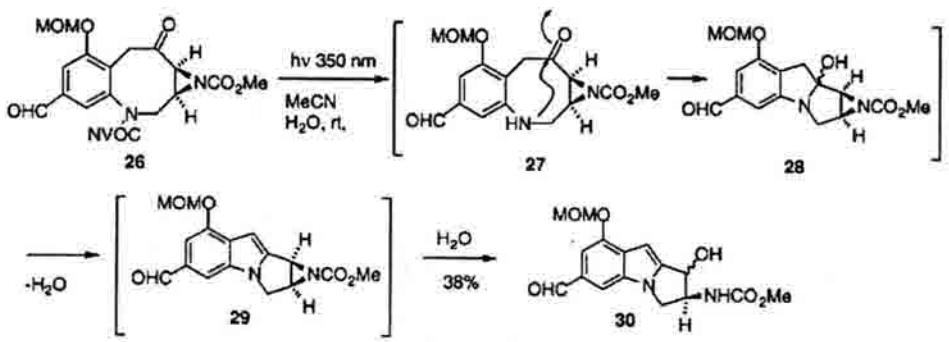
aziridine afforded **16** (88%, for the three steps). Removal of the O-TBS ether from **16** with tetra-*n*-butyl ammonium fluoride gave the corresponding alcohol (86% yield) which was converted to the corresponding aldehyde (**17**) with Dess–Martin periodinane[16] in 95% yield.

Following literature procedures, commercially available 3,5-dinitro-*p*-toluic acid was transformed into methyl 3-methoxymethyloxy-4-methyl-5-nitrobenzoate (**18**).[12b,12f,17] Deprotonation of nitro toluene **18** and nucleophilic addition[12a] 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 the corresponding O-TBS ether (96%). The oxidative removal of the O-*p*-methoxybenzyl group[18] gave the primary alcohol (93%) which was subjected to Dess–Martin oxidation[16] to afford aldehyde **20** (82%) with an overall yield from **19** to **20** of 73%. Reduction of the nitro group was accomplished by catalytic hydrogenation to afford the labile aniline **21**.

As expected, cyclization of **21** to the eight-membered ring substance **23** 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 molecular sieves under dilute conditions (~0.002 M). After 24 h, the crude imine was reduced with $NaCNBH_3$ to give **23** in 60% overall yield from **20**. It should be noted that the imine-forming cyclization reaction is somewhat capricious with variable amounts of dimer derived from **22** being observed.

Acylation of **23** with 6-nitroveratryl chloroformate (NVocCl) produced the corresponding carbamate (88%) as a mixture of conformational isomers (^{1}H NMR analysis).[20] Reduction of the methyl ester and removal of the carbomethoxy group in one step with DIBAH gave **24** (61%).[13b] It was observed that the TBS ether of **24** could be removed only via prior deprotection of the aziridine nitrogen atom. Thus, following decarbomethoxylation of the aziridine, the O-TBS ether was smoothly removed with TBAF to afford the corresponding diol. Selective re-protection of the aziridine gave **25** in 89% overall yield from **24**.[13b] Finally, Dess–Martin oxidation[16] of the primary and secondary alcohols produced keto-aldehyde **26** in 83% yield (Scheme 3).

With the 'pro-mitosene' model system (**26**) in hand, we examined removal of the NVOC group photochemically under various conditions to explore the proof-of-principle for the photochemical generation of a mitosene (Scheme 4). This was best effected by subjecting **26** (λ_{max}=345 nm, ϵ=6800; 295 nm, ϵ=7740; 238 nm, ϵ=17 300; 217 nm, ϵ=18 500, CH_3CN) to 350 nm irradiation for 24 h at room temperature in a 3:1 solution of CH_3CN/H_2O.[19] The sole isolable product was the ring-opened mitosene **30** as a 1:1 mixture of secondary alcohol diastereomers (38%). This substance must have arisen by the stepwise trans-annular cyclization of the secondary amine (**27**) on the ketone to give the tetracyclic carbinolamine **28** followed by dehydration to the mitosene **29**. This substance (**29**) proved too reactive to isolate from the aqueous milieu and we were



Scheme 4.

only able to obtain and characterize the water trapping adducts **30**.

Synthesis of **26** and the selective production of **30** from this material by photochemical activation 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 are under investigation in these laboratories and will be reported on in due course.

Experimental

General procedures

Unless otherwise noted materials were obtained from commercially available sources and used without further purification. Diethyl ether (Et_2O) and THF were distilled from sodium benzophenone ketyl under a nitrogen atmosphere. Methylene chloride, triethylamine, pyridine, acetonitrile, and methanol were distilled under a nitrogen atmosphere from calcium hydride. Dimethyl formamide was dried over activated 4 Å molecular sieves. All reactions involving hygroscopic substances were conducted with flame or oven dried glassware under an inert atmosphere (Ar) dried by passage of atmospheric gases through a column packed with $CaSO_4$. Filtrations of organic extracts were conducted with a cotton plug using gravity, and concentration of the resultant filtrate was performed under reduced pressure (aspirator) using a rotary evaporator. Chromatographic separations were performed with EM Science TLC plates (silica-gel 60, F_{254}, 20×20 cm×250 μm) or with EM Science 230–400 mesh silica gel using positive air pressure. Reactions and chromatographic fractions were monitored and analyzed with EM Science TLC plates. Visualization on TLC was achieved with ultraviolet light or heating of TLC plates submerged in a 5% solution of phosphomolybdic acid in 95% ethanol. Radial chromatography employed a Chromatotron Model 7954 using 2 or 4 mm silica plates as needed. Melting points were determined in open-ended capillary tubes with a Mel-Temp apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 1600 series FTIR as thin films from dichloromethane and are reported as λ_{max} in wavenumbers (cm^{-1}). Optical rotations were obtained on a Rudolph Research Autopol III automatic polarimeter at a wave length of 589 nm (sodium 'D' line) with a 1.0 dm cell with a volume of 1 mL. Specific rotations, $[\alpha]_D^{25}$ are reported at the specified temperature and concentration (c) given in grams per 100 mL in the specified solvent. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are accurate to within ±0.4% of the calculated values. High resolution mass spectra were obtained on a Fisons VG-7070 at University of California Riverside. Nuclear Magnetic Resonance (NMR) spectra were acquired using a Bruker AC-300 or JS-300 spectrometer.

4,7-Dihydro-2-(4-methoxyphenyl)-1,3-dioxepin. A mixture of *p*-anisaldehyde (136 g, 1.0 mol, 1.0 equiv.), *cis*-2-butene-1,4-diol (**12**) (105 g, 1.2 mol, 1.2 equiv.), and *p*-TsOH (0.20 g, 1.1 mmol, 0.11 mol%) in 450 mL of benzene was refluxed with azeotropic removal of water. After 1.5 days, the dark brown mixture was cooled to room temperature, diluted with 500 mL of benzene, washed sequentially with 3×125 mL H_2O and 1×200 mL sat. $NaCl_{(aq)}$. The organic solution was concentrated in vacuo, and the resulting oil was fractionally distilled under vacuum (1 mmHg, ~160°C) to yield 120 g (58% yield) of 4,7-dihydro-2-(4-methoxyphenyl)-1,3-dioxepin as a clear, colorless, viscous oil (>95% pure). R_f=0.50 (5:1 Hex/EtOAc). ^1H NMR (300 MHz, $CDCl_3$) δ TMS: 3.79 (3H, s); 4.23 (2H, dd, J=1.7, 15.0 Hz); 4.36 (2H, dd, J=1.7, 15.0 Hz); 5.74 (2H, t, J=1.7 Hz); 5.81 (1H, s); 6.88 (2H, d, J=8.8 Hz); 7.43 (2H, d, J=8.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 55.2 (q), 64.3 (t), 101.9 (d), 113.4 (d), 127.6 (d), 129.9 (d), 131.1 (s), 159.6 (s). IR (NaCl, neat): 3030, 2938, 2855, 1613, 1586, 1513, 1445 cm^{-1}. Anal. calcd for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 69.68; H, 6.69.

(*Z*)-4-[(4-Methoxyyphenyl)methoxy]-2-buten-1-ol. The acetal (4,7-dihydro-2-(4-methoxyphenyl)-1,3-dioxepin) prepared as described above (11.8 g, 57.2 mmol, 1 equiv.) and 180 mL of dry DMF were added to a 1 L flask. A solution of $NaCNBH_3$ (10.6 g, 168.7 mmol, 3 equiv.) and TFA (22.7 mL, 294.6 mmol, 5 equiv.) in 90 mL of dry DMF (prepared at 0°C) was transferred via cannula into the flask under negative pressure at ambient temperature. The

resulting solution was stirred 2.0 h until TLC analysis (1:1 EtOAc/Hex) showed the reaction to be complete. The reaction was quenched by dropwise addition of 1 M $NaOH_{(aq)}$ until the solution reached a pH equal to 7. Following concentration in vacuo, the resulting oil was redissolved in 200 mL of CH_2Cl_2 and washed with 2×100 mL H_2O and 1×150 mL sat. $NaCl_{(aq)}$. The solution was dried over $MgSO_4$, filtered, concentrated, and dried under vacuum for 12 h. The crude oil was purified by distillation using a kugelrohr apparatus (~1 mmHg, 160°C) to yield 10.0 g of the allylic alcohol (85% yield) as a clear colorless oil (>95% pure). R_f=0.40 1:1 Hex/EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 2.04 (1H, br, D_2O exch.); 3.78 (3H, s); 4.04 (2H, d, J=6.0 Hz); 4.14 (2H, d, J=6.0 Hz); 4.44 (2H, s); 5.76 (2H, m); 6.86 (2H, d, J=8.6 Hz); 7.23 (2H, d, J=8.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 55.2 (q), 58.7 (t), 65.3 (t), 72.1 (t), 113.8 (d), 128.4 (d), 129.5 (d), 129.9 (s), 132.3 (d), 159.3 (s). IR (NaCl, neat): 3406, 3022, 2934, 2860, 1613, 1586, 1513, 1464 cm^{-1}. Anal. calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 68.94; H, 7.56.

(2*S*-*cis*)-3-[[(4-Methoxyphenyl)methoxy]methyl]-oxiranemethanol (13). Freshly distilled CH_2Cl_2 (200 mL) was added to a 1 L three-neck round bottom flask and cooled to −24°C before addition of 5 g of powdered 4 Å molecular sieves. Next, freshly distilled (+)-diethyl-L-tartrate (8.2 mL, 48.5 mmol, 1.3 equiv.), freshly distilled titanium isopropoxide (12.2 mL, 41.0 mmol, 1.1 equiv.), and 3.0 M *tert*-butyl hydroperoxide in toluene (25 mL, 74.6 mmol, 2.0 equiv.) were added to the flask. The mixture was stirred for 30 min to let the catalyst age. The allylic alcohol ((*Z*)-4-[(4-methoxyyphenyl)methoxy]-2-buten-1-ol) prepared as described above (7.5 g, 36.0 mmol, 1.0 equiv.) in ~10 mL of CH_2Cl_2 (dried over 4 Å molecular sieves) was added dropwise to the mixture over 20 min. The reaction was stirred at −24°C for 2 days. After TLC analysis (1:1 Hex/EtOAc) showed no sign of starting material, the reaction was placed on a −45°C acetonitrile/CO_2 bath and quenched with 100 mL of 10% aqueous tartaric acid. The two-phase solution was stirred with a mechanical stirrer for 30 min and then allowed to warm to room temp over 1 h. Approximately 200 mL of water was added to the mixture, and the aqueous solution was extracted. During the extraction, the emulsion due to the molecular sieves was removed by filtering the aqueous solution through a cotton plug. The combined organic extracts were dried over $MgSO_4$ and immediately passed through a Celite pad. The concentrated oil was dissolved in 150 mL Et_2O and cooled to 0°C on an ice bath. Next, 50 mL of 1 M $NaOH_{(aq)}$, pre-cooled to 0°C, was added to the organic solution. The biphasic mixture was stirred vigorously for 1.5 h. The organic layer was separated, washed with 1×H_2O, and 1×sat. $NaCl_{(aq)}$, and dried over Na_2SO_4. The resulting oil was dried overnight under vacuum. Crystalization of the product from Et_2O at −33°C afforded 6.05 g (75% yield) of epoxide **13** as a white solid (>95% pure). R_f=0.31 (1:1 Hex/EtOAc) $[\alpha]_D^{25}$= −25.5 (c=1.0, $CHCl_3$). R_f=0.31 (1:1 Hex/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 2.04 (1H, br, D_2O exch.); 3.22–3.28 (2H, m); 3.62 (1H, dd, J=5.0, 11.0 Hz); 3.71 (1H, dd, J=5.9, 11.0 Hz); 3.65–3.80 (2H, m); 3.80 (3H, s); 4.46 (1H, d, J=11.4 Hz); 4.55 (1H, d, J=11.4 Hz); 6.87 (2H, d, J=8.6 Hz); 7.26 (2H, d, J=8.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 54.7 (d); 55.2 (q); 55.6 (d); 60.7 (t); 67.7 (t); 73.1 (t); 113.9 (d); 129.4 (s); 129.5 (d); 159.4 (s). IR (NaCl, neat): 3424, 2935, 1612, 1585, 1513, 1463 cm^{-1}. Anal. calcd for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found: C, 64.49; H, 7.10.

Mosher ester of epoxide 13. Alcohol **13** (~0.10 mmol, 1.0 equiv.) and 500 μL of CH_2Cl_2 were added to a 10 mL conical flask. The solution was stirred until the alcohol had completely dissolved, and DMAP (1.0 equiv.) and Et_3N (4.0 equiv.) were added. After stirring for another 2 min, (+)-MTPA-Cl (1.2 equiv.) was added to the solution. An immediate change to orange was seen, and the reaction was stirred until the reaction was complete by TLC analysis (4:1 Hex/EtOAc). The excess acid chloride was quenched by the addition of dimethylaminopropylamine (5.0 equiv.), and the mixture was stirred for another 15 min. The mixture was concentrated and passed through a short plug of silica gel (4:1 Hex/EtOAc). The crude oil was analyzed by ^{19}F NMR without further purification. R_f=0.60 (4:1 Hex/EtOAc). ^{19}F NMR (282 MHz, $CDCl_3$) (ref CF_3CH_2OH −80 ppm) δ TMS: −74.54 (CF_3); −74.60 (CF_3). 87% *ee* (±2% *ee*).

[*S*-(*R*,*S*)]-2-Azido-4-[(4-methoxyphenyl)methoxy]-1,3-butanediol and [*R*-(*R*,*S*)]-3-azido-4-[(4-methoxyphenyl)-methoxy]-1,2-butanediol. Epoxide **13** (1.52 g, 6.78 mmol, 1.0 equiv.), NH_4Cl (0.72 g, 13.5 mmol, 2.0 equiv.), NaN_3 (2.20 g, 33.9 mmol, 5.0 equiv.), 40 mL $CH_3OCH_2CH_2OH$, and 5 mL distilled H_2O were added to a 100 mL flask. The stirred reaction mixture was heated at reflux for 4 h when TLC analysis (EtOAc) showed complete loss of starting material. The cooled mixture was concentrated in vacuo. The resulting orange solid was dissolved in EtOAc, passed through a short plug of silica gel using EtOAc as eluant, concentrated, and dried overnight under vacuum. The cloudy orange oil was used without further purification. For analytical purposes, the mixture of regioisomers was further purified (>95% pure) by silica gel column chromatography (EtOAc). R_f=0.56; 0.47 (EtOAc). Mixture of isomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 2.1–2.3 (1H, br, D_2O exch.); 2.4–2.7 (1H, br, D_2O exch.); 3.52 (1H, d, J=1.8 Hz); 3.53 (1H, d, J=2.3 Hz); 3.59 (1/2H, m); 3.64 (1/2H, d, J=5.0 Hz); 3.71 (1/2H, d, J=5.1 Hz); 3.77 (3/2H, m); 3.79 (3H, s); 3.82 (1/2H, d, J=4.8 Hz); 3.94 (1/2H, q, J=4.3 Hz); 4.47 (1H, s); 4.49 (1H, s); 6.87 (2H, d, J=8.6 Hz); 7.24 (2H, d, J=8.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: Major isomer, 55.2 (q), 62.6 (t), 64.1 (d), 70.6 (d), 70.7 (t), 73.2 (t), 113.9 (d), 129.3 (s), 129.5 (d), 159.4 (s). Minor isomer, 55.2 (q), 62.2 (d), 63.6 (t), 69.8 (t), 71.8 (d), 73.2 (t), 113.9 (d), 129.3 (s), 129.4 (d), 159.4 (s). IR (NaCl, neat): 3410, 2935, 2865, 2104, 1612, 1586, 1514, 1464 cm^{-1}. Anal. calcd for the mixture $C_{12}H_{17}N_3O_4$: C, 53.92; H, 6.41; N, 15.72. Found: C, 53.71; H, 6.36; N, 15.49.

[*S*-(*R*,*S*)]-3-Azido-4-[[(1,1-dimethlethyl)dimethylsilyl]oxy]-1-[(4-methoxyphenyl)methoxy]-2-butanol (14) and [*R*-(*R*,*S*)]-3-azido-1-[[(1,1-dimethlethyl)dimethylsilyl]oxy]-4-[(4-methoxyphenyl)methoxy]-2-butanol (15). The diol (as a mixture of isomers) from the previous reaction (1.15 g, 4.31 mmol, 1.0 equiv.), and 17 mL of CH_2Cl_2 were added to a 50 mL conical flask. The stirred mixture was cooled on an ice bath for 10 min when Et_3N (1.20 mL,

8.62 mmol, 2.0 equiv.), TBSCl (943 mg, 6.25 mmol, 1.4 equiv.), and DMAP (53 mg, 0.43 mmol, 0.1 equiv.) were added. After stirring for 1 h, the mixture was placed in the refrigerator at 4°C. After 15.5 h, TLC analysis of the crude reaction mixture (EtOAc) showed complete loss of starting material. The reaction mixture was concentrated in vacuo and passed through a short plug of silica gel using 4:1 Hex/EtOAc as eluant to yield 1.60 g (90% from **13**) of **14** and **15** as a cloudy orange oil which was used without further purification. For analytical purposes, the mixture was further purified (>95% pure) by silica gel column chromatography (4:1 Hex/EtOAc). Mixture of isomers: R_f=0.34; 0.27 (1:1 Hex/ EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 0.05 (1.5H, s); 0.06 (1.5H, s); 0.08 (3H, s); 0.88 (4.5 H, s); 0.89 (4.5H, s); 2.5 (1H, br, D_2O exch.); 3.78 (3H, s); 3.46–3.92 (6H, m); 4.47 (1H, s); 4.50 (1H, s); 6.87 (2H, d, J=8.6 Hz); 7.23 (1H, d, J=8.6 Hz); 7.26 (1H, d, J=8.6 Hz). IR (NaCl, neat): 3424, 3005, 2935, 2838, 2103, 1613, 1586, 1514 cm^{-1}. Anal. calcd for $C_{18}H_{31}N_3O_4Si$: C, 56.66; H, 8.19; N, 11.01. Found: C, 56.48; H, 7.89; N, 10.79.

[*S*-(*R*,*S*)]-3-Azido-4-[[(1,1-dimethlethyl)dimethylsilyl]oxy]-1-[(4-methoxyphenyl)methoxy]-methanesulfonate-2-butanol and [*R*-(*R*,*S*)]-3-azido-1-[[(1,1-dimethlethyl)dimethylsilyl]oxy]-4-[(4-methoxyphenyl)methoxy]-methanesulfonate-2-butanol. Alcohols **14** and **15** (15.8 g, 41.4 mmol, 1.0 equiv.) and 414 mL of CH_2Cl_2 were added to a 1 L conical flask. The stirred solution was placed on an ice bath for 15 min when Et_3N (17.3 mL, 124.1 mmol, 3.0 equiv.) was added. The mixture was stirred for another 5 min when methanesulfonyl chloride (4.8 mL, 62.0 mmol, 1.5 equiv.) was added to the flask dropwise over a minute. After 30 min, TLC analysis (2:1:2 CH_2Cl_2/Et_2O/Hex) of the reaction showed complete loss of the starting material. To the reaction mixture was added 200 mL of sat $NaHCO_{3(aq)}$, and the bilayer solution was stirred vigorously for 10 min. Following the addition of 100 mL of H_2O, the two layers were separated. The aqueous layer was extracted with 2×150 mL EtOAc, and the combined organic layers were washed with 1×200 mL sat $NaCl_{(aq)}$, and dried over Na_2SO_4. The reaction mixture was concentrated in vacuo and passed through a short plug of silica gel using 4:1 Hex/EtOAc as eluant to yield 19.0 g (96% yield) of the product as a light yellow oil which was used without further purification. For analytical purposes, the mixture was further purified (>95% pure) by column chromatography (10:1:10 CH_2Cl_2/Et_2O/Hex). Mixture of isomers: R_f=0.44 (2:1:2 CH_2Cl_2/Et_2O/Hex). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 0.03, 0.05, 0.06 (6H, s); 0.86, 0.87 (9H, s); 3.04, 3.05 (3H, s); 3.79 (3H, s); 3.64-3.88 (6H, m); 4.47, 4.48 (2H, ABq, J=11.4 Hz); 4.70, 4.80 (1H, q, J=5 Hz); 6.86, 6.87 (2H, d, J=8.6 Hz); 7.22, 7.25 (2H, d, J=8.6 Hz). IR (NaCl, neat): 2955, 2932, 2108, 1613, 1515, 1465 cm^{-1}. Anal. calcd for $C_{19}H_{33}N_3O_6SSi$: C, 49.65; H, 7.24; N, 9.14. Found: C, 49.86; H, 7.06; N, 8.98.

(2*S*-*cis*)-Methyl ester 2-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-3-[(4-methoxypheny)methoxylmethyl]-1-aziridinecarboxylic acid (16). The mesylate compound described above (19.0 g, 41.3 mmol, 1 equiv.) and 440 mL of EtOH were added to a 1 L flask. To the resulting solution was added hydrazine monohydrate (34.3 mL, 707.1 mmol, 17.1 equiv.) followed by approximately 3 g of Raney Nickel. The reaction mixture was stirred for 3 h under argon atmosphere when TLC analysis (1:1 EtOAc/Hex) showed the reaction to be complete. The reaction was filtered through a pad of Celite with EtOH and concentrated. The resulting oil was dissolved in 400 mL of EtOAc and washed with 1×$NaCl_{(aq)}$ and dried over Na_2SO_4. The mixture was filtered, concentrated, and placed under vacuum for 12 h. The clear yellow oil was dissolved in 300 mL of CH_2Cl_2 and cooled on an ice bath for ~15 min while stirring. Pyridine (10.0 mL, 20.8 mmol, 3.1 equiv.) was added to the mixture, and the mixture was stirred for another 5 min when methyl chloroformate (6.4 mL, 82.8 mmol, 2.0 equiv.) was added dropwise over 2 min. The reaction was stirred for 20 min when TLC analysis showed a complete loss of the unprotected aziridine (R_f=0.73 10:1 CH_2Cl_2/MeOH). To the reaction mixture was added 300 mL sat $NaHCO_{3(aq)}$, and the bilayer was stirred vigorously for 10 min. The two layers were separated. The aqueous layer was extracted with 2×150 mL EtOAc, and the combined organic layers were washed 1×200 mL sat $NaCl_{(aq)}$. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by column chromatography (4:1 Hex/EtOAc) to yield 15.0 g (88% overall yield from **14** and **15**) of **16** as a light yellow oil (>95% pure). $[\alpha]_D^{25}$=+9.6 (c=2.1, $CHCl_3$). R_f=0.50 (2:1 hexane/EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ TMS: 0.03 (3H, s); 0.05 (3H, s); 0.86 (9H, s); 2.69 (1H, dd, J=6.0, 6.0 Hz); 2.77 (1H, dd, J=6.0, 6.0 Hz); 3.55 (1H, dd, J=5.5, 11.2 Hz); 3.58 (1H, dd, J=6.3, 11.2 Hz); 3.60 (1H, dd, J=6.1, 11.4 Hz); 3.71 (3H, s); 3.77 (1H, dd, J=5.9, 11.4 Hz); 3.78 (3H, s); 4.46 (1H, d, J=11.5 Hz); 4.59 (1H, d, J=11.5 Hz); 6.85 (2H, d, J=8.6 Hz); 7.26 (2H, d, J=8.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: −5.4 (q); −5.3 (q); 18.2 (s); 25.7 (q); 40.4 (d); 41.8 (d); 53.5 (q); 55.1 (q); 61.2 (t); 67.1 (t); 72.4 (t); 113.7 (d); 129.4 (d); 129.9 (s); 159.2 (s); 163.5 (s). IR (NaCl, neat): 3436, 3001, 2954, 2931, 1732, 1613, 1514, 1464, 1439 cm^{-1}. Anal. calcd for $C_{20}H_{33}NO_5Si$: C, 60.73; H, 8.41; N, 3.54. Found: C, 60.60; H, 8.62; N, 3.45.

(2*S*-*cis*)-Methyl ester 2-(hydroxymethyl)-3-[(4-methoxypheny)methoxylmethyl]-1-aziridinecarboxylic acid. Aziridine **16** (2.02 g, 5.1 mmol, 1.0 equiv.) and 50 mL of THF were added to a 200 mL flask. The stirred solution was cooled on an ice bath for 15 min, and 1 M TBAF in THF (6.1 mL, 6.1 mmol, 1.2 equiv.) was added. After 30 min, the reaction was complete by TLC analysis (4:1 Hex/EtOAc). The reaction mixture was removed from the ice bath, quenched by the addition of 25 mL sat $NH_4Cl_{(aq)}$, and stirred vigorously for 5 min. The THF was evaporated, and the aqueous solution was diluted with 25 mL H_2O and extracted with Et_2O (5×30 mL). The combined organic layers were dried over Na_2SO_4 overnight. The filtered solution was concentrated, and the resulting oil was purified by column chromatography (2:1 CH_2Cl_2/Et_2O) to yield 1.24 g (86% yield) of the alcohol as light yellow oil (>95% pure). $[\alpha]_D^{25}$=+36.6 (c=1.3, $CHCl_3$). R_f=0.21 (2:1 CH_2Cl_2/Et_2O). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 2.37 (1H, br, D_2O exch.); 2.81 (2H, m); 3.40 (1H, dd, J=7.2, 10.7 Hz); 3.51 (1H, m); 3.70 (3H, s); 3.78 (3H, s); 3.82 (1H, m); 3.84 (1H, dd, J=5.5, 10.7 Hz); 4.44 (1H, d, J=11.4 Hz); 4.52 (1H, d, J=11.4 Hz); 6.86 (2H, d, J=8.7 Hz); 7.23 (2H, d,

J=8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 39.5 (d); 41.5 (d); 53.7 (q); 55.2 (q); 60.5 (t); 67.4 (t); 73.0 (t); 113.9 (d); 129.2 (s); 129.6 (d); 159.5 (s); 163.2 (s). IR (NaCl, neat): 3430, 3003, 2955, 1728, 1613, 1586, 1514, 1440 cm^{-1}. Anal. calcd for $C_{14}H_{19}NO_5$: C, 59.78; H, 6.81; N, 4.98. Found: C, 59.80; H, 7.02; N, 4.82.

(2*S*-*cis*)-Methyl ester 2-formyl-3-[(4-methoxypheny)-methoxylmethyl]-1-aziridinecarboxylic acid (17). The alcohol described above (1.29 g, 4.58 mmol, 1.0 equiv.) and 45 mL CH_2Cl_2 were added to a 200 mL flask. The mixture was stirred for 5 min when Dess–Martin reagent[16] (3.5 g, 7.3 mmol, 1.6 equiv.) was added to the flask in one portion. The mixture was stirred for 2.5 h when TLC analysis (2:1 CH_2Cl_2/Et_2O) showed complete loss of starting material. The reaction mixture was dissolved in 150 mL Et_2O and poured into a solution of 150 mL sat $NaHCO_{3(aq)}$ with seven-fold excess of $Na_2S_2O_3{\cdot}5H_2O$ (9.0 g). The biphasic mixture was vigorously stirred for 15 min while the milky color of the organic layer slowly disappeared The two layers were separated. The organic layer was washed with 1×25 mL sat $NaHCO_{3(aq)}$ and 1×25 mL H_2O. The combined aqueous layers were back extracted with 5×30 mL Et_2O. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by column chromatography (1.5:1 Hex/EtOAc) to yield 1.20 g (92% yield) of **17** as a clear colorless oil (>95% pure). $[\alpha]_D^{25}$=−80.6 (c=1.3, $CHCl_3$). R_f=0.60 (2:1 CH_2Cl_2/Et_2O). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 3.04 (1H, ddd, J=4.3, 4.4, 6.9 Hz); 3.10 (1H, dd, J=4.5, 6.9 Hz); 3.64 (1H, dd, J=4.3, 11.2 Hz); 3.73 (1H, dd, J=4.4, 11.2 Hz); 3.75 (3H, s); 3.78 (3H, s); 4.45 (1H, d, J=11.5 Hz); 4.47 (1H, d, J=11.5 Hz); 6.85 (2H, d, J=8.7 Hz); 7.19 (2H, d, J=8.7 Hz); 9.31 (1H, d, J=4.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 43.5 (d); 44.7 (d); 54.1 (q); 55.2 (q); 65.8 (q); 73.0 (q); 113.9 (d); 129.2 (s); 129.5 (d); 159.4 (s); 161.8 (s); 196.3 (d). IR (NaCl, neat): 3006, 2953, 2834, 1719, 1612, 1586, 1513, 1438 cm^{-1}. Anal. calcd for $C_{14}H_{17}NO_5$: C, 60.21; H, 6.14; N, 5.02. Found: C, 60.45; H, 6.21; N, 4.96.

[2*S*-(2α,3α)]-Methyl ester 2-[1-hydroxy-2-[4-(methoxy-carbonyl)-2-(methoxymethoxy)-6-nitrophenyl]ethyl]-3-[[(4-methoxyphenyl)methoxy]methyl]-1-aziridinecar-boxylic acid (19). Compound **18** (4.69 g, 18.4 mmol, 2.0 equiv.) and 20 mL of DMF were added to a 50 mL flask. The stirred mixture was cooled on an ice bath for 20 min when 0.5 M NaOMe in MeOH (1.8 mL, 0.9 mmol, 0.1 equiv.) was added. The clear solution immediately turned dark purple. After the addition of base, **17** (2.58 g, 9.2 mmol, 1.0 equiv.) in 10 mL of DMF was added to the reaction mixture in 1 mL aliquots every 5 min. After the additions were complete (50 min), the reaction was stirred for another 3.5 h and quenched with 35 mL of sat $NH_4Cl_{(aq)}$. After 10 min, the reaction was diluted with 20 mL water, and the aqueous mixture was extracted with 6×50 mL Et_2O, 1×25 mL CH_2Cl_2, and 1×25 mL EtOAc. The combined organic extracts were washed with 1×45 mL sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered and concentrated. The crude oil was purified by column chromatography (1:1 Hex/EtOAc) to yield 4.2 g (85–90% yield) of **19** as a yellow oil (4:1 mixture of separable diastereomers) (>95% pure).

Major diastereomer **19**: $[\alpha]_D^{25}$=−38.4 (c=1.3, $CHCl_3$). R_f=0.50 (2:1 CH_2Cl_2/Et_2O). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 2.65 (1H, dd, J=6.5, 8.4 Hz); 2.80 (1H, ddd, J=5.7, 6.5, 7.9 Hz); 2.98 (1H, br, D_2O exch.); 3.33 (1H, dd, J=7.9, 10.5 Hz); 3.40 (2H, m); 3.42 (3H, s); 3.60 (1H, m); 3.64 (3H, s); 3.76 (3H, s); 3.85 (1H, dd, J=5.7, 10.5 Hz); 3.92 (3H, s); 4.41 (1H, d, J=11.5 Hz); 4.46 (1H, d, J=11.5 Hz); 5.25 (2H, app. sing.); 6.79 (2H, d, J=8.6 Hz); 7.17 (2H, d, J=8.6 Hz); 7.91 (1H, d, J=1.5 Hz); 8.10 (1H, d, J=1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 30.4 (t), 39.2 (d), 44.8 (d), 52.4 (q), 53.5 (q), 54.9 (q), 56.3 (q), 67.3 (t), 69.1 (d), 72.7 (t), 94.7 (t), 113.6 (d), 117.6 (d), 118.1 (d), 126.8 (s), 128.8 (s), 129.4 (d), 129.7 (s), 151.5 (s), 156.0 (s), 159.2 (s), 162.7 (s), 164.7 (s). IR (NaCl, neat): 3509, 2956, 2923, 2854, 1728, 1613, 1538, 1514, 1438 cm^{-1}. Anal. for the mixture of diastereo-mers: calcd for $C_{25}H_{30}N_2O_{11}$: C, 56.18; H, 5.66; N, 5.24. Found: C, 55.93; H, 5.83; N, 5.04.

Minor diastereomer **19**: $[\alpha]_D^{25}$=+14.2 (c=1.3, $CHCl_3$). R_f=0.45 (2:1 CH_2Cl_2/Et_2O). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: 2.33 (1H, br, D_2O exch.); 2.66 (1H, dd, J=6.8, 6.8 Hz); 2.81 (1H, ddd, J=5.2, 6.5, 6.8 Hz); 3.20 (1H, dd, J=5.2, 13.4 Hz); 3.36 (2H, m); 3.41 (3H, s); 3.50 (1H, dd, J=6.5, 11.0 Hz); 3.73 (3H, s); 3.78 (3H, s); 3.84 (1H, m); 3.92 (3H, s); 4.43 (1H, d, J=11.4 Hz); 4.50 (1H, d, J=11.4 Hz); 5.21 (2H, app. sing.); 6.84 (2H, d, J=8.6 Hz); 7.21 (2H, d, J=8.6 Hz); 7.92 (1H, d, J=1.5 Hz); 8.08 (1H, d, J=1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 30.9 (t), 41.3 (d), 45.8 (d), 52.7 (q), 53.9 (q), 55.2 (q), 56.6 (q), 66.9 (t), 68.9 (d), 72.6 (t), 95.0 (t), 113.8 (d), 118.0 (d), 118.5 (d), 126.3 (s), 129.4 (d), 129.7 (s), 130.3 (s), 151.5 (s), 156.3 (s), 159.3 (s), 163.3 (s), 164.7 (s). IR (NaCl, neat): 3509, 2956, 2855, 1728, 1613, 1538, 1514, 1438 cm^{-1}.

[2*S*-(2α,3α)]-Methyl ester 2-[1-[[(1,1-dimethylethyl)di-methylsilyl]oxy]-2-[4-(methoxycarbonyl)-2-(methoxy-methoxy)-6-nitrophenyl]ethyl]-3-[[(4-methoxyphenyl)-methoxy]methyl]-1-aziridinecarboxylic acid. Compound **19** (337 mg, 0.70 mmol, 1.0 equiv.), and 350 μL of DMF were added to a 10 mL conical flask. Once **19** had completely dissolved, imidazole (167 mg, 2.46 mmol, 3.5 equiv.), and TBSCl (212 mg, 1.41 mmol, 2.0 equiv.) were added to the flask. After stirring for 24 h, TLC analysis (1:1 Hex/EtOAc) of the crude reaction showed complete loss of starting material, and the reaction was diluted with 15 mL Et_2O. The organic solution was washed with 10 mL water, and the two layers were separated. The aqueous layer was back extracted with 6×15 mL Et_2O. The combined organic layers were washed with 15 mL sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by column chromatography (1.5:1 Hex/EtOAc) to give 437 mg (96% yield) of product as a clear yellow oil (>95% pure).

Major diastereomer: $[\alpha]_D^{25}$=−27.6 (c=1.6, $CHCl_3$). R_f=0.50 (1:1 Hex/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: −0.40 (3H, s); −0.11 (3H, s); 0.69 (9H, s); 2.59 (1H, dd, J=5.5, 6.3 Hz); 2.74 (1H, ddd, J=4.6, 6.3, 6.8 Hz); 3.22 (1H, dd, J=4.8, 13.5 Hz); 3.41 (1H, dd, J=9.0, 13.5 Hz); 3.43 (3H, s); 3.60 (1H, dd, J=6.8, 11.0 Hz); 3.68 (3H, s); 3.68 (1H, dd, J=4.6, 11.0 Hz); 3.77 (3H, s); 3.91 (3H, s);

4.12 (1H, ddd, J=4.8, 5.5, 9.0 Hz); 4.50 (1H, d, J=11.4 Hz); 4.60 (1H, d, J=11.4 Hz); 5.21 (2H, app. sing.); 6.85 (2H, d, J=8.5 Hz); 7.27 (2H, d, J=8.5 Hz); 7.90 (1H, d, J=1.5 Hz); 8.06 (1H, d, J=1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: −0.53 (q), −0.48 (q), 17.8 (s), 25.6 (q), 32.2 (t), 41.1 (d), 45.1 (d), 52.6 (q), 53.6 (q), 55.2 (q), 56.6 (q), 67.3 (t), 68.0 (d), 72.5 (t), 94.9 (t), 113.7 (d), 117.6 (d), 118.4 (d), 127.3 (s), 129.5 (d), 129.9 (s), 130.0 (s), 151.7 (s), 156.6 (s), 159.2 (s), 163.5 (s), 164.9 (s). IR (NaCl, neat): 3001, 2954, 2856, 1731, 1613, 1537, 1514, 1438 cm^{-1}. Anal. for the mixture of diastereomers: calcd for $C_{31}H_{44}N_2O_{11}Si$: C, 57.39; H, 6.84; N, 4.32. Found: C, 57.50; H, 6.91; N, 4.50.

Minor diastereomer: $[\alpha]_D^{25}$=+10.5 (c=1.1, $CHCl_3$). R_f=0.5 (1:1 Hex/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: −0.31 (3H, s); 0.03 (3H, s); 0.80 (9H, s); 2.61 (1H, ddd, J=4.4, 6.7, 7.1 Hz); 2.69 (1H, dd, J=6.7, 8.5 Hz); 2.98 (1H, dd, J=4.4, 11.1 Hz); 3.11 (1H, dd, J=6.6, 13.2 Hz); 3.19 (1H, dd, J=7.1, 11.1 Hz); 3.23 (1H, dd, J=7.6, 13.2 Hz); 3.39 (3H, s); 3.69 (3H, s); 3.77 (3H, s); 3.79 (1H, m); 3.91 (3H, s); 4.40 (1H, d, J=11.5 Hz); 4.50 (1H, d, J=11.5 Hz); 5.17 (2H, apparent singlet); 6.82 (2H, d, J=8.6 Hz); 7.18 (2H, d, J=8.6 Hz); 7.87 (1H, d, J=1.5 Hz); 8.01 (1H, d, J=1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: −5.3 (q), −4.8 (q), 17.7 (s), 25.6 (q), 31.8 (t), 40.9 (d), 46.0 (d), 52.6 (q), 53.4 (q), 55.1 (q), 56.5 (q), 66.9 (t), 70.3 (d), 72.1 (t), 94.7 (t), 113.6 (d), 117.4 (d), 118.2 (d), 126.1 (s), 129.4 (d), 129.6 (s), 130.1 (s), 151.5 (s), 156.6 (s), 159.1 (s), 163.2 (s), 164.6 (s). IR (NaCl, neat): 2964, 1732, 1614, 1538, 1514, 1438 cm^{-1}.

[2*S*-(2α,3α)]-Methyl ester 2-[1-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-2-[4-(methoxycarbonyl)-2-(methoxymethoxy)-6-nitrophenyl]ethyl]-3-(hydroxymethyl)-1-aziridinecarboxylic acid. The silyl ether described above (205 mg, 0.32 mmol, 1.0 equiv.), 2.7 mL of CH_2Cl_2, and 150 μL of H_2O were added to a 25 mL flask. After stirring for 5 min, DDQ (93 mg, 0.41 mmol, 1.3 equiv.) was added to the mixture in one portion. The reaction mixture immediately turned dark green, and over the course of the next 1.5 h, the mixture slowly turned bright orange. After 1.5 h, the crude reaction mixture was passed through a short plug of activated alumina using 10:1 CH_2Cl_2/MeOH as eluant. After concentration in vacuo, the crude oil was purified by column chromatography (1:1 Hex/EtOAc) to give 160 mg (93% yield) of product as a clear orange oil (>95% pure).

Major diastereomer: $[\alpha]_D^{25}$=−55.6 (c=1.2, CH_2Cl_2). R_f=0.30 (1:1 Hex/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: −0.34 (3H, s); −0.06 (3H, s); 0.73 (9H, s); 1.95 (1H, br, D_2O exch.); 2.61 (1H, dd, J=5.7, 6.2 Hz); 2.72 (1H, ddd, J=4.5, 6.4, 6.4 Hz); 3.26 (1H, dd, J=5.1, 13.4 Hz); 3.42 (1H, dd, J=8.8, 13.4 Hz); 3.49 (3H, s); 3.67 (3H, s); 3.90 (2H, m); 3.92 (3H, s); 4.25 (1H, ddd, J=5.1, 5.7, 8.8 Hz); 5.27 (1H, d, J=6.9 Hz); 5.29 (1H, d, J=6.9 Hz); 7.92 (1H, d, J=1.5 Hz); 8.09 (1H, d, J=1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: −5.2 (q), −4.9 (q), 17.8 (s); 25.5 (q); 32.3 (t); 42.9 (d)45.9 (d); 52.6 (q); 53.7 (q); 56.6 (q); 60.2 (t); 68.1 (d); 95.0 (t); 117.6 (d); 118.4 (d); 127.0 (s); 129.9 (s); 151.6 (s); 156.6 (s); 163.5 (s); 164.8 (s). IR (NaCl, neat): 3510, 2955, 2856, 1730, 1540, 1438 cm^{-1}. Anal. for the mixture of diastereomers: calcd for $C_{23}H_{36}N_2O_{10}Si$: C, 52.26; H, 6.86; N, 5.30. Found: C, 52.22; H, 6.66; N, 5.19.

Minor diastereomer: $[\alpha]_D^{25}$=+8.8 (c=2.8, CH_2Cl_2). R_f=0.30 (1:1 Hex/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: −0.24 (3H, s); 0.02 (3H, s); 0.82 (9H, s); 1.78 (1H, br, D_2O exch.); 2.56 (1H, ddd, J=4.4, 6.6, 6.7 Hz); 2.74 (1H, dd, J=6.6, 8.7 Hz); 3.13 (3H, m); 3.25 (1H, dd, J=7.2, 13.2 Hz); 3.47 (3H, s); 3.68 (3H, s); 3.90 (1H, m); 3.92 (3H, s); 5.27 (1H, d, J=7.0 Hz); 5.29 (1H, d, J=7.0 Hz); 7.92 (1H, d, J=1.5 Hz); 8.05 (1H, d, J=1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: −5.3 (q), −4.8 (q), 17.7 (s), 25.5 (q), 31.9 (t), 42.8 (d), 46.9 (d), 52.6 (q), 53.4 (q), 56.6 (q), 60.0 (t), 70.0 (d), 94.9 (t), 117.4 (d), 118.1 (d), 125.9 (s), 130.2 (s), 151.5 (s), 156.6 (s), 163.3 (s), 164.6 (s). IR (NaCl, neat): 3503, 2954, 2857, 1732, 1538, 1438 cm^{-1}.

[2*S*-(2α,3α)]-Methyl ester 2-[1-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-2-[4-(methoxycarbonyl)-2-(methoxymethoxy)-6-nitrophenyl]ethyl]-3-formyl-1-aziridinecarboxylic acid (20). The alcohol described in the previous experiment (98 mg, 0.18 mmol, 1.0 equiv.), and 1.5 mL of CH_2Cl_2 were added to a 25 mL flask. The mixture was stirred for 5 min and Dess–Martin reagent[16] (118 mg, 0.32 mmol, 1.8 equiv.) was added to the flask in one portion. After stirring for 2.5 h, the cloudy white mixture was diluted in 10 mL Et_2O and poured into a solution of 20 mL sat $NaHCO_{3(aq)}$ with 8.0 equiv. of $Na_2S_2O_3{\cdot}5H_2O$ (435 mg). The milky biphasic mixture turned clear after 15 min of vigorous stirring. The two layers were separated, and the organic layer was washed with 1×10 mL $NaHCO_{3(aq)}$, and 1×10 mL H_2O. The combined aqueous layers were extracted with 3×15 mL Et_2O. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by flash chromatography (2:1 Hex/EtOAc) to give 81 mg (82% yield) of **20** as a clear colorless oil (>95% pure).

Major diastereomer **20**: $[\alpha]_D^{25}$=+5.4 (c=1.1, CH_2Cl_2). R_f=0.42 (1:1 Hex/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: −0.18 (3H, s); 0.00 (3H, s); 0.76 (9H, s); 2.79 (1H, dd, J=3.5, 6.8 Hz); 3.00 (1H, dd, J=4.6, 6.8 Hz); 3.14 (1H, dd, J=6.8, 13.2 Hz); 3.23 (1H, dd, J=7.4, 13.2 Hz); 3.49 (3H, s); 3.68 (3H, s); 3.92 (3H, s); 4.50 (1H, ddd, J=3.5, 6.8, 7.4 Hz); 5.30 (2H, s); 7.94 (1H, d, J=1.5 Hz); 8.12 (1H, d, J=1.5 Hz); 9.50 (1H, d, J=4.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: −5.3 (q), −4.7 (q), 17.9 (s), 25.6 (q), 32.1 (t), 45.1 (d), 48.7 (d), 52.7 (q), 53.9 (q), 56.7 (q), 67.7 (d), 94.9 (t), 118.0 (d), 118.4 (d), 125.8 (s), 130.4 (s), 151.2 (s), 156.7 (s), 161.9 (s), 164.6 (s), 196.9 (d). IR (NaCl, neat): 2962, 2863, 1730, 1537, 1437 cm^{-1}. Anal. for the mixture of diastereomers: calcd for $C_{23}H_{34}N_2O_{10}Si$: C, 52.46; H, 6.51; N, 5.32. Found: C, 52.64; H, 6.61; N, 5.30.

Minor diastereomer **20**: $[\alpha]_D^{25}$=+112 (c=2.0, CH_2Cl_2). R_f=0.42 (1:1 Hex/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ TMS: −0.23 (3H, s); 0.08 (3H, s); 0.85 (9H, s); 3.01 (2H, m); 3.05 (1H, dd, J=6.7, 13.3 Hz); 3.23 (1H, dd, J=7.3, 13.3 Hz); 3.47 (3H, s); 3.72 (3H, s); 3.93 (3H, s); 3.96 (1H, m); 5.26 (1H, d, J=7.0 Hz); 5.28 (1H, d, J=7.0 Hz); 7.91 (1H, d, J=1.5 Hz); 8.06 (1H, d, J=1.5 Hz); 8.95 (1H, d, J=5.5 Hz). ^{13}C NMR (75 MHz, CDCl3) δ TMS: −5.3 (q), −4.8 (q), 17.7 (s), 25.5 (q), 31.7 (t), 45.6 (d), 48.7 (d), 52.6

(q), 53.8 (q), 56.6 (q), 69.7 (d), 94.8 (t), 117.6 (d), 118.3 (d), 125.1 (s), 130.5 (s), 151.4 (s), 156.3 (s), 161.4 (s), 164.5 (s), 195.0 (d). IR (NaCl, neat): 2956, 2858, 1732, 1538, 1439 cm^{-1}.

(1a*S*,9a*S*)-Dimethyl ester 9-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-1H-azirino[2,3-*c*][1]benzazocine-1,5-dicarboxylic acid (23). 40 mL of MeOH, freshly distilled from CaH_2, was added to a 100 mL flask. The stirred solution was degassed with H_2 for 30 min using a 20 gauge needle connected directly to a H_2 cylinder. The flask was then flushed with argon, and 5% Pd/C (200 mg, 0.095 mmol, 0.25 equiv.) was added in one portion. The mixture was degassed with H_2 for another 30 min and then kept under H_2 for another 30 min. Nitroaldehyde **20** (200 mg, 0.38 mmol, 1.0 equiv.) in 2 mL of MeOH was added to the mixture dropwise over 1 min. After 8 min, TLC analysis (1:1 Hex/EtOAc) of the reaction showed complete loss of **20**. The reaction was diluted with MeOH and passed through a short pad of Celite using MeOH, and the filtrate was concentrated in vacuo. The residue was filtered through a short plug of Celite using CH_2Cl_2, and the filtrate was concentrated again. The residue was dissolved in 200 mL of CH_2Cl_2. Activated 4 Å molecular sieves (~30 pieces) and $MgSO_4$ (2 g) were added to the solution. The stirred mixture was heated to reflux for 24–36 h. The cooled mixture was filtered through a pad of Celite using 10:1 CH_2Cl_2/MeOH (500 mL). The filtrate was concentrated, and the residue was immediately dissolved in solution of 2:1 CH_2Cl_2/MeOH (12 mL). After the mixture was cooled on an ice bath for 10 min, $NaCNBH_3$ (23 mg, 0.38 mmol, 1.0 equiv.) and TFA (29 μL, 0.38 mmol, 1.0 equiv.) were added in one portion. After 4 min, TLC analysis of the reaction (1:1 Hex/EtOAc) showed no signs of the starting material, and the reaction was quenched with 30 mL sat $NaHCO_{3(aq)}$. The two layers were separated, and the aqueous layer was extracted with 3×CH_2Cl_2. The combined organic layers were washed with 1×sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated, and purified by radial silica PTLC (4 mm plate, 2:1 Hex/EtOAc) to give 110 mg (40–60% yield from **20**) of **23** as a clear yellow oil (>95% pure).

[2*S*-(2α,3α)]-Methyl ester 2-[2-[2-amino-4-(methoxycarbonyl)-6-(methoxymethoxy)phenyl]-1-[[(1,1-dimethylethyl)dimethylsilyl]oxy]ethyl]-3-formyl-1-aziridinecarboxylic acid (21). Major diastereomer **21**: R_f=0.43 (1:1 Hex/EtOAc). ^1H NMR (300 MHz, $CDCl_3$) δ TMS: −0.13 (3H, s); 0.10 (3H, s); 0.82 (9H, s); 2.69 (1H, dd, *J*=6.5, 13.8 Hz); 2.82 (1H, dd, *J*=7.8, 13.7 Hz); 2.88 (1H, dd, *J*=2.6, 6.9 Hz); 3.05 (1H, dd, *J*=4.0, 6.9 Hz); 3.47 (3H, s); 3.73 (3H, s); 3.84 (3H, s); 3.89 (2H, br, D_2O exch.); 4.44 (1H, m); 5.21 (2H, s); 7.03 (1H, d, *J*=1.4 Hz); 7.11 (1H, d, *J*=1.4 Hz); 9.54 (1H, d, *J*=4.0 Hz). IR (NaCl, neat): 3466, 3381, 2954, 2857, 1718, 1586, 1437 cm^{-1}.

Intermediate imine (22). Major diastereomer **22**: R_f=0.49 (1:1 Hex/EtOAc). ^1H NMR (300 MHz, $CDCl_3$) δ TMS: 0.07 (3H, s); 0.18 (3H, s); 0.96 (9H, s); 2.29 (1H, dd, *J*=11.0, 12.9 Hz); 2.56 (1H, dd, *J*=5.9, 5.9 Hz); 2.86 (1H, d, *J*=6.0 Hz); 3.00 (1H, dd, *J*=4.3, 12.9 Hz); 3.46 (3H, s); 3.73 (3H, s); 3.87 (3H, s); 4.28 (1H, ddd, *J*=4.5, 6.0, 10.7 Hz); 5.22 (1H, d, *J*=6.9 Hz); 5.28 (1H, d, *J*=6.9 Hz); 7.36 (1H, d, *J*=1.4 Hz); 7.50 (1H, d, *J*=1.4 Hz); 8.09 (1H, s).

(1a*S*,9a*S*)-Dimethyl ester 9-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-1H-azirino[2,3-*c*][1]benzazocine-1,5-dicarboxylic acid (23). Major diastereomer **23**: $[\alpha]_D^{25}$=+48.9 (*c*=0.9, CH_2Cl_2). R_f=0.42 (1:1 Hex/EtOAc). ^1H NMR (300 MHz, $CDCl_3$) δ TMS: 0.15 (3H, s); 0.17 (3H, s); 0.94 (9H, s); 2.52 (2H, m); 2.84 (1H, dd, *J*=10.5, 13.9 Hz); 3.18 (1H, dd, *J*=5.3, 13.9 Hz); 3.46 (3H, s); 3.59 (1H, m); 3.67 (3H, s); 3.78 (1H, m); 3.84 (3H, s); 4.06 (1H, br, D_2O exch.); 4.47 (1H, ddd, *J*=5.3, 5.3, 5.3 Hz); 5.19 (1H, d, *J*=6.6 Hz); 5.23 (1H, d, *J*=6.6 Hz); 7.04 (1H, d, *J*=1.5 Hz); 7.18 (1H, d, *J*=1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: −5.1 (q), −4.9 (q), 18.4 (s), 25.8 (q), 31.0 (t), 41.4 (d), 43.0 (d), 47.3 (t), 52.0 (q), 53.3 (q), 56.3 (q), 69.0 (d), 94.3 (t), 105.3 (d), 114.3 (d), 118.3 (s), 129.4 (s), 148.3 (s), 156.2 (s), 163.9 (s), 166.8 (s). IR (NaCl, neat): 3394, 2952, 2855, 1724, 1587, 1438 cm^{-1}. Mass spectrum (ES+) *m/z*: 481 (M+H). Anal. for the mixture of diastereomers: calcd for $C_{23}H_{36}N_2O_7Si$: C, 57.48; H, 7.55; N, 5.83. Found: C, 57.77; H, 7.86; N 5.64.

Minor diastereomer **23**: $[\alpha]_D^{25}$=+127 (*c*=0.9, CH_2Cl_2). R_f=0.50 (1:1 Hex/EtOAc). ^1H NMR (300 MHz, $CDCl_3$) δ TMS: 0.10 (3H, s); 0.11 (3H, s); 0.92 (9H, s); 2.58 (1H, m); 2.66 (1H, dd, *J*=5.1, 6.9 Hz); 3.06 (1H, dd, *J*=8.7, 15.0 Hz); 3.12 (1H, d, *J*=4.5 Hz); 3.13 (1H, d, *J*=4.5 Hz); 3.45 (3H, s); 3.62 (1H, dd, *J*=3.6, 8.1 Hz); 3.66 (3H, s); 3.83 (1H, br, D_2O exch.); 3.84 (3H, s); 4.23 (1H, ddd, *J*=4.5, 4.5, 4.5 Hz); 5.16 (1H, d, *J*=6.6 Hz); 5.19 (1H, d, *J*=6.6 Hz); 7.07 (1H, d, *J*=1.5 Hz); 7.30 (1H, d, *J*=1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: −5.0 (q), −4.9 (q), 18.2 (s), 25.7 (q), 33.5 (t), 40.2 (d), 48.2 (t), 48.4 (d), 52.0 (q), 53.4 (q), 56.2 (q), 72.9 (d), 94.6 (t), 107.7 (d), 115.6 (d), 123.3 (s), 129.0 (s), 149.3 (s), 156.4 (s), 163.3 (s), 166.8 (s). IR (NaCl, neat): 3387, 2953, 2856, 1724, 1585, 1437 cm^{-1}. Mass spectrum (ES+) *m/z*: 481 (M+H).

(1a*S*,9a*S*)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 1,5 dimethyl ester 9-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-1H-azirino[2,3-*c*][1]benzazocine-1,3,5(2H)-tricarboxylic acid. Compound **23** (140 mg, 290 μmol, 1.0 equiv.) and 3.5 mL of CH_2Cl_2 were added to a 25 mL conical flask. The solution was stirred for 3 min when *N,N*-diisopropylethylamine (152 μL, 870 μmol, 3.0 equiv.), 6-nitroveratryl chloroformate (200 mg, 730 μmol, 2.5 equiv.), and DMAP (36 mg, 290 μmol, 1.0 equiv.) were added. After 4 h, TLC analysis (1:1 Hex/EtOAc) of the reaction showed no starting material. The reaction was diluted with 15 mL sat $NaHCO_{3(aq)}$ and extracted with 3×EtOAc. The combined organic layers were washed with 1×sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated, and purified using radial silica gel PTLC (2:1 Hex/EtOAc, 2 mm plate) to give 185 mg (88% yield) of product as a clear yellow oil.

Major diastereomer: $[\alpha]_D^{25}$=+26.9 (*c*=1.2, CH_2Cl_2). R_f=0.40 (1:1 Hex/EtOAc). ^1H NMR (300 MHz, d_6-DMSO, 383 K) δ TMS: 0.12 (3H, s); 0.14 (3H, s); 0.88 (9H, s); 2.68 (2H, br); 2.88 (2H, s); 2.93 (1H, br); 3.07 (1H, br); 3.46 (3H, s); 3.62 (3H, s); 3.80 (3H, br); 3.86 (3H, s); 3.87 (3H, s); 4.34 (1H, br); 5.29 (2H, s); 5.41 (2H, s); 6.92

(1H, s): 7.46 (1H, d, J=1.5 Hz); 7.63 (1H, d, J=1.5 Hz); 7.65 (1H, s). IR (NaCl, neat): 2953, 2856, 1726, 1581, 1522, 1440 cm^{-1}. Mass spectrum (ES+) m/z (relative intensity): 720 (M+H) (100%). Exact mass: (FAB) calcd for $C_{33}H_{46}N_3O_{13}Si$ 720.2799. Found: 720.2786. Anal. calcd for $C_{33}H_{45}N_3O_{13}Si$: C, 55.06; H, 6.30; N, 5.84. Found: C, 54.93; H, 6.48; N, 5.66.

(1a*S*,9a*S*)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 5-methanol-9-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-1H-azirino[2,3-*c*][1]benzazocine-3-carboxylic acid (24). Nitroveratryl carbamate described above (76 mg, 0.105 mmol, 1.0 equiv.) and 1.5 mL CH_2Cl_2 were added to a 25 mL round bottom flask. The stirred solution was cooled to −78°C on a CO_2/acetone bath for 10 min when 1.0 M DIBAL in hexane (528 μL, 0.528 mmol, 5.5 equiv.) was added in dropwise portions with 5 min between each addition. After 5 h, the reaction was quenched at −78°C by the addition of one drop of MeOH and two drops of sat $NaCl_{(aq)}$. After removing from the bath and coming to room temperature, the solution was filtered through a short plug of Celite with CH_2Cl_2. The two layers were separated, and the aqueous layer was extracted with 3×CH_2Cl_2. The combined organic layers were dried over Na_2SO_4. The solution was filtered, concentrated, and purified by radial silica gel PTLC (2 mm plate, 22:1 CH_2Cl_2/MeOH) to give 39 mg (61% yield) of aziridine **24** as a clear yellow oil.

Major diastereomer: $[\alpha]_D^{25}$=+25.4 (c=2.6, CH_2Cl_2). R_f=0.41 (10:1 CH_2Cl_2/MeOH). ^1H NMR (300 MHz, d_6-DMSO, 373 K) δ TMS: 0.12 (3H, s); 0.13 (3H, s); 0.91 (9H, s); 2.02 (2H, s); 2.86 (2H, s); 2.93 (2H, s); 3.45 (3H, s); 3.83 (3H, s); 3.87 (3H, s); 4.28 (1H, s); 4.46 (2H, s); 4.71 (1H, s, D_2O exch.); 5.19 (1H, d, J=6.6 Hz); 5.22 (1H, d, J=6.6 Hz); 5.41 (2H, s); 6.81 (1H, s); 7.03 (2H, s); 7.66 (1H, s). IR (NaCl, neat): 3368, 2954, 2856, 1713, 1582, 1524, 1441 cm^{-1}. Mass spectrum (ES+) m/z (relative intensity): 634 (M+H, 100%). Exact mass: (FAB) calcd for $C_{30}H_{44}N_3O_{10}Si$: 634.2796. Found: 634.2760. Anal. calcd for $C_{30}H_{43}N_3O_{10}Si$: C, 56.85; H, 6.84; N, 6.63. Found: C, 56.53; H, 7.07; N, 6.37.

(1a*S*,9a*S*)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 5-methanol-1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-9-hydroxy-1H-azirino[2,3-*c*][1]benzazocine-3-carboxylic acid. Compound **24** (50 mg, 0.079 mol, 1.0 equiv.) and 1 mL THF were added to a 10 mL conical flask. The solution was stirred for 5 min on an ice bath when 1.0 M TBAF in THF (135 μL, 0.135 mmol, 1.65 equiv.) was added dropwise over 1 min. After the addition was complete, the reaction was allowed to warm to room temp. After 4 h, TLC analysis (10:1 CH_2Cl_2/MeOH) showed no sign of starting material. The reaction was diluted with water, and the THF was removed in vacuo. The aqueous solution was extracted with 3×EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, concentrated, and purified by radial silica gel PTLC (2 mm plate, 10:1 CH_2Cl_2/MeOH) to give 35 mg (85% yield) of the diol as a foamy yellow oil. The unstable diol was immediately taken on to the next step without further purification.

Major diastereomer: R_f=0.23 (10:1 CH_2Cl_2/MeOH). IR (NaCl, neat): 3429 br, 3314 br, 2928, 2854, 1704, 1581, 1524, 1440 cm^{-1}. Mass spectrum (ES+) m/z (relative intensity): 520 (M+H, 100%).

(1a*S*,9a*S*)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 1-methyl ester 5-methanol-1a,2,3,8,9,9a-hexahydro-7-(methoxymethoxy)-9-hydroxy-1H-azirino[2,3-*c*][1]benzazocine-1,3(2H)-dicarboxylic acid (25). The diol described above (15 mg, 29 μmol, 1.0 equiv.) and 300 μL pyridine were added to a 10 mL flask. After stirring for 5 min, *N*-((methoxy)carbonyloxy)succinimide (5 mg, 29 μmol, 1.0 equiv.) was added in one portion. After 2.5 h, TLC analysis (10:1 CH_2Cl_2/MeOH) showed complete loss of starting material, and the reaction was diluted with water and sat $NH_4Cl_{(aq)}$. The aqueous solution was extracted with 3×EtOAc. The combined organic layers were washed with 1×sat $NaHCO_{3(aq)}$ and 1×sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated, and purified by radial silica gel PTLC (10:1 CH_2Cl_2/MeOH, 2 mm plate) to give 14 mg (89% yield from **24**) of **25** as a foamy yellow oil.

Major diastereomer: $[\alpha]_D^{25}$=+30.6 (c=1.5, CH_2Cl_2). R_f=0.38 (10:1 CH_2Cl_2/MeOH). ^1H NMR (300 MHz, d_6-DMSO, 378 K) δ TMS: 2.64 (2H, s); 2.84 (2H, s); 2.91 (2H, s); 3.44 (3H, s); 3.62 (3H, s); 3.78 (3H, s); 3.85 (3H, s); 4.06 (1H, s); 4.41 (1H, br, D_2O exch.); 4.45 (2H, s); 4.72 (1H, D_2O exch.); 5.21 (2H, s); 5.37 (2H, s); 6.81 (1H, s); 6.90 (1H, s); 7.03 (1H, s); 7.65 (1H, s). IR (NaCl, neat): 3741, 2954, 2852, 1731, 1715, 1614, 1582, 1520, 1442 cm^{-1}. Mass spectrum (ES+) m/z (relative intensity): 578 (M+H, 100%). Exact mass: (FAB) calcd for $C_{26}H_{32}N_3O_{12}$: 578.1986. Found: 578.1954. Anal. calcd for $C_{26}H_{32}N_3O_{12}\cdot 0.6H_2O$: C, 53.07; H, 5.51; N, 7.14. Found: C, 53.39; H, 5.71; N, 6.75.

(1a*S*-*cis*)-3-[(4,5-Dimethoxy-2-nitrophenyl)methyl] 1-methyl ester 5-formyl-1a,8,9,9a-tetrahydro-7-(methoxymethoxy)-9-oxo-1H-azirino[2,3-*c*][1]benzazocine-1,3(2H)-dicarboxylic acid (26). Diol **25** (35 mg, 62 μmol, 1.0 equiv.) and 600 μL CH_2Cl_2 were added to a 10 mL conical flask. The solution was stirred for 5 min when Dess–Martin periodinane[16] (68 mg, 160 μmol, 2.6 equiv.) was added in one portion. The reaction immediately became cloudy and white. After 1.5 h, additional amounts of Dess–Martin reagent (55 mg) and 150 μL CH_2Cl_2 were added to the reaction. After another 0.5 h, TLC analysis (10:1 CH_2Cl_2/MeOH) showed no sign of staring material. The reaction was diluted with Et_2O and added to a solution of sat $NaHCO_{3(aq)}$ and $Na_2S_2O_3\cdot 5H_2O$ (123 mg, 8 equiv.). The biphasic mixture was vigorously stirred for 15 min. The organic layer was diluted with EtOAc and separated from the aqueous layer. The organic layer was washed with 1×sat $NaHCO_{3(aq)}$ and 1×H_2O. The combined aqueous layers were back extracted with 2×EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, concentrated, and purified using radial silica gel PTLC (2 mm plate, 2:1 CH_2Cl_2/Et_2O) to give 28 mg (83% yield) of ketone **26** as a clear foamy oil.

Major diastereomer: $[\alpha]_D^{25}$=−40.3 (c=1.3, CH_2Cl_2). R_f=0.80 (10:1 CH_2Cl_2/MeOH). ^1H NMR (300 MHz, d_6-DMSO, 378 K) δ TMS: 2.91 (4H, s); 3.37 (1H, s); 3.38 (1H, s); 3.44 (3H, s); 3.60 (3H, s); 3.76 (3H, s); 3.85 (3H, s); 5.34 (4H, m); 6.82 (1H, s); 7.27 (1H, s); 7.58 (1H, s); 7.63

(1H, s); 9.93 (1H, s). IR (NaCl, neat): 2954, 2847, 1729, 1702, 1581, 1521, 1443 cm^{-1}. UV λ_{max} (CH_3CN) nm (ϵ): 345 (6800), 298 (7740), 238 (18 500). Mass spectrum (FAB) *m/z* (relative intensity): 574 (M+H, 100%). Exact mass: (FAB) calcd for $C_{26}H_{28}N_3O_{12}$: 574.1673. Found: 574.1702.

(2*S*)-Methyl ester [6-formyl-2,3-dihydro-1-hydroxy-8-(methoxymethoxy)-1H-pyrrolo[1,2-*a*]indol-2-yl]-carbamic acid (30). Ketone **26** (15 mg, 26 μmol, 1.0 equiv.), 3 mL CH_3CN, and 1 mL H_2O were added to a 5 mL pyrex tube. The test tube was placed in a 50 mL pyrex test tube. The 50 mL tube was stoppered and placed in a Rayonet™ photochemical reactor and exposed to 350 nm light. Over the course of the reaction, the solution slowly turned dark orange. After 24 h, the reaction mixture was removed from the photo reactor, and the CH_3CN was removed in vacuo. The resulting aqueous solution was diluted with water and extracted with 3×EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The orange residue was purified by PTLC to yield 3.0 mg of **30** (38% yield) as brown solids in a 1:1 mixture of isomers. The stereochemistry was tentatively assigned by ^1H NMR correlation with similar diastereomers.[21]

trans-Diastereomer: $[\alpha]_D^{25}=+15.2$ (c=0.25, CH_2Cl_2). R_f=0.42 (20:20:1 CH_2Cl_2/Et_2O/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ TMS: 2.34 (1H, br, D_2O exch.); 3.52 (3H, s); 3.74 (3H, s); 3.92 (1H, dd, J=8.0, 10.0 Hz); 4.55 (1H, dd, J=8.0, 10.0 Hz); 4.88 (1H, m); 5.19 (1H, d, J=4.8 Hz); 5.34 (1H, d, J=6.6 Hz); 5.37 (1H, d, J=6.6 Hz); 5.70 (1H, d, J=7.2 Hz); 6.65 (1H, s); 7.23 (1H, d, J=1.2 Hz); 7.41 (1H, d, J=1.2 Hz); 9.88 (1H, s). IR (NaCl, neat): 3354, 2956, 2923, 1716, 1682, 1558, 1538, 1456 cm^{-1}. ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 47.9 (t), 52.6 (d), 56.3 (q), 56.5 (q), 66.6 (d), 77.1 (s), 94.1 (d), 94.5 (t), 102.2 (d), 109.4 (d), 127.8 (s), 132.3 (s), 133.5 (s), 144.9 (s), 151.2 (s), 192.0 (d). Mass spectrum (ES+) *m/z* (relative intensity): 335 (M+H, 100%). Exact mass: (FAB) calcd for $C_{16}H_{19}N_2O_6$: 335.1243. Found: 335.1229.

cis-Diastereomer: $[\alpha]_D^{25}=-21.6$ (c=0.25, CH_2Cl_2). R_f=0.27 (20:20:1 CH_2Cl_2/Et_2O/MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ TMS: 2.81 (1H, br, D_2O exch.); 3.53 (3H, s); 3.73 (3H, s); 3.93 (1H, m); 4.61 (2H, m); 5.06 (1H, br, D_2O exch.); 5.22 (1H, d, J=3.9 Hz); 5.35 (2H, s); 6.64 (1H, s); 7.25 (1H, d, J=1.2 Hz); 7.45 (1H, d, J=1.2 Hz); 9.93 (1H, s). IR (NaCl, neat): 3332, 2923, 2852, 1704, 1682, 1568, 1532, 1455 cm^{-1}. ^{13}C NMR (75 MHz, $CDCl_3$) δ TMS: 48.4 (t), 52.7 (d), 56.3 (q), 63.3 (q), 74.2 (d), 77.1 (s), 94.0 (d), 94.9 (t), 103.3 (d), 108.5 (d), 128.7 (s), 132.4 (s), 133.6 (s), 145.2 (s), 151.2 (s), 191.8 (d). Mass spectrum (ES+) *m/z* (relative intensity): 335 (M+H, 100%). Exact mass: (FAB) calcd for $C_{16}H_{19}N_2O_6$: 335.1243. Found: 335.1244.

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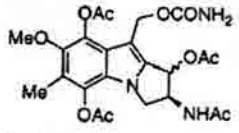
19. A control experiment where, incubation of **29** in the dark for 24 h in 3:1 CH_3CN/H_2O at room temperature led to no detectable loss of the starting material.

20. From this point forward, only the major diastereomer series was carried through.

21. The stereochemistry of tetraacetates *i* and *ii* has previously been determined: Egbertson, M. S. PhD Dissertation, Yale University, 1989.



i, cis

ii, trans

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

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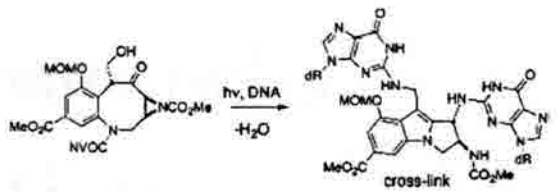
ABSTRACT



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

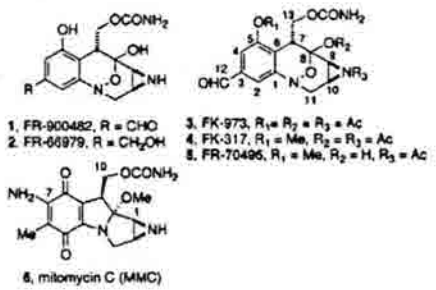


Figure 1.

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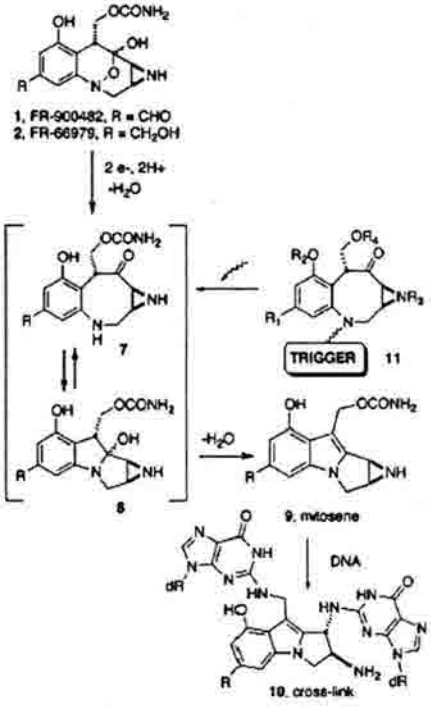
activity in human clinical trials. FK317 is now in advanced human clinical trials in Japan[6] and holds significant promise for replacing the structurally related and widely used antitumor drug mitomycin C (MMC, **6**).[8] Additionally, FK317 does not induce vascular leak syndrome, a serious side effect 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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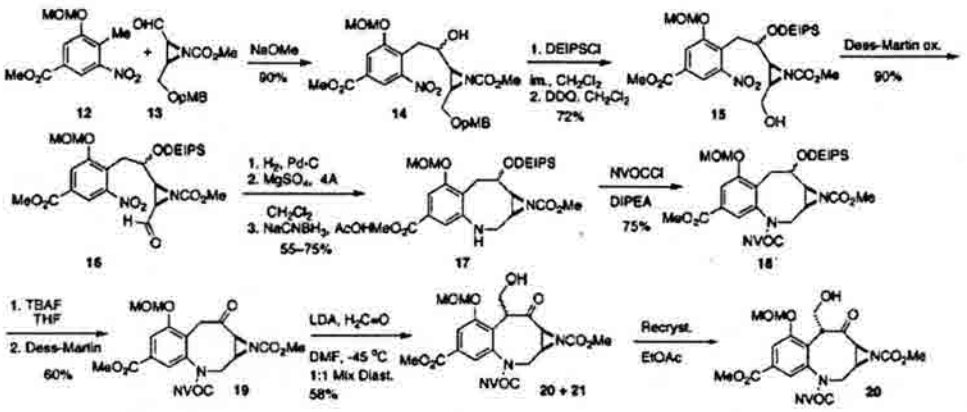
Scheme 2



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

(17) Rodriguez, L.; Lu, N.; Yang, N.-L. *Synlett* **1990**, 227.
(18) Cech, T. R. *Biochemistry* **1981**, *20*, 1431.

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

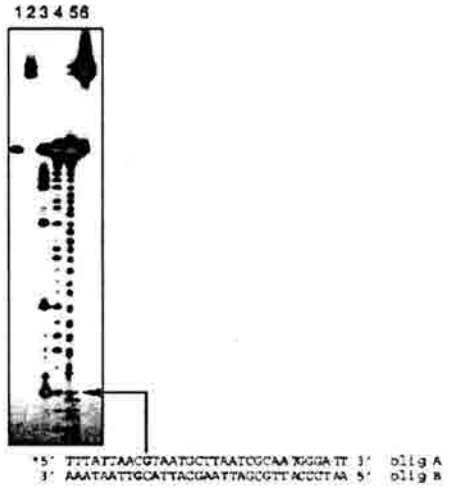
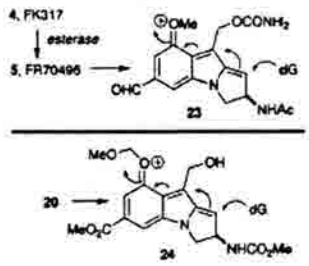


Figure 3. Autoradiogram of Fe(II)−EDTA footprinting of cross-linked A/B duplex (labeled at the 5′-terminus of oligo A). Lane 1: DNA standard (oligo A). Lane 2: cross-linked **1**. Lane 3: Maxam−Gilbert G. Lane 4: Maxam−Gilbert G + A. Lane 5: 1 mM Fe(II)−EDTA digestion of oligo A (control). Lane 6: 1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

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**.

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

Scheme 3



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.

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.

Acknowledgment. This work was supported by the National Institutes of Health (CA51875). Mass spectra were obtained on instruments supported by the NIH Shared Instrumentation Grant GM49631. We are indebted to Fujisawa Pharmaceutical Company of Japan for the gift of authentic FR-900482. We also acknowledge Fellowship support from Boehringer-Ingelheim (to T.C.J.).

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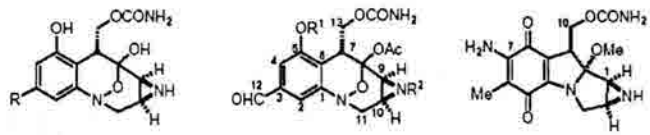
(19) The faster mobility of the cross-linked DNA at the 10 μM concentration (lane 6, Figure 1) relative to that for the 1 μM concentration (lane 7 Figure 1) reaction is attributed to extensive (multiple) cross-links at the higher concentration.

Concise Enantioselective Synthesis of (+)-FR66979 and (+)-FR900482: Dimethyldioxirane-Mediated Construction of the Hydroxylamine Hemiketal**

Ted C. Judd and Robert M. Williams*

Dedicated to Professor Albert I. Meyers on the occasion of his 70th birthday.



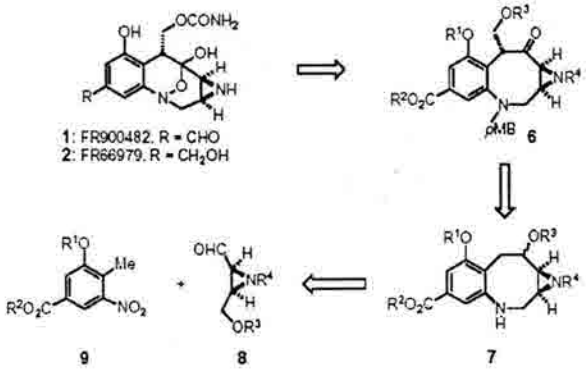
1: FR900482, R = CHO
2: FR66979, R = CH_2OH
3: FK973, $R^1 = R^2 = Ac$
4: FK317, R^1 = Me, R^2 = Ac
5: mitomycin C

Scheme 1. Structures of FR900482 (1) and congeners.

The antitumor antibiotic natural products FR900482 (1) and FR66979 (2) were isolated from *Streptomyces sandaensis* No. 6897 by the Fujisawa Pharmaceutical Co. in 1987.[1] Both compounds have been shown to crosslink DNA preferentially at 5'CpG3' steps in the minor groove following reductive activation.[2-4] Additionally, recent studies from our laboratory have demonstrated that FR900482 (1) and FK317 (4) crosslink the minor groove-binding HMGA1 oncoprotein to DNA in vivo, which has very significant implications for the mode of action of these agents.[5] Both FK973 (3)[6] and FK317 (4),[7] semisynthetic derivatives of FR900482 (1), have shown highly promising antitumor activity in human clinical trials in Japan[6] and hold significant promise to replace the structurally related and widely used antitumor drug mitomycin C (5).[8] Notably, FK317 (4) has been shown not to induce vascular leak syndrome (VLS), a highly detrimental side effect observed in human clinical trials with the natural products FR900482 (1), FR66979 (2), and the semisynthetic derivative FK973 (3). The mechanistic basis for the anomalous difference between 1 and 4 in causing VLS has been revealed at a biochemical level, but the structural and chemical basis for these very important phenomena remains unclear (Scheme 1).[5b]

In conjunction with these studies, research from our laboratories has focused on a concise enantioselective total synthesis of the natural products 1 and 2 that would be amenable to the preparation of biologically useful analogues. To date, there have been three total syntheses of FR900492 (1) reported;[9] of these, only one was asymmetric, but required a 57-step sequence.[9c-e] Additionally, a formal total synthesis was disclosed recently, applicable to an enantioselective variation.[10] In addition to the above-mentioned syntheses, several other synthetic approaches have been reported since the isolation of 1.[11] Herein, we describe a concise, enantioselective total synthesis of 1 and 2. This sequence is the shortest total synthesis of 1 and 2 reported to date and features a new method to construct the hydroxylamine hemiketal ring system unique to this family of natural substances.

Our approach to the construction of 1 and 2 was predicated on two bold strategies: 1) the labile aziridine would be installed in the very beginning and carried intact to the end, and 2) a simultaneous oxidative deprotection of an eight-membered-ring aminoketone 6 was envisioned to form the hydroxylamine hemiketal functionality in a single operation (Scheme 2). Ketone 6 could in turn be obtained from the eight-membered-ring amine 7, which would ultimately be derived from the coupling of the optically active aziridine 8 and the trisubstituted nitrobenzene 9.



1: FR900482, R = CHO
2: FR66979, R = CH_2OH
6
9
8
7

Scheme 2. Retrosynthesis of FR900482 (1) and FR66979 (2). *p*MB = *p*-methoxybenzyl.

Aziridine 14 was prepared according to a recent report from this laboratory in 21 steps from the commercially available reagents 10 and 12 (Scheme 3).[12,13]

Reaction of 14 with *p*-methoxybenzyl bromide, followed by removal of the DEIPS group with TASF in DMF/H_2O[14] and subsequent oxidation with Dess–Martin periodinane[15] afforded ketone 15, which corresponds to 6. Treatment of 15 with LDA in dry DMF at −45 °C followed by the addition of an anhydrous formaldehyde solution in THF[16] furnished the aldol adducts 16 and 17 as a ~1:1 mixture of diastereomers in 50% yield (45% recovery of unreacted starting material). Separation of 16 and 17 by preparative thin-layer chromatography (PTLC) followed by treatment of the undesired adduct 17 with DBU in toluene afforded a 2.5:1 mixture of epimers favoring 16, which has the desired configuration at C7. Treatment of the primary alcohol of 16 with TBSOTf and

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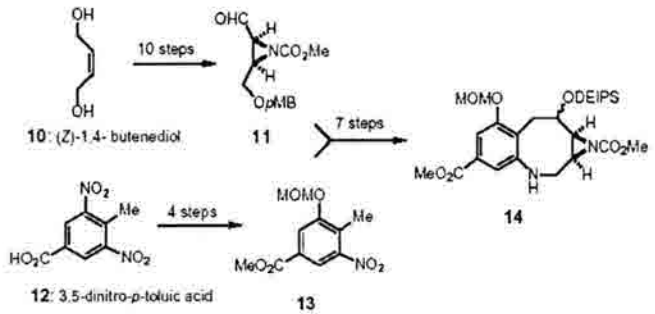
[**] This work was supported by the National Institutes of Health (Grant CA51875). We are grateful to Boehringer-Ingelheim for fellowship support to T.C.J. Mass spectra were obtained on instruments supported by the National Institutes of Health Shared Instrumentation Grant GM49631. We are grateful to Fujisawa Pharmaceutical Co., Japan, for the generous gift of natural FR900482.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author. Spectroscopic data for all new compounds is included.



Scheme 3. Synthesis of aziridine precursor **14**.[12]

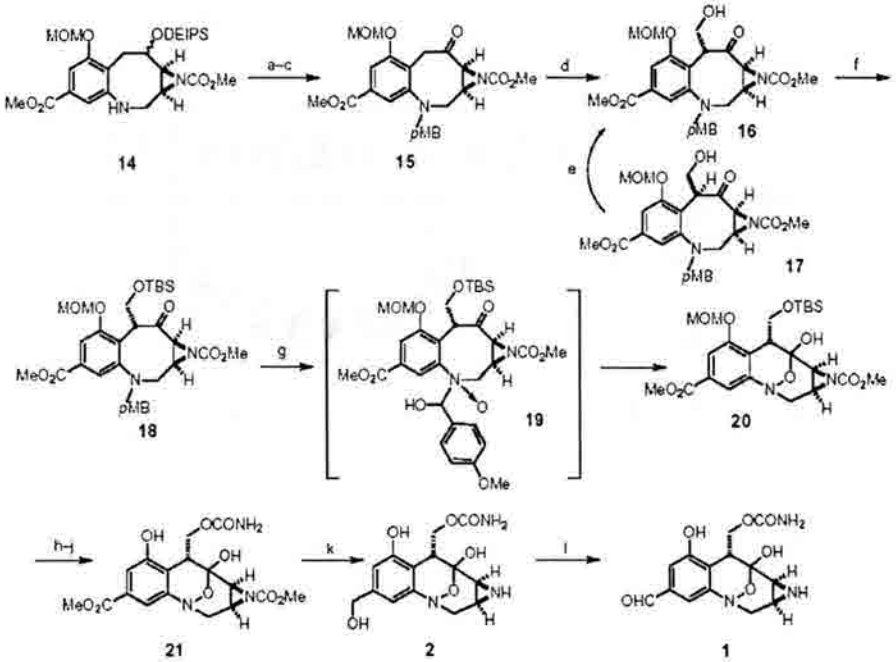
2,6-lutidine gave the silyl ether **18** in essentially quantitative yield (Scheme 4).

For the construction of **20**, a one-step protocol was employed that both cleaved the *N*-*p*-methoxybenzyl residue and oxidized the amine to the corresponding hydroxylamine, thus forming the desired hydroxylamine hemiketal. Reaction of **18** with excess dimethyldioxirane (DMDO)[17] in a 1:1 mixture of CH_2Cl_2 and saturated aqueous K_2CO_3 furnished **20** as the only isolated product in 30–50% yield, along with recovered starting material (40–50%). Attempts to drive this reaction to completion by varying the stoichiometry of DMDO, time, temperature, etc., proved unsuccessful. The very clean nature of this reaction allowed the practical recycling of recovered **18**.[18]

The mechanism for the formation of **20** from **18** is presumed to involve initial insertion of the dioxirane into the C–H bond of the *N*-*p*-methoxybenzyl methylene residue to form a methanolamine species.[19] The hydroxy group of the methanolamine is invoked to direct the DMDO oxidation of the amine to the corresponding *N*-oxide species **19**.[20] Subsequent collapse of the methanolamine with concomitant loss of *p*-anisaldehyde and transannular closure of the incipient hydroxylamine on the ketone furnishes **20**.[21]

Removal of the TBS protecting group followed by reaction of the primary hydroxy group with trichloroacetyl isocyanate (methanol/silica gel workup)[22] installed the urethane moiety at C13. TMSBr effected removal of the methoxymethyl ether (MOM) in the presence of the acid-sensitive aziridine functionality at −45 °C over 3 h to afford **21** in 60% yield.[23]

Final reduction of both carbomethoxy groups with $LiBH_4$/MeOH in THF[24] followed by Pd-catalyzed cleavage of the resulting borane amine complex,[25] furnished the natural product FR66979 (**2**) in 78% yield. Synthetic **2** was identical to the natural substance (^1H NMR spectra, mobility on TLC, mass spectra (ES^+), optical rotation, and IR spectra). Finally, the natural product FR900482 (**1**) can obtained by Swern oxidation of **2** in 33% yield.[26,27]



Scheme 4. Synthesis of **1** and **2**. Reagents and conditions: a) *p*-methoxybenzyl bromide, iPr_2NEt, CH_2Cl_2 (86%); b) TASF, DMF/H_2O, room temperature; c) Dess–Martin oxidation (75%, 2 steps); d) LDA, DMF, −45 °C; CH_2O/THF, −45 °C, (50%; **16**/**17** 1:1); e) DBU, toluene (70% + 30% starting material); f) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78→0 °C (96%); g) DMDO, aqueous K_2CO_3/CH_2Cl_2, 0 °C→RT (30–50%); h) TBAF, THF, 0 °C (92%); i) $Cl_3CCONCO$, CH_2Cl_2, 0 °C; then MeOH, silica gel, room temperature (86%); j) TMSBr, CH_2Cl_2, −45 °C (60%); k) $LiBH_4$/MeOH, THF, room temperature (78%); l) $(COCl)_2$, Me_2SO, THF, −78→−40 °C; then Et_3N (33%). DEIPS = diethylisopropylsilyl, TASF = tris(dimethylamino)sulfonium difluorotrimethylsilicate, DMF = *N*,*N*-dimethylformamide, LDA = lithium diisopropylamide, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, TBSOTf = *tert*-butyldimethylsilyl triflate, DMDO = dimethyldioxirane, TBAF = tetrabutylammonium fluoride, TMS = trimethylsilyl.

The chemistry described herein represents the most concise total synthesis of either (+)-FR66979 (**2**) or (+)-FR900482 (**1**) reported to date.[28] Future efforts in the preparation and biological evaluation of synthetic analogues are currently underway and will be reported in due course.

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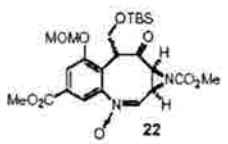
[18] In the absence of the saturated aqueous base, exclusive formation of nitrone **22** was the only product observed. Alternative oxidation systems resulted in no reaction (*m*-chloroperoxybenzoic acid, Davis' reagent) or direct oxidative cleavage of the *p*-methoxybenzyl group to a mitosene ($[VO(acac)_2]$ or $[Mo(CO)_6]$ and *t*BuOOH; $[MeReO_3]$ and H_2O_2).



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[27] A previous report (see reference [9c]) demonstrated the successful oxidation of monoacetylated FR66979 by Swern oxidation.

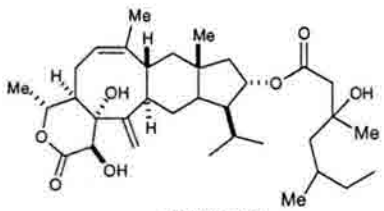
[28] After submission of our manuscript, we recently learned that Professor Tohru Fukuyama and co-workers (Tokyo University) and Prof. Marco Ciufolini and co-workers (Université Claude Bernard Lyon 1) have independently completed syntheses of FR900482 and FR66979, respectively. We appreciate receiving preprints of their manuscripts. See the following communications: M. Suzuki, M. Kambe, H. Tokuyama, T. Fukuyama, *Angew. Chem.* **2002**, *114*, 4880; *Angew. Chem. Int. Ed.* **2002**, *41*, 4686; R. Ducray, M. A. Ciufolini, *Angew. Chem.* **2002**, *114*, 4882; *Angew. Chem. Int. Ed.* **2002**, *41*, 4688.

Appendix 3

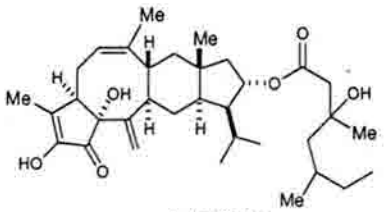
Research Proposal

Specific Aims

The primary objective of the proposed research includes the total synthesis of the natural products YW3548 (**1**) and YW3699 (**2**) in a manner amiable to the synthesis of biologically significant analogs. Both the conformation of the structure and the absolute stereochemistry will be determined.

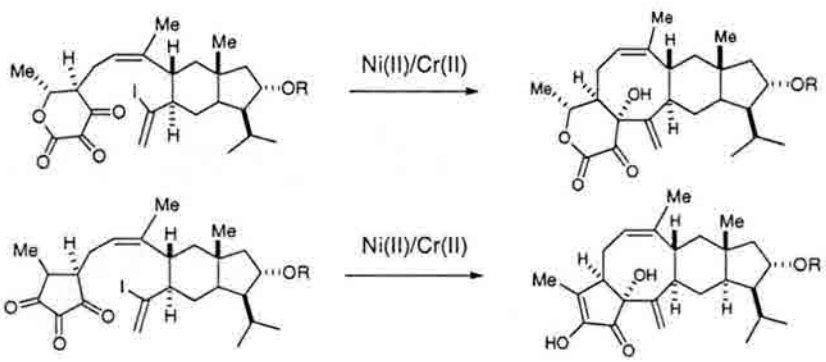


1. YW3548



2. YW3699

The proposed synthetic strategy includes a key Ni(II)/Cr(II)-mediated cyclization by reaction of a vinyl iodide with an electronically activated ketone. The goal of this synthetic sequence is to expand the general utility of this reaction to include a broader range of possible substrates following the recent advancements that have been established in the reaction conditions.



In addition, the synthesis includes the methodological development for the stereoselective construction of the challenging *(Z)* trisubstituted olefin functionality. In connection with the proposed synthesis, the development of a catalytic asymmetric version of the Ni(II)/Cr(II) reaction is planned.

Background and Significance

The natural products YW3548 and YW3669 were isolated from the fungus *Paecilomyces inflatus* and *Codinaea simplex* in 1998 following screening for inhibitors of glycosylphosphatidylinositol (GPI)-anchoring in yeast[1]. Both natural products are described as having an unusual tricarbocyclic sesterterpenoid skeleton with YW3548 additionally possessing a δ-lactone. The relative stereochemistry of the tetracyclic core for both natural products was determined by spectroscopic means including FAB-MS, ESI-HR-MS, ^{1}HNMR, ^{13}CNMR, DQ-COSY, ROESY, HSQC, and HMBC. However, the stereochemistry of the 3-hydroxyl-3,5-dimethyl-heptanoic fragment along with the overall absolute stereochemistry remains undetermined.

The biological significance of these natural products lies in their ability to inhibit GPI-anchor biosynthesis.[2] GPI-anchors are used as an alternative way to anchor proteins to cell membranes in eukaryotes, and are more commonly employed by protozoan over higher eukaryotes.[2] In addition to this function, GPIs play a broader role in various aspects of signal transduction, the extent of which is only recently being understood. One particular example of clinical significance is the role of the prevalent GPIs in parasitic protozoa in inducing the release of tumor necrosis factor α (TNF-α) in their hosts.[2] High levels of TNF-α have been linked to the damaging and fatal symptoms during malaria infection, and have been directly attributed to the parasitic toxin, malarial GPI.[2] In this regard, the development of compounds that selectively inhibit the biosynthesis of GPIs in protozoan over higher eukaryotes remains an important goal. Interestingly, the GPI-anchor consists of a conserved core structure among all eukaryotes; this includes an

ethanolamine phosphate attached to three sequential mannose rings followed by a glucosamine attached to a phosphatidylinositol (EtN-PO_4-Man-α-(1-2)-Man-α-(1-6)-Man- α-(1-4)-Glc*N*-Ino-PO_4-CH_2-lipid). However, further additions including the attachment of other glycosides or ethanolamine phosphate to the mannoside rings differ between species.[2]

Inhibitors of GPI synthesis, remains an important tool in elucidating the biosynthetic pathway for discovering potential drug targets. Several known compounds have been successfully utilized in this manner, resulting in increased knowledge of GPI synthesis.[2] The natural products YW3548 and YW3699 have been demonstrated to inhibit GPI biosynthesis in mammalian, yeast, and certain fungi.[3] Specifically, the compounds inhibit the addition of the third mannose prior to the attachment of the ethanolamine phosphate in the common structure.[3] However this inhibition was not seen in various protozoan species and demonstrates a subtle, but important difference, in the GPI synthesis with these lower eukaryotes. The inhibitory activity of the two natural products differed significantly, YW3699 showing a 1000 fold decrease in activity compared to YW3548.[1b] Likewise, small changes in the core skeleton of YW3548 by semi-synthesis, resulted in a similar decrease in activity.[1a, 3] The exact reason for the discrepancy in activity between the protozoa and other eukaryotes is not known. Initially, the possible differences in the acyl groups on the inositol portion between species was thought to provide a different presentation to the GPI α-1,2 mannosyltransferase, one in which the natural products better mimics.[3] An alternative explanation is based on the observation of an ethanolamine phosphate moiety attached to the primary mannose ring of the GPI in yeast and mammalian cells, but not in protozoa. The natural products YW3699 and YW3548 may

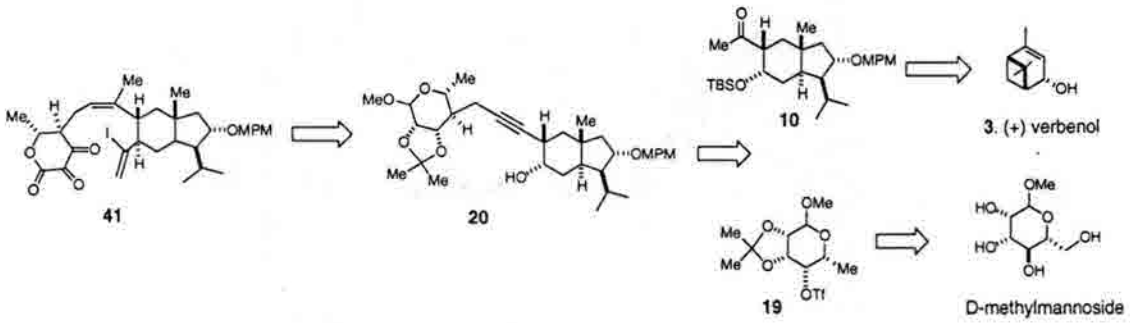
inhibit the addition of this ethanolamine phosphate, which may be necessary prior to the addition of the third mannoside.[2]

In place of the ethanolamine phosphate, many species of parasitic protozoa attach glycosidic units (consisting of galactose and/or *N*-acetyl galactose) to the primary mannose ring.[2] During what stage of the biosynthesis this is accomplished is unknown, but may occur after the addition of the third mannoside. The possible inhibition of these modifications may lead to potential drug targets. Analogs of the natural product YW3548 and YW3699 may provide possible probes for this purpose. Specifically, the additional incorporation of highly oxygenated rings, similar to the δ-lactone in YW3548, may provide mimics of later stage intermediates in the biosynthetic path. Inhibition of these later stages may also provide new information on the biosynthetic pathway of GPIs in protozoa, and valuable information for clinical studies. A concise total synthesis of both natural products that can be readily adapted to incorporate these functionalities will make this possible.

In a broader context, the development of the Ni(II)/Cr(II)-mediated reaction[4] for the use with ketones in structurally complex intermediates will find great value in the synthesis of bioactive molecules. The multiple use of this reaction in the synthesis of the clinically significant halichondrin natural products by the sponsor's group stands as an example of this idea.[4] In this connection, the development of an asymmetric version of the reaction, catalytic in Cr(II), would be highly desirable for synthesis of complex molecules for clinical investigation. The diminished quanities of the pharmacologically active Cr(II) salts in a catalytic process would make the reaction more applicable for this purpose; this concept has recently been noted.[5]

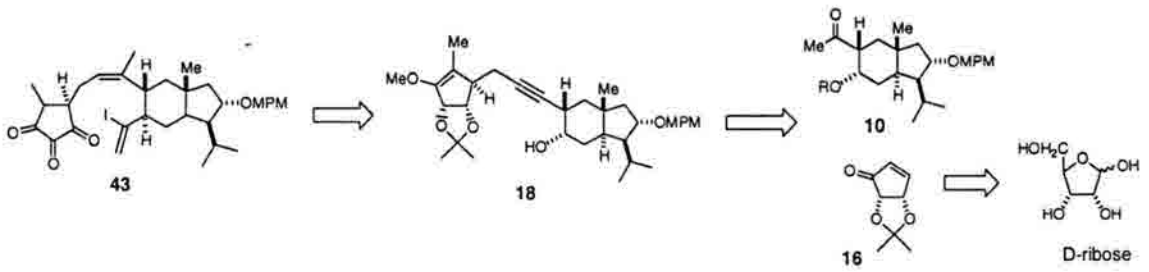
30b. Research Design and Methods

The overall synthetic scheme for the total synthesis of YW3548 and YW3699 focuses on construction of the tricarbonyl precursors for the key Ni(II)/Cr(II) mediated ring-forming reaction (Schemes 1 and 2).



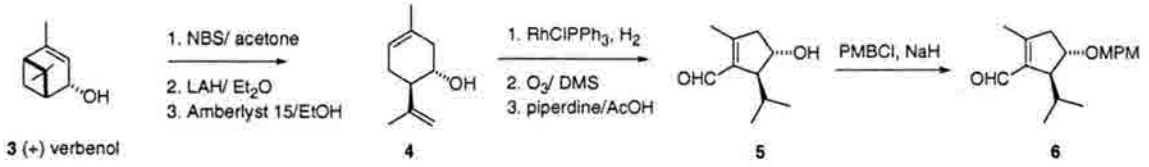
Scheme 1. Retrosynthesis Scheme of Tricarbonyl Intermediate for YW3548.

The retro-synthetic analysis demonstrates that both of the target compounds can be derived from a common intermediate ketone **10**, which can ultimately be derived in optically pure form from (+) verbenol (**3**). The tricarbonyl fragment for each precursor can be derived from the appropriate commercially available carbohydrate, and subsequently coupled to the aforementioned methyl ketone **10**. For the construction of the *(Z)* trisubstituted olefin from alkyne **20**, the development of new methodology is planned based on reported metallo-mediated carboaluminations. Several potential methods may be explored for the formation of this difficult bond with both stereo and regio-control. The proposed synthetic strategy outlined should provide both natural products in a concise manner along with high degree of stereocontrol.

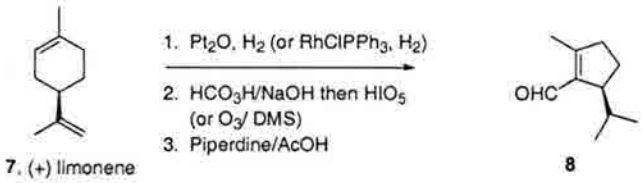


Scheme 2. Retrosynthetic Scheme of Tricarbonyl Intermediate for YW3699.

Optically pure (+)-*trans*-*p*-mentha-1,8-dien-5-ol **4** is available from (+) verbenol (**3**) (derived in two steps from commercially available (+) α-pinene (99% ee)[6]) following the reported synthesis of its antipode (Scheme 3).[7] Paralleling the precedent in the conversion of (+) limonene to the α,β unsaturated aldehyde **8** (Scheme 4),[8] intermediate **6** should be readily obtainable.



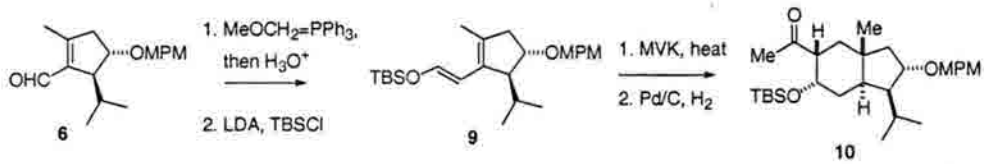
Scheme 3



Scheme 4. Reported Synthesis of Aldehyde **8** from Limonene[8]

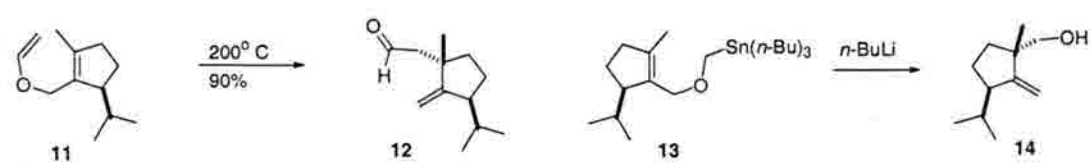
The secondary alcohol will remain protected until a late stage of the synthesis for incorporation of the 3,5 dimethyl heptanoic acid portion. The ambiguous stereochemical assignment [1a,b] of this fragment in the natural products necessitates its installment at a later stage. A Diels-Alder reaction is planned for the stereospecific formation of methyl ketone **10**. In this regard, α,β unsaturated aldehyde **6** will undergo reaction with

(methoxymethylene)triphenylphosphorane, and following hydrolysis, will be converted to the silyl enol ether **9** (Scheme 5). The steric interactions of the isopropyl and vinyl methyl groups are expected to direct the formation of the *(E)* over the *(Z)* alkene. Reaction of the resulting diene with methyl vinyl ketone (MVK) should provide the Diels-Alder adduct with both regio and stereocontrol.



Scheme 5

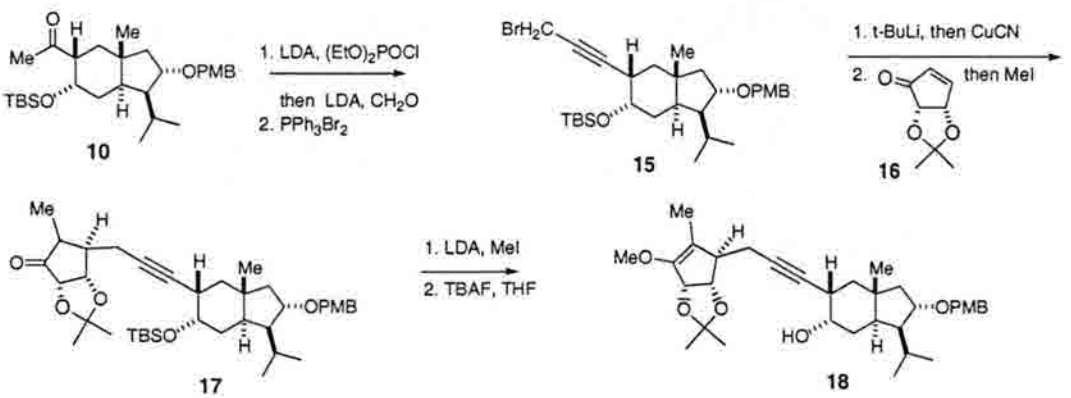
The regio-chemical outcome is anticipated to follow well established patterns in the reaction of a diene with a strong electron-donating group in the primary position, with an electron deficient dieneophile. Additionally, the methyl ketone functionality and the silyl ether will be obtained in *cis*-fashion as determined by the *(E)*- configuration of the silyl enol ether diene and presumed *endo*-transition state of the cycloaddition. The adjacent isopropyl group will be used to direct the overall enatioselectivity of the reaction. The basis for this prediction is supported by entioselective sigmatropic reactions of similar substrates.[9, 10] For example a stereospecific Claisen reaction with substrate **11** (Scheme 6) was performed in synthetic studies of the dolastane diterpenes.[9] Likewise, a Still reaction with substrate **13** gave alcohol **14** as a single enantiomer in the enatioselective synthesis of (-)-retigeranic acid A.[10]



Scheme 6. Examples of Previously Reported Stereospecific Reactions.

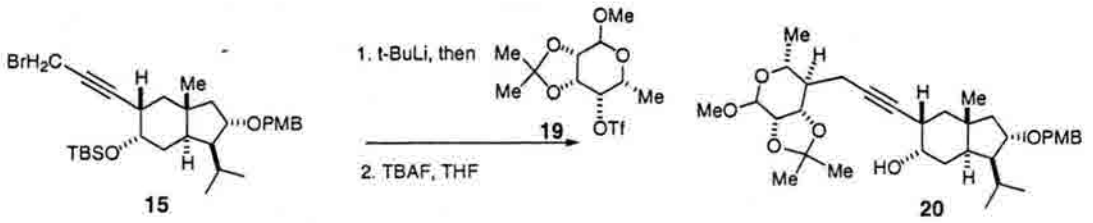
Hydrogenation of the alkene at the ring junction should provide the intermediate methyl ketone **10** with all the required stereochemistry (Scheme 5). It is anticipated that the isopropyl group will again direct the stereoselectivity of the reaction. Hydrogenation with Pd/C afforded the single *trans* isomer in the synthesis of hyrdindanones resulting from the steric bulk of the isopropyl group in the α-position.[11]

Conversion of the methyl ketone to the propargyl alcohol through known methods[12] would allow for an opportunity to explore a selective carboalumination or hydroalumination in construction of the trisubstituted olefin (Scheme 7). Two options are available for synthesis of substrates for this methodological study. Initially, the propargyl alcohol will be converted to the alkynyl bromide **15** and subsequently coupled to either carbohydrate derivative. Halogen-lithium exchange of the bromide and formation of the cuprate would allow for 1,4 addition to the known enone **16** (synthesized in 3 steps from D-ribose as a single enatiomer according to Borchardt *et al.*[13]). Reaction of the intermediate enolate with methyl iodide should afford the desired ketone **17** as a precursor for the synthesis of YW3699.



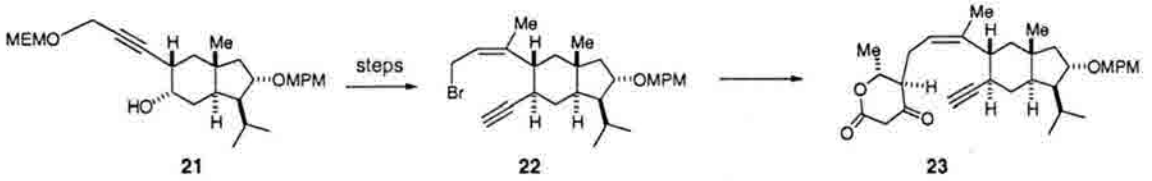
Scheme 7

For the natural product YW3548, coupling with the known triflate **19** (prepared in six steps from D-mannose[14]) is planned following halogen lithium exchange.



Scheme 8

This coupling strategy may prove problematic with possible side reactions including elimination of the triflate. An alternative strategy involves utilizing the protected propargyl alcohol **21** for formation of the *(Z)*-trisubstituted olefin and coupling the multi-carbonyl fragment to a later stage intermediate (*vide infra*). This scenario would involve the reaction of allyl bromide **22** (Scheme 9) with the anion of the pyrone **24** (Scheme 10).



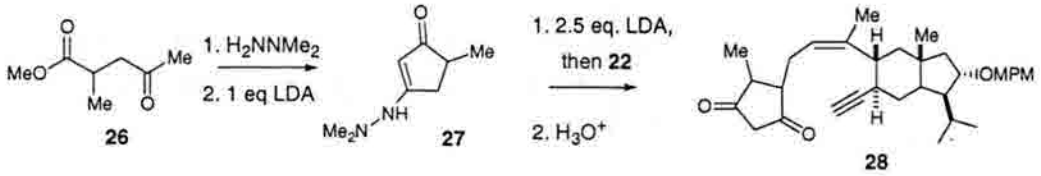
Scheme 9

Following the precedent of Gilbert and co-workers[15], the anion of pyrone **24** (synthesized in 2 steps from *(R)*-(-)-methyl 3-hydroxy butanote[16]) would react in SN^2 fashion with allylic bromide **22** to afford the desired dicarbonyl after hydrolysis.

Scheme 10. Pyrone and Dimethylhydrazone

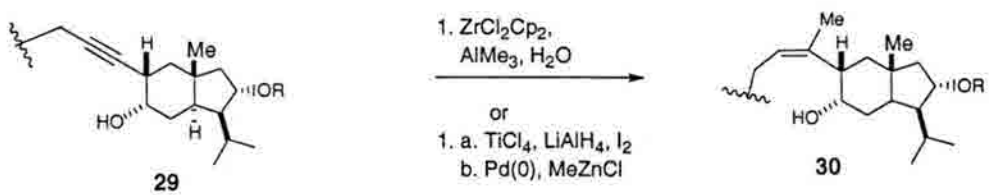
The dianion of dimethylhydrazones such as **25** (Scheme 10) have been reported to selectively add to carbon-4 as shown, and following hydrolysis, could provide the diketone as an alternative method.[17] The dianion of dimethylhydrazone **27**, derived from

the known[18] keto-ester **26**, would provide the complimentary substrate for the synthesis of YW3699 (Scheme 11). It should be noted that the dianion of dimethylhydrazones of β-diketo cyclopentanones have additionally been shown to selectively add at carbon-4 in the desired fashion.[17]



Scheme 11

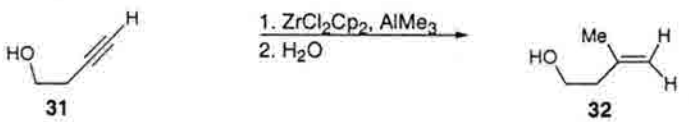
With either strategy employed, the *(Z)*-olefin will be constructed through the β-hydroxy alkyne **29** (Scheme 12). The stereoselective synthesis of *(Z)*-trisubstituted olefins continues to represent a challenge in synthetic chemistry. This difficulty is evident in the synthesis of insect pheromones where numerous synthetic efforts have resulted in poor or non-selective formation of this functionality. A current review in this area highlights these disappointing results.[19] This challenging transformation has been noted in a recent communication with a multi-step substitution and cross-coupling process as one solution to this problem.[20] The general intermediate β-hydroxy disubstituted alkyne **29** presents an opportunity to develop this area. Specifically, the β-hydroxyl group may assist in either a regio and stereoselective zirconium catalysed carboalumination or a titanium-mediated hydroalumination.



Scheme 12. Strategies for Synthesis of the *(Z)*-Trisubstituted Olefin.

The Cl_2ZrCp_2 catalyzed carboaluminations of terminal alkynes have been well documented to give both regio-control as well as exclusive *cis*-addition across the triple bond.[21] Additionally, the reaction has been shown to afford high yields with internal alkynes, however the regioselectivity of the reaction was ambiguous since symmetrical alkynes were used.[21a] Due to the large steric differences in the proposed disubstituted alkyne, perhaps a high degree of regioselectivity will be observed on this principle alone (the related Schwartz reagent has been found to show high selectivity from steric effects of internal alkynes[22]).

Interestingly, Cl_2ZrCp_2 catalyzed carboaluminations have been shown to be both high yielding and regioselective with terminal propargyl and homopropargy alkynes, even with the free hydroxyl (Scheme 13).[21]



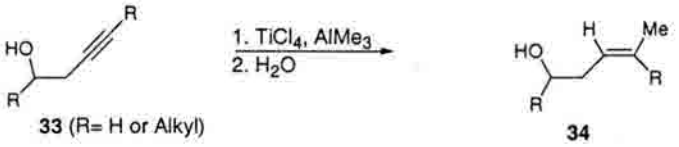
31 32

Scheme 13

However, it is difficult to ascertain the role of the hydroxyl group in directing the regio-chemical outcome since the selectivity is nearly identical with the unfunctionalized terminal alkynes, although some complex with the hydroxyl and $AlMe_3$ would be expected. Although the exact mechanism of the reaction needs to be elucidated, recent studies have suggested a zirconium-assisted carboalumination rather then a methyl-

zirconium intermediate being responsible for the addition.[21b] In this context, the β-hydroxyl group of the proposed substrate may complex the trimethyl aluminum thereby directing the regio-chemistry to the internal alkyne.

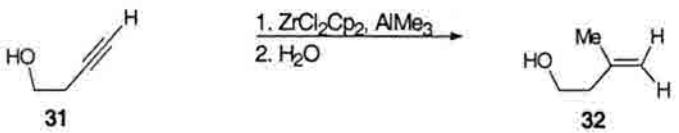
A separate report demonstrates the stereo- and regioselective carboalumination of homopropargyl alcohols with $TiCl_4$ and $AlMe_3$.[23] Although the regioselectivity is opposite that of the zirconium catalyzed process, complete regioselectivity was seen with both terminal and disubstituted alkynes in addition to the *cis* addition (Scheme 14). The authors propose that the free hydroxyl group in these substrates complexes with the titanium.[23]



33 (R= H or Alkyl) **34**

Scheme 14

Titanium complexes with aluminum hydrides have additionally been shown to give predominately *cis* addition to alkynes.[24] Although $LiAlH_4$ by itself normally provides the *trans*-vinyl aluminum species, the use of $LiAlH_4$ in combination with $TiCl_4$ has been shown to give nearly exclusive *cis*-reduction of internal alkynes.[24a] Likewise, Cp_2TiCl_2 has been shown to give complete *cis*-addition with a variety of aluminum hydride reagents with internal alkynes providing the alkene with aqueous workup or the vinyl halide following reaction with iodine (Scheme 15).[24b]

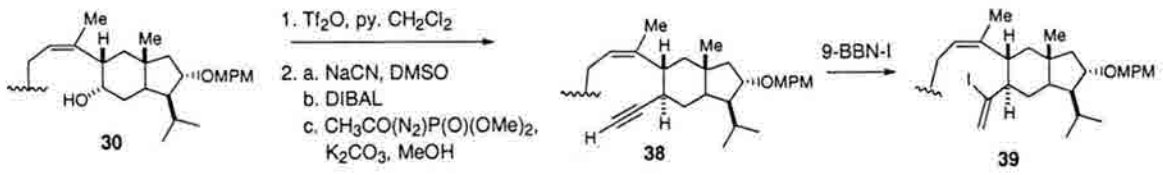


31 **32**

Scheme 15

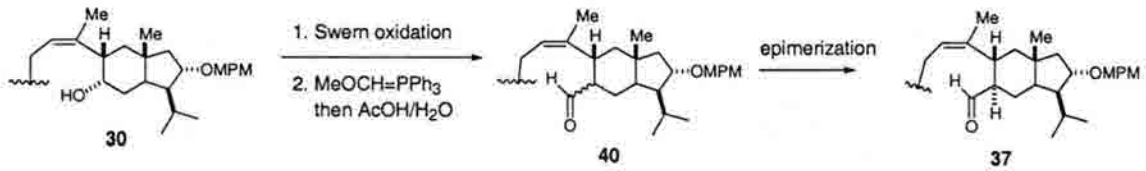
Based on the results of the above studies, reaction of the proposed substrate with $TiCl_4$ and $LiAlH_4$ (or other aluminum hydride reagent) followed by I_2 quench is expected to afford the vinyl iodide in a regioselective manner. The vinyl iodide can be readily converted to the desired methyl tri-substituted alkene following standard organometallic-mediated cross-coupling reactions. The utilization of the latter sequence of reagents in this regard has yet to be published in the literature, and may represent a novel way for stereoselctive synthesis of *(Z)*-trisubstituted olefins from β-hydroxy alkynes.

With the trisubsituted olefin in place, conversion of the secondary alcohol to the vinyl iodide will follow. Ideally, the hydroxyl group might be displaced by conversion to the triflate and subsequent nucleophilic displacement by sodium cyanide or related reagent (Scheme 16). Direct nitrile substitution of the hydroxyl group may be possible with recently reported modified Mitsonobu reagents.[25] Substitution of a secondary alcohol on a fused six-membered ring with a nitrile, was accomplished in 73% yield with these conditions. Following standard protocol, the nitrile can be reduced to the aldehyde and converted to the terminal alkyne.[26] Reaction with B-iodo-9-borabicyclo[3.3.1]nonane (9-BBN-I) should provide the desired vinyl iodide. Iodoboration of terminal alkynes with 9-BBN-I is known to be both regioselective and chemoselective with respect to internal alkenes;[27] this concept was recently demonstrated in the synthesis of (-)-7-deacatoxyalcyonin acetate.[28]



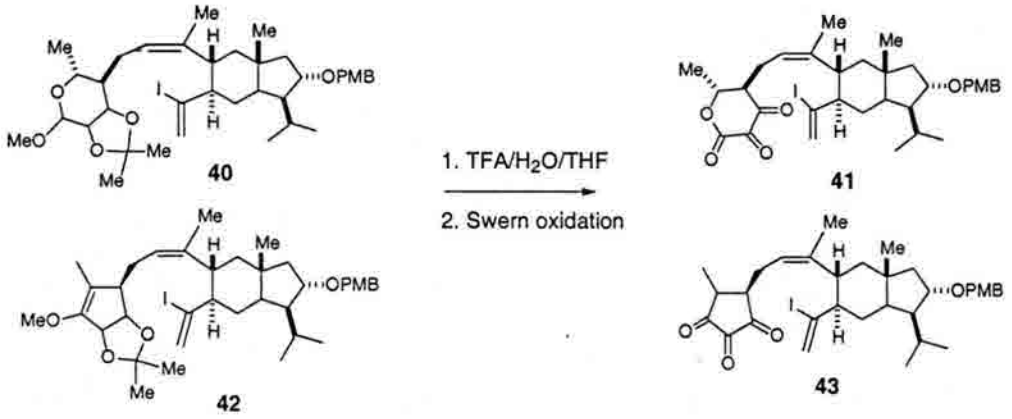
Scheme 16

It should be noted that possible competitive elimination could make the desired substitution of the hydroxyl group problematic. In the event that satisfactory conditions cannot be found, an alternative procedure would include oxidation of the secondary alcohol to the ketone and carbon homoglation by reaction with (methoxymethylene)triphenylphosphorane (Scheme 17). Hydrolysis of the resulting enol ether should result in the positioning of the aldehyde favoring the *anti*-orientation to the *(Z)*-olefin functionality (molecular mechanics calculations support this hypothisis[29]). Further epimerization with sodium methoxide or other mild base may be necessary to obtain a favorable ratio.



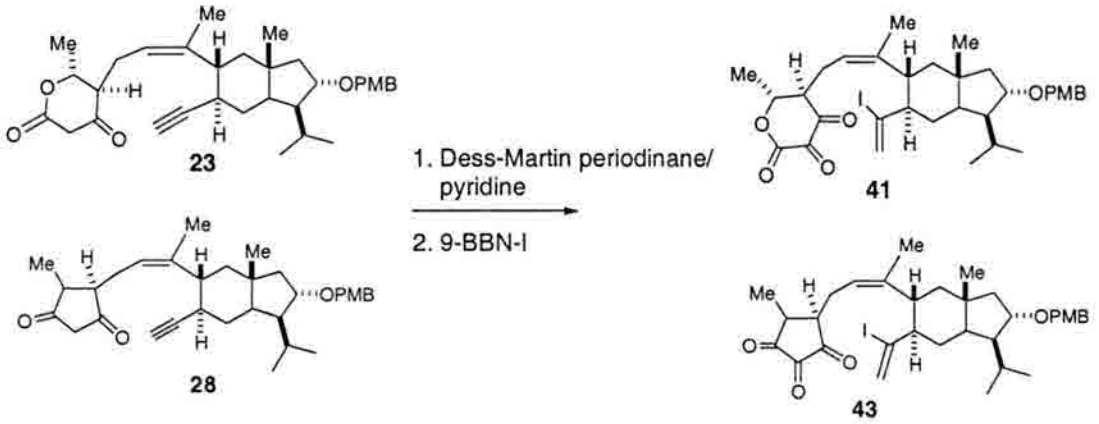
Scheme 17 Alternative Strategy for Conversion to the Alkyne.

With the installment of the vinyl iodide functionality, the second half of the molecule will be prepared for the key Ni(II)/Cr(II)-mediated coupling reaction. Acidic hydrolysis of the acetonide group in either natural product precursor followed by Swern oxidation[30] will provide the tricarbonyl unit necessary for the key reaction (Scheme 18). The choice of a Swern oxidation is based on the successful employment of these conditions for the tricarbonyl system in the synthetic studies and the total synthesis of FK506.[31]



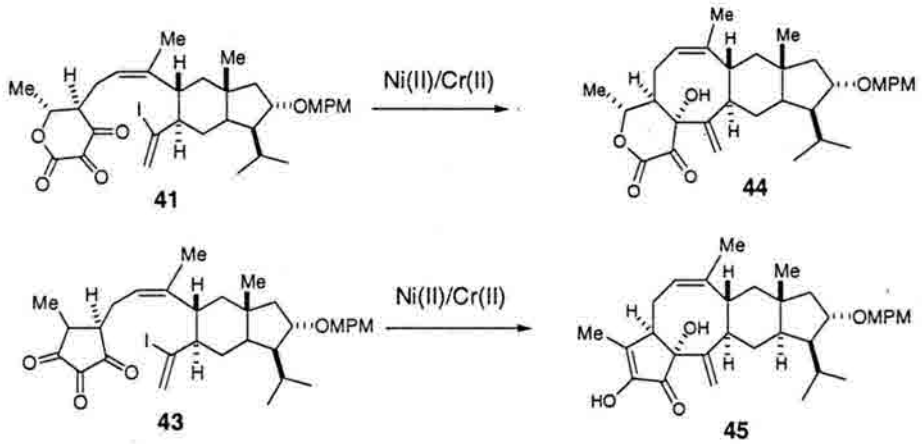
Scheme 18

In the event that the alternative coupling strategy is used (Scheme 9 and 11), the resulting β-dicarbonyl compound will be converted to the tricarbonyl substrate in a single step with the pyridine/Dess-Martin periodinane conditions reported by Golec *et al.*[32] or by the two-step protocol by Wasserman *et al.*[33] (Scheme 19). It should be noted that the dimethyl dioxirane (DMDO) oxidation of the intermediate α-bromo-β-dicarbonyl by the latter method should be selective over the alkene and alkyne. This prediction is based on a similar DMDO protocol for the oxidation of the triphenyl phosphine ylid reported by the same authors.[34]



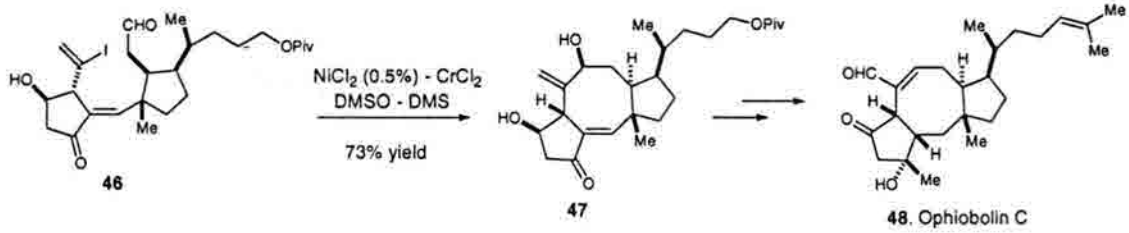
Scheme 19

The synthesis of the tricarbonyl intermediate for both natural products will allow for the exploration of the Ni(II)/Cr(II)-mediated ring forming reaction. Several factors support the validity of this proposed step (Scheme 20).



Scheme 20

Since its discovery by the sponsor's research group during the total synthesis of palytoxin[4], the Ni(II)/Cr(II)-mediated coupling reaction has found numerous applications in the synthesis of natural and non-natural products by the sponsor's group and others.[4,5] The mild conditions necessary for the reaction and the tolerance of a variety of other functionalities allow for the reaction's utility in key bond-forming steps during the late stages of total synthesis. In addition to intermolecular couplings of vinyl iodides or triflates to aldehydes, the utility of this reaction for intramolecular reactions has proved to be a reliable method for formation of various sized rings.[4, 5] In relation to the proposed ring-forming sequence, the Ni(II)/Cr(II) mediated reaction was employed by the sponsor's group in the total synthesis of ophiobolin C (**48**).[35] The formation of the eight membered ring **47** was constructed in the late stages of the synthesis by intramolecular coupling of a vinyl iodide with the aldehyde (Scheme 21).

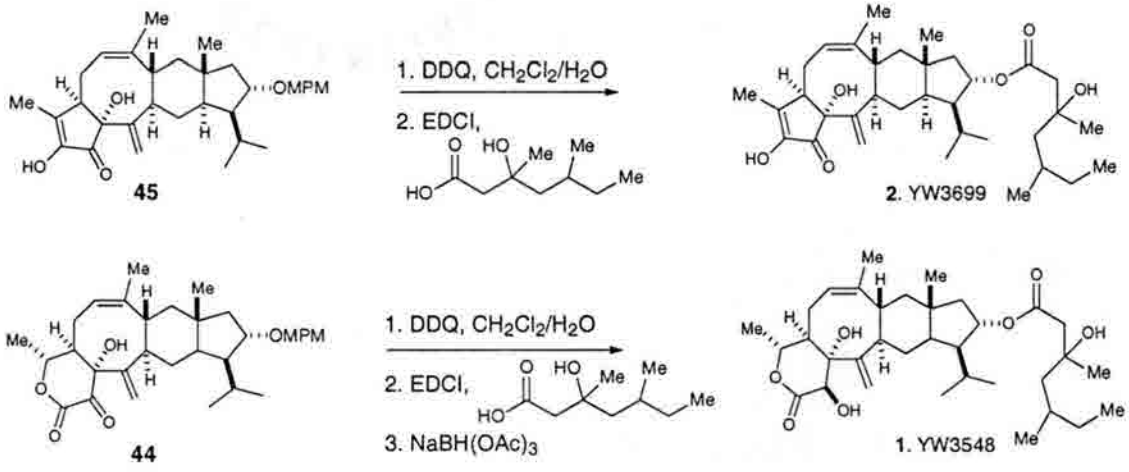


Scheme 21. Synthesis of Ophiobolin C by Ni(II)/Cr(II)-mediated Intramolecular Cyclization.

In addition to the 73% yield, the reaction afforded a single diastereomer, the identity of which was predicted on the basis of steric considerations. Based on this precedent, the proposed coupling reaction for the synthesis of YW3669 and YW3548 is anticipated to furnish exclusively the desired stereoisomer. The significant difference between the proposed couplings and the successful ring-forming reaction in the synthesis of ophiobolin C, is the reaction of a vinyl iodide with a ketone in place of an aldehyde. Until recently, the Ni(II)/Cr(II)-mediated couplings were limited to reactions with aldehydes. This was largely due to the necessity of having to use minimal amounts of Ni(II); larger portions of which resulted in homocoupling reactions between the vinyl iodide (triflate) reactants.[4] During the course of investigating the use of chiral ligands for the development of an asymmetric process, the sponsor's group discovered that the addition of pyridine and dipyridyl ligands completely suppressed the homocoupling reaction.[36] This discovery allowed the use of a 1:2 mixture of $NiCl_2$ and $CrCl_2$ in the reactions, which in turn dramatically enhanced the rate of couplings between substrates. Furthermore, the coupling reaction of iodoolefins was found to be successful with ketones, even enolizable ketones. The use of 4-*tert*-butylpyridine in a more recent report both suppressed the homocoupling and homogenized the reaction, thus allowing for

successful couplings with previously unreactive substrates.[37] These improvements in the reaction conditions should allow for the successful ring-closing step in the proposed scheme. Additionally, it should be noted that the proposed ketone in both substrates are far more electronically activated then a normal ketone.

The exclusive formation of the eight-membered ring is anticipated as this would seem to be the most accessible based on steric constraints. Although the central ketone in the tricarbonyl system is the most electronically active, a cyclization to the nine-membered ring would seem unfavorable based on the steric strain of this undesired product. Even in the event that the Cr(III) species does initially react with the central carbonyl, the resulting intermediate may isomerize to the more stable eight-membered ring on this basis.

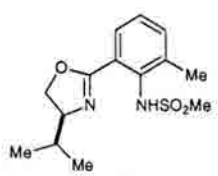


Scheme 22. Completion of Natural Products

Following a successful intramolecular cyclization, the tricarbocyclic sesterterpenoid skeletons **44** and **45** will require only a few trivial synthetic manipulations for completion of the synthesis. Deprotection of the *p*-methoxy benzyl (MPM) group and subsequent coupling with the heptanoic fragment will complete the synthesis of YW3699

from substrate **45**. With the additional stereoselective reduction of the α-hydroxy ketone **44** by standard methods,[38] YW3548 will be completed in a similar manner. Since the stereochemical assignment of the 3-hydroxyl-3,5-dimethyl-heptanoic portion of the molecule remains unassigned, all four possible stereoisomers will have to synthesized and individually coupled to the intermediate secondary alcohol. Direct comparison to the spectroscopic data of the natural product will be used to reveal the correct stereochemistry of this portion of the natural products.

In conjunction with the total synthesis of YW3699 and YW3548, further investigation of the Ni(II)/Cr(II)-mediated C-C bond-formation is planned as part of the proposed research. Specifically, this includes the development of a catalytic asymmetric version of the reaction. Recent efforts by the sponsor's group has led to the development of an asymmetric Ni(II)/Cr(II) coupling reaction. These results follow the preliminary studies using the aforementioned dipyridyl ligands.[36] Using the chiral sulfonamide ligand such as **49**, useful levels of asymmetric induction have been achieved.[39]



49

Scheme 23. Chiral Sulfonamide Ligand.

In connection with this result, a catalytic chromium version of this asymmetric process would be the next stage in further expanding these reactions which already exhibit broad potential. Recent developments of catalytic versions of this reaction have been reported.[40a- c] These initial breakthroughs include the use of manganese[40a] or aluminum[40b] as a reductant in conjugation with trimethyl silyl chloride (TMSCl),

enabling the use of a catalytic amount of Cr(II) and Ni(II). Additionally, an electroreduction process using lithium salts or TMSCl has been described which is both catalytic in Pd(0) and Cr(II).[40c] Further development of these initial systems, or other catalytic versions of the reaction, combined with the newly discovered asymmetric process, will provide a reaction with far greater synthetic utility. This will be especially important in the area of synthesis of highly complex bioactive molecules for clinical studies.

The proposed enatioselective synthesis of both natural products will provide a means for the preparation of biologically significant analogs. Furthermore, several methodological developments in connection with this work will contribute to the field of organic synthesis.

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