DISSERTATION

MITOMYCIN ALKALOIDS: SYNTHETIC STUDIES

Submitted by

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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 DANIEL ALAN GUBLER ENTITLED MITOMYCIN ALKALOIDS: SYNTHETIC STUDIES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

MITOMYCIN ALKALOIDS: SYNTHETIC STUDIES

Documented herein are efforts towards the first asymmetric total synthesis of the mitomycin family of natural products. Methods have been developed that efficiently construct eight-membered ring precursors of the natural products. Additionally, a tetracyclic mitosane compound containing all the core features of the mitomycins except the C9a methyl aminal has been constructed.

The above-mentioned synthetic efforts help set the stage for future completion of the asymmetric total synthesis of this family of compounds. The studies mentioned herein, while not totally successful, shed new light on the reactivity of the mitomycins as well as the remarkable electronic effect of the electron-rich arene ring.

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List of Abbreviations

ACCN	Azobis(cyclohexanecarbonitrile)
Ac_2O	Acetic anhydride
AcOH	Acetic acid
AgOTf	Silver trifluoromethanesulfonate
AIBN	Azobisisobutyronitrile
AllocCl	Allyl chloroformate
9-BBN	9-Borabicyclo[3.3.1]nonane
Bn	Benzyl
BnOH	Benzyl alcohol
BnBr	Benzyl bromide
Boc	*tert*-Butoxycarbonyl
Boc_2O	Di-*tert*-butyldicarbonate
BOMCl	Benzyloxymethyl chloride
BsCl	Benzenesulfonyl chloride
CAN	Ceric ammonium nitrate
CbzCl	Benzyl chloroformate
$Cu(acac)_2$	Copper(II) acetylacetonate
DCE	Dichloroethane
DDQ	2,3-Dichloro-5,6-dicyanobenzoquinone
DEAD	Diethyl azodicarboxylate

DEIPSCl	Diethylisopropylsilyl chloride
Dess-Martin Periodinane	Triacetoxy *o*-iodoxybenzoic acid
DET	Diethyl tartrate
DMAP	4-(Dimethylamino)-pyridine
DMF	Dimethylformamide
DMDO	Dimethyldioxirane
DPM	Diphenyl methyl
Et_3N	Triethylamine
EtOAc	Ethyl Acetate
Et_2O	Diethyl ether
Frémy's salt	Potassium nitrosodisulfonate
HBF_4	Fluoroboric acid
IBX	*o*-iodoxybenzoic acid
Imid	Imidazole
LDA	Lithium *N,N*-diisopropylamide
*m*CPBA	*m*-Chloroperbenzoic acid
MeCN	Acetonitrile
MeI	Methyl iodide
MOMCl	Methyl chloromethyl ether
MsCl	Methanesulfonyl chloride
MTAD	4-Methyl-1,2,4-triazoline-3,5-dione
NaHMDS	Sodium bis(trimethylsilyl)amide
NBS	N-Bromosuccinimide

NMO	4-Methyl morpholine *N*-oxide
NsCl	2-Nitrobenzenesulfonyl chloride
dNsCl	2,4-Dinitorbenzenesulfonyl chloride
NVOC	6-Nitroveratryl chloroformate
PDC	Pyridinium dichromate
$Pd(OAc)_2$	Palladium(II) acetate
PCC	Pyridinium chlorochromate
Pd/C	Palladium on carbon
$Pd_2(dba)_3$	Tris(dibenzylideneacetone)dipalladium
Ph	Phenyl
$PhI(OAc)_2$	Iodobenzene diacetate
PPTS	Pyridinium *p*-toluenesulfonate
2-PrOH	Isopropanol
Py. or Pyr.	Pyridine
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
SAM	S-Adenosyl methionine
$Sc(OTf)_3$	Scandium(III) trifluoromethylsulfonate
SEMCl	2-(Trimethylsilyl)ethoxymethyl chloride
TASF	Tris(dimethylamino)sulfonium difluorotrimethylsilicate
TBAF	Tetrabutyl ammonium fluoride
TBDMSCl or TBSCl	*tert*-Butyldimethylsilyl chloride
TBSOTf	*tert*-Butyldimethylsilyl trifluoromethanesulfonate

TBHP	*tert*-Butyl hydrogen peroxide
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy, free radical
TFA	Trifluoroacetic acid
Tf_2O	Trifluoromethanesulfonyl anhydride
THF	Tetrahydrofuran
TMAD	*N,N,N',N'*-Tetramethylazodicarboxamide
TMSCl	Trimethylsilyl chloride
TMSOK	Potassiumtrimethyl silanoate
TMSOTf	Trimethylsilyl trifluoromethylsulfonate
TPAP	Tetrapropylammonium perruthenate
Triton B	Benzyltrimethylammonium hydroxide
TrocCl	Trichloroethyl chloroformate
TsCl	*p*-Toluenesulfonyl chloride
p-TsOH	*p*-Toluenesulfonic acid

Chapter 1: Introduction and Biological Activity

1.1: Introduction

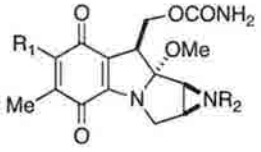
1.1.1: Background

The mitomycin family of natural products (Figure 1) have been the subject of intense research in the scientific community over the last fifty years due to their intriguing structure and unique biological activity. This family consists of the mitomycins (mitomycins A-K, porifomycin, isomitomycin A, albomitomycin A), as well as FR900482 and related congeners. Mitomycin C (MMC) has been used in the clinic for over 40 years and has been particularly effective in the treatment of solid tumors. The mode of action of MMC and the other members of the family has been linked to their ability to form sequence-specific covalent interstand DNA cross-links at the 5'-CG-3' sequence in the minor groove.[1] Additionally, these compounds have also been shown to form covalent cross-links between DNA and proteins.[2]

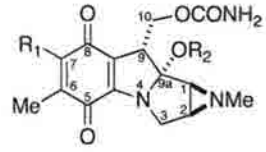
1.1.2: Isolation of Mitomycins and FR900482

Mitomycins A and B were first isolated by Hata and co-worker at Kyowa Hakko company in 1956 from the bacterial strain *Streptomyces caespitosus* found in Japanese soil samples.[3] This antibiotic was shown to have a strong inhibitory activity against a variety of microorganisms. Additionally, mitomycin was shown to be active against Ehrlich ascites tumor cells. Two years later researchers at Kyowa Hakko identified 4 different components of the mitomycin antibiotic, designating them mitomycins A-C, and

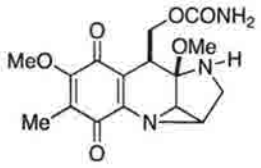
porifomycin (Figure 1).[4] Webb and co-workers at Lederle Laboratories later investigated the structure of the mitomycins in detail and assigned the pertinent stereochemistry for each of the four compounds.[5] Webb also detailed the conversion of mitomycin A to mitomycin C by treatment with aqueous ammonia in methanol and elucidated the stereochemical relationship of the C9 carbon relative to the aziridine. It was found that mitomycins, A, C, and porifomycin have the C9 carbon cis to the aziridine, whereas C9 is trans to the aziridine in mitomycins B and D. Tulinsky later confirmed the proposed structure of mitomycin A by X-ray analysis.[6]



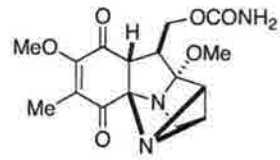
	R_1	R_2
Mitomycin C	NH_2	H
Mitomycin A	OMe	H
Mitomycin F	OMe	Me
Porifomycin	NH_2	Me



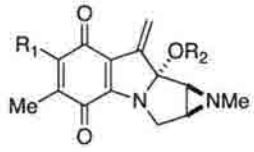
	R_1	R_2
Mitomycin B	OMe	H
Mitomycin D	NH_2	H



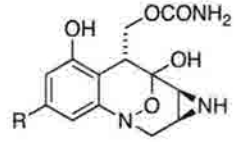
Isomitomycin A



Albomitomycin A



	R_1	R_2
Mitomycin G	NH_2	H
Mitomycin H	OMe	H
Mitomycin K	OMe	Me

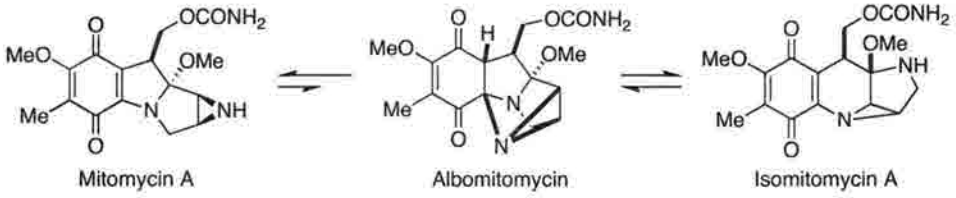


FR-900482 R = CHO
FR-66979 R = CH_2OH

Figure 1: The Mitomycin Family of Natural Products

Isomitomycin and Albomitomycin (Figure 1) were isolated from the fermentation broth of mitomycins by Kono and co-workers.[7] Isomitomycin A appears to be the result

of a Michael addition of the free aziridine, followed by a retro-Michael addition to release the secondary amine. Albomitomycin appears to be the result of a Michael addition of the free aziridine of mitomycin A. Additionally these two natural products have also been found in mitomycin broths of various streptomyces bacteria.[8] It was shown that isomitomycin A would slowly convert to mitomycin A, with albomitomycin as an intermediate. This conversion was termed by the authors the "mitomycin rearrangement".[7] Fukuyama and co-workers used this rearrangement in their total synthesis of mitomycins A and C (discussed in Chapter 2.1.3).



Scheme 1: The Mitomycin Rearrangement

Other members of the mitomycin family of natural products include mitomycins G, H, and K where elimination of the carbamate group has resulted in an exo-methylene group at C9-C10.[9] These variants were found to not be as potent as their counterparts bearing the carbamate moiety. Nevertheless, mitomycins G-K represent a unique class of Mitomycins that could bear a novel mode of action. One could envision mono-alkylation of DNA by these types of compounds without the need for reductive activation.

FR900482 (Figure 1), a unique antibiotic with structural similarities to the mitomycins was isolated in 1987 from a *Streptomyces sandaenesis* strain.[10] This compound proved to be even more active than mitomycin C against solid tumors without the toxicity associated with the mitomycins. It was demonstrated that FR900482 also formed covalent cross-links with DNA via bio-reduction of the prodrug as seen in the

mitomycins.[11] Moreover, the DNA sequence specificity was found to be the same as for the mitomycins.[12]

1.1.3: Structure and Terminology

Prominent features of a generic mitomycin (Figure 2) include a quinone, an aziridine, and a aminal linkage. All of these functionalities are incorporated into a very dense, highly functionalized tetracyclic skeleton. It is known that the mitomycins are unstable to strongly acidic or reductive conditions, and stable to basic and oxidative conditions.

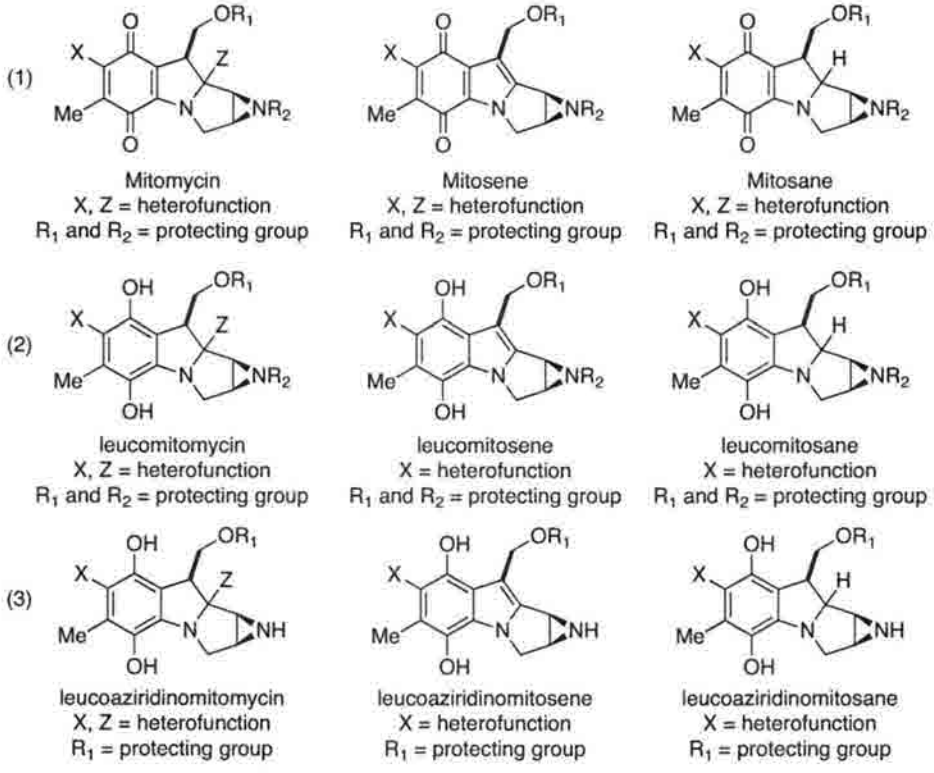


Figure 2: Terminology of the Mitomycins and their Derivatives

Shown in Figure 2 is pertinent terminology that is helpful in a discussion of the mitomycins. Row 1 of Figure 2 depicts the general structure of a mitomycin as mentioned previously. Elimination of oxygen at C9a and concomitant loss of a proton to mitomycin would give rise to a structure, with an indole-like olefin, known as a mitosene (Figure 2,

Row 1, center). It is through the mitosene that the mitomycins exert their biological activity. A mitomycin where a hydrogen atom has replaced the oxygen functionality at C9a is known as a mitosane (Figure 2, Row 1, right). Most mitosanes reported in the literature are devoid of biological activity. Reduction of the quinone in the three structures in Row 1 of Figure 2 provides the corresponding hydroquinone compounds (Row 2 of Figure 2) referred to as a leucomitomycin, a leucomitosene, and a leucomitosane respectively. Removal of the protecting group on the aziridine gives rise to the "aziridino" compounds shown in Row 3 of Figure 2.

1.1.4: Biosynthesis

Since their isolation, there has been an ongoing effort to elucidate how the mitomycins and FR900482[13] are made biosynthetically. Feeding studies, done primarily in the 1970's, have established the constituent building blocks in the biosynthesis of the mitomycins (Figure 3).[14] These building blocks were identified to be: D-erythrose, S-adenosyl methionine (SAM), carbamoyl phosphate, pyruvate[15], D-glucosamine[16], and 3-amino-5-hydroxy benzoic acid (AHBA)[17,18] Although the constituents are known, the exact order in which each of the building blocks are assembled and combined remains a mystery. One intriguing question that remains to be answered is the following: does the same biosynthetic machinery make the mitomycins and FR900482? If this is the case, then at what point does the path diverge to give the respective natural products? Another interesting question that remains unanswered is the source defining the stereochemistry of the C9 carbon to be cis to the aziridine in some of the mitomycins but then trans to the

aziridine in other mitomycins and FR900482. This observation is unusual due to the high selectivity inherent in enzymatic processes.

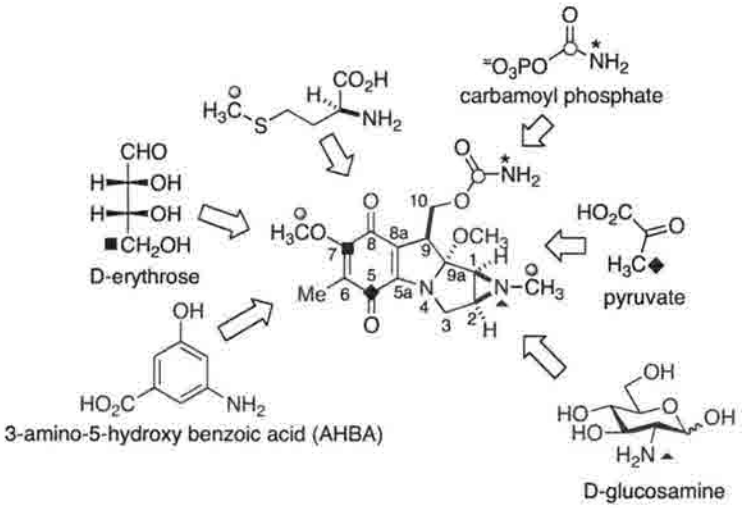
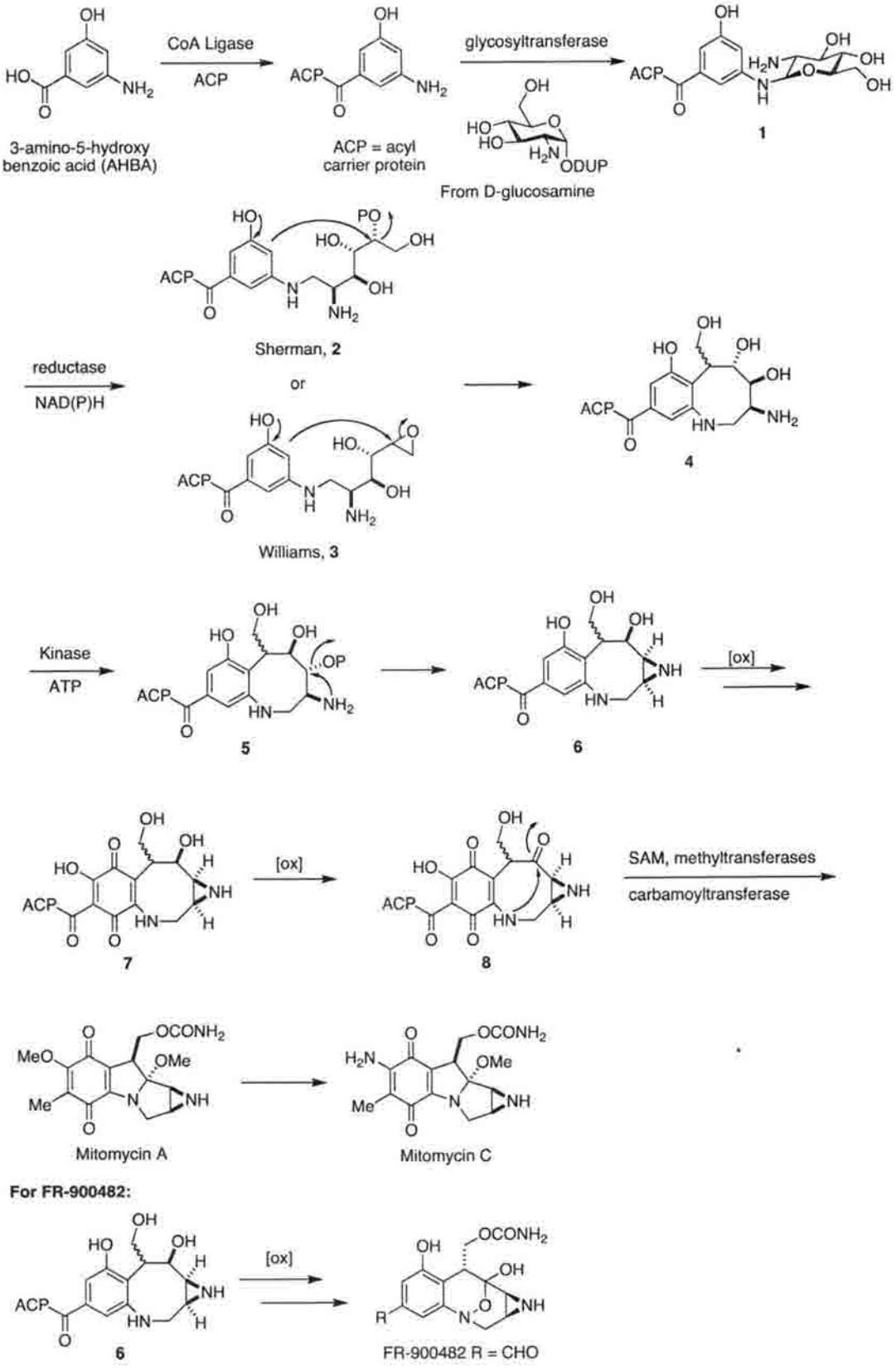


Figure 3: Constituents in the Biosynthesis of the Mitomycins

Our research group, in collaboration with the research group of Professor David Sherman at the University of Michigan, has undertaken a project to try and answer some of the questions mentioned above. Shown in Scheme 2 is a pathway proposed by Williams and Sherman for the biosynthesis of the mitomycins and FR900482. First AHBA is coupled to an acyl carrier protein and then reacted with D-glucosamine to give coupled adduct **1**. The synthesis of **1** has been accomplished in our group and is currently being studied by Sherman and co-workers. The next key step in the proposed biosynthesis of the mitomycins and FR900482 involves the ring opening of the glucosamine to give a ring-opened adduct. Possible ring-opened adducts include those proposed by Sherman **2** or Williams **3** (Scheme 2). This ring-opened adduct is then proposed to undergo a Friedel-Crafts alkylation to give benzazocine **4**. The exact nature of the ring-opened adduct should determine the selectivity of C9 in the ring closure to provide benzazocine **4**. The ring-opened adduct of **1** has also been made in our group.



Scheme 2: Proposed Biosynthetic Pathway for the Mitomycins and FR900482

Williams and Sherman propose that both the mitomycins and FR900482 arise from the same precursor such as benzazocine **6**. Oxidation to the quinone and the ketone (i.e. **7** to **8**) would furnish the necessary oxidation for the mitomycins. Alternatively one could envision oxidation of benzazocine **6** to the hydroxylamine hemiketal system requisite in FR900482 (Scheme 2). It is hoped that details to the mechanism of mitomycin and FR900482 biosynthesis will be answered in due course.

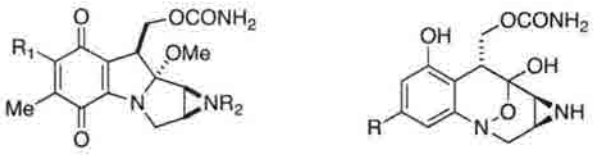
Isolation and elucidation of the gene cluster involved in the biosynthesis of the mitomycins and FR900482 has also been under study primarily by Sherman and co-workers.[19] Elucidation of the structure and function of the individual enzymes in the biosynthetic pathways of natural products could provide an alternative to chemical synthesis and could have far-reaching implications in the future.

1.2: Biological Activity and Mode of Action

1.2.1: Biological Activity

One of the major driving forces for interest and research in the mitomycin family of natural products (subset of the family shown in Figure 4) is due to their potent and unique biological activity. Mitomycin C (MMC) has been used in clinical cancer chemotherapy since the 1960's. MMC was found to be effective against solid tumors, and was shown to be selective for the hypoxic regions of these tumors.[20] MMC is an important component in many of the chemotherapeutic regiments used today. Both colorectal and bladder cancer are commonly treated with MMC, as it remains one of the most potent drugs for each of these cancers.[21] MMC is also the drug of choice for treatment of non-small cell lung cancer.[22] Recently the general clinical use of MMC has

diminished slightly due to its inherent toxicity and the discovery of new anti-cancer drugs that are less toxic.



	R_1	R_2
Mitomycin C	NH_2	H
Mitomycin A	OMe	H
Porifomycin	NH_2	Me

FR-900482 R = CHO
FR-66979 R = CH_2OH

Figure 4: Selected Members of the Mitomycin Family of Natural Products

In addition to its antitumor activity, MMC has been shown to have a variety of other effects on mammalian cells. MMC has been shown to inhibit DNA synthesis, DNA repair, and sister chromatid exchange.[23] Suppression of P-glycoprotein and multidrug resistance in cancer cells was also shown.[24] Several reports have shown that MMC has a significant effect on the overall gene expression of an organism, although the mechanisms involved are unknown at present.

FR900482 and FR66979 (Figure 4) have been shown to have similar biological activity to the mitomycins without the excess toxicity that has hampered the clinical utility of MMC. One of the contributors to the toxicity of MMC has been shown to be the quinone moiety. Quinone containing compounds are known to undergo redox cycling *in vivo* resulting in the formation of superoxide and other reactive radical species that can cause indiscriminate damage to DNA. Due to the absence of a quinone and the different mode of activation, FR900482 and FR66979 do not undergo redox cycling and have proven to be less toxic to mammalian cells.

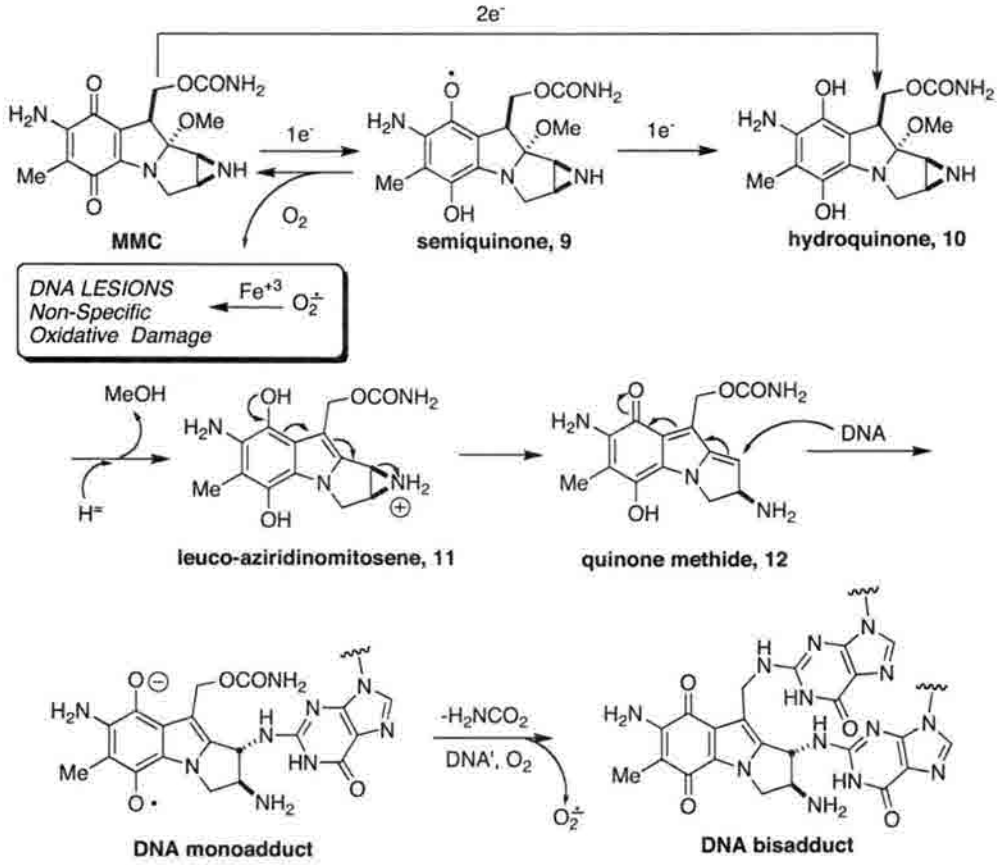
1.2.2: Mode of Action

The unique biological activity of this family of natural products have been traced to the ability of these compounds to form covalent cross-links with complementary strands of DNA.[25] Monoalkylation adducts of MMC to DNA as well as a DNA *interstrand* cross-link has also been discovered.[26] At the time of the discovery, the mitomycins were the first natural product know to cross-link DNA. Simple bifunctional synthetic alkylating agents were reported two years earlier.[25] Since then other natural products have also been found to display this mode of action.[27] Cross-linking of DNA has been shown to be an extremely potent method in inducing cell death and is more effective than monoalkylation of DNA. It was reported that a single cross-link per genome was enough to cause cell death.[25] Reasons for this selectivity are not completely understood. It is thought that cross-linking at a replication fork would be much more detrimental to the cell than monoalkylation. Another hypothesis proposes that the mechanisms inherent to the repair of damaged DNA are more able to mend a lesion caused by monoalkylation as opposed to a lesion caused by cross-linking.

A current understanding of the mechanism of DNA cross-linking by the mitomycins is shown in Scheme 3. It should be noted that MMC is a prodrug that, upon bioreduction, gives the active mitosene species. Bioreductive prodrugs have been an intense area of interest in an effort to develop drugs with higher degrees of selectivity. [28]

MMC has shown to be able to be reduced *via* either a one or two-electron process to ultimately give hydroquinone **10** (Scheme 3).[29] One-electron reduction of MMC *in vivo* gives semiquinone **9**, which upon addition of a proton and electron yields hydroquinone **10**. Conversion of MMC to semiquinone is a reversible process in the

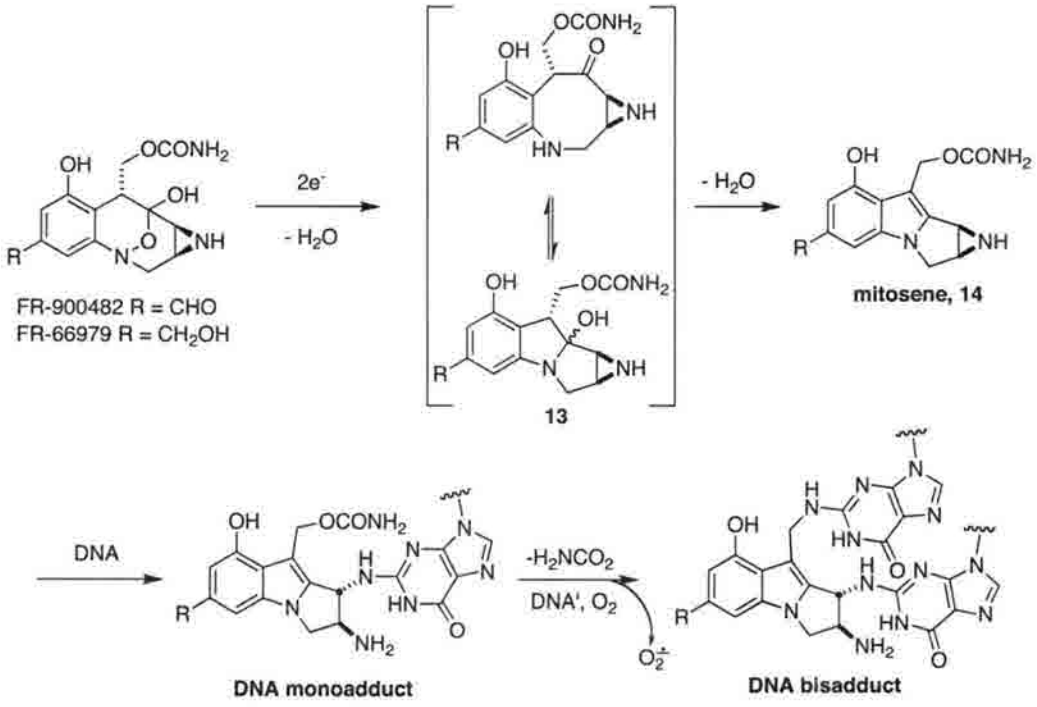
presence of oxygen and yields MMC and superoxide (source of the toxicity associated with the mitomycins). This finding lends support to evidence that MMC is much more active under hypoxic conditions.



Scheme 3: Proposed Mechanism of the Cross-Linking of DNA by the Mitomycins

Alternatively, two-electron reduction of MMC plus two protons would also yield hydroquinone **10** (Scheme 3). Loss of methanol and addition of a proton to hydroquinone **10** would give leuco-aziridinomitosene **11**. Ring opening of **11** would form the highly reactive *o*-quinone methide species **12**. Nucleophilic attack of DNA to **11** would then give the DNA monoadduct. Loss of the carbamate would reveal a highly reactive iminium species that, upon attack from DNA, would give the DNA bisadduct.

FR900482 and FR66979 also possess the ability to from DNA-interstrand cross-links and react *via* a similar mechanism as the mitomycins. These compounds, like the mitomycins, also need to be reductively activated *in vivo*. However in the case of FR9004982 and FR66979, the trigger is a hydroxylamine hemiketal moiety as opposed to the quinone used in the mitomycins. It has been shown *in vitro* that chemical reducing agents such as thiols and sodium dithionite can induce this transformation.[30] A mechanism for the reductive activation of these compounds was proposed to proceed *via* a two-electron reduction to give alcohol **13** that would collapse to form mitosene **14** (Scheme 4).[31] Reaction of mitosene **14** as in the case of the mitomycins would give both the DNA monoadduct and DNA bisadduct.



Scheme 4: Mode of Action of FR900482 and FR66979

In spite of a working knowledge of the mechanism of the bioreduction of both MMC and FR900482 as discussed above, several questions remain unanswered. One of these questions is what enzymes are responsible for the bioreduction of these compounds *in vivo*? Much research has been done in attempting to elucidate what enzyme(s) are responsible for these reductions. Answering of this question is important because if the reductase responsible for these transformations could be identified, then several approaches could be imagined centered on the concept of developing enzyme-directed chemotherapy for both MMC and FR900482. Findings have shown that DT-diaphorase[32], NADPH-cytochrome c reductase[33], and other enzymes[34] all can activate MMC *in vivo*. In short, it has been shown that there is no one enzyme solely responsible for activation of MMC and FR900482 *in vivo*.

It was originally thought that MMC was intercalated into DNA, however further studies demonstrated that MMC formed a covalent bond[35] to DNA and bound in the minor groove.[36] NMR studies of an MMC-DNA cross-linked adduct remarkably, showed very little perturbation in comparison with the native form.[37] These results and others showed a slightly wider minor groove bearing covalently bound MMC with the major groove being slightly contracted.

The nature of the mitomycin-DNA cross-link has been elucidated and has been found to be selective for 5-CG-3' sequences.[38] Specifically, it has been demonstrated that guanidine in the 5'-CG-3' sequence are highly preferred sites for the first alkylation and are absolutely specific for the second alkylation.[39] A model for the observed sequence specificity was proposed by Tomasz and co-workers and is shown in Figure 5. It was proposed that C10 of mitomycin forms a specific hydrogen bond with the 2-amino group

of the guanidine on the opposite strand. The authors propose it is this interaction that positions the mitomycin for formation of a covalent bond with the 2-amino group of the guanidine on the target strand. This proposal was verified by observation of such a hydrogen bond in the NMR of the DNA monoadduct.[40] It was also shown that mitomycin derivatives devoid of C10 functionality lacked the 5'-CG-3' specificity observed with the natural product.[41] All of these results seem to suggest that mitomycin C first recognizes 5'-CG-3' sequences non-covalently by hydrogen bonding prior to formation of the first covalent bond.

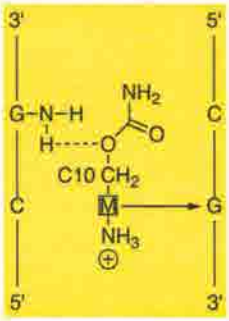


Figure 5: Rationale for 5'-CG-3' Specificity in Mitomycin/DNA Cross-links. Boxed M = mitomycin, C10 = Carbon 10 on the mitomycin skeleton.

FR900482 and FR66979 have also shown to be selective for 5'-CG-3' sequences. Interestingly, it was shown that FR66979 showed no specificity for monoalkylation as observed with mitomycin C.[42] Recent research has shown that FR66979 is capable of forming DNA-protein cross-links in addition to DNA-DNA cross-links. Williams and Rajski reported that FR66979 could efficiently cross-link the binding domain of the high mobility group (HMG) protein I/Y (HMG I/Y) with DNA.[43] HMG proteins are found in the minor groove of DNA, are over-expressed in cancerous cells, and thus act as markers for cancer.[44] The above finding was monumental in that it suggests an alternate mode of action than DNA-interstrand cross-linking alone.

The possibility of the mitomycin family of compounds forming cross-links with nuclear proteins and chromatin was further validated in a recent report by the groups of Luger and Williams.[45] In this report the authors show that when DNA is organized into nucleosomes, essentially no DNA-mitosene cross-linked adduct was found. The compound used for this study is a derivative of the FR900482 family of compounds that forms the active mitosene species upon photochemical activation. Monoalkylation was found to be the major adduct in all cases. Organization of DNA into nucleosomes seems to be a novel way of protecting the DNA from drug-mediated cross-linking. This result makes sense when considering the spatial requirement for cross-linking to occur and the radically different spatial arrangement of nucleosomal-bound DNA. The above result lends credence to the idea of alternate pathways for the biological mode of action of this class of compounds.

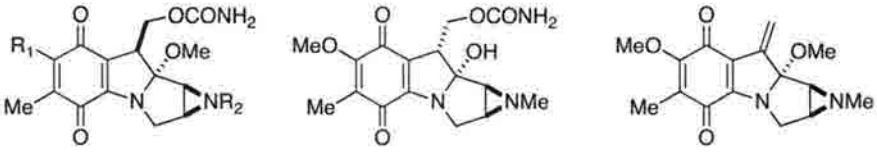
Mentioned in this chapter has been an introduction to the mitomycin family of natural products. Details about the background, isolation, and terminology of these compounds have been discussed. The proposed biogenesis of these compounds has been addressed as well as recent efforts in this area. Additionally, the biological activity and the mode of action of these compounds were presented. As can be seen from the material presented above, despite the multitude of reports concerning the mode of action of these compounds, the exact nature of how these compounds exert their biological activity is not completely understood. It is anticipated that continued study in this area would illuminate more details on this subject.

Chapter 2: Mitomycin Synthetic Studies Reported in the Literature

2.1: Previous Total Syntheses of the Mitomycins

2.1.1: Synthetic Challenges Associated with the Mitomycins

Due to the intriguing structure and potent activity, it is not surprising that the mitomycins have caught the attention of the synthetic community over the past 40 years. Despite the numerous synthetic routes that have been attempted towards these compounds, there have only been four successful approaches to this family of natural products. In 1977, Kishi reported the first total synthesis of any members of the mitomycins and made mitomycins A, B, C, and porifomycin (Figure 6). Ten years later, Fukuyama reported the successful completion of mitomycins A, and C. Additionally, Danishefsky and Jimenez have both completed total syntheses of mitomycin K (Figure 6). *To date, all total syntheses of the mitomycins have been racemic.*

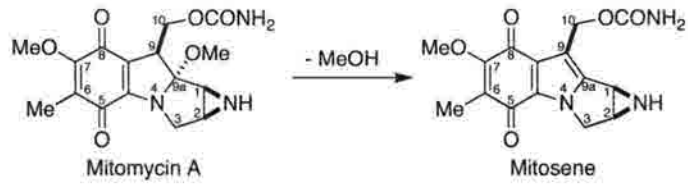


	R_1	R_2
Mitomycin C	NH_2	H
Mitomycin A	OMe	H
Porfiromycin	NH_2	Me

Figure 6: Members of the Mitomycins that have Succumbed to Total Synthesis

One of the challenges associated with the synthesis of these compounds is the hemiaminal at the C9a position. Under various conditions mitomycins will suffer rapid elimination of methanol to form the corresponding mitosene (Scheme 5). This

transformation appears to be even more facile in certain leucomitomycin systems. With the mitosene in place, the tetracycle is more prone to opening of the aziridine.



Mitomycin A Mitosene

Scheme 5: Elimination of Methanol from a Mitomycin to form the Mitosene

Another challenge with the mitomycins is the installation and preservation of the aziridine ring. Given the mode of action of these compounds as presented in Chapter 1, it is not surprising that the aziridine is prone to opening in certain cases. One might think of it as going through a synthetic sequence with a spring-loaded compound, ready to unleash itself to its active and more stable form at any moment. The fact that no asymmetric total synthesis of the mitomycins has been accomplished is largely due to the inability to introduce the aziridine at an early stage and in an asymmetric fashion.

The quinone moiety in the mitomycins also presents a problem. Once the quinone is in place, certain transformations (like removal of a protecting group from the aziridine) are difficult. Additionally as a synthesis unfolds, it is easy to get differences in oxidation states throughout the system that make for a challenge.

Arguably the most difficult challenge in the total synthesis is the compact, densely functionalized nature of these compounds. Danishefsky eloquently put the predicament this way:

> "The complexity of the problem arises from the need to accommodate highly interactive functionality in a rather compact matrix and to orchestrate the chemical progression such as to expose and maintain vulnerable structural

elements as the synthesis unfolds. *The synthesis of a mitomycin is the chemical equivalent of walking on egg shells.*"[46] (emphasis added)

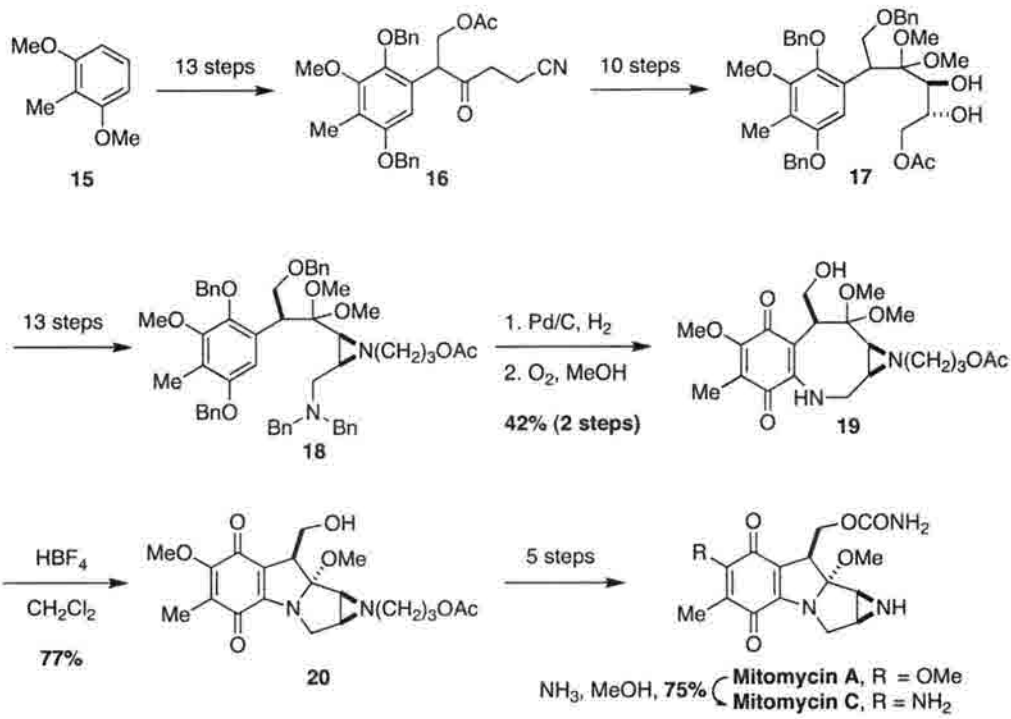
These problems mentioned above will manifest themselves as the total syntheses of mitomycins are presented.

2.1.2: Kishi's Total Synthesis of Mitomycins A, B, C and Porfiomycin[47]

In 1977 Kishi and co-workers reported their landmark total synthesis of mitomycins A and C. This synthesis is very impressive even by today's standards and represented a quantum leap in the field of natural product synthesis. The synthesis started with commercially available 2,6-dimethoxy toluene **15** (Scheme 6), and was elaborated thirteen steps to arrive at ketone **16**. From ketone **16**, ten more steps were required to from the ketal, install the diol, and reduce the terminal cyanide to the corresponding acetate to give diol **17**. Many of these steps were various protecting group manipulations in order to get the right functionality in place for subsequent transformations.

With diol **17** in hand, the next task was installation of the aziridine ring and conversion to a suitable cyclization precursor (Scheme 6). Thirteen steps were needed to accomplish these tasks and obtain ketal **18**. The majority of these thirteen steps were employed in the synthesis of the protected aziridine found in **18**. Due to the nature of the precursors, **18** could not be prepared enantioselectively. Ketal **18** was ready to be subjected to the key steps in the synthesis. Treatment of **18** under hydrogenation conditions simultaneously removed the benzyl groups on the aromatic, the hydroxyl group adjacent to C10 and the terminal amine. Treatment of the intermediate hydroquinone/primary amine compound under oxygen in methanol promoted quinone

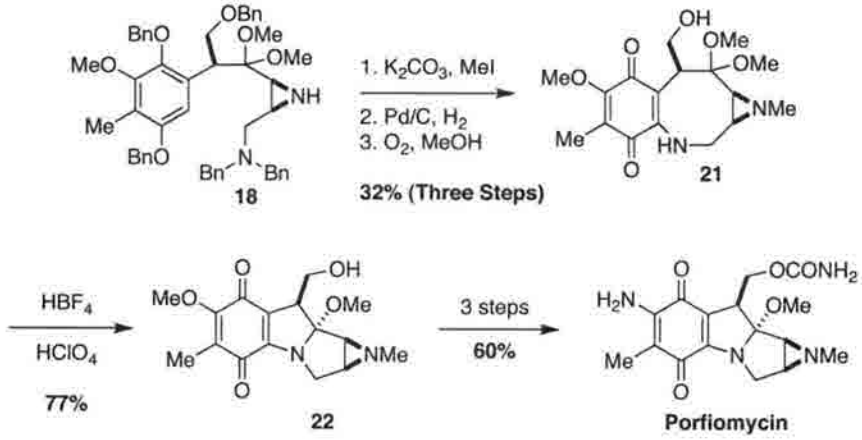
formation and Michael addition of the primary amine into the quinone to provide the protected benzazocane **19** in moderate yield (42% for two steps).



Scheme 6: Kishi's Total Synthesis of Mitomycins A and C

The final key step of the synthesis entailed conversion of protected benzazocane **19** to tetracycle **20** (Scheme 6). This transformation was accomplished by treatment of **19** with a Lewis acid (HBF_4) in dichloromethane to provide tetracycle **20** in good yield. With tetracycle **20** in hand, all that remained was conversion of the alcohol to the carbamate and removal of the protecting group on the aziridine. The carbamate was made in two steps and the free aziridine was obtained through a 3 step procedure involving removal of the acetate, oxidation of the alcohol to the aldehyde, and treatment with a Lewis Acid to effect a retro-Michael addition and furnish mitomycin A in a 44 step linear sequence with a overall yield of 0.19%. Conversion of mitomycin A to mitomycin C was accomplished according to the procedure of Webb and co-workers.[5]

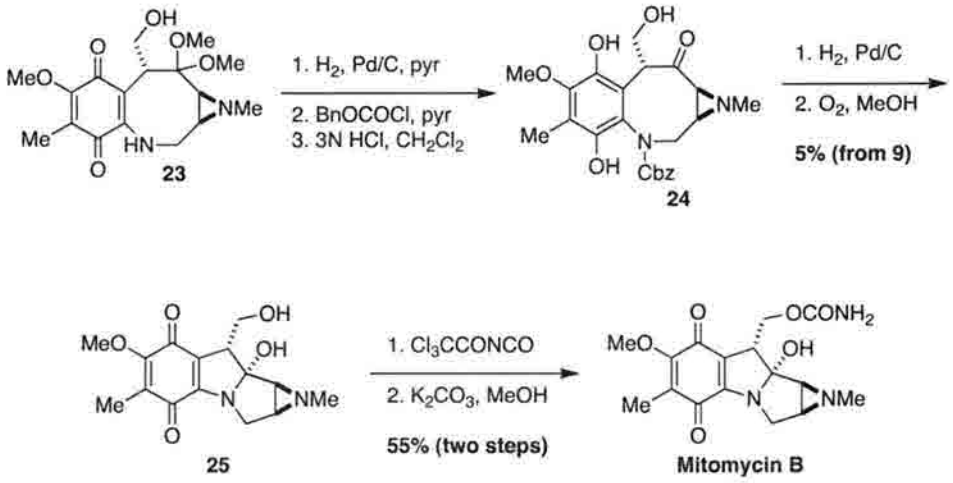
This method was also applied to the total synthesis of porfiomycin (Scheme 7) starting from common intermediate **18**. Methylation of the free aziridine followed by quinone formation and Michael addition as before provided quinone **21**. Lewis acid promoted transannular cyclization under the previously developed conditions provided tetracycle **22**, which was subsequently converted to porfiomycin in three additional steps involving carbamate and amine formation. The overall yield for porfiomycin is about the same as for mitomycin A.



Scheme 7: Total Synthesis of Porfiomycin by Kishi and Coworkers

Kishi also accomplished the total synthesis of mitomycin B in a similar manner as mitomycins A, C and porfiomycin (Scheme 8). At the outset of the synthesis, it was not known which diastereomer of C10, resulting from a hydroxymethylation reaction, corresponded to mitomycins A and B respectively. Having established the correct diastereomer for the total synthesis of mitomycins A and C, the other diastereomer was brought through the same sequence of steps to arrive at quinone **23**. Reduction of **23** by hydrogenation followed by protection of the benzazocane nitrogen with benzyl chloroformate gave hydroquinone **24**. Treatment of **24** to hydrogenation conditions and then oxygen effected removal of the Cbz carbamate, oxidation to the quinone and

subsequent cyclization to give tetracycle **25** in a poor overall yield (5%) from quinone **25**. Conversion of tetracycle **25** to mitomycin B proved difficult, but it was eventually found that addition of potassium carbonate in methanol in the second step did provide mitomycin B in 55% yield for the two steps.



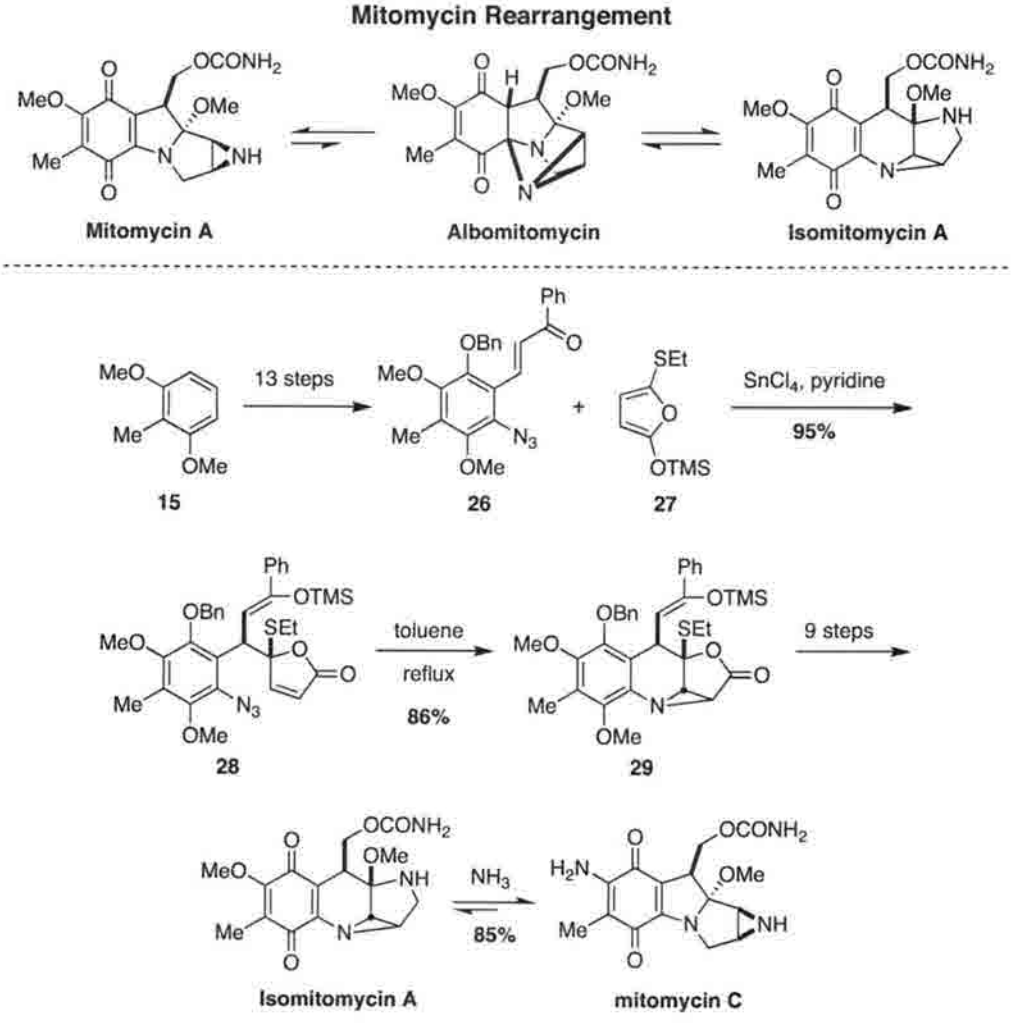
Scheme 8: Kishi's Total Synthesis of Mitomycin B

Kishi's total synthesis of the mitomycins represented a landmark achievement at the time and stands respectable still today. The synthetic route, though lengthy and linear, allowed for the synthesis of multiple members of the mitomycins from common intermediates.

2.1.3: Fukuyama's Total Synthesis of Isomitomycins A, C and Mitomycins A, C[48]

In 1987 and later in 1989 Fukuyama and Yang published a racemic total synthesis of isomitomycin A, mitomycin A, and mitomycin C. Their approach took advantage of the mitomycin rearrangement reaction discovered earlier by Kono and co-workers (Scheme 9).[7] This novel approach could be advantageous with regard of being able to avoid the unstable tetracyclic skeleton of the mitomycins until the last step, at which the stable natural product would be formed.[49]

The synthesis commenced by use of 2,6-dimethoxy toluene **15**, the same starting material used by Kishi, and elaborated thirteen steps to chalcone **26**. Chalcone **26** was then reacted with siloxy furan **27** (prepared in one step) with tin tetrachloride at -78 °C to cleanly give (after the addition of pyridine) azide **28** in excellent yield. Azide **28** was then heated in toluene at reflux to induce an intramolecular azide-olefin cycloaddition that smoothly gave the tetracyclic aziridine **29** in good yield. Ten steps were used to transform tetracyclic aziridine **29** to isomitomycin A.



Scheme 9: Fukuyama's Total Synthesis of Isomitomycin A and Mitomycin C

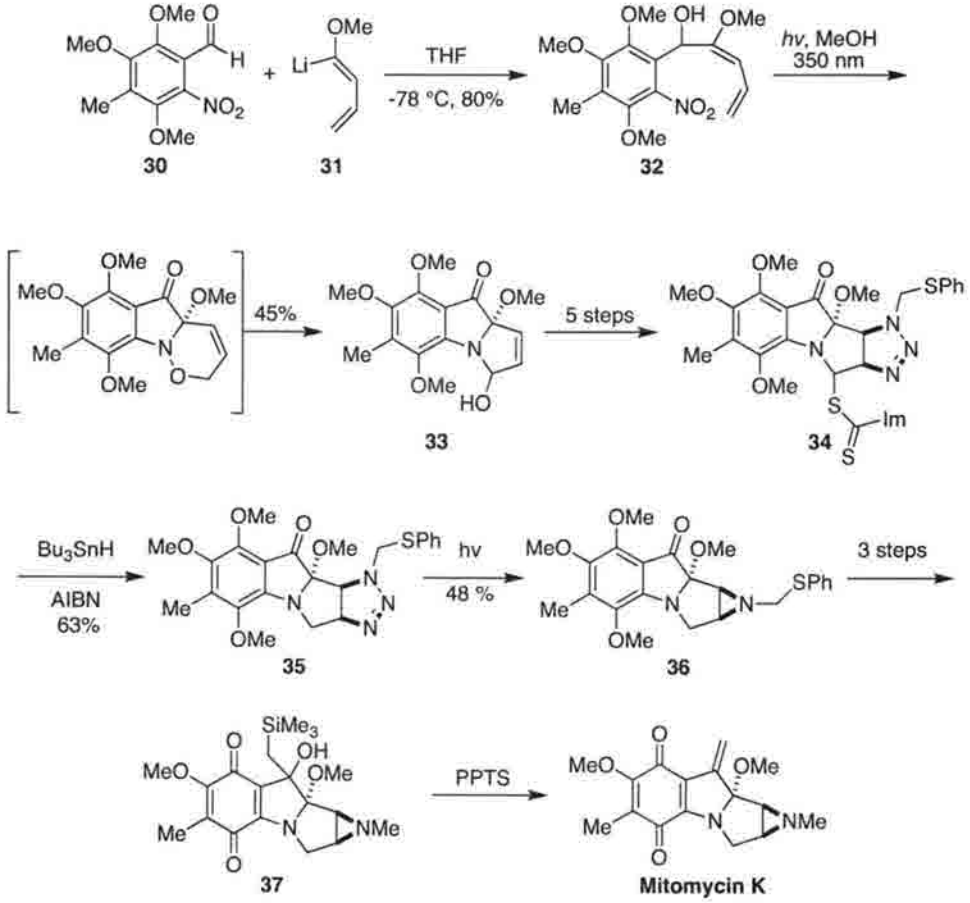
It was found that subjection of isomitomycin A to ammonia in methanol provided cleanly mitomycin C (presumably through isomitomycin C as an intermediate) in 26 steps and 10% overall yield. Mitomycin A was also obtained by treatment of isomitomycin A to the mitomycin rearrangement conditions ($(AlO{i}Pr)_3$, MeOH, room temperature, 2 days) in 91% yield. This elegant synthesis has set the benchmark for total synthesis of the mitomycins that has yet to be matched. The authors also reported that each step in the synthesis is scalable, allowing for large amounts of the natural products to be made.

2.1.4: Danishefsky's Total Synthesis of Mitomycin K[50]

Danishefsky and his colleagues have contributed significantly to both the synthesis of the mitomycins and their analogues as well as their mode of action.[46] As part of this work, Danishefsky developed a synthesis of mitomycin K using novel transformations never before seen on the mitomycins. The synthesis starts from nitro aldehyde **30** (prepared in 6 steps from commercial material) and reacting it with the known 1-methoxylithiobutadiene **31** to form alcohol **32** (Scheme 10). Reaction of **32** under intramolecular Diels-Alder (IMDA) conditions provided tricycle **33** in modest yield. Presumably the nitrosodieneone is the active IMDA substrate and forms the fused intermediate (shown in brackets, Scheme 10), which upon rearrangement gives ketone **33**. Ketone **33** was smoothly converted to triazoline **34** in five more steps.

The tetracyclic core of the mitomycins was formed first by elimination of the thiocarbonyl moiety to form ketone **35** (Scheme 10). Formation of the aziridine was accomplished by photolysis of **35** to provide aziridine **36**. De-sulfurization to give the N-

methyl aziridine, reaction of the ketone with trimethylsilylmethyl lithium, and quinone formation provided quinone **37**. It should be noted that oxidation to the quinone with the para-methoxy groups was accomplished albeit in poor yield (8-16%). Treatment of **37** under PPTS conditions effected Peterson elimination and provided mitomycin K in 21 steps and 0.1% overall yield.



Scheme 10: Total Synthesis of Mitomycin K by Danishefsky

Despite the low yield in the quinone-forming reaction, the synthesis of mitomycin K by Danishefsky is an elegant piece of work that employs several novel transformations. Key steps in the synthesis include formation of the tricyclic core by an IMDA reaction, aziridine formation by photolysis of triazoline **35**, and Peterson elimination to form the exocyclic olefin found in mitomycin K.

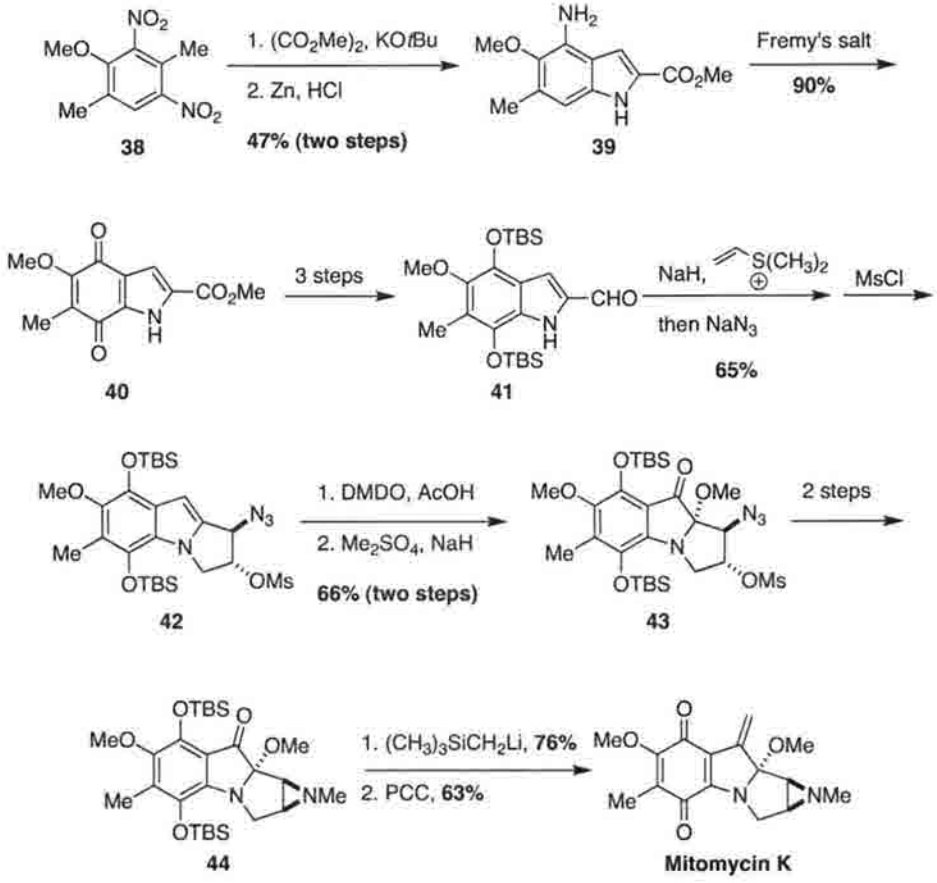
2.1.5: Jimenez's Total Synthesis of Mitomycin K[51]

The most recent completed total synthesis of a mitomycin is the synthesis of mitomycin K by Jimenez and Wang (Scheme 11). The synthesis starts from dinitro arene **38** (prepared in one step from 2,5-dimethylanisole) and, after alkylation and reduction, provided indole **39**. Reaction of indole **39** with Fremy's salt gave quinone **40**, which was subsequently reduced to the hydroquinone, protected as the bis-TBS arene, followed by reduction of the methyl ester to give aldehyde **41**. Reaction of aldehyde **41** with dimethylvinylsulfonium iodide in the presence of sodium hydride, followed by ring opening of the tetracyclic epoxide with sodium azide furnished tricyclic azido alcohol. Mesylation of the tricyclic azido alcohol provided indole **42**, which was ready for the last key step of the synthesis.

The last key step of the synthesis entailed oxidation of the indole double bond of **42**. It was found that treatment of indole **42** with dimethyldioxirane and acetic acid followed by methylation of the resulting alcohol gave ketone **43** in good yield for the two steps as a single diastereomer (Scheme 11). Staudinger reaction of **43** to form the free aziridine and methylation gave tetracycle **44**. Reacting tetracycle **44** to the conditions used by Danishefsky effected formation of both the quinone and the exocyclic olefin to provide mitomycin K in 15 steps and 9% overall yield.

Jimenez and Wang accomplished the total synthesis of mitomycin K in a straightforward and efficient manner without the excess use of protecting groups. The key step in the synthesis is the oxidation of the C2-C3 indole double bond in **42** to form ketone **43** with concomitant installation of the requisite methylaminal. This novel

transformation gave access to substrates where chemistry developed earlier by Danishefsky and coworkers could be used to complete the synthesis.



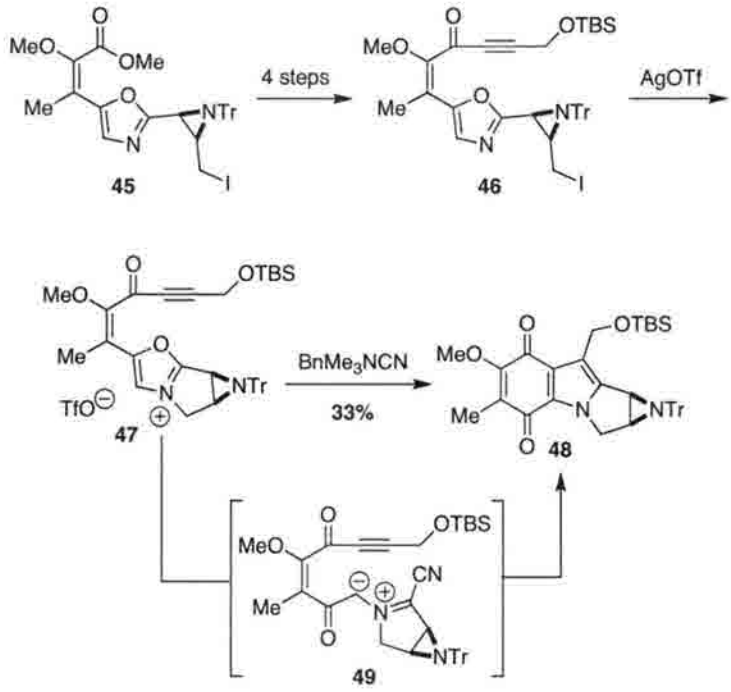
Scheme 11: Synthesis of Mitomycin K by Jimenez and Wang

2.2: Synthetic Studies Towards the Mitomycins

Listed in this section are several studies towards the synthesis of the mitomycins. Because of the large amount of literature concerning synthetic efforts towards the mitomycins, this is not a comprehensive review. Rather, included are several of the most recent synthetic efforts, accounts where advanced intermediates were made, and reports that helped to guide our thinking in the development of our own synthesis towards these natural products.

2.2.1: Synthesis of Aziridinomitosenes by Vedejs[52]

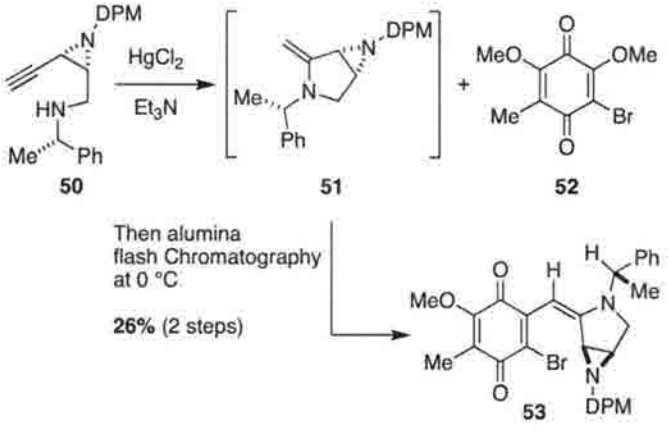
In a fresh approach to access the tetracyclic skeleton of the mitomycins, Vedejs and coworkers developed a dipolar cycloaddition approach as shown in Scheme 12. The key step of the synthesis was the treatment of oxazole **46** (prepared in 4 steps from **45**) with silver triflate to give tricycle **47**. Tricycle **47** was not isolated but was treated in situ with the organic soluble cyanide source $BnMe_3N^+CN^-$, which gave aziridinomitosene **48** in 33% yield. It is presumed that aziridinomitosene **48** goes through intermediate **49**, upon which a dipolar cycloaddition would give the desired product. This is a very nice and concise synthetic sequence that is in further development by Vedejs and coworkers.



Scheme 12: Dipolar Cycloaddition Approach to Mitosenes by Vedejs

2.2.2: Johnston's Synthesis of an Advanced Intermediate[53]

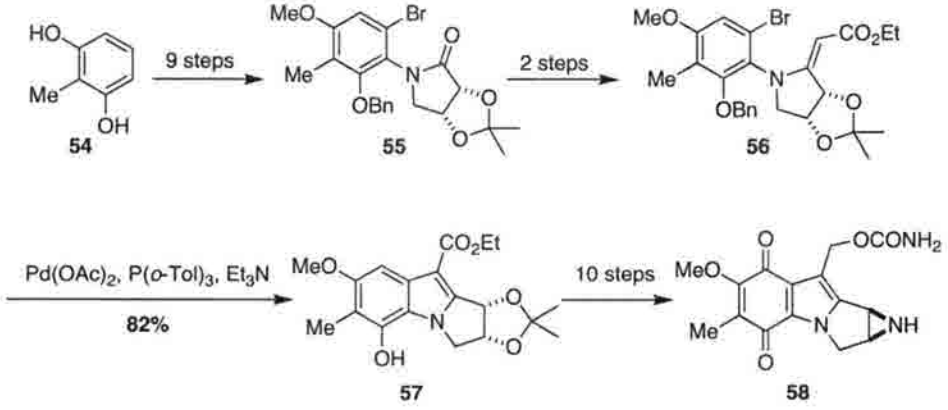
Johnston and coworkers have recently developed an efficient approach to an advanced intermediate **53** by regioselective coupling of enamine **51** with quinone **52** (Scheme 13). Enamine **51** is formed in situ from the alkynylamine **50**, which is formed by a base-promoted aza-Darzens reaction with the diphenylmethyl amine Shiff base of ethyl glyoxylate with *tert*-butyl chloroacetate. Despite the low yield of **53** (26% over 2 steps), the synthetic sequence requires only 9 steps from commercially available ethyl glyoxylate. If successful this synthesis would be a huge step forward in the quest to find a synthetically useful sequence for the short and efficient construction of analogues. One drawback to the synthesis is the instability of intermediate **53** ($t_{1/2}$ = 1.5 days at -15 °C). This will undoubtedly make the next steps in the synthesis quite challenging. Hurdles yet to overcome in the synthesis include removal of the protecting groups on both the aziridine and pyrrolidine nitrogen's, as well as installation of the C10 hydroxymethyl moiety.



Scheme 13: Johnston's Synthesis of Advanced Intermediate **53**

2.2.3: Michael's Format Total Synthesis of a 7-Methoxyaziridinomitosene[54]

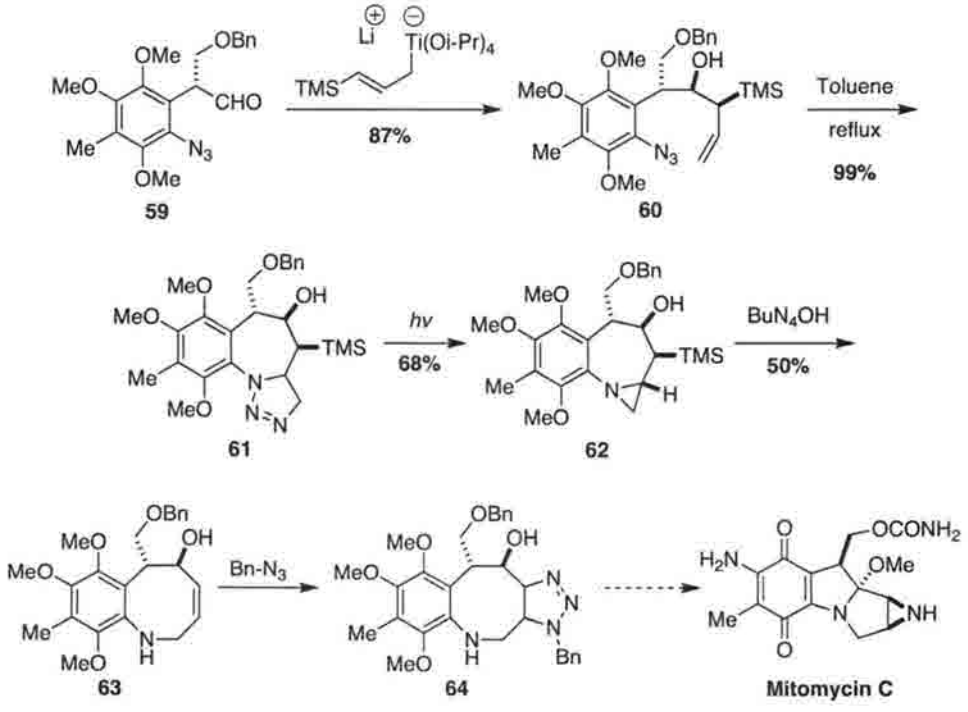
In 2006, Michael and coworkers disclosed a formal synthesis of 7-methoxy-aziridinomitosene as shown in Scheme 14. The synthesis was actually a formal total synthesis relying on the work done by Jimenez and Dong to perform the last step of aziridine formation.[55] The synthesis started from 2-methylresorcinol **54** and was converted to lactam **55** in nine steps. The first key step of the sequence was lactam formation accomplished by mesylation of the primary alcohol and subsequent nucleophilic attack and ring closure by the amide nitrogen. Two more steps were required to make enamine **56**. The next key step entailed treatment of enamine **56** to intramolecular Heck cyclization conditions, which afforded tetracycle **57** in 82% yield. Ten more steps involving aziridine, quinone, and carbamate formation were required to access 7-methoxyaziridinomitosene **58**. This is a nice synthesis that builds off of earlier precedence in the mitomycin literature where enamines were used in tetracycle formation.[56]



Scheme 14: Michael's Synthesis of 7-Methoxyaziridinomitosene **58**

2.2.4: Ciufolini's Approach to the Mitomycins[57]

Ciufolini recently disclosed their approach to the mitomycins that takes advantage of the homo-Brook methodology for eight-membered ring formation previously used in their total synthesis of FR66979.[58] The synthesis starts with aldehyde **59** and is converted to aziridine **62** in three steps and readied for the key step of the synthesis (Scheme 15). Homo-Brook rearrangement on aziridine **62** proceeds smoothly by treatment with tetrabutylammonium hydroxide and, as in the case of FR66979, and provides benzazocine **63** in reasonable yield. Reaction of benzazocine **63** with benzyl azide smoothly produced triazoline **64**, whereupon photolysis of **64** should yield the *N*-benzyl aziridine. From this point, a number of straightforward transformations should give the mitomycins.

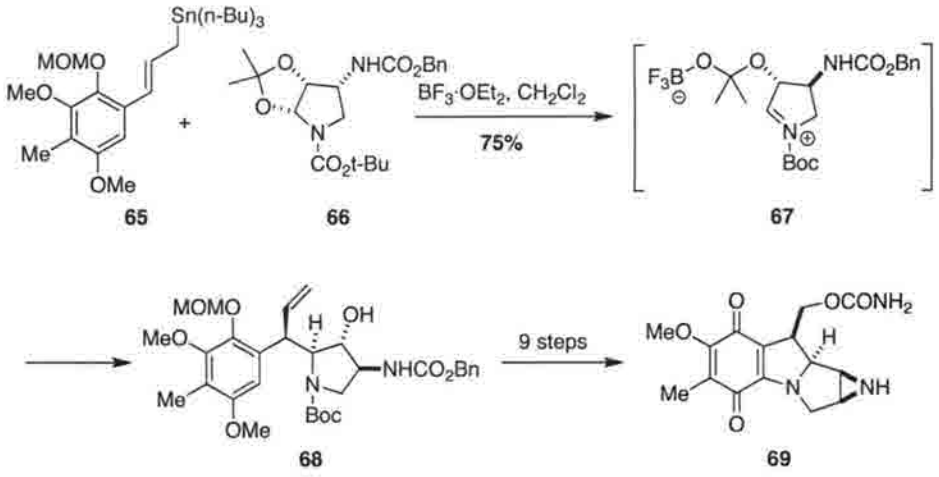


Scheme 15: Ciufolini's Approach to the Mitomycins

A few potential problems with this route are the following. First, the use of a hydroquinone protected as the bis-methoxy ether substrate that might make quinone formation difficult. Second, oxidation of benzazocine **63** or **64** to the benzazocane might be difficult in the presence of the free N-H bond in the ring. Third, epimerization of the C10 hydroxymethyl moiety to that desired for mitomycin C might also be difficult. Nonetheless, this approach demonstrates the effective application of the homo-Brook rearrangement methodology to access the core structure of the mitomycins.

2.2.5: Coleman's Asymmetric Synthesis of (+)-9a-Desmethoxymitomycin A [59]

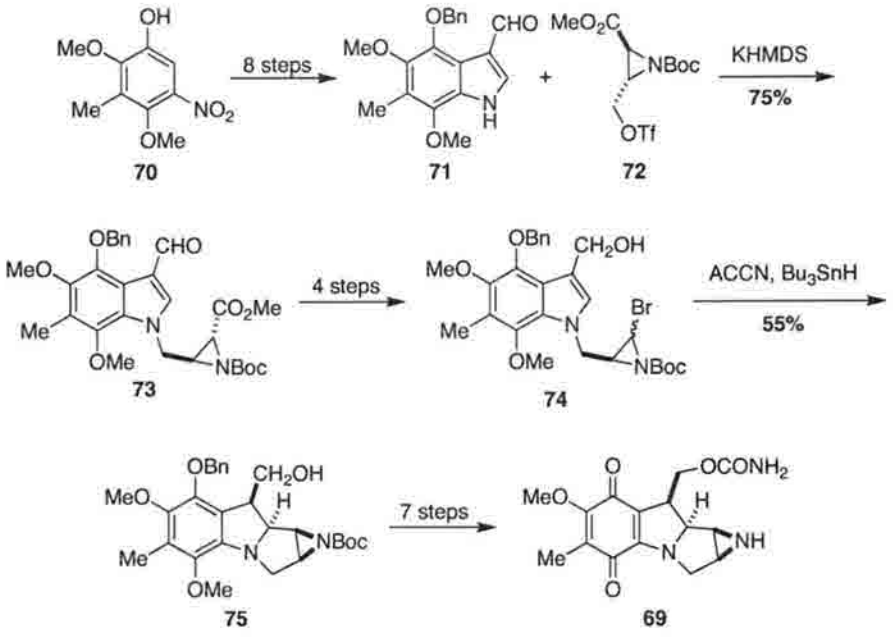
In 2004 Coleman and coworkers reported the asymmetric total synthesis (=)-9a-desmethoxymitomycin A **69**. As can be seen, **69** contains all the required functionality for mitomycin A except for the C9a methoxy moiety (Scheme 16). The key step of the sequence is the addition of alkylstannane **65** to iminium ion **67** (formed in situ from pyrrolidine **66**) to form alcohol **68**. Aziridine formation by use of a Mitsunobu reaction, carbamate formation, Boc removal, quinone formation, and cyclization gave aziridinomitosane **69** in good overall yield. The author's report that attempts to install the required C9a oxygen for completion of the synthesis is now in progress. If late-stage introduction of oxygen fails, significant re-tooling of the synthetic route would be required so as to account for earlier installment of the required oxygen.



Scheme 16: Coleman's Asymmetric Synthesis of Aziridinomitosane **69**

2.2.5: Ziegler's Synthesis of (+)-9a-Desmethoxymitomycin A[60]

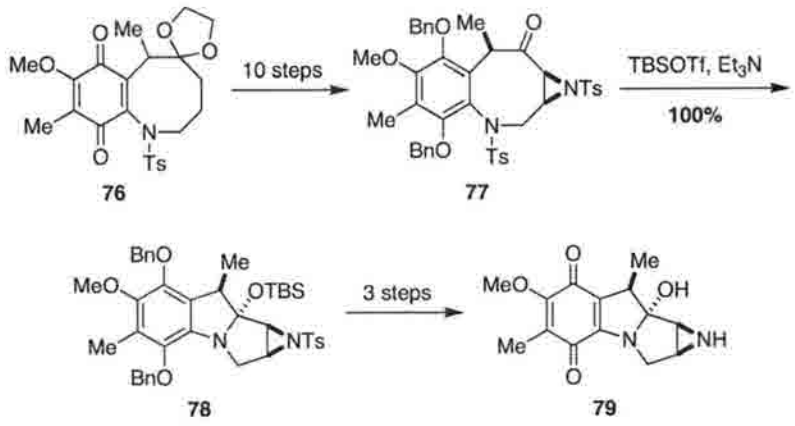
Before the successful synthesis of desmethoxymitomycin A **69** by Coleman and coworkers (Scheme 16), Ziegler and Berlin reported the asymmetric synthesis of the same molecule by use of a aziridinyl radical cyclization route. This same approach was successfully used in the synthesis of the FR900482 core structure.[61] The synthesis, shown in Scheme 17, commenced with nitro arene **70**, and elaborated eight steps to indole **71**. Indole **71** was coupled with ester **72** to afford coupled product **73** and elaborated four more steps to give bromoaziridine **74**. The key step of the synthesis was treatment of bromoaziridine **74** under aziridinyl radical-forming conditions, which cyclized onto the indole olefin to give tetracycle **75** in decent yield. It is interesting to note that conversion of **74** to tetracycle **75** is completely diastereoselective for the diastereomer shown in **75**. Further manipulation of **75** did provide desmethoxymitomycin A **69** in seven more synthetic transformations.



Scheme 17: Synthesis of (+)-Desmethoxymitomycin A by Ziegler and Berlin

2.2.6: Approach to the Mitomycins by Ban and Coworkers[62]

A synthetic approach of keen interest to us in our initial search of the literature was the synthesis of tetracycle **79** by Ban and coworkers (Scheme 18). Quinone **76** (obtained by a criss-cross annulation reaction as previously reported[63]) was elaborated to benzazocane **77** in a straightforward manner requiring ten steps. The key step of the synthesis involved treatment of benzazocane **77** with TBSOTf and triethyl amine, which effected a transannular cyclization reaction and provided tetracycle **78** in quantitative yield. Three more steps involving quinone formation and deprotection of protecting groups gave tetracycle **79** in good overall yield. The step of interest to us was the key step (transannular cyclization), and plans were made to see if this transformation might be able to be incorporated into our own synthesis of the mitomycins.



Scheme 18: Ban's Synthesis of Tetracycle **79** by Transannular Cyclization

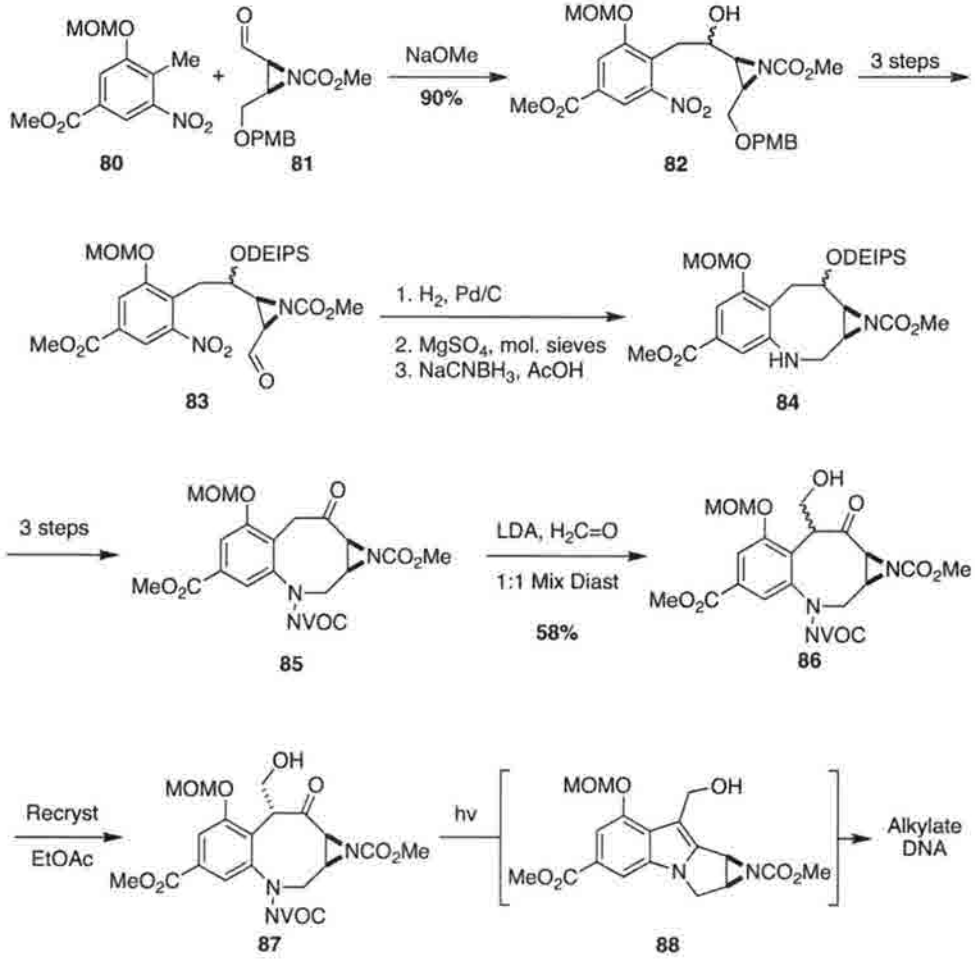
2.3: Synthetic Studies on FR900482 in the Williams Group

2.3.1: Synthesis of a Photo-Triggered Pro-Mitosene Compound[64]

The most useful previous synthetic studies that guided our thinking were not those mentioned above, but were those from our own research group in our synthetic studies of FR900482. The first study that was very useful in our own synthesis was the synthesis of photo-triggered pro-mitosene **87**. This compound was shown to efficiently crosslink DNA upon photoactivation (presumably through the formation of mitosene **88**). Pro-mitosene **87** was the first synthetic compound of its type that forms the reactive mitosene upon activation.

The synthesis of **87** commenced with aldol coupling of the known nitro arene **80** with aziridne aldehyde **81** (prepared in ten steps from commercial material) to afford alcohol **82** as a 2:1 mixture of diastereomers (Scheme 19). Silyl protection, PMB ether removal, and oxidation of the primary alcohol to the aldehyde gave aldehyde **83** in good yield. Benzazocine **84** was formed by first reduction of the nitro moiety in **83** using hydrogenation conditions to give the corresponding aniline. The anilino aldehyde

intermediate was found to be unstable and was directly taken on to the reductive amination sequence shown in Scheme 19 to afford benzazocine **84** in good yield. Hydroxymethylation of benzazocane **85** (obtained in three steps from benzazocine **84**) with strong base (LDA) and anhydrous formaldehyde did provide the desired alcohols **86** as a 1:1 mixture of diastereomers. It was found that the diastereomer **87**, bearing an anti relationship to the aziridine, could be obtained from recrystallization in ethyl acetate.



Scheme 19: Synthesis of a Photo-Triggered Pro-Mitosene

In summary, pro-mitosene precursor **87** could be obtained by an efficient synthetic sequence shown in Scheme 19. Key steps to the synthesis include coupling of nitro arene **80** with aziridine aldehyde **81**, reductive amination to afford benzazocine **84**,

and hydroxymethylation/recrystallization to give the desired promitosene **87**. As will be discussed in Chapter 3, we thought the above synthetic sequence might also be amendable to the synthesis of the mitomycins.

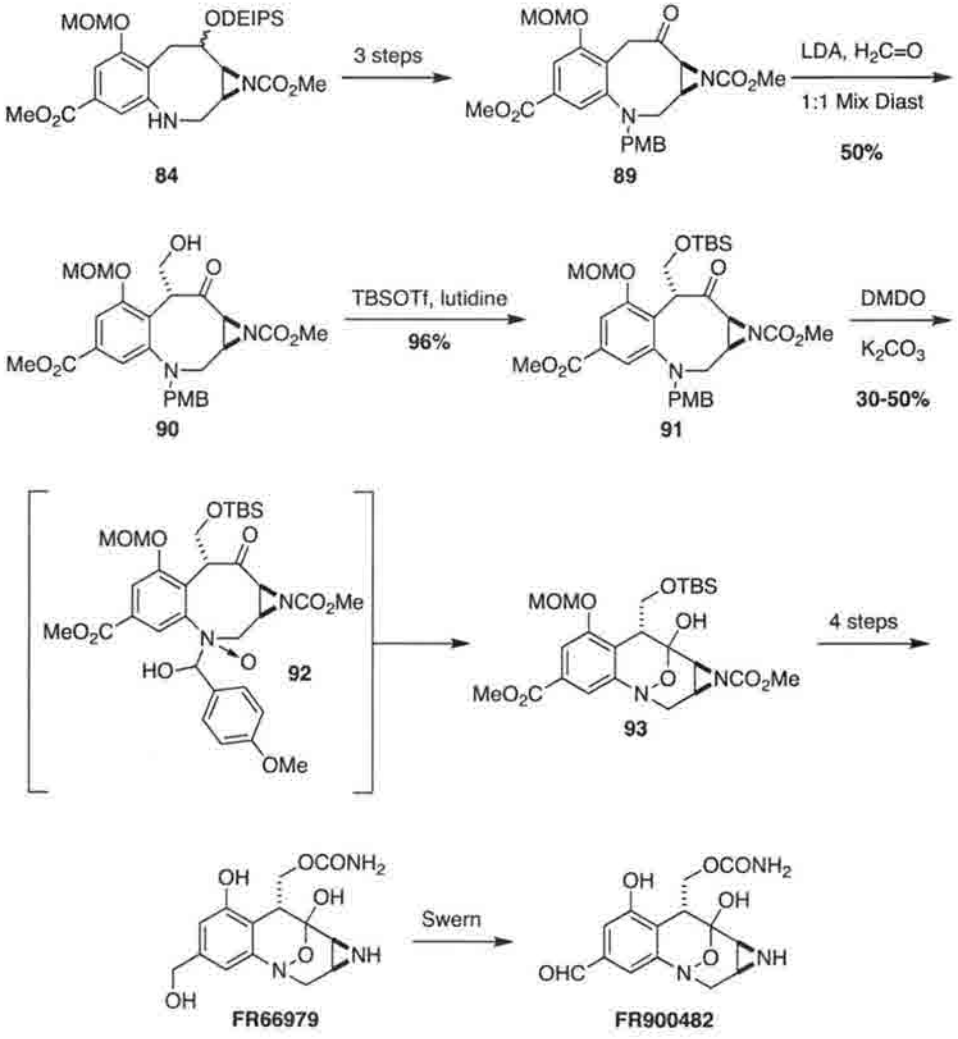
2.3.2: Asymmetric Total Synthesis of (+)-FR900482 and (+)-FR66979[65]

The synthetic method developed in our group for the synthesis of pro-mitosene **87** (shown in Scheme 19) was also used in the asymmetric total synthesis of (+)-FR900482 and (+)-FR66979. The synthesis started from benzazocine **84** (Scheme 19) and was converted to benzazocane **89** in three steps as seen in Scheme 20. Hydroxymethylation proceeded as previously described, and it was found that the disfavored diastereomer could be epimerized and converted to the desired diastereomer **90**. Protection of alcohol **90** as the TBS silyl ether gave benzazocane **91**.

The key step of the synthesis entailed treatment of benzazocane **91** with dimethyldioxirane, which did provide tetracycle **93** in moderate yield (Scheme 20). It is presumed that the reaction is proceeding through formation of *N*-oxide **92**, which then attacks the ketone followed by concomitant loss of *p*-anisaldehyde to give the desired tetracycle (Scheme 20). Four more steps were required to access (+)-FR669979 followed by oxidation of the primary alcohol using the Swern protocol to provide (+)-FR900482. This is a very nice synthesis that employs a novel transformation for formation of the hydroxylamine hemiketal moiety. This synthesis provided us with many ideas as we were planning our efforts towards the mitomycins.

This chapter has summarized various approaches, both successful and not, towards the synthesis of mitomycins. In addition to the interesting biological activity and

mode of action of these compounds, the framework of the mitomycins is an interesting scaffold upon which novel reactions have been (see above) and will continue to be developed. We have also described previous synthetic efforts in our group that particularly guided our thinking in the development of our first generation approach to the mitomycins. This approach is presented and discussed in detailed in the next chapter.



Scheme 20: Asymmetric Total Synthesis of (+)-FR900482 and (+)-FR66979

Chapter 3: First Generation Approach to the Mitomycins

3.1: Goals, General Strategy, and Early Studies

3.1.1: Goals For the Project

At the outset of this project, we were intent on accomplishing the three major goals shown below:

Goal #1: Complete the Asymmetric Total Syntheses of Mitomycins A, B, C, and K.

At the start of this project, Ted Judd in the Williams group had recently completed the asymmetric total synthesis of FR900482 and FR66979. This was accomplished via a highly convergent route (see section 2.3.2 for more details). It was thought that this same strategy could be used for the asymmetric total synthesis of the mitomycins to get to the core of the molecule by employment of a different aromatic piece. It was thought that the chemistry would be more "plug and chug" and should proceed as before to make the benzazocane core, whereupon new chemistry would need to be developed in order to access the tetracyclic skeleton of the mitomycins. It turns out there were some major assumptions made that turned out to be wrong as the synthesis progressed.

Goal #2: Complete the Synthesis of Putative Biosynthetic Intermediates of the Mitomycins

Once the asymmetric total synthesis of the mitomycins was completed, we expected to use the synthetic route we had developed to make putative biosynthetic intermediates to be tested in the laboratories of Professor David Sherman at the

University of Michigan (see section 1.1.4 for the proposed biosynthesis of the mitomycins and FR900482). Early-stage intermediates in the proposed biosynthesis of the mitomycins and FR900482 have recently been synthesized in our group that are currently under investigation.

Goal #3: Investigate the Existence and structure of Mitomycin DNA-HMG I/Y Cross-links.

After synthesis of the natural products and biosynthetic intermediates was accomplished, it was hoped to then turn our attention to the identification of DNA-HMG I/Y protein cross-links in mitomycin C and FR669779. It was hoped to expand upon the findings of Rajski by *elucidating the exact structure of this complex.* It was hoped that use of NMR, mass spectrometry, as well as other methods would help to accomplish this feat.

3.1.2: General Synthetic Strategy

In designing a route towards the total synthesis of the mitomycins, there were a few items that were central to our plan. First, we wanted a synthesis that was highly convergent that would readily allow for the synthesis of analogues, and proposed putative biosynthetic intermediates of the mitomycins. A convergent synthesis would be convenient should the inevitable need arise to go back and modify our route.

Second, we wanted to install the aziridine at an early stage so that the asymmetry might be intact before arriving at the more challenging steps of the synthesis. As seen in Chapter 2, a major reason there hasn't been an asymmetric total synthesis of the mitomycins to date is due to the late stage installment of the aziridine moiety with a lack

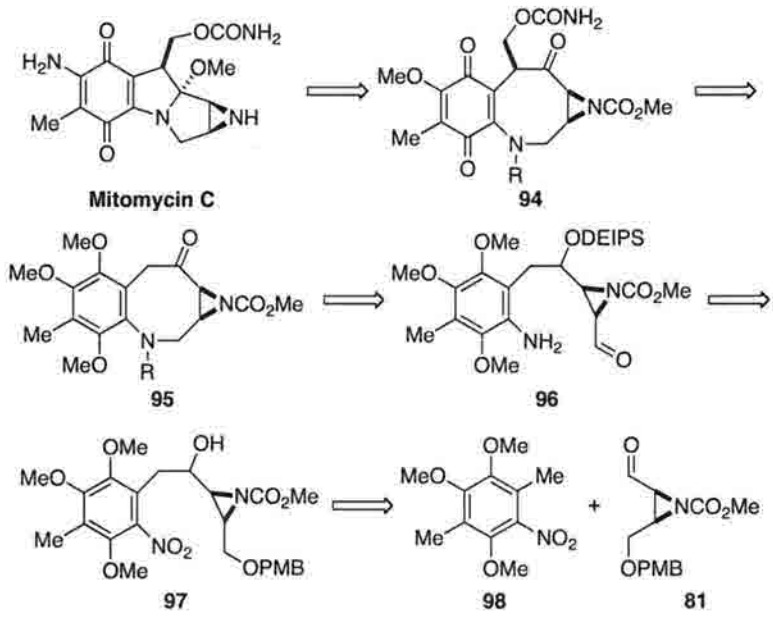
of stereoselectivity in each case. Carrying an aziridine moiety through a multi-step complex synthesis is an enormous challenge in its own right. However, we were encouraged by the fact that this was successfully done in Judd and Williams' elegant total synthesis of FR900482 and FR66979.

Lastly, we wanted to formulate the synthesis to be flexible enough so that we might access all the members of the mitomycins through a common intermediate. This would involve judicial use of protecting groups on the aziridine, the C9a hemiaminal oxygen, and the hydroxymethyl group. Another challenge in this regard is the construction of intermediates such that both diastereomers of C9-C10 might be obtained in order to access all members of the family. Elimination of the hydroxymethyl moiety on C10 would also be required in order to access mitomycins G-K bearing the exocyclic olefin.

3.1.3: Retrosynthetic Analysis

With the goals and criteria for the synthesis laid out above, we created our first retrosynthetic analysis (Scheme 21). It was hoped that MMC could come from quinone **94**. The key step involved would be transannular cyclization of the benzazocane nitrogen onto the carbonyl (or a suitable surrogate like a ketal) to give the tetracyclic core of the mitomycins. Removal of the methyl carbamate protecting the aziridine would then give MMC. The choice of a methyl carbamate as a protecting group for the aziridine was chosen based on its successful outcome in the total synthesis of FR900482 by our group and demonstrated by Danishefsky. It was thought that quinone **94** could be formed *via* hydroxymethylation to install C10 and then quinone formation of benzazocane **95**. It was

postulated that benzazocane **95** could be accessed by a reductive amination of aniline **96,** as previously performed by Judd in the FR900482 synthesis, followed by removal of the silyl group and oxidation to the ketone. Aniline **96** could arise from alcohol **97** *via* protection of the secondary alcohol, removal of the PMB ether and oxidation to the aldehyde. Alcohol **97** would come from a coupling reaction of nitro arene **98** and aziridine aldehyde **81** used by Judd in the synthesis of FR900482.

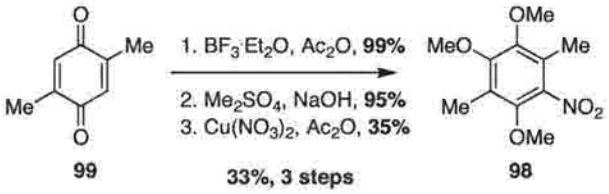


Scheme 21: Retrosynthetic Analysis for the Total Synthesis of Mitomycin C

3.1.4: Synthesis of Nitro Arene and Aziridine Aldehyde:

The first task that was undertaken towards the synthesis of the mitomycins was the development of efficient routes to access nitro arene **98** and aziridine aldehyde **81** respectively. Synthesis of nitro arene **98** (Scheme 22) commenced from commercially available 2,5-dimethylquinone **99**. Acetylation and subsequent methylation provided the trimethoxy arene precursor by following a previously reported procedure.[66] Nitration of the trimethoxy arene proved to be more difficult than anticipated. The best method for the

nitration was found to be copper nitrate in acetic anhydride and gave nitro arene **98** in 35% yield (33% for three steps). The yield for the nitration reaction is comparable with similar systems where cupric nitrate has been employed (40% for the bis-benzyl derivative). The major product of the nitration reaction was 2,5-dimethyl-3-methoxy quinone. It was found that the byproduct could be recycled to by a three-step procedure involving reduction to the hydroquinone, protection as the methyl ethers, and re-subjecting the bis-methoxy arene to the nitration conditions.

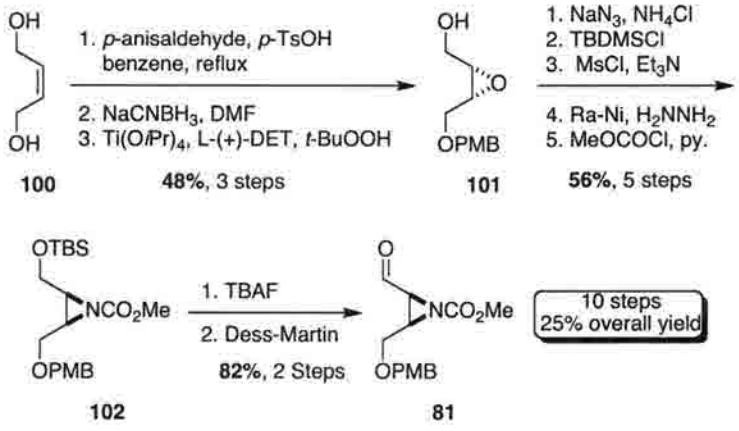


99 **33%, 3 steps** **98**

Scheme 22: Synthesis of Nitro Arene **98**

The synthesis of aziridine aldehyde **81** was performed as previously reported by our research group in the total synthesis of FR900482 and is shown in Scheme 23.[64] The synthesis started with the commercially available cis-butene-1,4-diol **100** and was converted to epoxide **101** in three straightforward transformations. The key step in the sequence was enantioselective formation of the aziridine *via* a Sharpless asymmetric epoxidation reaction (87% ee, 98% after recrystallization). In the original procedure, the yield of the Sharpless epoxidation suffered as a result of a complicated workup. In our studies we introduced a workup that is easier, faster, and resulted in quantitative yield of the epoxide. Details of this new workup are included in the experimental portion. As mentioned previously, early installation of the aziridine seems to be key in accessing the mitomycins in an asymmetric fashion. Epoxide **101** was carried through five transformations resulting in formation of the aziridine protected as the methyl carbamate

and protection of the primary alcohol to afford aziridine **102**. Removal of the silyl ether and oxidation of the alcohol furnished aziridine aldehyde **81** in 10 steps overall with a yield of 25%. This sequence of steps is highly amendable to large scale with over 10 grams of **81** being easily prepared in under 2 weeks time.

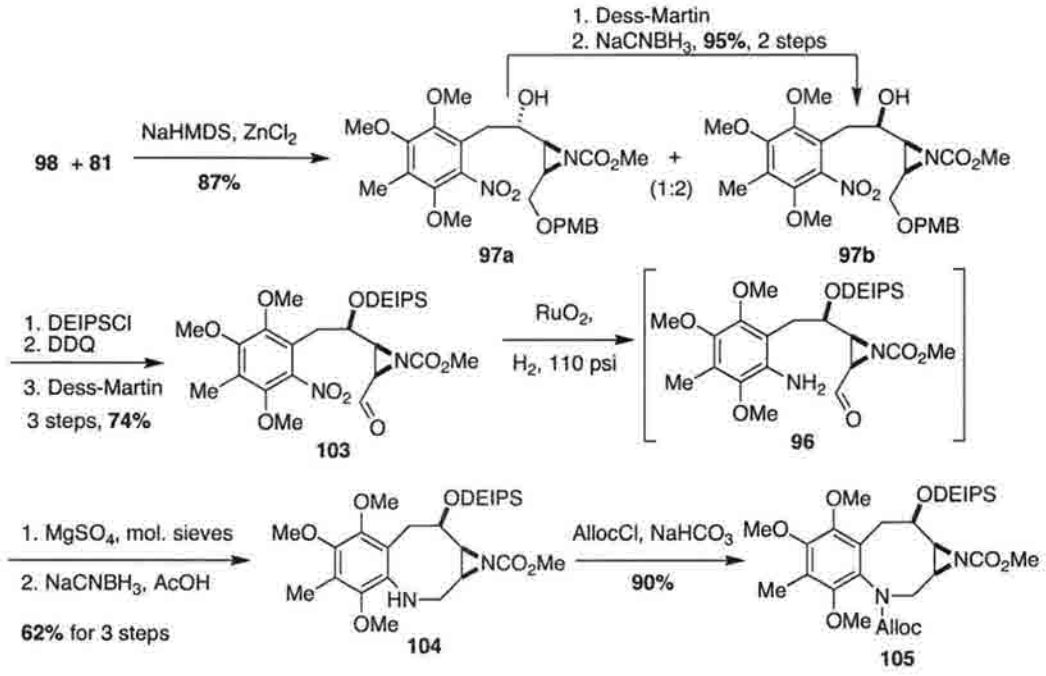


Scheme 23: Synthesis of Aziridine Aldehyde **81**

3.1.5: Synthesis of Benzazocines *Via* Reductive Amination

Having accomplished efficient routes to both nitro arene **98** and aziridine aldehyde **81**, the next task at hand was the coupling of these two components to produce alcohol **97** (Scheme 24). Coupling of **98** and **81** proved to be more difficult than the coupling reaction performed previously in our group in the FR900482 series. The major difficulty of this transformation was due to the extreme propensity for nitro arene **98** to dimerize in the presence of both base and oxygen. Accordingly, both **98** and **81** were azeotroped with anhydrous toluene and deoxygenated by use of the freeze-pump-thaw method. Contrary to the FR900482 system, it was found that a Lewis acid was needed for the aziridine aldehyde in order to increase its electrophilicity and allow the reaction to occur. Zinc chloride was found to be an effective Lewis acid for this purpose; however,

great care was needed in the fusing and use of zinc chloride so as to not introduce water in the reaction due to the highly hygroscopic nature of this compound. After much optimization, we were able to obtain alcohol **97** as a 1:2 mixture of diastereomers (**97a** and **97b**). This same ratio of diastereomers was observed in the FR900482 case as well. Two-dimensional NMR as well as coupling constant analysis found the major diastereomer having the alcohol syn to the protected aziridine (**97b**) while the minor diastereomer **97a** had the alcohol anti to the aziridine. It was expected that nitro arene **98** would attack the less hindered face of aziridine aldehyde **81** according to the Cram chelate model, thus providing **97b** as the major product. It was also found that the minor diastereomer **97a** could be readily converted to **97b** *via* oxidation of **97a** with Dess-Martin periodinane, followed by reduction of the ketone with sodium cyanoborohydride as shown in Scheme 24.



Scheme 24: Synthesis of *N*-Alloc Benzazocine **105** *via* Reductive Amination

The next major hurdle in the synthesis was reduction of the nitro functionality and subsequent reductive amination of aldehyde **96** to furnish benzazocine **104** (Scheme 24). Aldehyde **103** was obtained by subjecting alcohol **97b** to a three-step sequence consisting of silyl protection of the alcohol, removal of the PMB-ether protecting group, and oxidation of the resultant primary alcohol to afford **103** in good overall yield for the three steps (74%). In preparation for the key step of ring closure by reductive amination, reduction of the nitro group in **103** to the corresponding anilino aldehyde **96** was required. Reduction of the nitro group using the same procedure as in the total synthesis of FR900482 (10% Pd/C in methanol) failed to give **96**. The electron-rich nature of the aromatic ring in our system altered the electronics of the aniline enough that new conditions were needed to accomplish this transformation. After screening a barrage of reducing agents, it was found that ruthenium (IV) oxide under high-pressure hydrogen would perform this reaction in near quantitative yield.

In spite of the excellent yield of the anilino aldehyde **96**, the use of ruthenium oxide had some significant drawbacks. The reaction was found to be extremely capricious and was dependant on the type of ruthenium oxide used (purity and supplier). Upon original discovery of this reduction, an old bottle of ruthenium (IV) oxide found in the laboratory effected this transformation beautifully in quantitative yield. Eventually this supply of ruthenium oxide was expended and a new bottle of ruthenium (IV) oxide was needed. However, the new bottle of ruthenium oxide failed to catalyze this process in any appreciable yield and resulted in recovery of virtually all the starting material. It was found that ruthenium (IV) oxide from different suppliers gave different results.

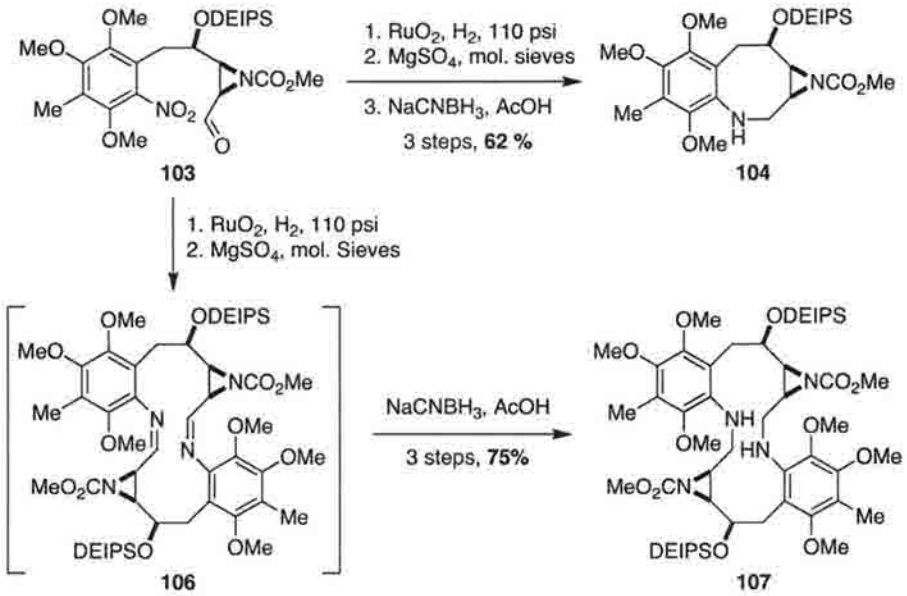
Ruthenium oxide from Aldrich Co. proved to be more reactive and effective than ruthenium oxide from Strem Co.

It was found that the water content of the ruthenium oxide had a remarkable impact on the reactivity of the catalyst. Addition of a small amount of water was found to drive the reaction to completion in cases where the reaction was either not progressing or sluggish. This realization helped to account for the difference in reactivity of ruthenium oxide from different suppliers. It was postulated that the ruthenium oxide from Aldrich was a better catalyst for the system because of the increased water content as compared to the same reagent obtained from Strem. Although this realization allowed the reaction to go to completion, the *amount* of water in the reaction was also found to be crucial. Addition of excess water in the reaction made the catalyst so reactive that reduction of the aldehyde to the corresponding alcohol would also occur in addition to nitro reduction to give the anilino alcohol. Efforts to oxidize the alcohol to the anilino aldehyde in this system were fruitless. As illustrated by the difficulties mentioned above, reduction of the nitro group in **103** to the corresponding anilino aldehyde **96** was extremely capricious and gave inconsistent yields.

Reductive amination of anilino aldehyde **96** also proved troublesome. The major difficulty associated with this reaction was the extreme propensity of the intermediate imine to dimerize to give macrocycle **107** (obtained by reduction of bis-imine macrocycle **106**) as shown in Scheme 25. In order to help circumvent this problem, this reaction was run under very dilute concentrations (0.001 M). Despite the dilute conditions, dimerization was still observed in most cases. It is still not clear what factors lead to dimerization as opposed to the desired benzazocine product. Each time the reaction was

performed, extreme care was taken to make sure the reaction was both water and oxygen-free. It was found that upon carefully controlling the reaction conditions, benzazocine **104** could be obtained in 62% yield for three steps. Treatment of **104** with allyl chloroformate and sodium bicarbonate provided *N*-Alloc benzazocine **105** in good yield.

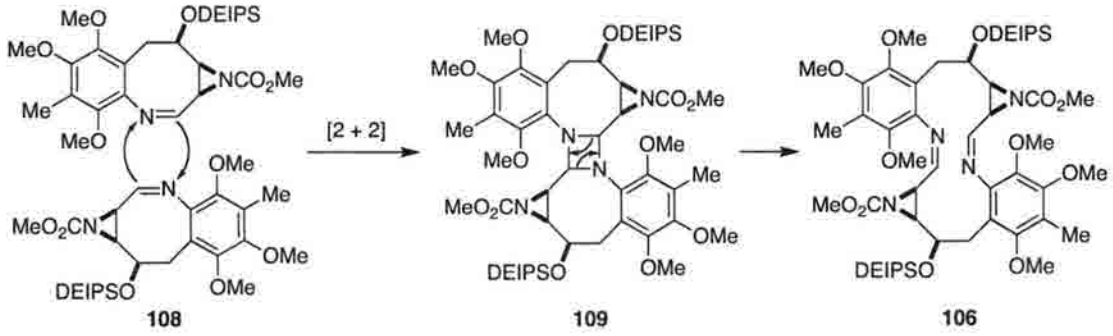
Another major drawback to the reductive amination approach is the inability to scale-up the reaction to anything resembling the useful domain in organic synthesis. It was found that the largest scale possible using this sequence was 200 milligrams. Attempts to try this sequence using more anilino aldehyde **96** (>200 milligrams) resulted in exclusive formation of dimer **107**. The inability to scale-up this sequence of steps made it very tedious to bring up synthetically useful amounts of material. As result, large amounts of time were spent repeatedly conducting the three-step reductive amination sequence (i.e. **103** to **104**, Scheme 25) until a sufficient amount of material could be obtained in order to continue on with the synthesis.



Scheme 25: Dimerization of the Intermediate Imine

Another drawback to this sequence of steps was the long length of reaction times for conversion (6 days over two steps). This process could be expedited somewhat by setting up multiple reductions in concert with each other. However, the workup and separation of dimer **107** from benzazocine **104** was difficult and laborious. Thus, it proved very difficult to be able to make large quantities of benzazocine **104** in a relatively short amount of time with minimal workup and purification.

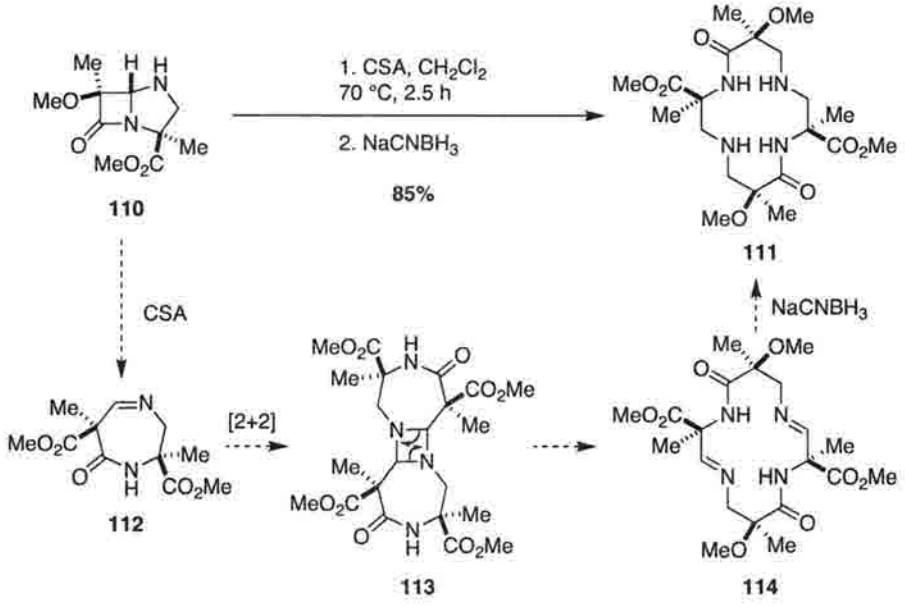
The exact mechanism by which dimerization occurs is unknown, however a proposed mechanism is shown in Scheme 26 starting from imine **108**. It is proposed that intermediate imine **108**, instead of undergoing reduction of the imine to give the desired benzazocine **104,** could undergo a [2+2] thermal cycloaddition to give intermediate **109**. Rearrangement/ring opening of intermediate **109** would provide imine **106**, which is a stable compound that can be easily isolated and handled. Reduction of imine **106** provided macrocycle **107** in good yield as illustrated in Scheme 25.



Scheme 26: Proposed Mechanism for Dimer Formation

Support for the proposed mechanism shown above comes from the work of Hegedus and coworkers. In their work (Scheme 27) they showed that treatment of azapenam **110** to acidic conditions followed by treatment with $NaCNBH_3$ produced dioxycyclam **111** in good yield.[67] The authors propose that acidic conditions convert

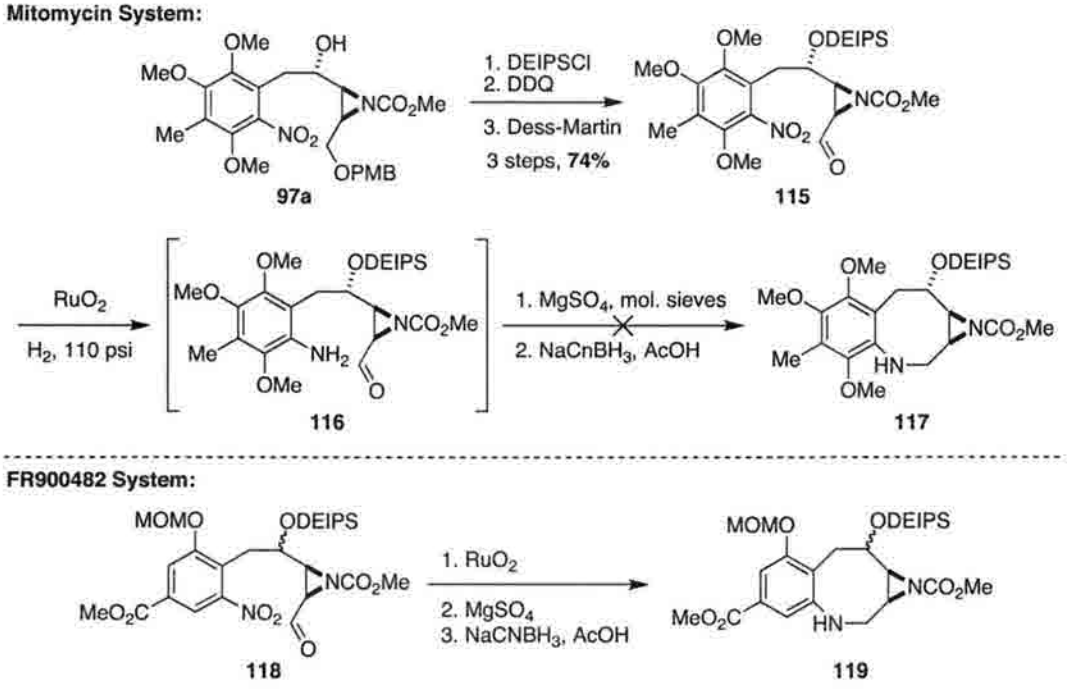
azapenam **110** to imine **112**. Imine **112** then undergoes a [2+2] cycloaddition to give intermediate **113**. Ring opening of **113** gives bis-imine **114**, which, upon treatment with $NaCNBH_3$, gives dioxycyclam **111**. To the best of our knowledge our system is the only example in the literature that displays similar reactivity to the system reported by Hegedus. An alternate mechanism for dimerization might also be drawn in a step-wise fashion to arrive at bis-imine **114**.



Scheme 27: Precedent for Proposed Dimerization Mechanism

Another interesting finding in this reductive amination reaction was that only the major diastereomer from the coupling reaction of nitroarene **98** and aziridine aldehyde **81** (i.e. **97b**) underwent cyclization to provide benzazocine **104** (Scheme 24). Alcohol **97a** was converted to aldehyde **115** by the same three-step procedure used for **97b**. Treatment of the anilino aldehyde **116** (prepared by reduction of **115** with ruthenium oxide) under identical reductive amination conditions gave no detectable amounts of benzazocine **117** (Scheme 28). This result was unexpected due to the observation that in the FR900482 system, both diastereomers of aldehyde **118** undergo the reductive amination reaction to

give benzazocine **119** as a mixture of diastereomers as shown in Scheme 28. The exact reason for the failure of the minor diastereomer is unknown at this point in time.



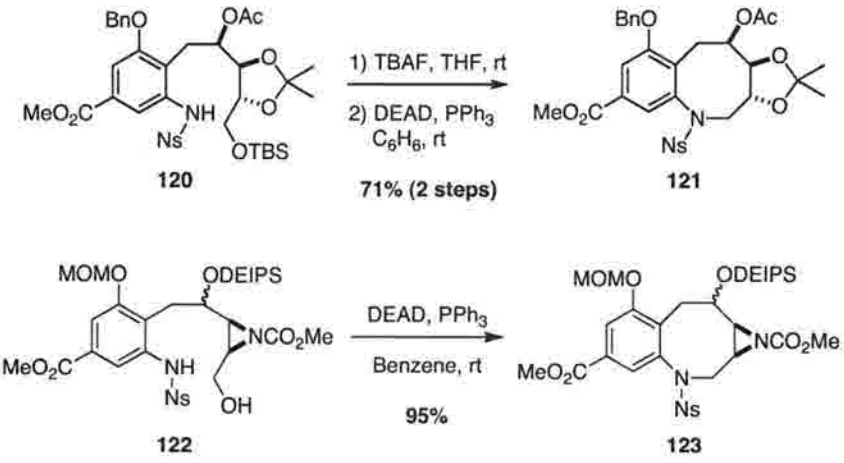
Scheme 28: Minor Diastereomer from Coupling Reaction does not Cyclize

3.2: Formation of Benzazocines *via* Mitsunobu Cyclization

3.2.1: Previous Studies and Key Concepts

Due to the difficult nature of the reductive amination approach mentioned above, other methods to access benzazocines were explored. Upon searching the literature, a method that immediately caught our eye was the use of a Mitsunobu reaction by Fukuyama and coworkers in the asymmetric total synthesis of FR900482 (Scheme 29). The authors report that desilylation of nosyl sulfonamide **120** followed by treatment of the resultant alcohol under standard Mitsunobu conditions gave benzazocine **121** in good yield over two steps. This protocol was also demonstrated to be effective on a large scale with no decrease in overall yield. Following this precedent, Dr. Pascal Ducept (a postdoc

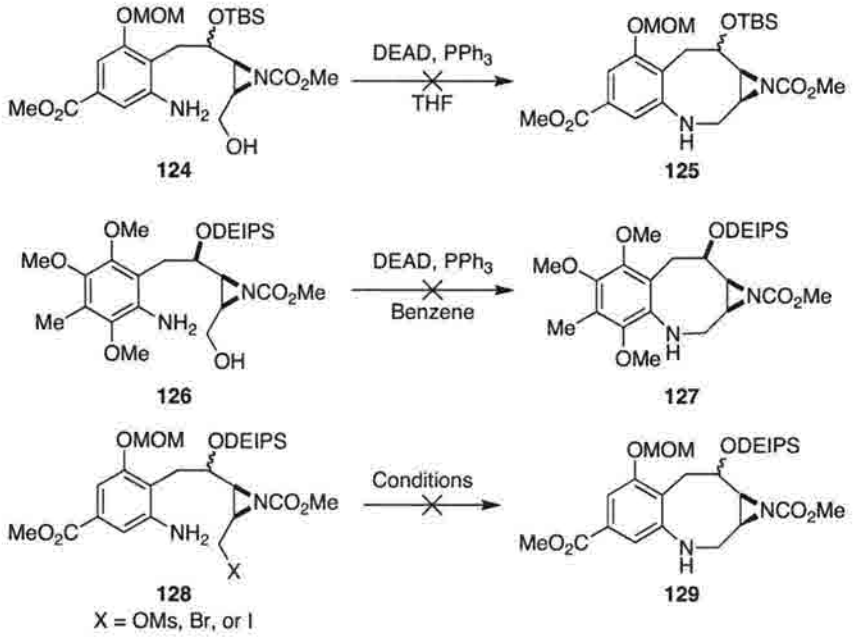
in the Williams group working on putative biosynthetic intermediates of FR900482) took alcohol **122** (readily available from the reductive amination sequence) and upon treatment under standard Mitsunobu conditions obtained the desired benzazocine **123** in 95% yield. The Mitsunobu reaction went to completion quickly (30 minutes), and was performed on a five gram scale with no observed decrease in the yield.



Scheme 29: Literature Precedence for Mitsunobu Cyclization

It should be mentioned that a Mitsunobu cyclization strategy had previously been attempted in our group before on both the FR900482 and mitomycin system as shown with the preparation of primary alcohols **124** and **126** in Scheme 30.[68] In either case none of the desired benzazocines **125** or **127** were obtained with only starting material recovered even after prolonged reaction times and increased temperature. Use of an S_N2 strategy by conversion of the alcohol to a good leaving group **128** such as a mesylate or halide also gave no reaction to benzazocine **129** (Scheme 30).[69] From Fukuyama's successful result and the previous failed results from our group, it became clear that the sulfonamide of the nitrogen was activating the aniline by lowering the pK_a of the sulfonamide proton and facilitating the reaction. The sulfonamide also served as a protecting group during additional synthetic sequences that could be readily removed

when desired. This strategy fit our synthetic sequence perfectly as protection of the benzazocine nitrogen would be required.

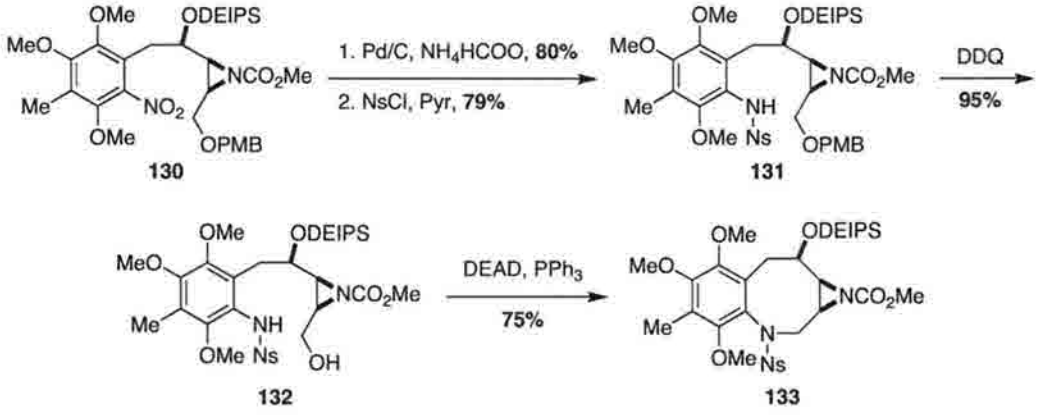


Scheme 30: Previous Cyclization Attempts in the Williams Group

3.2.2: Mitsunobu Cyclization in Formation of Benzazocines

From the successful outcomes shown above, we wanted to see if this same method could be used for benzazocine formation en route to the mitomycins. We were interested to see the effect (if any) the electron rich aromatic of the mitomycins might have as opposed to electron deficient (relative to the mitomycins) aromatic ring present in FR900482. The sequence (shown in Scheme 31) started with the same nitro arene **130** used in our reductive amination approach and was converted to the sulfonamide by a two-step sequence of nitro reduction and subsequent protection of the resulting aniline as the nosyl sulfonamide **131**. Treatment of **131** with DDQ removed the PMB ether to give alcohol **132** in excellent yield and provided the key intermediate to try the Mitsunobu

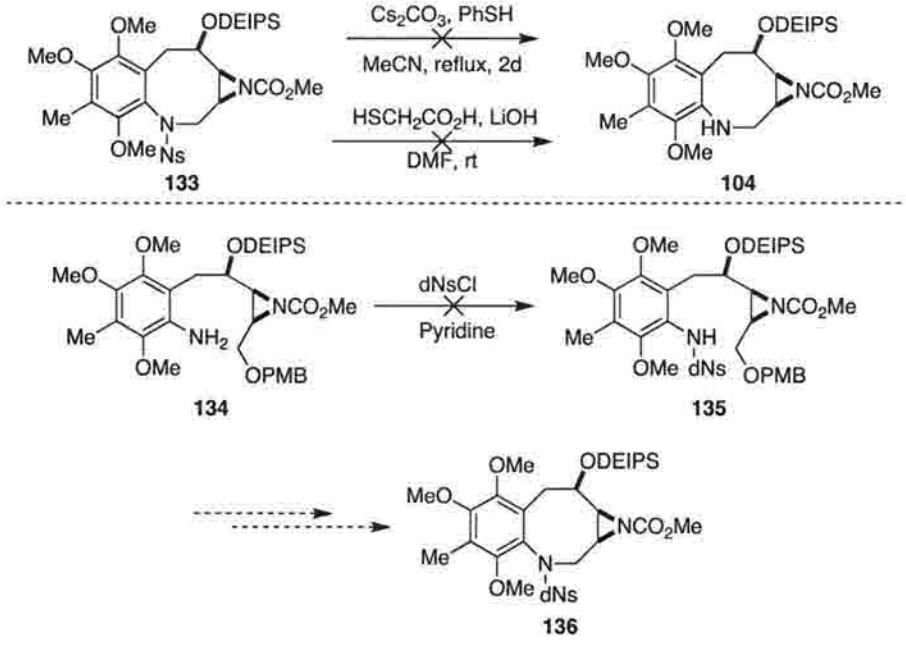
cyclization reaction. To our delight, treatment of alcohol **132** under standard Mitsunobu conditions did provide benzazocine **133** in 75% yield. This reaction had gone to completion in 45 minutes, proved to be scalable (reaction performed on ~1 gram), and could be run at high concentrations (0.1 M) unlike the reductive amination protocol.



Scheme 31: Formation of Benzazocine **133** by Mitsunobu Cyclization

With an efficient route to benzazocine **133** in hand we wanted to be able to remove the nosyl group so that we might be able to replace it with another protecting group that might be more useful in our synthesis (such as a carbamate). Unfortunately, all attempts to remove the nosyl sulfonamide from **133** failed under standard conditions using a variety of solvents and temperatures to give benzazocine **104** (Scheme 32). We reasoned that the inability to remove the nosyl sulfonamide might be because it was too stable. Also observed was opening of the aziridine ring upon addition of excess thiol. We then set out to protect the aniline as the dinitrobenzene sulfonamide (dNs) **135**, with the intent to form benzazocine **136**. Reports in the literature have shown that dinitrobenzenesulfonamides are easier to remove than the nosyl variants. All attempts to form the desired sulfonamide **135** (Scheme 32) failed and gave mostly decomposition under the multiple conditions that were tried.

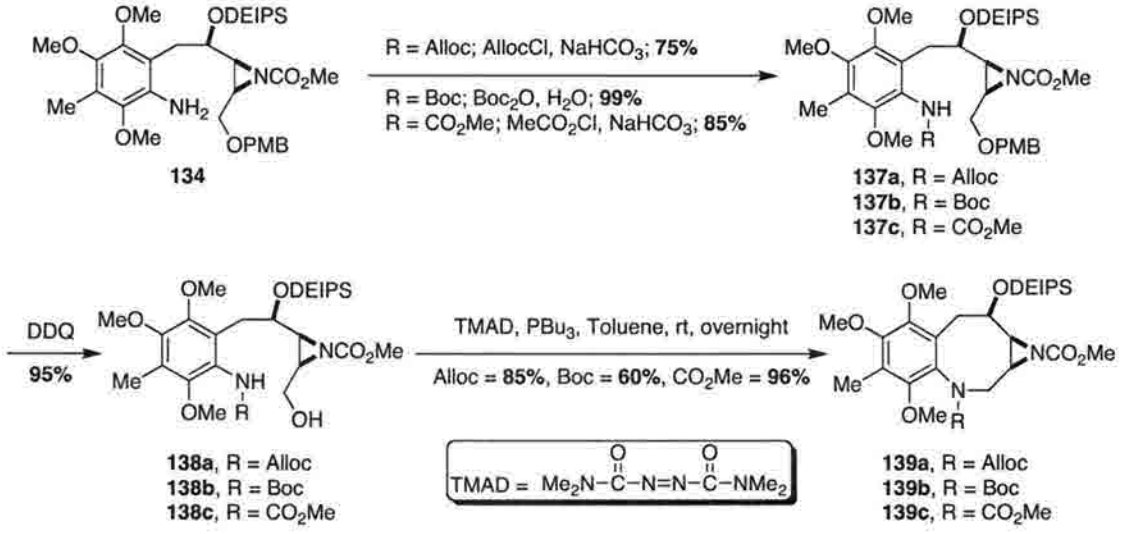
Due to the inability to remove the Ns sulfonamide from benzazocine **133**, it was postulated that Mitsunobu cyclization could still occur if the aniline nitrogen was protected with something that might lower the pK_a of the aniline like the sulfonamide did. Carbamates seemed to be the next logical choice due to their potential to lower the pK_a of the aniline like the sulfonamide and their desired use as a protecting group in the synthetic strategy. Mitsunobu reaction of the carbamate-protected anilines under standard conditions (DEAD, PPh_3) gave only small amounts of product after screening several different solvents and reaction temperatures. This lack of reactivity likely reflects the higher pK_a of the carbamate as compared to the sulfonamide.



Scheme 32: Inability to Remove Nosyl Sulfonamide and to Protect as dNs Sulfonamide

To overcome the lack of reactivity of the carbamate as mentioned above, different Mitsunobu systems were screened from the literature that are designed for nucleophiles possessing higher pK_a values (pK_a =11-13).[70] It was found that the TMAD/PBu_3 system developed by Ito and coworkers[71] worked very well and gave the desired benzazocine in

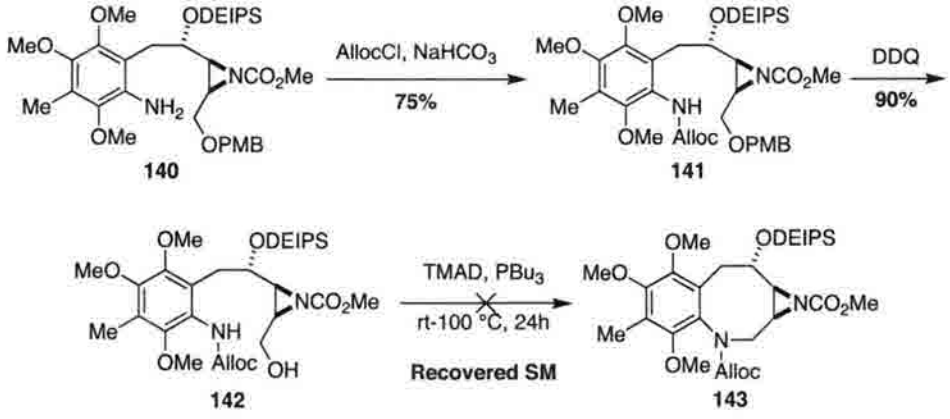
high yield. The synthetic sequence for the synthesis of these benzazocines (shown in Scheme 33) started with aniline **134** and was then protected as the allyl, *t*-butyl, or methyl carbamates to give carbamates **137a-c** in good yield. Removal of the PMB ether proceeded in high yield to furnish alcohols **138a-c** as the key precursor to the Mitsunobu reaction. As mentioned above, alcohols **138a-c** smoothly underwent conversion to benzazocines **139a-c** in time ranging from 6-12 hours at room temperature.[72] Observation of the yield of the cyclization reaction shows a direct correlation with the size of the carbamate. Smaller carbamates (methyl) provide the benzazocine in excellent yield, medium carbamates (allyl) in good yield, and large carbamates (*t*-butyl) in moderate yield. Workup of these reactions is very easy requiring only concentration of the reaction mixture followed by purification by column chromatography. An additional advantage of the TMAD/PBu_3 is the easy separation of benzazocines **139** from the Mitsunobu reaction reagents that was not always trivial in the DEAD/PPh_3 system.



Scheme 33: Benzazocine Formation by use of Mitsunobu Reaction with Carbamates

With this efficient protocol in hand, a question that remained to be answered was if the minor diastereomer from the coupling reaction of the aziridine aldehyde and nitro

arene would undergo cyclization under the Mitsunobu protocol. Accordingly, aniline **140** was protected with allyl chloroformate to yield allyl carbamate **141** in good yield (Scheme 34). PMB ether removal under standard conditions provided alcohol **142** in excellent yield and set the stage for the key cyclization reaction. Treatment of alcohol **142** under TMAD/PBu_3 conditions in temperatures ranging from room temperature to refluxing toluene for up to two days resulted in no formation of benzazocine **143** and quantitative recovery of the starting material. The remarkable resistance of the minor diastereomer to undergo cyclization was further supported by computations using density Functional Theory (DFT). These results showed a large barrier to rotation for the minor diastereomer of the mitomycins that is not present in the major diastereomer of the mitomycins or in either diastereomer of the alcohols in the FR900482 system. This is likely due to the highly functionalized nature of these substrates that locks the minor diastereomer in a particular conformation and prevents cyclization from occurring.



Scheme 34: Minor Diastereomer from Coupling Reaction Fails to Cyclize

The development of the ability to form benzazocines by Mitsunobu cyclization revolutionized the synthesis and allowed for more material to be pulled through so that development of reactions further in the synthesis could be accelerated. A comparison of

cyclization by the Mitsunobu reaction versus the original reductive amination approach is shown in Table 1. Although the two methods are comparable in the number of steps and overall yield (provided you have a good bottle of ruthenium oxide for the reductive amination protocol), the main benefit of the Mitsunobu protocol is the short reaction times and the ability to do the reaction on a large scale.

	Reductive Amination	**Mitsunobu Cyclization**
Number of Steps:	5	5
Overall Yield:	36%	41%
Total Reaction Time:	160 hours	36 hours
Maximum Scale	200 mg	> 2.0 g
Time to make 2 g	165 days	1.5 days
Solvent used for Key Step	12 L	100 mL

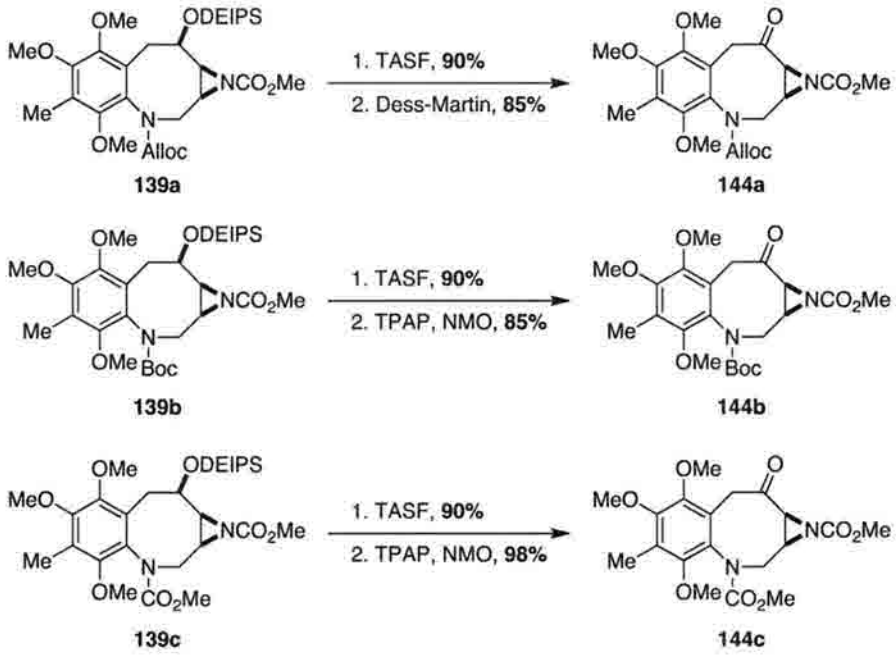
Table 1: Comparison of Reductive Amination and Mitsunobu Protocols

3.2.3: Synthesis of Benzazocanes from Benzazocines

With access to large amounts of benzazocines **139a-c** via the Mitsunobu cyclization protocol discussed above, the next objective was the formation of the corresponding benzazocanes **144a-c** (Scheme 35). This involved removal of the DEIPS silyl ether followed by oxidation to the ketone. Attempted removal of the silyl ether under standard TBAF conditions either resulted in decomposition or an epoxide resulting from Payne rearrangement involving attack of the alkoxide onto the aziridine. It was found that the more mild fluoride source tris(dimethylamino)sulfonium

difluorotrimethylsilicate (TASF) resulted in clean removal of the silyl ether in all three cases in great yield (90%).

Oxidation of the alcohol to the ketone proved to be straightforward using Dess-Martin periodinane as illustrated in the case of the *N*-Alloc benzazocine, albeit provided lower yields in the case of the *N*-Boc and *N*-CO_2Me benzazocines. The low yields were easily rectified by the use of Ley oxidation (TPAP, NMO), which provided benzazocanes **144b-c** in good yields as shown in Scheme 35. Benzazocanes **144a-c** all existed as a mixture of rotamers that failed to coalesce in VT-NMR experiments with temperatures up to 110 °C.

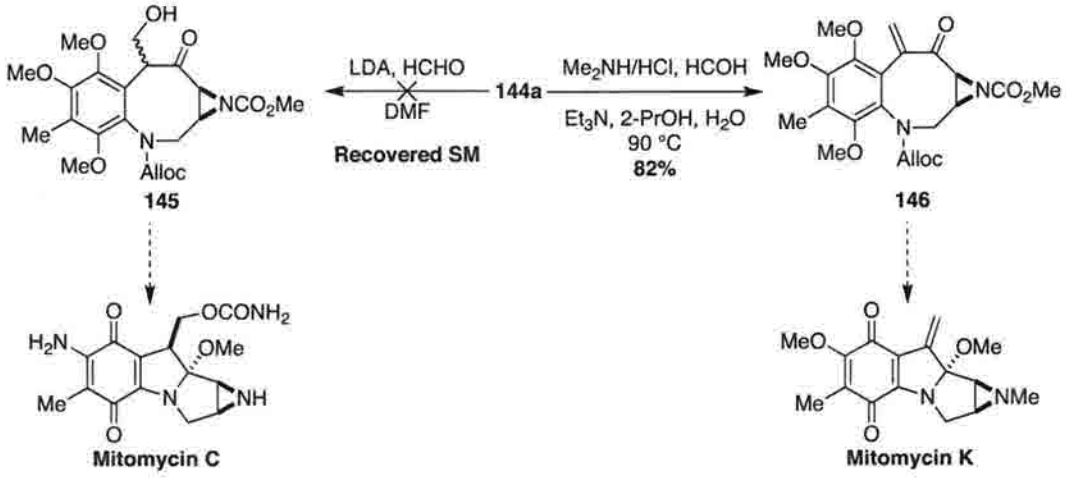


Scheme 35: Synthesis of Benzazocanes **144a-c** from Benzazocines **139a-c**

3.3: Attempted Manipulation of Benzazocanes into the Mitomycins

3.3.1: Attempted Hydroxymethylation of Benzazocanes

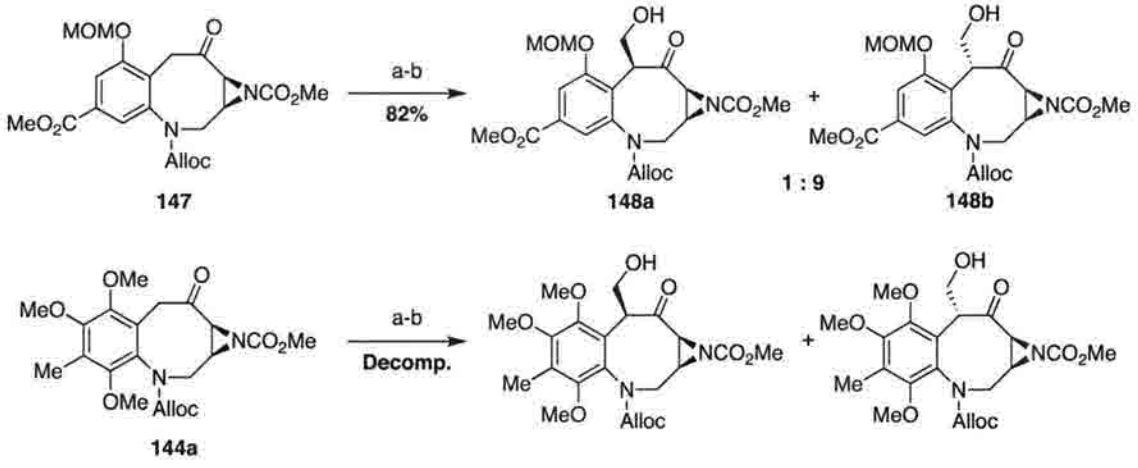
The next key step in our route towards the total synthesis of the mitomycins was the introduction of the C10 carbon as a hydoxymethyl group or the exocyclic olefin (Scheme 36). The hydroxymethylated product **145** would be required to access mitomycins A-C, while the exocyclic olefin product **146** was envisioned to be a precursor to the total synthesis of mitomycin K. The first attempt tried on benzazocane **144a** was hydroxymethylation using the successful protocol our group in the total synthesis of FR900482 (see section 2.3.2). These conditions (LDA, paraformaldehyde, DMF) failed to provide alcohol **145** as just the starting material was recovered. In experimenting with different hydroxymethylation conditions (such as L-proline and formalin) it was found that enone **146** was being produced. Treatment of benzazocane **144a** with Triton B and paraformaldehyde produced a 55% yield of **146**, which was improved to 82% upon treatment of **144a** with dimethylamine and formalin (scheme 36). Alcohol **145** was never produced in more than trace amounts in any of the conditions that were tried.



Scheme 36: Attempted Hydroxymethylation of **144a** and Formation of Enone **146**

In the preparation of putative biosynthetic intermediates in the biosynthesis of FR900482, new hydroxymethylation conditions were found for benzazocane **147** that delivered the desired hydroxymethylated products **148a-b** in both high yield and

diastereoselectivity (Scheme 37).[73] This method (based on methodology developed by Kobayashi[74] and coworkers), gave among the highest diastereoselectivity seen to date in these types of reactions and requires only a slight excess of formaldehyde. This is in contrast to other conditions used where over 100 equivalents of formaldehyde is required for the reaction to go to completion. Such a method is not practical especially when working with labeled substrates due to the exorbitant cost of ^{13}C-labelled formaldehyde[75]. This same method was tried on benzazocane **144a** but resulted only in decomposition (Scheme 37). Difficulties were encountered both in forming the silyl enol ether as well as the actual hydroxymethylation step.

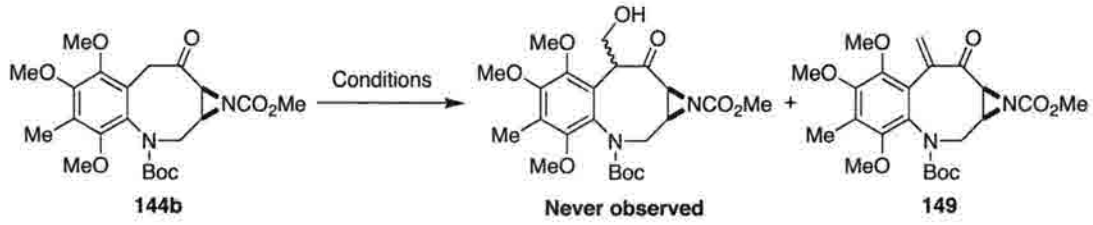


Conditions: a) Et_3N, TMSOTf; b) Formalin (1.5 equiv), $Sc(OTf)_3$ (0.1 equiv.), $THF:H_2O$ (9:1)

Scheme 37: Scandium Triflate Mediated Aldol Reaction

Due to the inability to hydroxymethylate *N*-Alloc benzazocane **144a** as well as other issues to be mentioned, we decided to switch to the *N*-Boc Benzazocane **144b** (the synthesis of which was shown in Schemes 33 and 35). Attempts to hydroxymethylate **144b** are shown in Table 2 and failed in every case to afford any of the hydroxymethylated product. Conditions tried on **144b** that were not performed previously include attempted alkylation with either SEMCl or ethyl formate. As seen previously, we

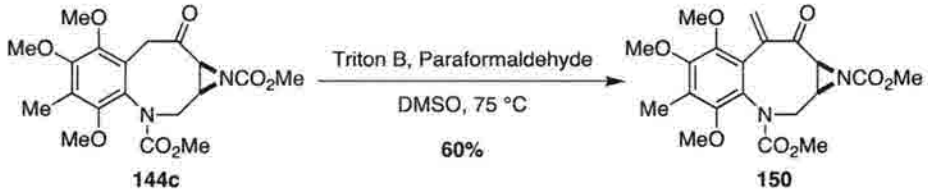
were able to find conditions in which enone **149** could be efficiently made. In this case the more basic conditions of triton B and paraformaldehyde provided the best yield (92%) for the formation of enone **149**.



Conditions	Result (%Yield)
LiOH, Formalin, THF / H_2O	No Reaction
Triton B, Paraformaldehyde, DMSO	Enone **149** (92%)
LHMDS, HCHO (Anhydrous), THF	Decomposition
LDA, Formalin, THF	No Reaction
LDA, SEMCl, THF	Decomposition
LHMDS, ethyl formate, THF	Decomposition

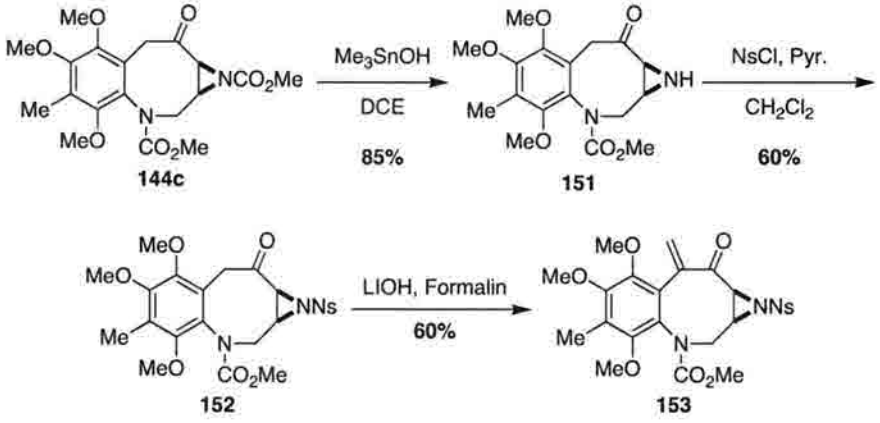
Table 2: Attempted Hydroxymethylation of *N*-Boc Benzazocane **144b**

N-methyl benzazocane **144c** reacted analogous to the Alloc and Boc benzazocanes already discussed. In all cases tried (similar to those previously discussed), the desired alcohol was never observed. Optimal methylenation conditions for **144c** was best accomplished using Triton B and paraformaldehyde and delivered enone **150** in 60% yield (Scheme 38). Alternate attempts to alkylate with different reagents such as ethyl formate, SEMCl, and BOMCl were all unsuccessful.



Scheme 38: Methylenation Conditions for Benzazocane **144c**

Due to the inability to hydroxymethylate in each case, we wanted to see if the carbamate protecting group on the aziridine was having an unwanted effect. In starting these studies with benzazocane **144c**, it was requisite to be able to remove the aziridine carbamate without touching the benzazocane carbamate. To our delight, it was found that treatment of **144c** with trimethyltin hydroxide in refluxing ethylene chloride gave the free aziridine **151** in excellent yield (Scheme 39). The conditions used in this reaction are identical to the ones pioneered by Nicolaou for the mild saponification of esters.[76] To the best of our knowledge this is the first example of carbamate cleavage using trimethyltin hydroxide. The next objective was to methylate free aziridine **151** so as to access the *N*-methyl aziridine moiety present in mitomycin K. Unfortunately, all attempts to methylate **151** failed and only starting material was recovered.

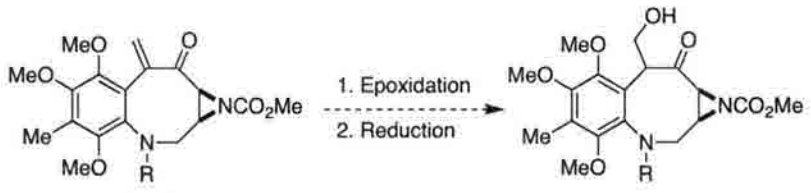


Scheme 39: Removal of Aziridine Protecting Group and Alkylation Attempts

We then decided to put a different protecting group on the aziridine so that we could selectively remove the methyl carbamate on the benzazocane nitrogen during the transannular cyclization reaction. This differentially protected benzazocane would allow access to the natural products with both the free aziridine as well as the *N*-methyl aziridine. To this end aziridine **151** was protected as the nosyl sulfonamide **152**.

Sulfonamide **152** behaved similarly to benzazocane **144c** in that only enone **153** (60% yield using LiOH and formalin) was obtained from the many alkylation attempts that were tried. This result lent credence to the idea that perhaps the electron-rich aromatic ring that is preventing formation of the hydroxymethyl moiety.

An avenue not fully explored in these studies that merits attention involves attempted epoxidation of enones **146**, **149**, and **150**. It is conceivable that ring-opening of the corresponding epoxides could give the desired alcohols **154-156** requisite in mitomycins A-C and originally tried to obtain from benzazocanes **144a-c** (Scheme 40). Rapoport and coworkers successfully used this approach in order to access the hydroxymethyl moiety in their synthesis of FR900482.[77]



146, R = Alloc
149, R = Boc
150, R = CO_2Me

154 R = Alloc
155, R = Boc
156, R = CO_2Me

Scheme 40: Proposed Route to Access Hydroxymethyl Group through the Enone

3.3.2: Transannular Cyclization Attempts

While not being able to hydroxymethylate benzazocanes **144a-c** was disappointing, enones **146**, **149**, and **150** still were promising substrates in order to complete the total synthesis of mitomycin K. The next task at hand was to induce a transannular cyclization by removal of the carbamate protecting group, hoping that it would cyclize onto the ketone to form the tetracycle. It was unknown how the enone

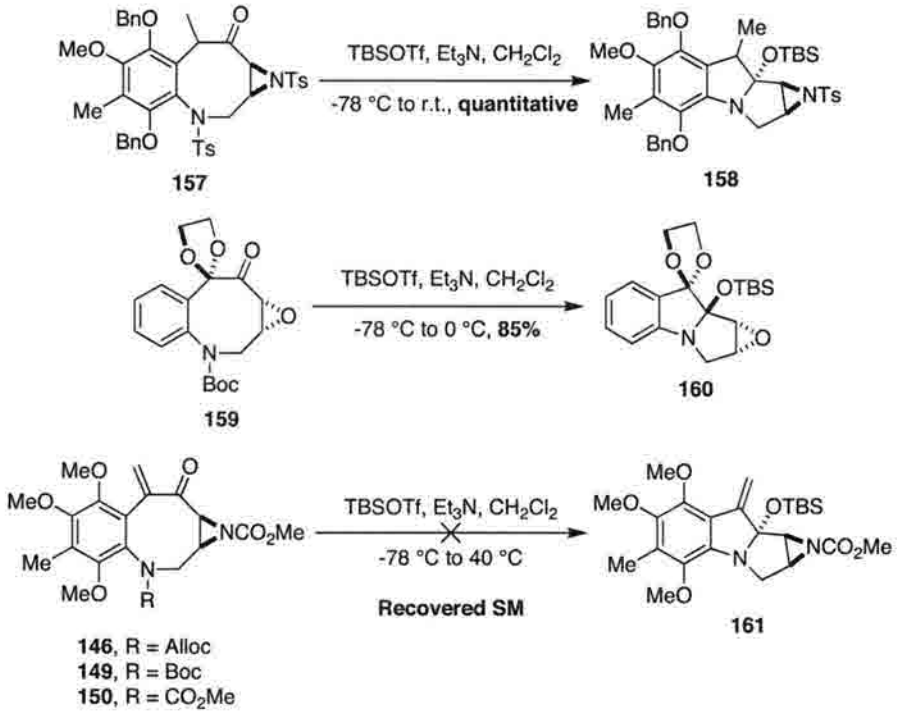
would react to cyclization conditions as well as if the C9a alcohol (if it formed) would be stable enough to isolate or would eliminate to the mitosene.

To date there is one example of this type of transannular cyclization that we wanted to attempt. This example is from Ban and coworkers[78] in which benzazocane **157** (Scheme 41) is treated with TBSOTf and triethylamine at -78 °C to give tetracycle **158** in quantitative yield. The authors report that this reaction takes place in about 10 minutes after warming the reaction mixture to room temperature. This is a curious result in which the tosyl sulfonamide is cleaved presumably due to the attack of the benzazocane nitrogen onto the TBS oxonium ion. Importantly, the authors note that this reaction only worked in their hands when the hydroquinone oxygens were protected as benzyl ethers. The authors reported that making the analogous benzazocane to **157** but with TBS ethers on the hydroquinone oxygens did not undergo the transannular cyclization reaction. The authors mention that in the IR spectra of **157** they noted the presence of a transannular effect with a C=O stretching frequency of 1700 cm^{-1}. In the IR spectra of the TBS ether substrate, the C=O stretching frequency was 1720 cm^{-1}.

A more recent example that validates the above reaction is from Miller and coworkers where they report that treatment of benzazocane **159** under the same conditions used by Shibasaki and Ban furnished tetracycle **160** in 85% yield (Scheme 41).[79] In this case the Boc carbamate attached to the benzazocane nitrogen is cleaved. Examination of the supporting information from the authors revealed a C=O stretching frequency of 1702 cm^{-1} for the ketone.

Based on these results we subjected enones **146**, **149**, and **150** to the identical conditions used in previous examples above and obtained none of the desired tetracycle

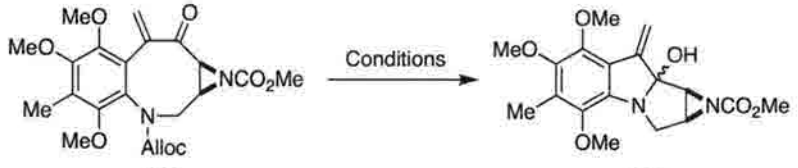
161. In all cases only starting material was recovered even when heated at 80 °C in a sealed tube overnight. In enones **146**, **149**, and **150**, the carbonyl stretching frequency is around 1730 cm^{-1}. These same conditions were tried on benzazocanes **144a-c** to see if a tetracycle might be formed. In each case only starting material was obtained as with the enone case. The exact reason for the lack of reactivity of these systems is not entirely understood.



Scheme 41: Precedence for Transannular Cyclization and Failed Attempts

With the failure of the transannular cyclization reaction catalyzed by TBSOTf as discussed above, it was decided to investigate conditions that would remove the carbamate protecting group on the nitrogen using more conventional methods. The first system we investigated was the possibility of removing the Alloc group from enone **146** using palladium to form tetracyclic alcohol **162** (Table 3). Standard palladium conditions used to remove Alloc groups caused decomposition of the starting material. One concern

with the use of palladium is that it might be reacting with the enone system as well, thus causing decomposition. A brief summary of the standard methods examined to remove the Alloc group is shown in Table 3.



146 162

Conditions:	Result:
PPh_3, $Pd(PPh_3)_4$	Decomposition
$Pd(PPh_3)_4$	Decomposition
$Pd_2(dba)_3$, PPh_3, HCOOH	SM

Table 3: Attempts of Removing the Allyl Carbamate

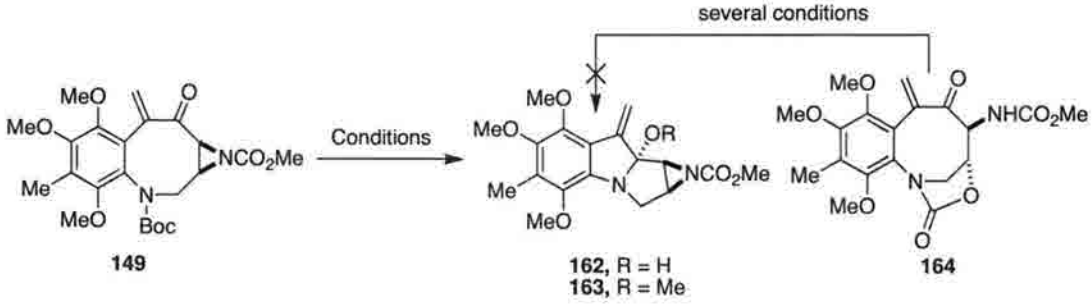
In choosing a protecting group for the benzazocane nitrogen, it is vital that the deprotection conditions will not react with other functionality in the molecule. This was an oversight in the choice of Alloc as the protecting group. The next carbamate protecting group we wanted to try was Boc. It was thought that with the myriad mild methods to remove a Boc carbamate reported in the literature, surely conditions could be found to efficiently perform the desired transformation. One of the methods we wanted to explore for Boc removal of enone **149** was thermal removal. It is well known that Boc groups will decarboxylate upon heating neat at 140-160 °C. This seemed to be an ideal protocol due to the fact that the reaction proceeds under essentially neutral conditions. It was observed that the Boc group was stable to heating neat until the temperature reached 150 °C, upon which **149** decomposed (Table 4). Heating **149** in toluene only gave starting material when heating to temperatures up to 190 °C, while heating in methanol caused rapid decomposition at 150 °C. Microwave conditions were also unsuccessful in

achieving this transformation. Many combinations of temperature and solvent were explored, all of which were unsuccessful.

Standard acidic conditions were then tried for Boc removal with some trepidation on what effect this might have on the aziridine ring. Treatment of enone **133** with TFA gave multiple products with the major product being a dimer by mass spectrometry as well the aziridine ring being opened up according to NMR analysis (Table 4). In scouring the literature for more mild ways of Boc removal, we came upon a protocol by Taylor[80] and coworkers where they report that Boc group can readily be removed with boron triflouoride diethyletherate in the presence of molecular sieves. Treatment of enone **146** to these conditions provided a clean reaction to a new product. During the characterization process of this new compound, several features suggested this was not the desired tetracycle **162**. Mass spectrometry did not match for the product but did match for the product mass plus carbon dioxide. ^{1}HNMR analysis revealed the unknown product still had enone peaks and two carbamate carbonyl peaks as well as the ketone peak were seen in the ^{13}CNMR spectra. Three carbonyl stretches were also seen in the IR spectra, confirming the presence of the enone as well as the carbamate carbonyl.

Based on the data the unknown compound was found to be cyclic carbamate **164** (Table 4). This telling result, though not desired, gave us insight into the electronics and characteristics of the system. This result suggested the strength of the nitrogen to carbon bond in the carbamate in that aziridine opening would be preferred over decarboxylation. Also inferred from this result that the electronics of the system might need to be changed in order for transannular cyclization to be successful. It was then postulated that perhaps cyclic carbamate **164** might still be useful in the synthesis if

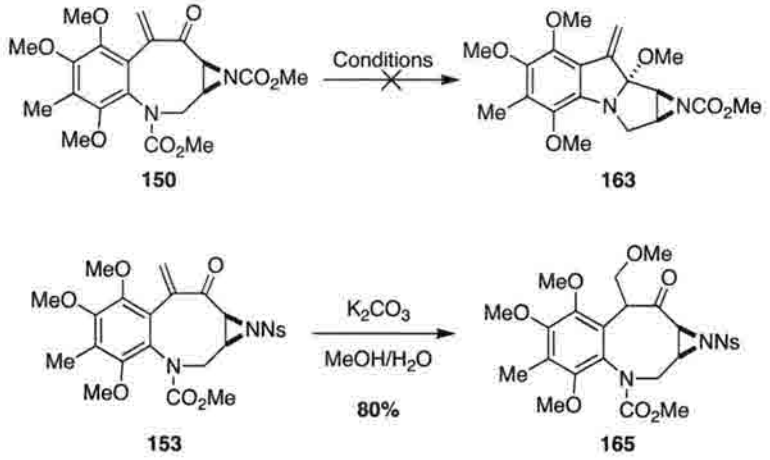
conditions could be found that might allow for aziridine ring closure, decarboxylation, and subsequent cyclization. However, all attempts to manipulate **164** under a variety of conditions (both acidic and basic) failed.



Conditions	Result (%Yield)
Heat, 150 °C	Decomposition
TFA, rt then Me_2SO_4	Unknown Product, dimer
TBSOTf, Then Me_2SO_4	Unknown Product, Dimer
$BF_3{\cdot}OEt_2$, CH_2Cl_2, 2h	**164 (87%)**
Microwave	Decomposition
TBSOTF, Lutidine	SM
ZnBr, CH_2Cl_2	SM + Br by Mass Spec (60%)

Table 4: Attempted Removal of the Boc Carbamate

Transannular cyclization attempts on methyl carbamate benzazocane **150** were equally disappointing (Scheme 42). Using the same conditions as with enones **146** and **149** as well as conditions specific to the methyl carbamate (basic conditions, TMSI, etc.) failed to provide any of tetracycle **163** and resulted mostly in decomposition. Interestingly, treatment of enone **153** under basic conditions did not induce cyclization but rather provided methyl ether **165** in 80% yield (Scheme 42). Using the identical conditions on **150** gave no reaction with the starting material being fully recovered. Attempts to add other alcohols (TBSOH, BnOH, etc.) other than methanol into **153** in an effort to make this result synthetically useful failed.



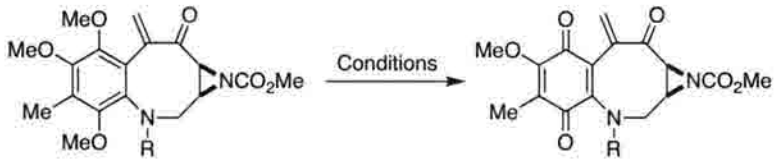
Scheme 42: Failed Transannular Cyclization with Methyl Carbamate

3.3.3: Efforts in Quinone Formation

Due to the inability to induce transannular cyclization on a number of different substrates as documented above, we sought to change the electronics of the system by formation of the quinone. We reasoned that formation of the quinone would significantly alter the electronics of the system, allowing for cleavage of the carbamate bond and subsequent cyclization.

Efforts to form quinones **166-168** from enones **146**, **149**, and **150** is summarized in Table 5. The standard conditions of CAN and AgO rapidly decomposed the substrates and gave multiple products. With *N*-Boc enone **149**, CAN conditions rapidly decomposed the substrate presumably due to the incompatibility of the Boc group to CAN conditions. Under conditions where both the time and temperature were carefully monitored, trace amounts of different products were able to be isolated. Unfortunately, none of the compounds analyzed had the characteristics of the desired quinone product. The use of dipicolinate ligands[81] (as used successfully by Danishefsky as seen below) with both

CAN and AgO were explored but gave no reaction in all cases examined. Hypervalent iodine reagents such as iodobenzene diacetate (Table 5) were also not successful.



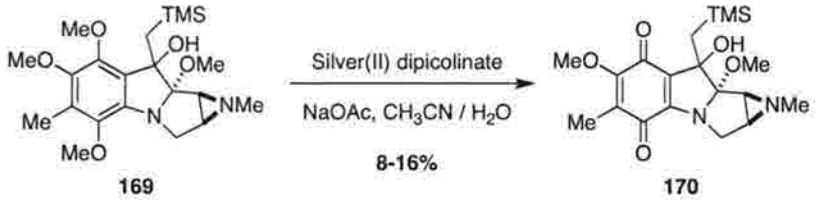
146, R = Alloc
149, R = Boc
150, R = CO_2Me

166, R = Alloc
167, R = Boc
168, R = CO_2Me

Condition	Result
CAN	Decomposition
AgO	Decomposition
CrO_3, H_5IO_6	Decomposition
CoF_3, dioxane	Recovered SM
$PhI(OAc)_2$	Recovered SM
HNO_3, AcOH	Decomposition
NBS, H_2SO_4	Decomposition

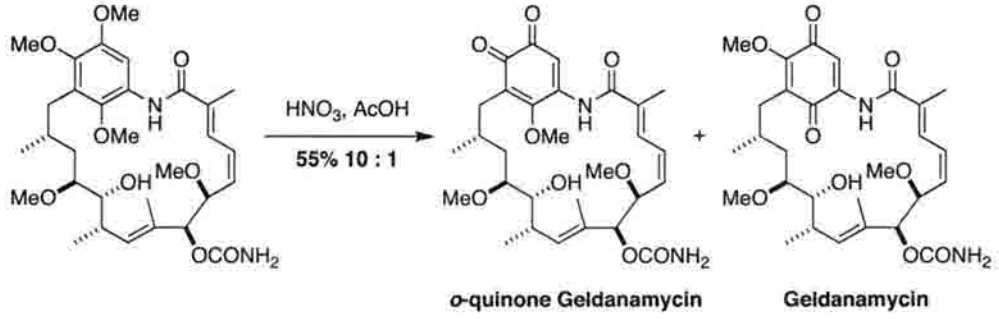
Table 5: Attempted Quinone Formation

The inability to induce quinone formation in the systems shown above, though disappointing, was not totally unexpected. In the synthesis of mitomycin K, Danishefsky and coworkers utilized the same bis-methoxy arene and ran into troubles with quinone oxidation as well.[82] They eventually found that treatment of the bis-methoxy arene **169** with silver (II) dipicolinate gave desired quinone **170** in a meager 8-16% yield (Scheme 43). Despite knowing this result before embarking on the synthesis, we were hopeful that quinone formation would work well enough to allow access to enough material to push through the final steps and complete the synthesis.



Scheme 43: Quinone Formation by Danishefsky and Coworkers

A more recent example lends support to the difficult nature of oxidizing the bis-methoxy aromatic piece. In their asymmetric total synthesis of geldanamycin, Andrus and coworkers utilized a macrocycle (containing a very similar aromatic to our substrates) and attempted oxidation to the natural product in the final step (Scheme 44).[83] After screening a variety of different conditions, they were only able to obtain geldanamycin in about 5% yield. The major product in this reaction was the ortho-quinone natural product shown in Scheme 44.



Scheme 44: Difficulty In Quinone Oxidation in the Synthesis of Geldanamycin

Aza-quinones are also a possibility with this type of aromatic. From the above two examples it is clear that oxidation of a bis-methoxy protected hydroquinone is difficult if nitrogen is appended to the ring. In order to solve this problem, a new arene that can be converted to the quinone under less harsh conditions is needed and will be the focus of the next chapter.

In this chapter we have shown the development of an efficient system to gain rapid access to both benzazocines and benzazocanes in an asymmetric fashion. The utility

of the synthesis was greatly enhanced by the development of an intramolecular Mitsunobu reaction that forms the benzazocines **144a-c** in high yield. Drawbacks to the current synthesis include the inability to hydroxymethylate our synthetic benzazocanes as well as failure to oxidize to the quinone.

Chapter 4: Second Generation Approach to the Mitomycins

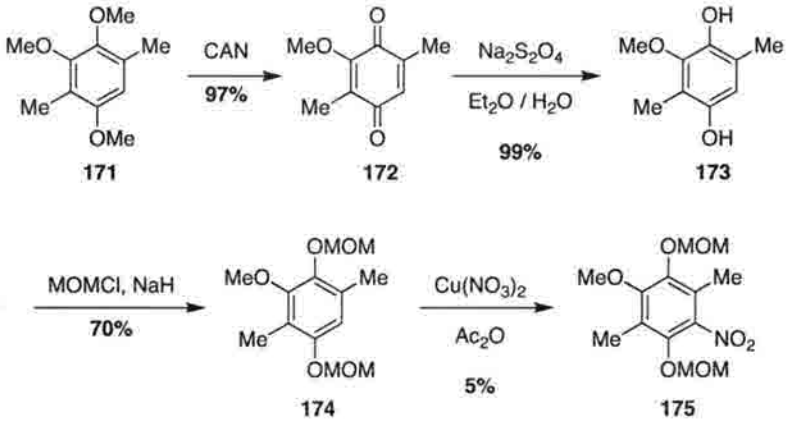
4.1: A New Aromatic Piece and Benzazocine Formation

4.1.1: Synthesis of a New Nitro Arene

Due to the failure to manipulate any of the furthest intermediates described in Chapter 3, it was necessary to investigate the synthesis of a new nitro arene with protecting groups on the hydroquinone oxygens that would be readily cleaved under a variety of mild conditions. The failure of the bis-methoxy substrates was due to the harsh conditions for removal (CAN, AgO, $PhI(OTf)_2$, etc.) that proved incompatible due to the nitrogen directly attached to the aromatic ring. Andrus and coworkers have published the synthesis of a bis-MOM arene where the MOM ethers are removed using sodium iodide and cerium trichloride to yield the hydroquinone followed by palladium catalysis with air to give the quinone found in geldanamycin in excellent yield.[84] This example demonstrates the feasibility of choosing a protecting other than the bis-methoxy ethers that readily form the quinone from an aromatic with nitrogen attached. This was a novel solution to the problem of the bis-methoxy groups that also plagued Andrus and coworkers in their synthesis of geldanamycin as mentioned in Chapter 3.

Accordingly, we set out on the synthesis of bis-MOM nitro arene **175** as shown in Scheme 45. The synthesis commenced with quinone formation of the previously synthesized trimethoxy arene **171** to afford quinone **172** in excellent yield. Reduction of quinone **172** with sodium dithionite gave hydroquinone **173**, which was immediately treated with MOMCl and sodium hydride to give bis-MOM arene **174** in decent yield.

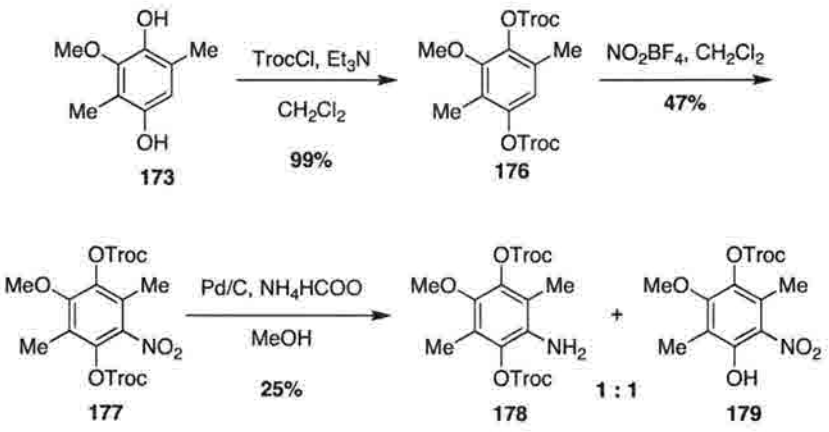
Treatment of **174** to our standard nitration conditions provided trace amounts of nitro arene **175**, with the bulk of the material being quinone **172**. From observation of the formation of quinone **172**, it became obvious that the MOM ethers were incompatible with the acidic conditions used in the nitro reduction. Other nitration methods were tried without success. At the same time we were working on the synthesis of **175**, we were also working on the synthesis of putative biosynthetic intermediates of FR900482 and the mitomycins. During the course of these studies, we found that sodium iodide and cerium trichloride (conditions used by Andrus for MOM removal) were not compatible with the aziridine ring present in our system. Because of this result, the optimization of the bis-MOM nitro arene **175** was not pursued further.



Scheme 45: Attempted Synthesis of bis-MOM Nitro Arene **175**

The next nitro arene we wanted to make was the bis-Troc nitro arene due to the wide range of mild conditions employed to remove Troc phenols. The synthesis of the bis-Troc nitro arene **177**, shown in Scheme 46, started with hydroquinone **173**. Bis-Troc protection proceeded smoothly to give **176**, and optimum nitration conditions were found by use of nitronium tetrafluoroborate (NO_2BF_4) to give nitro arene **177**. This reaction, however, was problematic on large scale and gave poor yields due to separation

difficulties. This is a major downside to this sequence as large amounts of nitro arene are needed for the synthesis (2.5 equivalents of nitro arene compared to 1 equivalent of aziridine aldehyde in the aldol reaction).

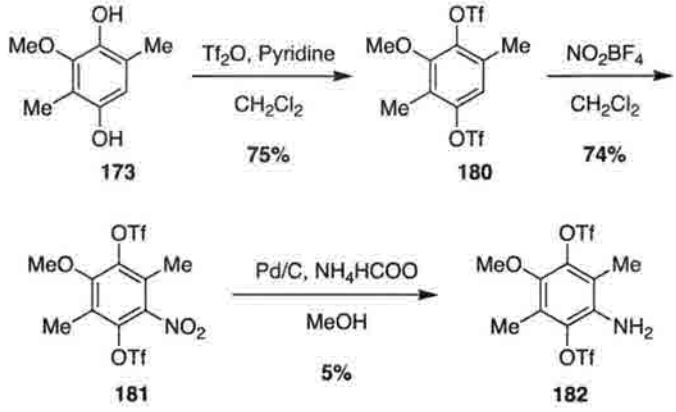


Scheme 46: Synthesis of bis-Troc Nitro Arene **177**

With the bis-Troc nitro arene **177** in hand, we then wanted to see if the nitro group could be reduced to aniline **178**. Standard transfer hydrogenation conditions used in our original system gave a 1:1 mixture of aniline **178** and phenol **179** (Scheme 46). Zinc dust and ammonium chloride has worked well in previous systems but is incompatible with Troc groups. Numerous other conditions were tried and all failed to produce decent amounts of aniline **178**. In light of these difficulties, new systems were investigated.

The next protecting group to be explored was the bis-triflate system, the preparation of which is shown in Scheme 47. From hydroquinone **173**, triflation and nitration both proceeded smoothly to give the desired nitro arene **181**. The possible advantages of this system could be the change in electronics. This could enable hydroxymethylation to give the desired alcohols instead of exclusively elimination and enone formation. In addition there are a number of other transformations that could

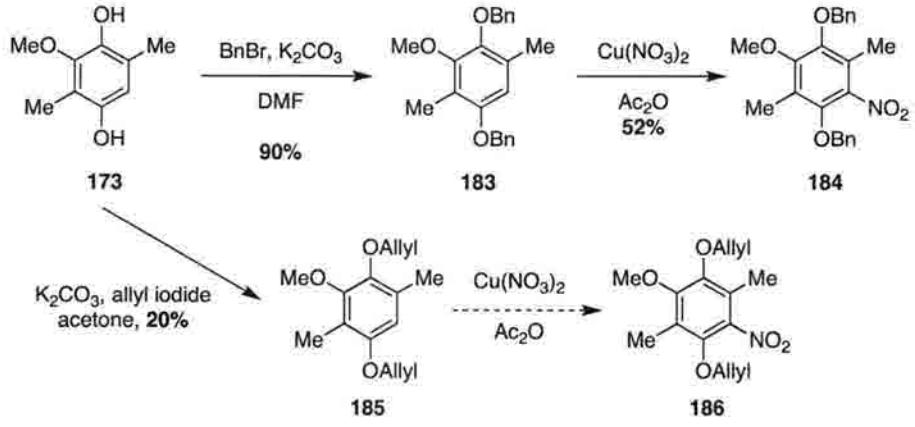
benefit from a more deactivated aromatic ring. However, there were some problems that arose in manipulation of the bis-triflate nitro arene **181**. Standard reduction conditions again failed to give the desired aniline **182** in any appreciable yield. Only starting material was observed that then started to decompose after two days. This result provided strong evidence that the bis-triflate nitro arene is too deactivating, and could cause major problems with the aldol reaction. As a result this substrate was dropped and alternate protecting groups were explored.



Scheme 47: Synthesis of bis-Troc Nitro Arene **181**

In the search for a suitable protecting group for hydroquinone **173**, two issues reigned paramount. First, it is essential that the protecting group be removable under a variety of diverse and mild conditions conducive to the functionality of the substrate. Second, the protecting group does not significantly alter the electronics of the system. An aromatic with similar electronic character as our original system would be expected to react similarly to our previously developed system. In theory this would allow us to make rapid progress to the key enone substrates where quinone formation would be attempted. Along these lines, the protecting groups we chose to pursue were the bis-benzyl and the bis-allyl ether substrates.

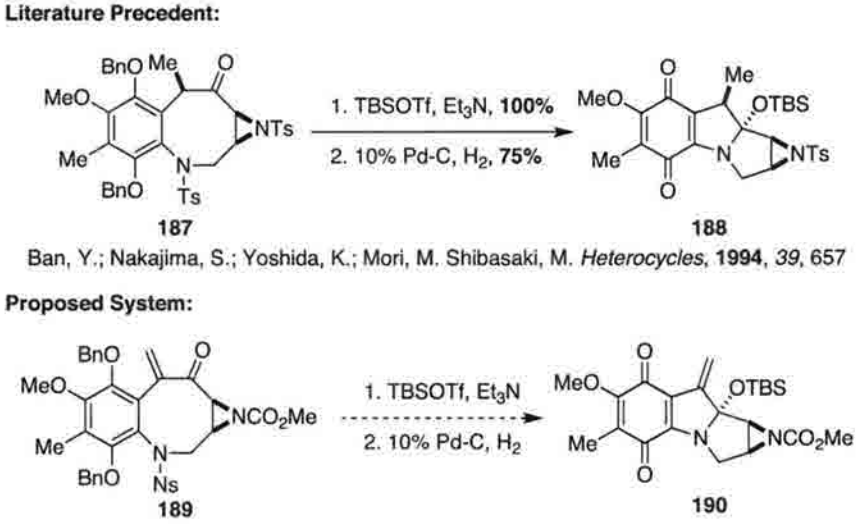
Shown in Scheme 48 are the initial attempts to form the bis-allyl and bis-benzyl ether nitro arenes starting from hydroquinone **173**. Reaction of **173** with potassium carbonate and either benzyl or allyl bromide in refluxing acetone gave the desired products **183** and **185** but in ~20% yield. It was found that DMF was a much better solvent for this reaction and could give the dibenzyl ether compound **183** in excellent yield (Scheme 48). Nitration of **183** under our standard conditions provided the desired bis-benzyl nitro arene **184** in good yield. Nitro arene **184** is also crystalline, which enable recrystallization from hexanes to give pure material for the aldol coupling reaction with the aziridine aldehyde. All characterization data were consistent with the data reported for **184** made by a different method.[85]



Scheme 48: Synthesis of bis-Benzyl and bis-Allyl Nitro Arene

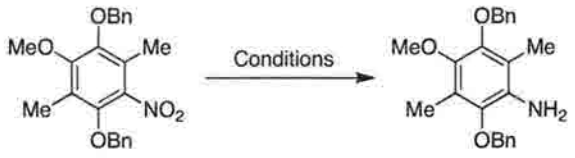
Once an efficient route to **184** was obtained, the work on making the bis-allyl nitro arene **186** was stopped. It is very likely that nitration of **186** would have worked using the same protocol shown above, however we decided to proceed forward with the benzyl ethers for two reasons. 1) The bis-allyl system might be problematic in the use of palladium for allyl ether removal just as removal the Alloc group in the presence of the enone was problematic in our first generation synthesis. 2) Through the bis-benzyl ether

substrate **184**, we might be able to construct a system (i.e. **189**, Scheme 49) that would be close enough to substrate **187** made by Shibasaki and Ban that transannular cyclization to tetracycle **190** might occur. It might be that the benzyl ethers are required for this transannular cyclization to proceed.



Scheme 49: Literature Precedent and Proposed System

Before aldol reaction of our new bis-benzyl nitro arene **184** with aziridine aldehyde **81**, it was important to find conditions that would reduce the nitro arene to the aniline. This was needed due to the incompatibility of using transfer hydrogenation to reduce the nitro moiety as had previously been done. After screening multiple conditions (Table 6) it was found that zinc dust and ammonium chloride effectively gave aniline **191** in almost quantitative yield.



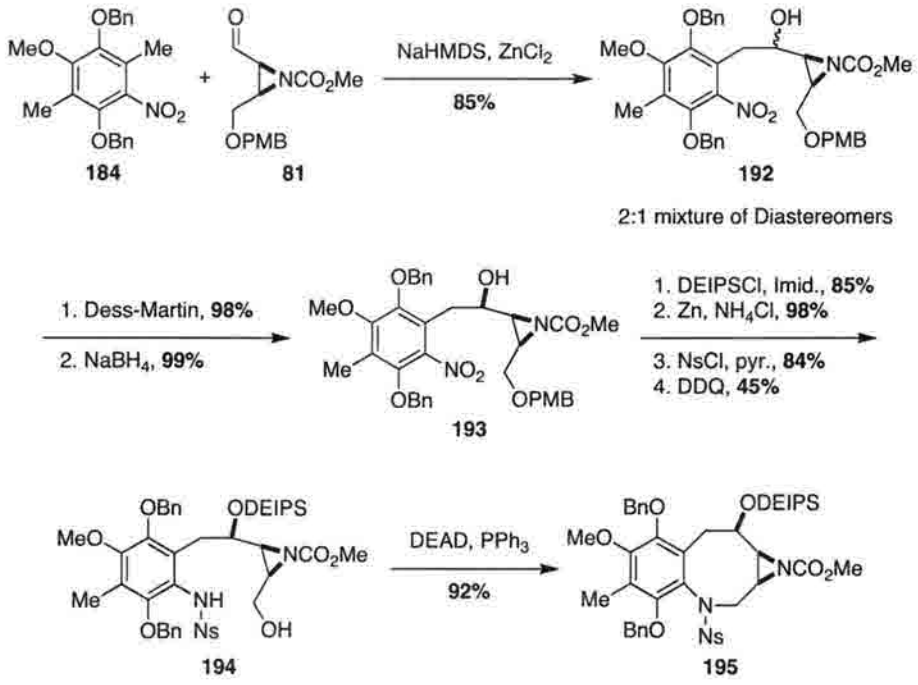
184 191

Conditions	Result (yield)
$Cu(acac)_2$, $NaBH_4$, rt, 24h	**191** (30%) + SM
$NaBH_4$, S, reflux, 48h	No Reaction
SBF_3, $NaBH_4$, rt, 12h	**191** (trace) + SM
Fe, NH_4Cl, rt, 24h	**191** (15%) + SM
$SnCl_2$, EtOH, reflux, 12h	**191** (trace) + SM
Zn, NH_4Cl, rt, 1h	**191 (98%)**

Table 6: Screening Conditions for Nitro Reduction of **184**

4.1.2: Benzazocane Formation with bis-Benzyl Analogues

Having developed an efficient sequence to bis-benzyl nitro arene **184** and discovered proficient conditions for the reduction of the nitro group, the next task at hand was aldol coupling of the two fragments. Aldol coupling of nitro arene **184** and aziridine aldehyde **81** using our previously developed conditions proceeded smoothly and gave almost the identical result as before as shown in Scheme 50 (85% yield of alcohols **192**, ~2:1 mixture of diastereomers). Oxidation of the secondary alcohols **192** to the ketone using Dess-Martin periodinane followed by reduction of the ketone with sodium borohydride provided alcohol **193** as a single diastereomer in excellent yield.



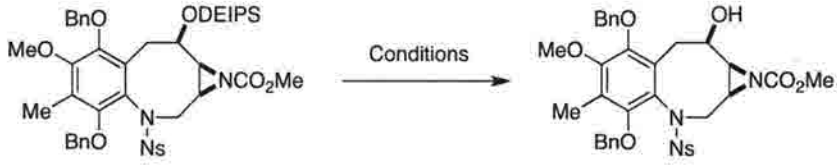
Scheme 50: Aldol Coupling and Benzazocine Formation

Formation of the DEIPS silyl ether was straightforward followed by nitro reduction using the zinc and ammonium chloride conditions previously described to provide the aniline in essentially quantitative yield. The aniline was then protected with nosyl chloride to give the requisite sulfonamide in good yield. PMB removal as before then gave the desired alcohol **194** but in poor yield (Scheme 50). It was found that the low yield in PMB removal is due to an inseparable byproduct in formation of sulfonamide. In spite of the low yield, this was a very fast and straightforward sequence to access alcohol **194** in order to try our key Mitsunobu cyclization reaction. It was found that standard Mitsunobu conditions (DEAD, PPh_3) over the course of 2 days provided a 92% yield of benzazocine **195**—one of the best yields in a Mitsunobu cyclization to date.

The sequence shown in Scheme 50 is extremely facile, high-yielding, and allowed efficient access to gram quantities of benzazocine **195**. We were interested in seeing what effect (if any) the bulky benzyl groups would have on the outcome of the Mitsunobu

cyclization reaction and were gratified to see that the reaction proceeds exceptionally well.

With large quantities of benzazocine **195** in hand, the next target was formation of the benzazocane generated first by silyl ether removal followed by oxidation of the resulting alcohol. Removal of the silyl protecting group, however, proved troublesome with previously developed conditions (TASF, DMF) furnishing alcohol **196** in only 30% yield. A number of conditions were examined (Table 7) in an effort to try and improve the yield of this transformation. Ammonium fluoride in methanol, conditions developed by Robins et. al.[86], provided **196** in fair yield on a small scale but then dropped down to ~40% when performed on a large scale. We then turned our attention to a protocol using HF·pyr, pyridine (forming pyridinium hydrofluoride in situ) developed by Trost[87] and co-workers and also used by Carreira and Du Bois[88]. This reaction, although providing **196** in slightly lower yield compared to ammonium fluoride, gave consistent yields of **172** on both large and small scale as shown in Table 7.

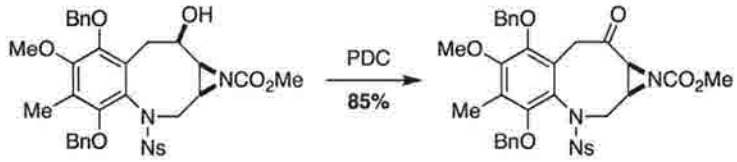


195 **196**

Conditions:	**Result:**
TASF, DMF	**30%**
TBAF, THF	**30%**
TBAF, AcOH, THF	Decomposition
HF, CH_3CN, 24h	**36% (50% BRSM)**
TBAF, NH_4F, THF	**20%**
THF/HCOOH/H_2O	Decomposition
NH_4F, MeOH, 42h (37 mg scale)	**68%**
NH_4F, MeOH, 42h (390 mg scale)	**45%**
HF·Pyr, Pyridine, THF (420 mg scale)	**52%**

Table 7: Conditions for Silyl Group Removal of Benzazocine **195** to Alcohol **196**

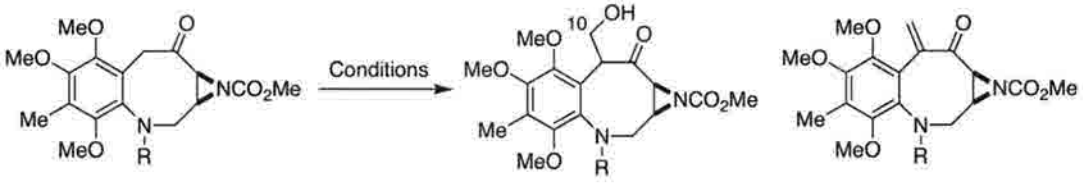
With an efficient way to make alcohol **196**, the only thing that remained in the synthesis of the benzazocane core was oxidation of the alcohol to ketone **197**. Standard conditions used previously including Dess-Martin periodinane or TPAP/NMO both provided ketone **197** albeit in modest yield at best (~50%). It was then that we came across a report by Shibasaki and Ban where PCC was used on a related substrate with good success. Accordingly, treatment of **196** with PDC overnight provided ketone **197** in 85% yield and completed the synthesis of the desired benzazocane (Scheme 51). Overall, the synthesis of benzazocane **197** proceeds smoothly and in good yield, and is also quite amendable to large scale with over 800 milligrams of **197** being prepared.



Scheme 51: Oxidation to Benzazocane **197**

The next major hurdle in our synthetic route, and one that has plagued this project throughout, is hydroxymethylation and installation of C10. Successful installation of the C10 carbon would mean that all the atoms are in place to complete the synthesis. As fully discussed in Chapter 3, all previous efforts towards construction of the hydroxymethyl moiety failed and resulted exclusively in enone formation (Scheme 52). It seems likely that the major problem here is the inability to deprotonate and also the propensity of the system to want to form the double bond and thus have a conjugated system. Due to the similar electronic nature of the system, it was anticipated that hydroxymethylation of

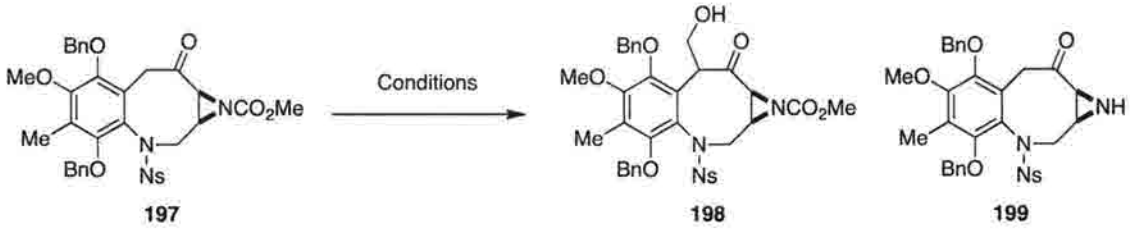
benzazocane **197** would not give the desired alcohol product. However, it was expected that enone formation would occur as before and could be carried on in the synthesis.



144a, R = Alloc
144b, R = Boc
144c, R = CO_2Me

Scheme 52: Failed Attempts for Hydroxymethylation and Enone Formation

Accordingly, hydroxymethylation of benzazocane **197** was attempted under standard conditions from the literature, especially those conditions that were successfully used on FR900482. A summary of a representative, but not exhaustive, sampling of conditions and reagents tried is shown in Table 8. Unfortunately the desired product of hydroxy-methylation, alcohol **198**, was not formed to any appreciable extent in all cases. Use of nucleophilic bases (LiOH, NaOH, KOH, etc.) only provided free aziridine **199** in modest yield with the remaining weight balance being recovered starting material. This was an interesting result in that aziridine cleavage is preferred with the catalytic amount of base even in the presence of over 100 equivalents of formaldehyde. Other conditions not shown in Table 8 led only to decomposition posing the question if alcohol **198** is a stable substrate. It could be possible that the desired compound **198** is forming but then decomposing or undergoing further reactions to other products.

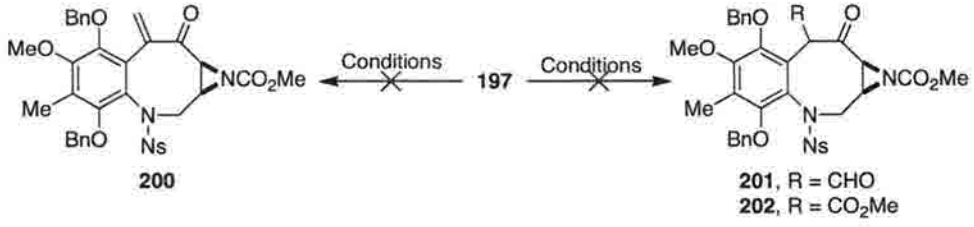


197 198 199

Conditions	Result (% Yield)
LiOH, NaOH, or KOH, Formalin	**199 (33%)**
LDA, paraformaldehyde, DMF	SM
NaH, paraformaldehyde, DMF	Decomposition
NaH, paraformaldehyde, THF	SM

Table 8: Failed Hydroxymethylation of Benzazocane **197**

In light of the inability to form desired alcohol **198** it was then thought to try to make enone **200** as previously performed, or to come in at a higher oxidation state as either the aldehyde **201** or ester **202** (Scheme 53). Attempts to form enone **200** failed and led in all cases to decomposition. This also led us to postulate whether the enone was again forming and, because of the reactive nature of enone **200**, rapidly decomposing to a number of other products. Standard conditions for enone formation that had worked in our previous methoxy-substituted aromatic system, failed on the corresponding bis-benzyl system **197**. The reasons and rationale for this are not clear to us. Maybe the presence of the electron-withdrawing sulfonamide on the benzazocane nitrogen is causing the problem.



200

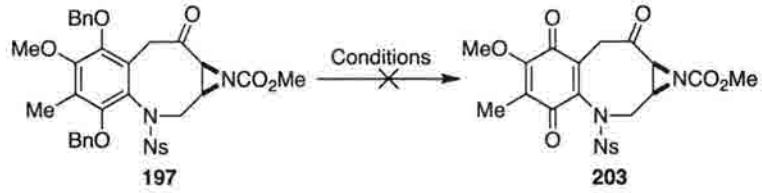
Scheme 53: Attempted Formation of Enone **200**, Aldehyde **201**, or Ester **202**

Also attempted was conversion first to the aldehyde **201** or ester **202** (Scheme 55), followed by subsequent reduction to provide alcohol **198**. Unfortunately neither of these methods proved fruitful under the several conditions examined for each of the desired transformation. This strategy was later abandoned because of the rationale that even if such a substrate could be formed, epimerization would be quite rapid. It was also envisioned that reduction of the β-keto ester could be problematic especially in the presence of the aziridine protected as the methyl carbamate.

The inability to alkylate ketone **197**, and especially the inability to invoke enone formation as previously performed on the bis-methoxy system, left us a bit puzzled. It was then investigated to see if alkylation could occur with other electrophiles. All efforts to alkylate benzazocane **197** using various electrophiles (MeI, Me_2SO_4, etc.) failed, returning starting material in every case. Deuterium-quenching studies revealed that the anion might not even be forming as no deuterium incorporation was observed after quenching with D_2O. In light of the results above, the most logical explanation for the inability to alkylate is due to the bulky nature of the benzyl groups. It is thought that having the bulky benzyl groups on the aromatic ring in an already sterically demanding system renders the system too hindered for alkylation to occur.

Attempts to form quinone **203** from benzazocane **197** were also unsuccessful and met with decomposition (Scheme 54). It was observed that removal of the benzyl group and subsequent oxidation to the quinone worked just fine on the arene alone before aldol coupling with the aziridine. This result led us to believe that the nitro group present in the nosyl protecting group is being reduced at the same time as the benzyl ethers, causing removal of the Ns group and subsequent decomposition. A slight amount of quinone **203**

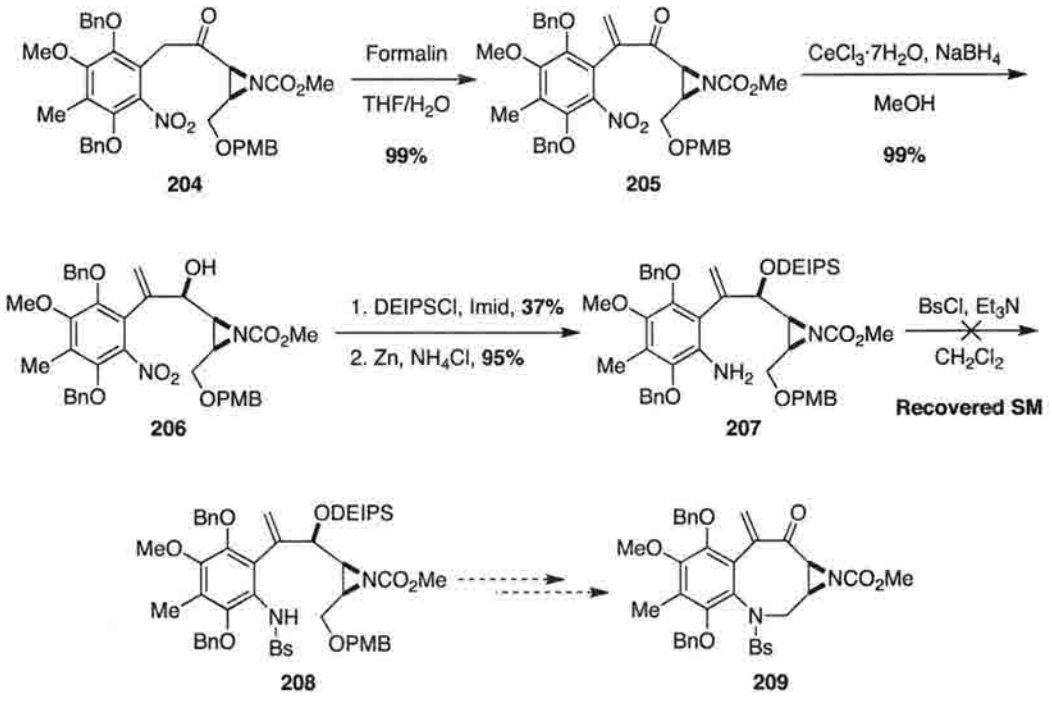
was produced by treatment of **197** with ceric ammonium nitrate (CAN), but not enough to be of any consequence. Even if conditions were found that provided quinone **203** in good yield, this would not be helpful due to the difficultly of trying to alkylate at the benzylic position of quinone **203**.



Scheme 54: Failed Quinone Formation

4.1.3: Benzazocine Formation with bis-Benzyl Analogues

Due to the inability to alkylate **197** as described above, we next thought about first alkylating the ketone in the acyclic form before ring closure and formation of the benzazocine. The major question with this proposed route was whether the Mitsunobu reaction would work with the olefin in place and in the presence of the bulky benzyl ethers. In order to address this question, we set out to synthesize benzazocane **209** (Scheme 55). Having the benzazocane nitrogen protected as the benzene sulfonamide (N-Bs) would be advantageous in that it should behave like the Ns group as shown previously, and be amenable to both benzyl ether removal and transannular cyclization conditions.

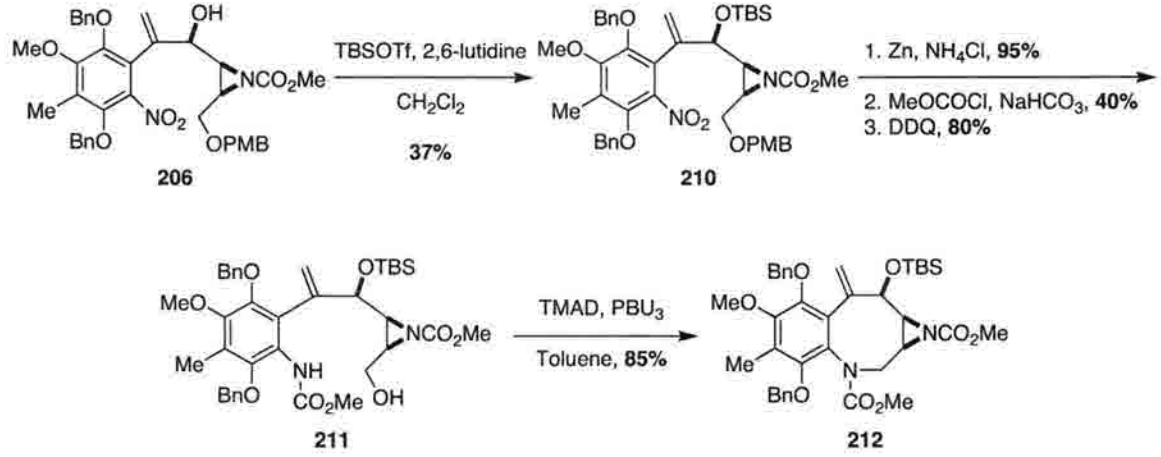


Scheme 55: Installation of Exocyclic Olefin Before Cyclization Strategy

Accordingly, the synthesis of **209** started from ketone **204**. Treatment of **204** under hydroxymethylation conditions did not provide any of the hydroxymethylated product, but rather provided enone **205** in essentially quantitative yield (Scheme 55). Other conditions tried in an attempt to obtain the hydroxymethylated compound without elimination failed. This result is probably due to the nitro group on the arene that likes to form a stable Michael acceptor. Luche reduction of enone **205** provided alcohol **206** in quantitative yield. Protection of alcohol **206** as the DEIPS ether resulted in poor yield of the desired silyl ether. This result is most likely due to the sterically demanding nature of the allylic alcohol. Reduction of **206** with zinc dust and ammonium chloride did provide the requisite aniline **207** in 95% yield. However, reaction of the aniline with benzene sulfonamide and triethylamine did not afford any of the desired sulfonamide **208**.

Due to the results shown above, we decided to see if we could alkylate aniline **207** with something we had used previously and was known to work. We also wanted to see if

silyl ether besides the DEIPS ether might be more easily removed in the synthesis. Accordingly, we decided to use methylchloroformate as the alkylating agent and to make the TBS silyl ether instead of DEIPS silyl ether. Protection of alcohol **206** as the TBS ether **210** was accomplished using TBSOTf and lutidine in moderate yield (Scheme 56). Efforts to optimize the reaction and increase the yield have been fruitless to this point. The low-yielding nature of this reaction is most likely due to the hindered accessibility and reactivity of alcohol **206**.

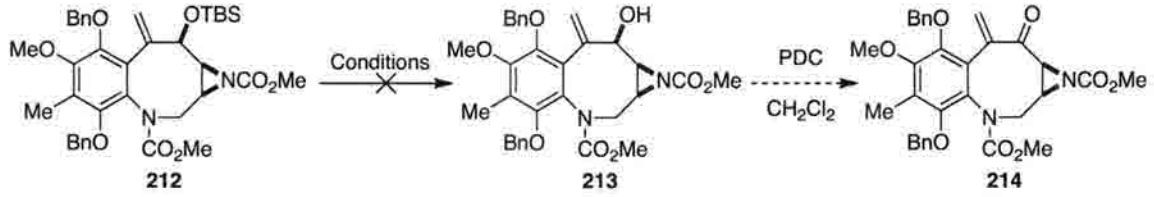


Scheme 56: Synthesis of Benzazocine **212**

Treatment of silyl ether **210** with zinc and ammonium chloride did provide the desired aniline in excellent yield and upon treatment with methylchloroformate gave the methyl carbamate product albeit in poor yield (Scheme 56). It appears that this system with the olefin already in place is much more sterically demanding than the corresponding unsubstituted system. Attempts to alkylate the aniline with agents larger than methylchloroformate such as BsCl, NsCl, and Boc either lead to extremely poor yields (< 5%) or no reaction at all. Removal of the PMB ether with DDQ worked well as before and gave alcohol **211** in good yield. To our delight, treatment of **211** under our standard Mitsunobu conditions of TMAD/PBu_3 gave the desired benzazocine **212** in 85%

yield! This result gives insight about the tolerance of the Mitsunobu cyclization in sterically demanding systems.

The next steps of the synthesis entailed removal of the TBS ether to supply alcohol **213**, followed by oxidation to the ketone to afford benzazocane **214**. Removal of the silyl group was more difficult than expected and failed to provide the desired alcohol **213**. Optimum conditions successfully used in other systems were not successful as shown in Table 9. Use of TBAF provided a new product but not alcohol **213**. The product was more polar than expected and also lacked a methoxy group by HNMR. Use of TASF, which had proved to be very effective with earlier substrates, failed to provide any reaction and only gave back starting material. Failure to desilylate benzazocine **212** is also likely due to sterics, with **212** being too bulky to allow for reaction with the various desilylating agents.



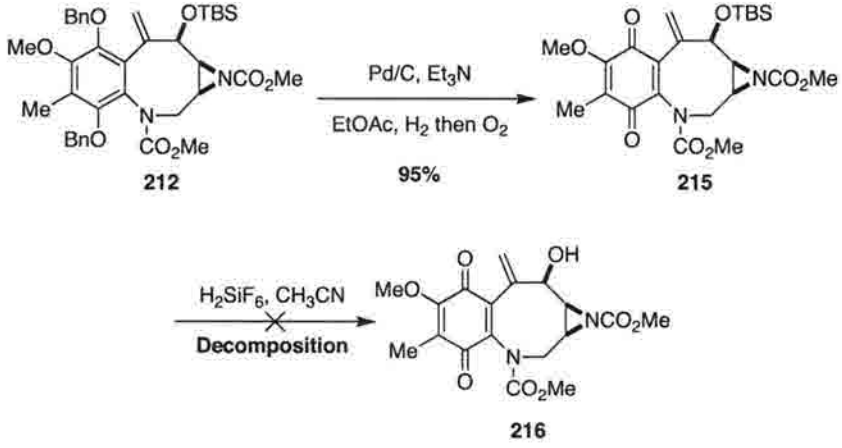
212 **213** **214**

Conditions	Result
TASF	No Rxn (Recovered SM)
TBAF	Unknown Product (Not **213**)
HF·Pyr., Pyr.	No Rxn (Recovered SM)
NH_4F, MeOH	No Rxn (Recovered SM)

Table 9: Failure to Remove the TBS Silyl Ether

One useful result that did come out of these substrates was the ability to oxidize benzazocine **212** to the desired quinone **215** in excellent yield (Scheme 57). This was an important "proof-of-principle" showing that the quinone could indeed be formed from the

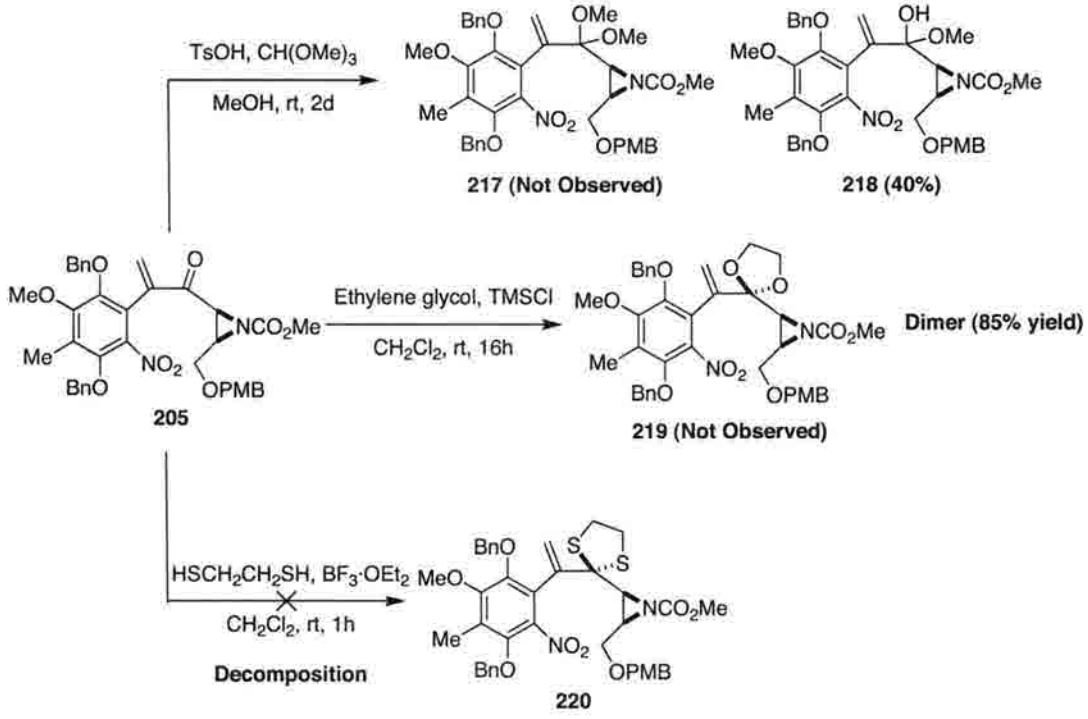
bis-benzyl aromatic system. From quinone **215** it was also tried to see if the silyl group could be removed at the quinone stage rather than the hydroquinone stage. Unfortunately, the substrate decomposed under the fluorosilic acid (H_2SiF_6) conditions tried (Scheme 57). H_2SiF_6 has previously been shown to be a very useful reagent for the mild removal of silyl groups in complex natural products. This result is most likely due to the unstable nature of the quinone to either acidic or strongly-nucleophillic conditions. The conditions mentioned in Table 9 were also tried on quinone **215** to no avail, as rapid decomposition was seen in each case. From the results above, it appears that silyl deprotection might need to occur at the protected hydroquinone stage before oxidation to the quinone. At this point in time, this route remains a dead end until conditions are found to remove the silyl group on either benzazocine **212** or quinone **215**.



Scheme 57: Quinone Formation and Failed Silyl Ether Removal

Owing to the difficulty in removing the TBS ether, alternate protecting group strategies were explored. One such strategy was that of protecting the carbonyl group of enone **205** directly without the need of reducing the ketone to the alcohol and re-oxidation to the enone at a later stage. The first protecting group tried on enone **205** was the dimethyl ketal as employed by Kishi in his successful total synthesis of mitomycin C

(Scheme 58). Multiple conditions were tried with ketal **217** not formed except for production of a small amount of hemi-ketal **218**. Subsequent reactions using **218** were tried and were found to not be compatible with the hemiaminal moiety. Methylation of hemiaminal **218** in an attempt to obtain ketal **217** was not successful. The poor yield also precluded any utility for **218**. Inability to make the ketal was not that surprising due to the difficulty Kishi encountered.

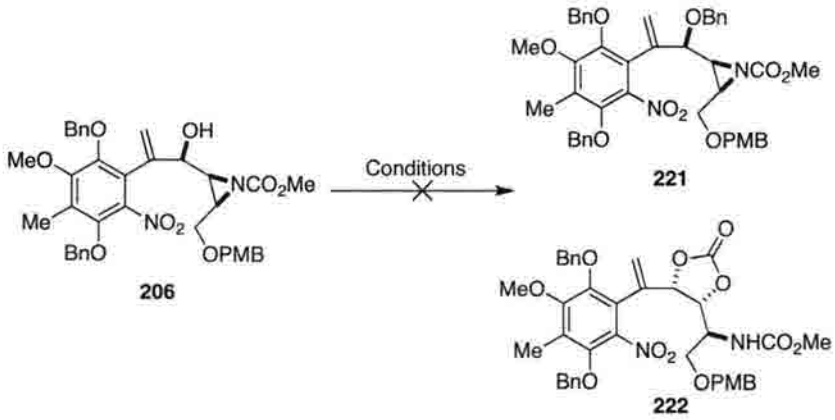


Scheme 58: Attempts to Protect the Carbonyl of Enone **205**

The next protecting group tried was to form the 1,3-dioxolane ketal **219**. After attempting several different conditions, it was found that TMSCl and ethylene glycol provided a clean product after stirring overnight (Scheme 58). The NMR spectra revealed a clean compound that could be reasonable for ketal **219**. However, HRMS revealed that the new compound was actually a dimer of **205** plus ethylene glycol. The actual structure of this compound is not known. Attempts were also made in an effort to construct the 1,3-

dithiolane derivative **220**, but gave decomposition (Scheme 58). This is not surprising considering the many Michael acceptors in the molecule and possible alternate reaction possibilities upon treatment of **205** with a strong nucleophile like a thiol.

Due to the failed attempts to form the ketal, a different protecting group for secondary alcohol **206** was examined. Due to the difficulty in removing the TBS ether, other protecting groups for the secondary alcohol were then investigated. We desired to pick a protecting group that might be able to be removed at the same time as quinone formation. Thus the benzyl protecting group was chosen. Attempts to form benzyl ether **221** from secondary alcohol **206** are shown in Table 10. Most conditions that were tried led to decomposition except treatment of **206** with potassium carbonate and sodium hydride, which led to formation of cyclic carbonate **222** in good yield. It was already known that alcohol **206** is not a good nucleophile and the reagents that were tried for benzylation just aren't electrophilic enough for the reaction to occur. Attempts to use more reactive benzylating agents such as benzyl triflate (Table 10) just gave decomposition.



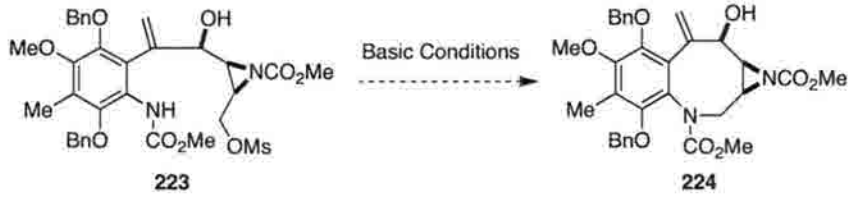
Conditions	Result (% Yield)
BnBr, NaH, DMF	Decomposition
BnBr, K_2CO_3, DMF	**222 (80%)**
BnOH, Tf_2O, Lutidine, CH_2Cl_2	Decomposition
$Cl_3C(NH)OBn$, TfOH	Decomposition

Table 10: Attempted Benzylation of Alcohol **206**

4.2: Synthesis of Benzazocines via Reductive Aminocyclization

4.2.1: Synthesis of Benzazocines

In light of protecting group difficulties related to secondary alcohol **206**, we wondered if we might be able to proceed with the synthesis without protection of the secondary alcohol in **206**. It was hoped that the sterically demanding environment of the alcohol that had originally made protection so difficult, might deter undesirable reactions of the alcohol throughout the synthesis. It was proposed that the primary alcohol be protected as the mesylate (i.e. **223** in Scheme 59), whereupon benzazocine formation could be accomplished under basic conditions as conducted by Tereshima et. al. in their synthesis of FR900482 to give benzazocine **224**.[89]

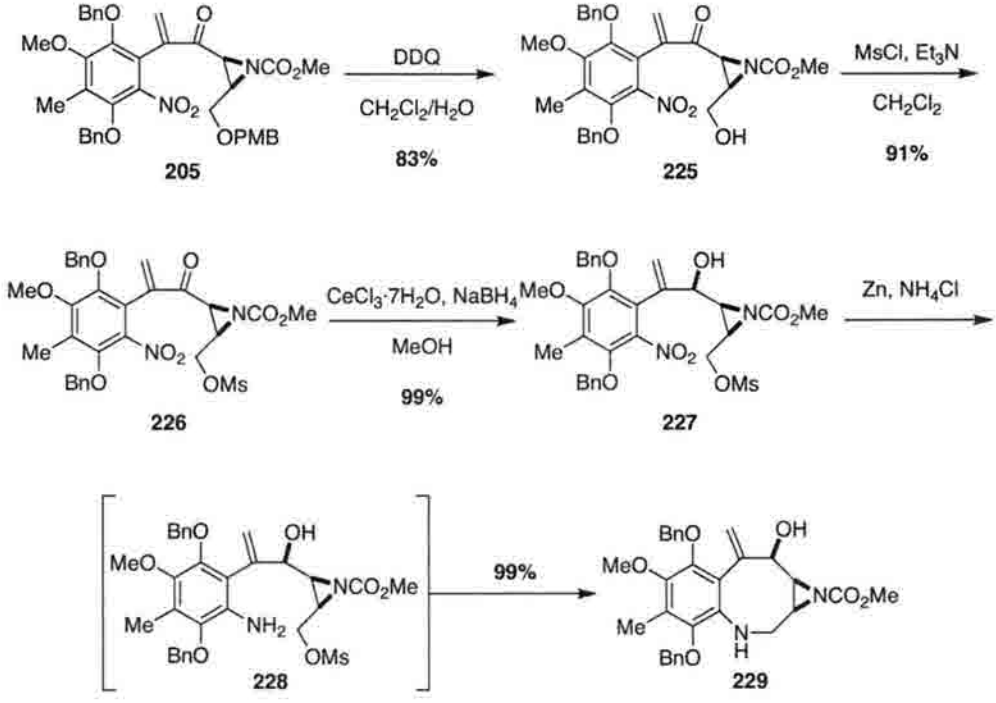


223 **224**

Scheme 59: Proposed Route with no Protection of the Secondary Alcohol

Accordingly, enone **205** was converted to alcohol **225** followed by treatment with mesyl chloride to provide mesylate **226** in good yield as shown in Scheme 60. Mesylate **226** then smoothly underwent a Luche reduction to give alcohol **227** in almost quantitative yield and good overall yield (75%) for the three steps. The next step was then to reduce the nitro group of alcohol **227** to the corresponding aniline **228**, and then to protect as the methyl carbamate as proposed in Scheme 59. Reduction of alcohol **227** under zinc/ammonium chloride conditions did provide the expected aniline **228** as well as

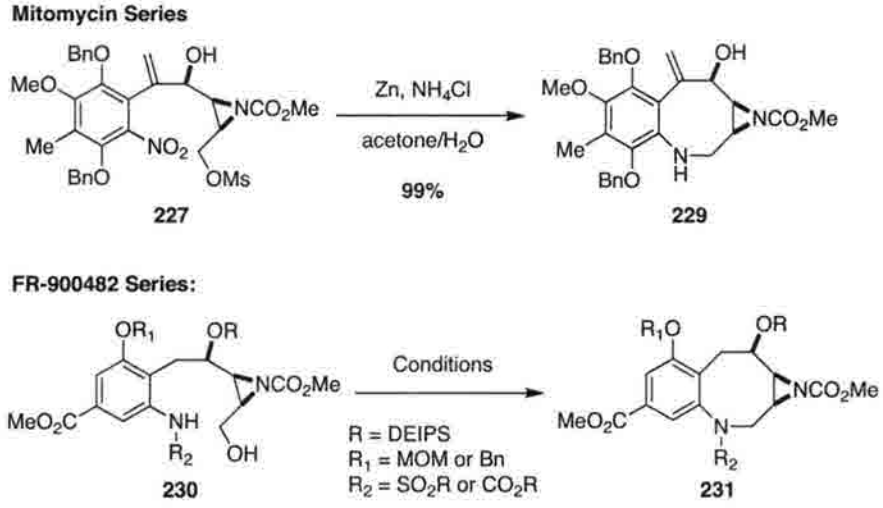
another compound that was later identified to be benzazocine **229** in a 1:1 ratio (Scheme 60). It was found that longer treatment of alcohol **227** under the nitro reduction conditions (3 hours as opposed to 30 min) gave exclusively benzazocine **229** in quantitative yield! Benzazocine **229** obtained from the reaction was extremely pure and did not require further purification.



Scheme 60: Carrying through Unprotected Material and Unexpected Cyclization

This result was quite unexpected due to previous studies on FR900482 by our group and others where they showed that the aniline must be activated (as a carbamate or sulfonamide) in order for closure to the benzazocine *via* a Mitsunobu reaction to occur (i.e. **230** to **231** in Scheme 61). However, as shown in Scheme 61, the aniline used in the FR-900482 series is not nearly as electron-rich as aniline **227** used in the synthesis of the mitomycins. It could be reasonable to assume that the aniline in the FR series must be activated in order to react, whereas aniline **227** is reactive enough that, under the right conditions, reaction to form the desired benzazocine can occur without the need for

additional activation. Upon further study of this unique cyclization reaction, it was found that acidic conditions were required for the cyclization to occur. Treatment of aniline **228** under a variety of basic conditions did not give benzazocine **229**. The mildly acidic conditions of an old bottle of deuterated chloroform were sufficient enough to catalyze the transformation of aniline **228** to benzazocine **229**.

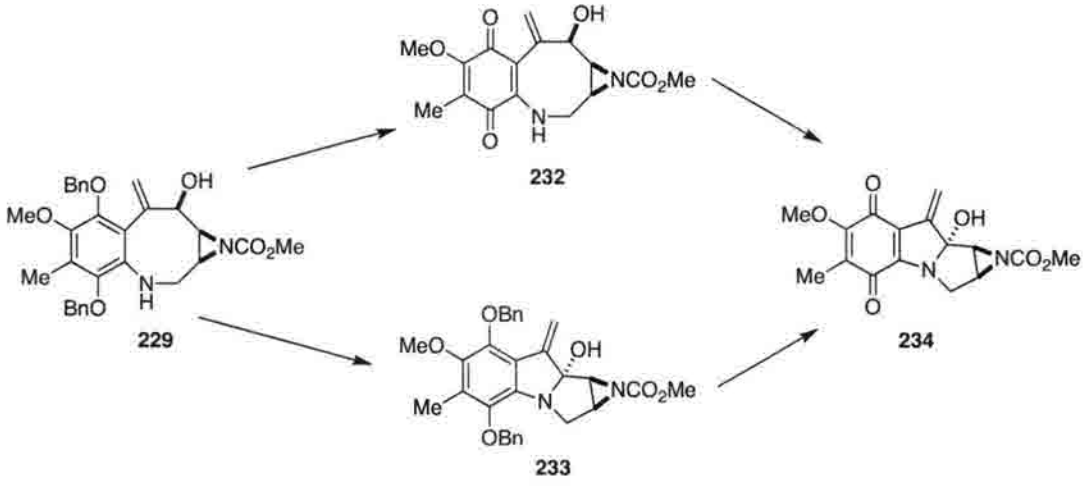


Scheme 61: Anilines used in Cyclization Reactions for Mitomycin and FR900482

In surveying the current mitomyicn/FR900482 literature, formation of benzazocine **229** is arguably the most efficient method for benzazocine formation reported. One of the major benefits of this approach is the elimination of the need for prior activation of the aniline. As seen in our first generation approach to the mitomycins, activation of the aniline was required for cyclization to occur and then the protecting/activating group was removed. The above approach saves two synthetic steps in gaining access to the same products as our first generation approach.

4.2.2: Attempted Conversion to the Mitomcyins

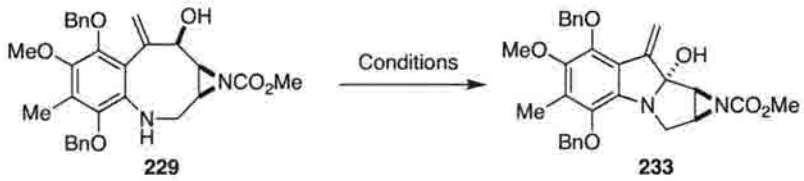
With benzazocine **229** in hand, the next transformations were either to remove the benzyl ethers and oxidize to quinone **232** or to oxidize the secondary alcohol to the ketone and hope it would cyclize to tetracycle **233** (Scheme 62). It was hoped from this sequence to be able to access tetracyclic alcohol **234**, which would be two steps away from an asymmetric total synthesis of mitomycin K.



Scheme 62: Planned Sequence of Steps Towards Mitomycin K

We first attempted to oxidize the alcohol of benzazocine **202** to the ketone, which should immediately close to tetracycle **233**. Shown in Table 11 is a sampling of oxidation conditions that were tried to affect this transformation. None of the conditions tried gave tetracycle **233**, and either saw decomposition or recovered starting material. As compounds like tetracycle **233** have not been reported in the literature, it is unsure how stable they might be. One might expect that compounds like **233,** with the hemi-aminal at the protected hydroquinone stage, might be unstable due to the proposed mode of action of these compounds. This might account for the observed decomposition of **229** under a variety of oxidation conditions. It is possible that the desired tetracycle **233** is forming

and then rapidly decomposing because of the inherent instability of this type of compound.

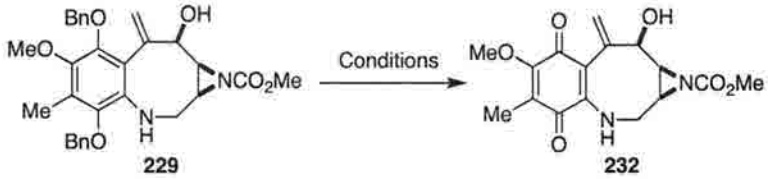


229 233

Conditions	Result
$(CoCl)_2$, DMSO, Et_3N	Decomposition
PDC	Decomposition
TPAP, NMO	Decomposition
Dess-Martin, pyridine	Decomposed on workup
Dess-Martin, Et_3N, TBSOTf	Decompositon
MnO_2, CH_2Cl_2	Recovered SM
TEMPO, CH_2Cl_2	Recovered SM
$BaMnO_4$, CH_2Cl_2	Recovered SM

Table 11: Attempts to Oxidize Benzazocine **229** to Tetracycle **233**

We next set out attempting to try the oxidation of benzazocine **229** to quinone **232**. It was hoped that quinone **232** might be more stable and allow us to access tetracyclic products. A list of conditions that were tried for quinone oxidation is shown in Table 12. It was found that the conditions previously used of Pd/C, Et_3N, EtOAc, H_2/O_2 gave a 52% yield of quinone **232**, provided the reaction mixture was kept free from air and water. From the reaction, it appears that the intermediate hydroquinone in the presence of both air and water is unstable and goes on to form other products. Other conditions mostly provided **232** in trace yield with multiple other products observed.

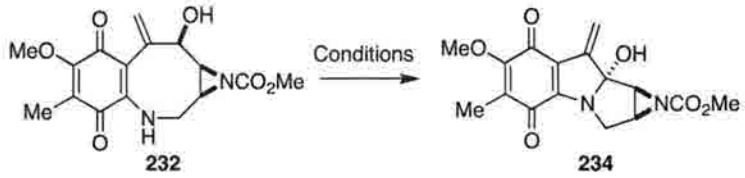


229 232

Conditions	Result (Yield)
10% Pd/C, Et_3N, EtOAc, H_2/O_2	**205** (62%)
10% Pd/C, THF	Byproduct
Li, Napthalene, THF, -78 °C	Recovered SM
5% Pd/C, NH_4HCOO, MeOH	Byproducts

Table 12: Attempted Oxidation of Benzazocine **229** to Quinone **232**

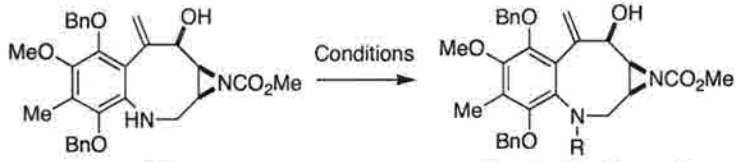
With Quinone **232** in hand, the next task was then to oxidize the secondary alcohol of **232** to the corresponding ketone. It was hoped that upon oxidation to the ketone, closure would be spontaneous to directly provide tetracycle **234**. It was postulated that hemiaminal moiety in **234** would be stable due to the stability of mitomycin H (differing from tetracycle **234** only by containing a *N*-methyl aziridine moiety). Shown in Table 13 are conditions that were tried to achieve this transformation, with none of the conditions being successful. In most cases, only starting material is observed. In the case of the Swern oxidation, an unknown product was observed. NMR and HRMS suggest this compound to be a dimer, although the exact structure of the dimer remains unknown. It also remains unclear how this dimer is forming and what steps might be done in order to circumvent its formation.



Conditions	Result
PDC, CH_2Cl_2, rt, 24h	S.M. & Decomposition
TPAP, NMO, CH_2Cl_2, MS, rt, 24h	Recovered S.M.
Dess-Martin, Pyridine, CH_2Cl_2, rt, 2h	Decompositon
$(COCl)_2$, DMSO, -78 °C, 1h	Unknown Product
MnO_2, toluene, reflux	Recovered SM
Dess-Martin, rt, 2h	Recovered SM
PCC, celite, CH_2Cl_2	Decomposition
TEMPO, CH_2Cl_2, rt, 24h	Recovered SM
IBX, Toluene/DMSO	New Spot (mass = 336)

Table 13: Attempted Oxidation of Quinone **232** to Tetracycle **234**

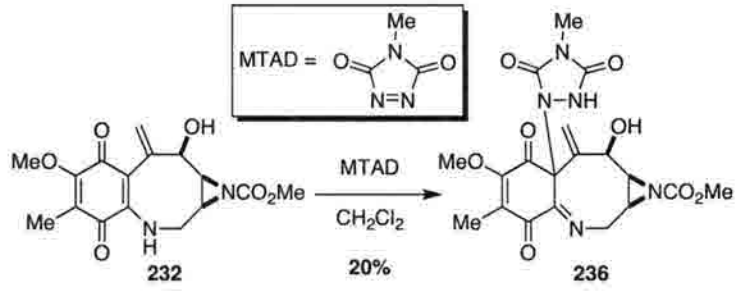
In analyzing the disappointing results shown above, it was thought that the free amine might be the source of our problems. We wanted to see if we might be able to protect the secondary nitrogen so that we might be able to form the ketone and then induce a transannular cyclization in order to get the tetracycle. Efforts to do this, all of which failed, are shown in Table 14. The last entry was surprising in that treatment under mesylation conditions did not react with either the amine or the secondary alcohol. These attempts helped to shed light on the inherent lack of reactivity of **229** towards either oxidation or alkylation.



Conditions	Result
TsCl, Pyr.	Decomposition
CbzCl, Pyr.	Recovered SM
MsCl, Et_3N	Recovered SM

Table 14: Efforts to Protect the Secondary Amine

Another attempt to mask the amine in an effort to see if oxidation would work was done using a protocol by Corey and Baran in the synthesis of a member of the okaramine family of natural products.[90] They employed the use of MTAD to mask the reactivity of a portion of their molecule during their key step, which employed a photochemical sequence. This was tried on quinone **232** (Scheme 63) and did result in the product **236** albeit in low yield. Additionally, the product was not stable and readily decomposed under attempting to oxidize to the ketone. Because of these difficulties, this approach was abandoned.



Scheme 63: Attempted use of MTAD to Mask Amine Functionality

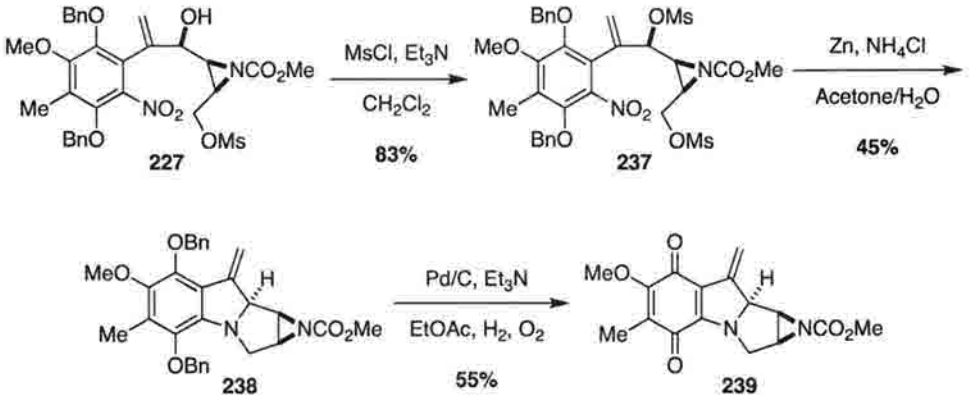
In conclusion, an efficient way to make benzazocines utilizing a reductive aminocyclization reaction has been developed. This is a very efficient method for the

synthesis of benzazocines that obviates the need for prior activation of the aniline. Unfortunately, all attempts to further manipulate benzazocine **229** or quinone **232** failed.

4.3: Synthesis of Mitosanes via Reductive Aminocyclization

4.3.1: Mitosane Formation

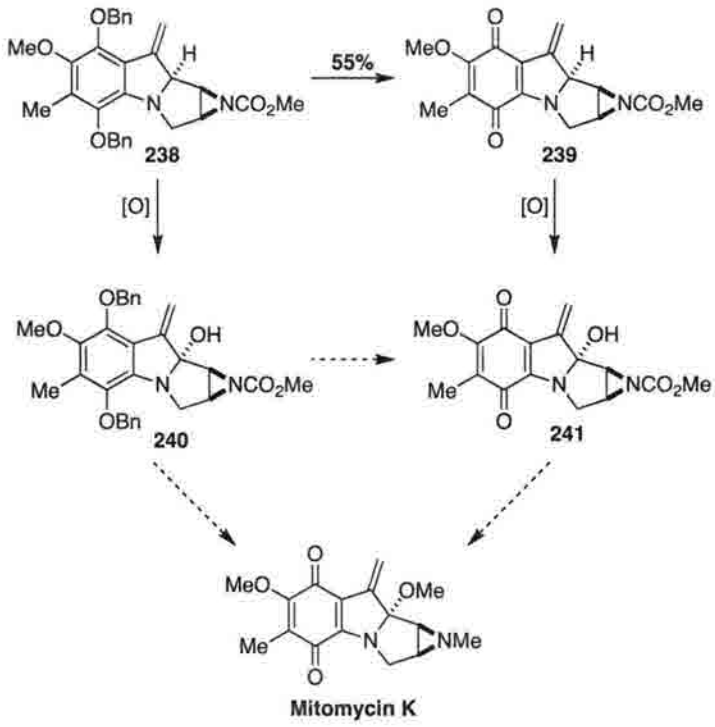
During the development of the formation of benzazocine **229** via a reductive aminocyclization reaction, we were intrigued with the possibility of being able to access the tetracyclic core of the mitomycins *via* a bis-mesylate compound (i.e. **237** in Scheme 64). Accordingly, bis-mesylate **237** was easily made from alcohol **227** under standard conditions and then subjected to reductive aminocyclization reaction conditions used in the synthesis of benzazocine **229**. After four days of stirring under these conditions, to our delight, a 45% yield of tetracycle **238** was obtained. The reaction proceeded very slowly with the low yield of tetracycle **238** likely due to competing decomposition under the reaction conditions. The slow rate and yield of this reaction is in stark contrast to the formation of benzazocine **229** (3 hours, quantitative). Nevertheless, this was the first tetracycle towards the mitomycins reported in our labs and one of few reports in the literature of a fully functionalized tetracycle. Additionally, treatment of **238** to standard hydrogenation conditions used previously gave mitosane **239** in moderate yield (55%, Scheme 64).



Scheme 64: Synthesis of Tetracycle **238** and Mitosane **239** from bis-Mesylate **237**

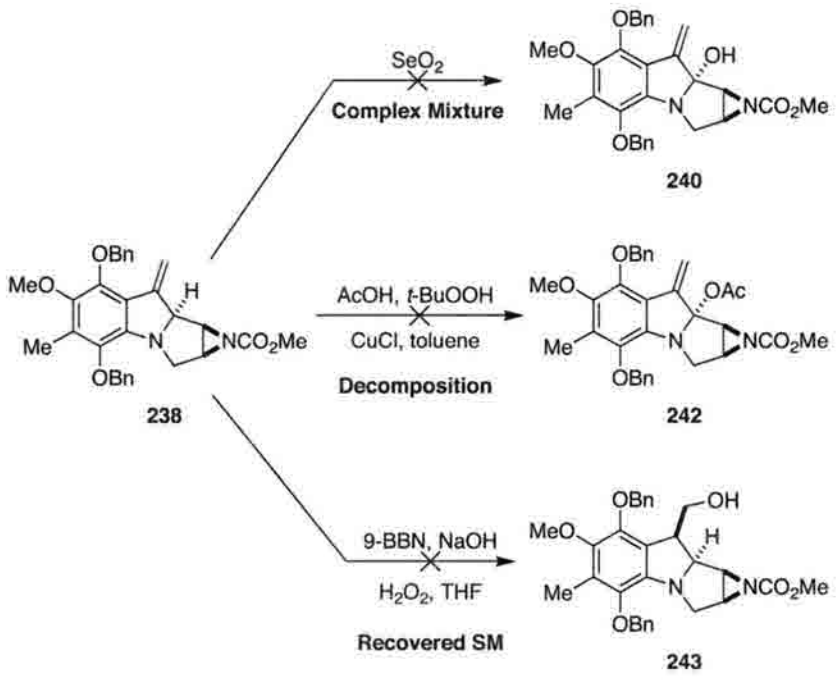
4.3.2: Attempted Conversion to Mitomycin K

As can be seen in Scheme 64, tetracycle **238** and mitosane **239** are an oxygenation at C9a away from achieving the complete tetracyclic core of the mitomycins (i.e. **240** and **241** respectively, Scheme 65). This type of transformation amounts to an allylic oxidation at C9a. There are no reports in the literature of an allylic oxidation of a mitomycin tetracycle like either **238** or **239** to give **240** or **241** respectively. Precious little is recorded in the literature regarding functionalization at the C9a position of mitosanes. Use of the Polonovsky reaction has been used in order functionalize the C9a position of FR900482[91] but was shown by Danishefsky[92] and coworkers to not be a viable route in the mitomycins. Despite the lack of literature precedent for such a transformation, we were interested in exploring the feasibility of this type of transformation with our material already in hand.



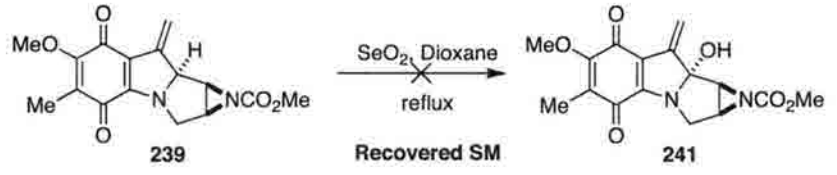
Scheme 65: Proposed Oxygenation of Tetracycle **238** and Mitosane **239**

Classic allylic oxidation conditions using selenium dioxide was first tried on tetracycle **238** but resulted in a complex mixture where multiple products were obtained (Scheme 66). Alcohol **240** could be among the products but, due to the small scale of the reaction, the individual products were not isolated. Next we explored the possibility of allylic oxidation of **238** to give acetate **242** by treatment under Kharasch oxidation conditions. These conditions resulted in total decomposition of tetracycle **238**. The Kharasch oxidation is known to go through radical intermediates and, as such, **238** might not have been the ideal substrate to try this reaction on. Tetracycle **238** was also subjected to hydroboration/oxidation conditions to see if we might be able to obtain the hydroxymethyl moiety necessary for mitomycins A and C (**243** in Scheme 66). These conditions resulted in no reaction with total recovery of the starting material.



Scheme 66: Attempts to Manipulate Tetracycle **238**

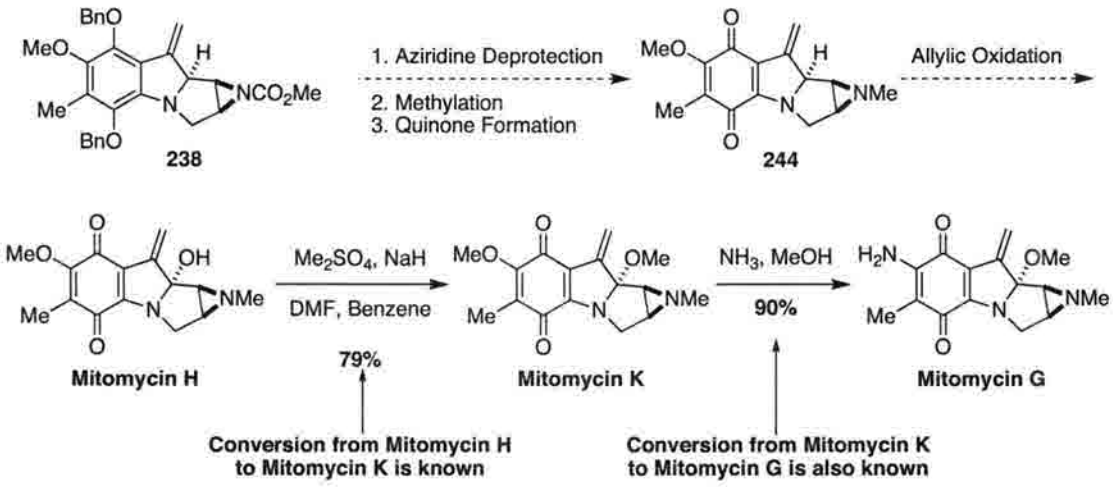
Mitosane **239** was also treated to the standard allylic oxidation conditions of selenium dioxide in refluxing dioxane to see if alcohol **241** could be obtained. However, only recovered starting material was observed in this case even after 3 days in refluxing dioxane (Scheme 67). This result lent further evidence to the extreme stability of the quinone tetracycles compared to the protected hydroquinone variants.



Scheme 67: Attempt to obtain alcohol **241**

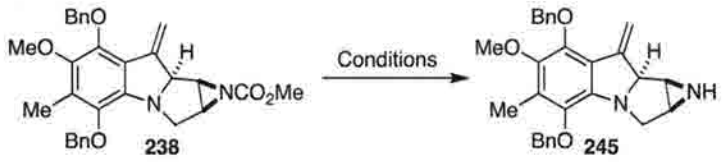
It was postulated that perhaps the bulky methyl carbamate protecting group on the aziridine nitrogen was preventing allylic oxidation. To test this theory it was desired to remove the protecting group on the aziridine and protect it as the *N*-methyl aziridine **244** (Scheme 68). As shown in Scheme 68, if quinone **244** could be made then allylic

oxidation would give mitomycin H and subsequently mitomycins K and G by reported procedures.[93] This route, if successful, would be a unique and novel approach to several of the mitomycins.



Scheme 68: Planned Synthesis of *N*-Methyl Aziridine **244** and Proposed Route

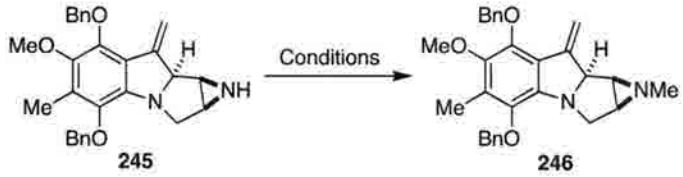
The first step in the sequence shown in Scheme 68 involved conversion of the methyl carbamate aziridine into the free aziridine and subsequent methylation to form the *N*-methyl aziridine moiety found in the natural products. It was anticipated that this sequence of steps would be relatively easy and would get us two steps closer to the natural product. As shown in Table 15, aziridine deprotection did not proceed smoothly at all. Under the conditions that were used, product **245** was formed in all instances but in very low yield. These reactions looked good when monitored by TLC but low yields were obtained upon workup and isolation. Complexation of the free aziridine to the metal used in the reduction seemed to be the major problem. Various workup conditions (including the use of Rochelle's salt) failed to provide the free aziridine **245** in higher yields than before. The use of Rochelle's salt actually gave a different compound by HNMR after workup.



Conditions	Result (% Yield)
L-Selectride, THF, rt	Product (**15-30%**)
$LiAlH_4$, 0 °C	Product (**20%**)
K_2CO_3, MeOH, rt, 5d	Product (**10%**)
TMSOK, MeOH, reflux	Product (**20%**)

Table 15: Attempts to Obtain Free Aziridine **245**

Despite the low yields of free aziridine formation, we decided to continue on and investigate methylation of free aziridine **245** to obtain *N*-methyl aziridine **246** (Table 16). All conditions used were unreactive even in the presence of large amounts of methylating agent and prolonged reaction times. One condition that has proved useful on other systems but hasn't been tried is that of butyl lithium and methyl triflate. These conditions will be attempted again upon making more of free aziridine **245**. Additionally, both the free aziridine **245** and the *N*-methyl aziridine **246** were found to be unstable to silica.

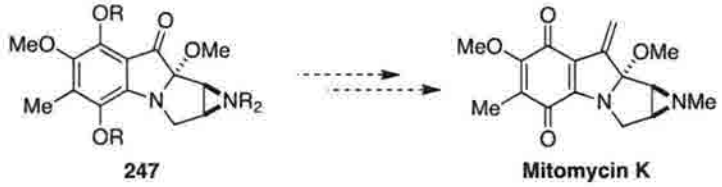


Conditions	Result (% Yield)
MeI, BuLi	Recovered SM
Formalin, $NaCNBH_3$	Recovered SM
Me_2SO_4, Pyridine	Trace amount of product

Table 16: Attempted Formation of *N*-Methyl Aziridine **246**

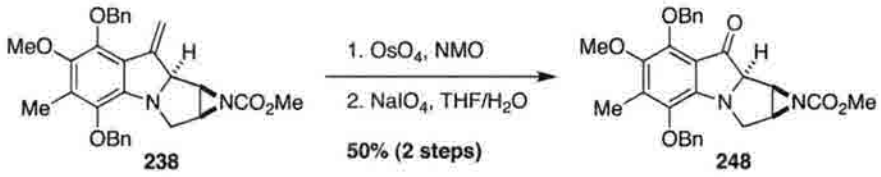
In both total syntheses of mitomycin K, Danishefsky and Jimenez accessed the exocyclic olefin of mitomycin K *via* ketone **247** (Scheme 69). It appears that the electronics of the tetracycle change completely by diverting the flow of electrons into the

ketone as opposed to opening the aziridine ring. This allowed the needed stability to be able to manipulate **247** at the protected hydroquinone without the decomposition problems we have faced in our research.



Scheme 69: Ketone **247** as an Intermediate in the Total Synthesis of Mitomycin K

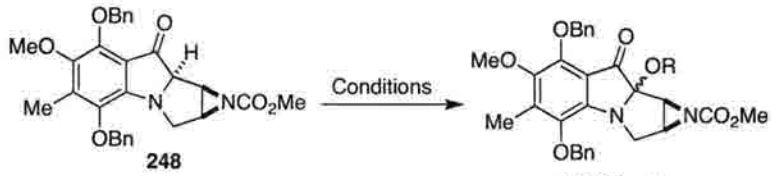
It light of this encouraging precedence shown above, we decided to see if conversion of tetracycle **238** to ketone **248** might be feasible (Scheme 70). It was found that this transformation could be carried out to yield ketone **248** by dihydroxylation of the exocyclic olefin and subsequent cleavage using sodium periodate in 50% yield for two steps.



Scheme 70: Synthesis of Ketone **248**

Our first attempt to oxidize ketone **248** was tried using Davis oxaziridine under basic conditions (NaHMDS) as shown in Table 17. From these reaction conditions a new product was obtained that looked by ^{1}HNMR analysis to be an aziridine-opened product and not the desired alcohol **249**. In light of the above result, we wanted to find conditions where alkylation could occur without charged intermediates that might open the aziridine. Rubottom oxidation seemed to fit these conditions and was tried on ketone **221**. Silyl enol ether formation went smoothly by use of TBSOTf but decomposed upon treatment with *m*CPBA. This result was not all that surprising in that treatment with *m*CPBA might

also be forming the *N*-oxide and causing rapid decomposition. Rubottom oxidation attempted using a freshly prepared solution of DMDO only gave back starting material and none of the desired tetracycle **250**. Despite the poor results, ketone **248** is a promising substrate that could be a key intermediate in a successful synthesis of mitomycin K.



Conditions:	Result:
Davis Oxaziridine, NaHMDS	Aziridine opened product
TBSOTf, then *m*CPBA	Decomposition
TBSOTf, then DMDO	Recovered SM

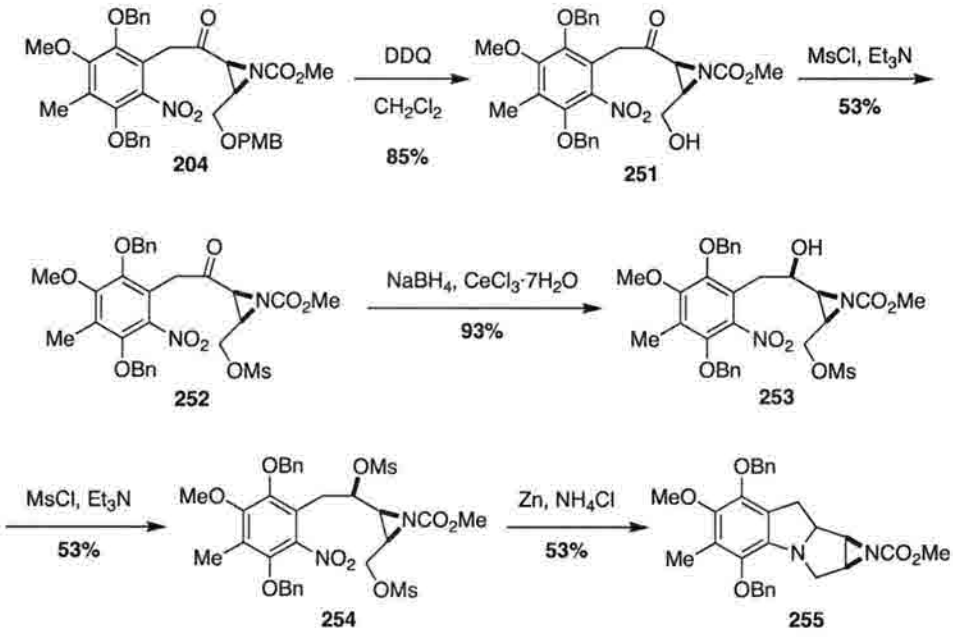
Table 17: Alkylation Attempts on Ketone **248**

4.4: Synthesis of Indolines *via* Reductive Aminocyclization

4.4.1: Indoline Formation and Attempted Manipulation

Having developed a route to the synthesis of benzazocines and mitosanes *via* a reductive aminocyclization reaction we wanted to try and expand the scope of the reaction. In particular, we wanted to see if the reductive aminocyclization reaction would work in the formation of indolines. The synthesis of a system to test this hypothesis started from ketone **204** obtained from oxidation of the mixture of alcohols obtained from the aldol coupling reaction. Removal of the PMB ether smoothly gave alcohol **251** and subsequent mesylation afforded mesylate **252** in fair yield due to a side reaction (Scheme 71). Reduction of the ketone in **252** gave alcohol **253** in great yield followed by a second

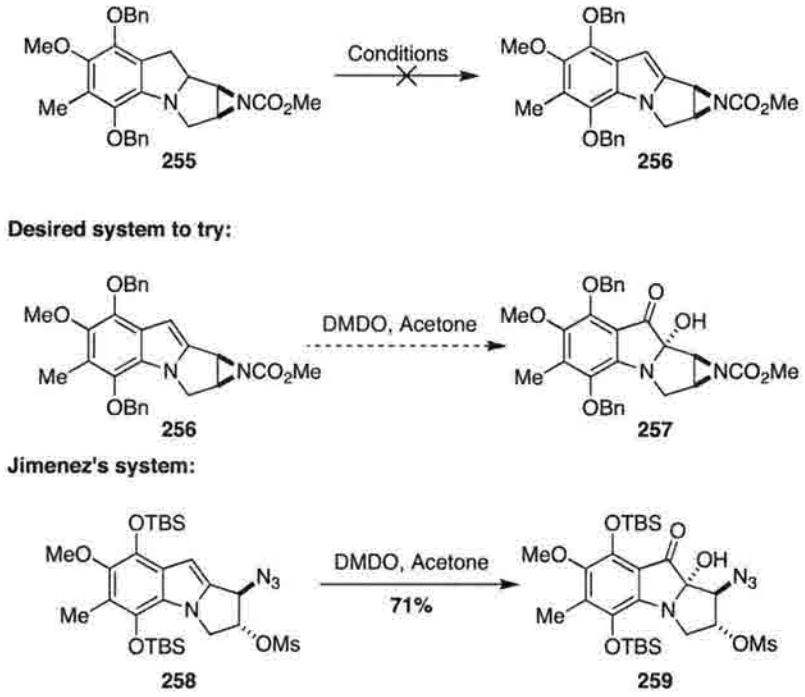
mesylation to produce bis-mesylate **254**. Poor yields were observed in both mesylation steps but provided enough material to try out the reductive aminocyclization reaction. We were pleased to find that subjection of bis-mesylate **254** to the same reductive aminocyclization conditions as before for 5 days at room temperature did give the desired indoline **255** in moderate yield (53%, Scheme 71).



Scheme 71: Synthesis of Indoline **255** *via* a Reductive Aminocyclization Reaction

It was desired to see if indoline **255** might be a useful intermediate towards the total synthesis of mitomycin K. Indoline **255** was treated with DDQ to see if indole **256** formation would occur, but was met only with decomposition (Scheme 72). Treatment of Indoline **255** to manganese dioxide conditions gave none of indole **256**, with starting material only recovered. This was a disappointing result in that we had hoped to treat indole **256** under the same conditions used by Jimenez an coworkers on indole **258** to give ketone **259** in their total synthesis of mitomycin K (Scheme 72). One potential pitfall to this approach is the presence of the aziridine in indole **256** whereas the aziridine is yet

to be formed in Jimenez's indole substrate **258**. Development of a suitable indoline made by the reductive aminocyclization reaction and without the presence of the aziridine ring could be a promising approach towards the synthesis of mitomycin K.



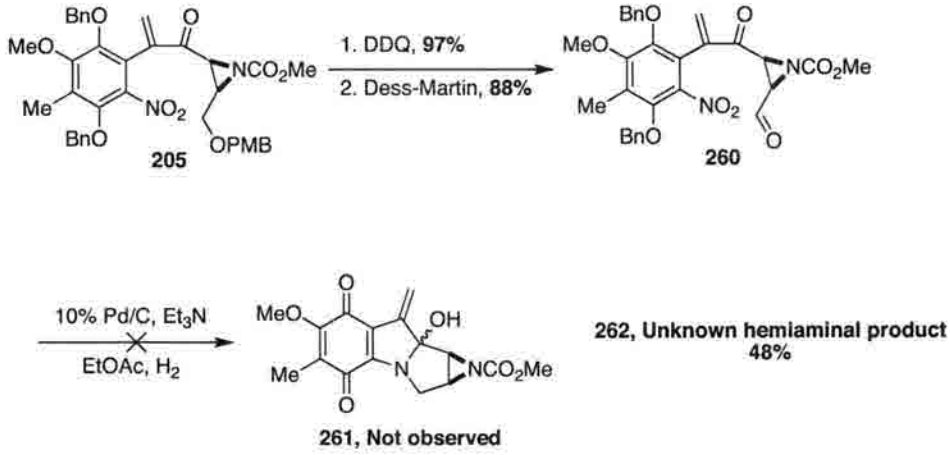
Scheme 72: Failed Indole Formation and Desired Oxidation

4.5: Other Approaches

4.5.1: Attempted Reductive Amination Route

During the thought process about new routes that might be successful for the synthesis of the mitomycins, a reductive amination route was postulated. It was thought that synthesis of aldehyde **260** with the bis-benzyl ethers might undergo a one pot reaction under hydrogenation conditions to remove the benzyl ethers, reduce the imine that could be formed, and oxidize to the quinone upon stirring with air (Scheme 73). Aldehyde **260** was easily prepared from enone **205** in two steps in excellent overall yield. Treatment of aldehyde **260** to hydrogenation conditions did after workup conditions

provide a quinone product in 48% yield that was missing the aldehyde and enone peaks by ^{1}HNMR. High resolution mass spectrometry (HRMS) revealed a compound with the same mass as **261**, and for a time it looked as though this reaction had been successful. However, careful analysis of the ^{13}CNMR spectra revealed the absence of the of the hemiaminal carbon that would be present in tetracycle **261**. Two-dimensional NMR experiments also showed the existence of a hemiaminal proton that is arising from the interaction of the aniline attached to the aromatic ring and the aldehyde. The exact structure of this compound **262** remains unclear, but we are certain this compound is not the desired tetracycle **261**.

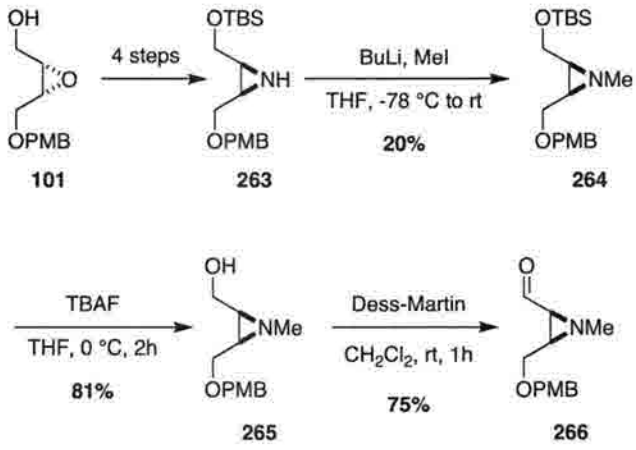


Scheme 73: Attempted Reductive Amination Attempt to the Mitomycins

4.5.2: *N*-Methyl Aziridine Aldehyde Synthesis

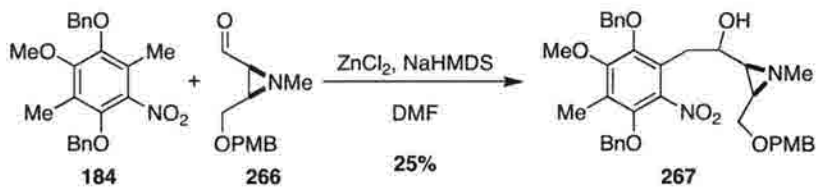
Due to the difficulties associated with the aziridine protected as the methyl carbamate and its removal, we investigated the possibility of installing the *N*-methyl aziridine moiety into the aziridine aldehyde before aldol coupling to the nitro arene. Synthesis of the *N*-methyl aziridine aldehyde **266** was accomplished successfully as shown in Scheme 74, but was problematic. Methylation of free aziridine **263** (prepared in

four steps from epoxide **101**) was difficult and gave *N*–methyl aziridine **264** in an optimized yield of 20% on a large scale. Removal of the TBS silyl ether to alcohol **265** and oxidation to aldehyde **266** were both difficult reactions in that the products existed as a mixture of invertomers about the aziridine center. It was found that one invertomer reacts considerably slower compared to the other one, and so prolonged reaction times were required. Nevertheless, enough material was obtained to take on to the aldol coupling step.



Scheme 74: Synthesis of *N*-Methyl Aziridine Aldehyde **266**

Aldol coupling of the *N*-methyl aziridine aldehyde **266** with bis-benzyl nitro arene **184** did proceed although in low yield (25%, Scheme 75). Surprisingly, a single diastereomer of alcohol **267** was obtained in the coupling reaction. At this point in time the configuration about the secondary alcohol of **267** is not known. Due to the poor yields of this sequence this route was dropped. This route would be impractical to continue 8-10 more steps if the yields for each step were as low as they are in the coupling step.



Scheme 75: Coupling of *N*-Methyl Aziridne Aldehyde **266** with Nitro Arene **184**

This chapter summarized our effort with the bis-benzyl nitro arene **184**. It was shown that although quinone formation was realized, we were unable to alkylate benzazocane **197** presumably due to the bulky benzyl groups on the aromatic ring. Installation of the exocyclic olefin before cyclization was successful but all attempts to remove the silyl ether on the benzazozocine oxygen on substrates such as **212** failed. These studies led to the discovery of an efficient system for the formation of benzazocines, mitosanes, and indolines *via* a reductive aminocyclization reaction. Unfortunately, all efforts to manipulate these compounds to the natural products failed. A reductive amination approach and utilization of a *N*-methyl aziridine aldehyde both failed to provide a feasible route to the mitomycins.

Chapter 5: Third Generation Approach to the Mitomycins

5.1: A Differentiated Nitro Arene Approach

5.1.1: Synthesis of Differentiated Nitro Arene

In an effort to retool the synthesis, we analyzed the intermediates we had previously made and why they failed (Figure 7). The first generation benzazocanes failed due to the inability to form the quinone with use of the methyl ethers to protect the hydroquinone. Switching to benzyl ethers to protect the hydroquinone allowed quinone formation to occur but shut down the ability to alkylate due to the bulky nature of the benzyl groups (second generation, Figure 7). For our third generation approach to the mitomycins, we wanted to make use of a differentiated nitro arene as shown in Figure 7. It was hoped that by having the methyl ether on the top of the molecule would allow enough space for alkylation to occur as in the first generation synthesis, while placement of a benzyl ether on the bottom of the molecule would provide a handle for quinone formation. Both Fukuyama and Ziegler used a differentiated nitro arene of this type successfully in their synthetic studies on the mitomycins (see Chapter 2).

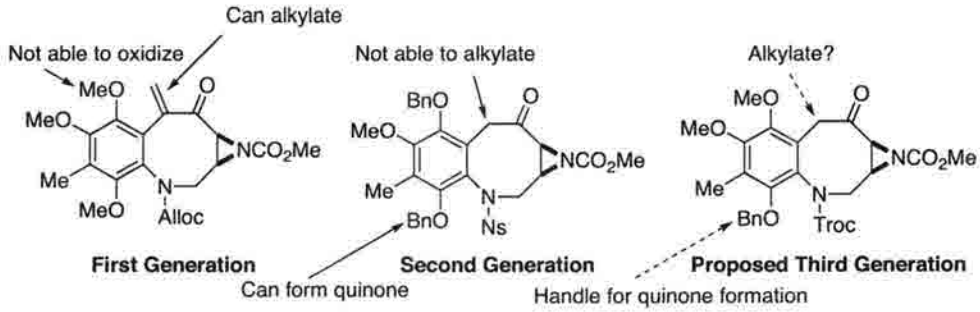
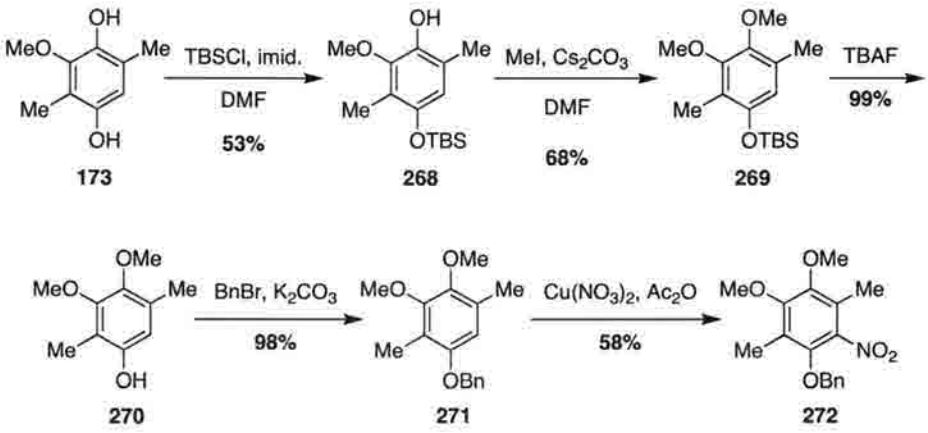


Figure 7: Proposed Third Generation Substrate

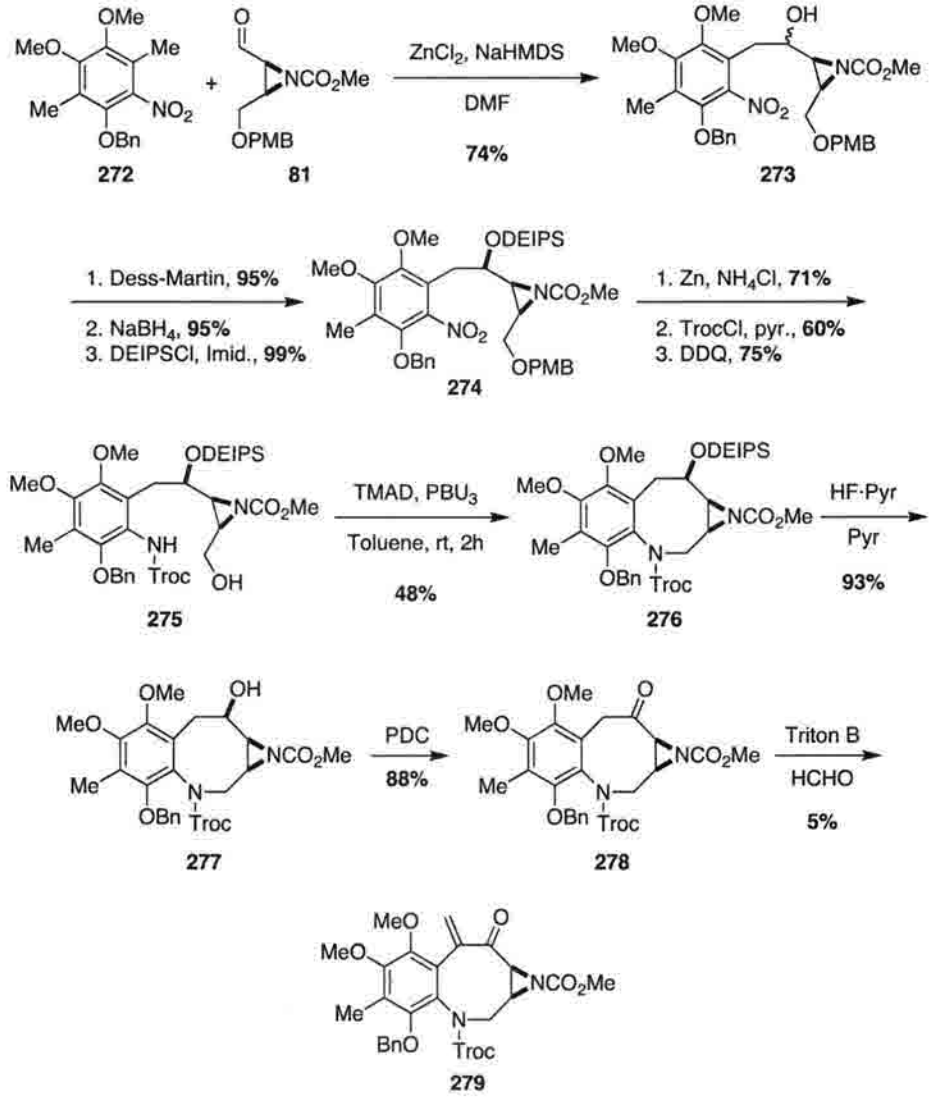
To test the above hypothesis we needed to develop a route to the synthesis of differentiated nitro arene **272** as shown in Scheme 76. All of the methods in the literature where similar arenes are obtained have the benzyl ether on the top of the molecule and so could not be used for this approach. The synthesis of nitro arene **272** commenced with hydroquinone **173**, which was used in the synthesis of the bis-benzyl nitro arene discussed in Chapter 4. Treatment of the hydroquinone **173** under reported conditions[94] selectively protected the less hindered alcohol to give phenol **268** in only 53% yield. Extensive attempts to oxidize this reaction were unsuccessful. The major byproduct of this reaction was the quinone resulting from oxidation of hydroquinone **173**. Methyl ether formation under standard methylation conditions gave methyl ether **269**, followed by removal of the silyl ether to afford phenol **270** in good yield. Benzylation under standard conditions provided benzyl ether **271** in good yield followed by nitration under our previously used conditions to give the desired nitro arene **272** in 58% yield (Scheme 76). Despite the low yield in the first step of the sequence, large amounts of nitro arene **272** could be made in short order.



Scheme 76: Synthesis of Differentiated Nitro Arene **272**

5.1.2: Synthesis of a Differentiated Benzazocane

Coupling of our differentiated nitro arene **272** with aziridine aldehyde **81** reacted in identical fashion to previous systems and gave alcohols **273** (as a 2:1 mixture of diastereomers) in good yield (Scheme 77). Oxidation to ketone, reduction to alcohol, and protection of the alcohol as the DEIPS silyl ether provided compound **274** in good yield.



Scheme 77: Aldol Coupling of Nitro Arene **272** and Benzazocane Formation

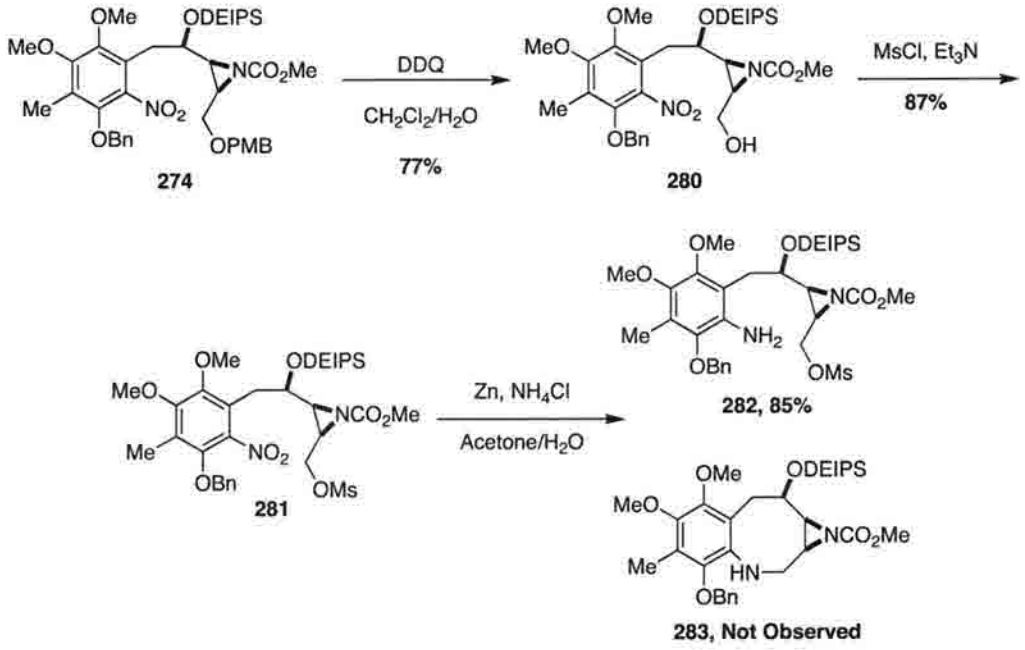
After much consideration, the carbamate-based protecting group chosen for aniline obtained by reduction of silyl ether **274** was the 2,2,2-trichloroethyl carbamate

(Troc). The selection for formation of the Troc carbamate was due to the mild methods reported in the literature for Troc removal that should be amenable to the other functionality present in our molecule (Scheme 77). Formation of the Troc carbamate proceeded in moderate yield followed by followed by PMB etehr removal to give alcohol **275**. We were delighted to find that treatment of alcohol **275** to our standard activated Mitsunobu conditions did afford benzazocine **276** in 48% yield. Optimization of the Mitsunobu reaction has not been performed, and we are confidant the yield of this reaction will improve. Treatment of benzazocine **276** to our previously developed conditions of HF·pyridine in excess pyridine gave alcohol **277** followed by treatment with PDC to afford benzazocane **278** in good yield.

With access to benzazocine **278**, we were then ready to test our hypothesis and see if alkylation could occur. Preliminary results revealed that alkylation is possible as small quantities of enone **279** were obtained using Triton B and paraformaldehyde. Other attempts to optimize the yield of this reaction thus far have been fruitless. Despite the low yield for the alkylation reaction, procurement of enone **279** provided evidence that this differentiated nitro arene system holds promise for the completion of the total synthesis of mitomycin K. Future studies in this area include optimization of the alkylation reaction followed by quinone formation and attempted cyclization.

One other avenue we wanted to explore was to see how general the reductive aminocyclization reaction developed in Chapter 4 is to different substrates. Accordingly, silyl ether **274** was converted to alcohol **280** and then mesylate **281** under standard conditions using the two-step procedure shown in Scheme 78. Treatment of mesylate **281** under standard conditions as performed previously did provide aniline **282** in good yield;

however, none of the desired benzazocine **283** was observed. This raises a question of what exactly is required in order for cyclization to occur. Perhaps it is the bulky silyl ether that is preventing aniline **282** from getting in the conformation needed for cyclization to occur.



Scheme 78: Attempted Reductive Aminocyclization Reaction

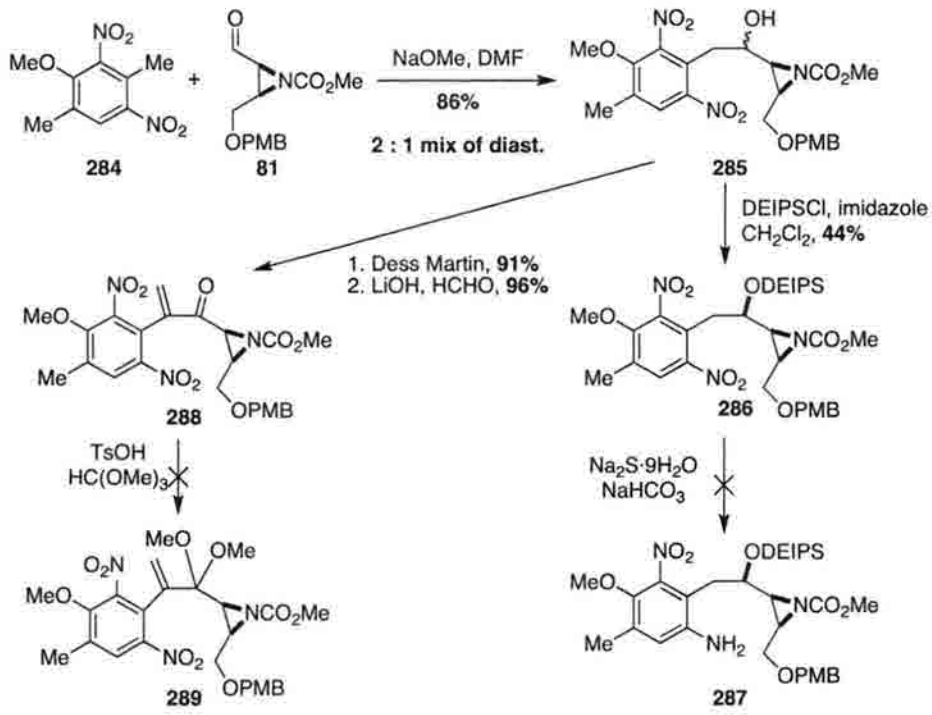
5.2: Miscellaneous Approaches to the Mitomycins

This section briefly reviews preliminary results on three other strategies towards the mitomycins. These strategies have not been fully explored and could merit some attention. These studies are documented in order to give the reader a full account of the various approaches tried that might be of value for future study of these molecules in the Williams group.

5.2.1: Use of bis-Nitro Benzene Aromatic

As our synthetic efforts were progressing, it became increasingly obvious that the electron-rich nature of the aromatic ring was causing problems on multiple fronts. To this end, a search was conducted in an attempt to find an arene with markedly different electronic properties. While conducting a search on the mitomycin literature, the bis-nitro aromatic (**284**, Scheme 79) used by Jimenez the synthesis of mitomycin K quickly caught our attention. Jimenez has shown that bis-nitro arene **284** can be made in one step from commercially available 2,5-dimethylanisole, and selective reduction of the less-hindered nitro group could be accomplished by use of sodium sulfide in refluxing ethanol.

With the encouraging precedent detailed above, we coupled bis-nitro arene **284** with the aziridine aldehyde piece **81** previously made to give the desired alcohol **285** in excellent yield (86%) as a 2:1 mixture of diastereomers (Scheme 79). Protection of the major diastereomer as the DEIPS ether was successful and gave the requisite product **286** in moderate yield. Unfortunately, selective reduction of the desired nitro group with sodium sulfide as previously demonstrated with just the aromatic piece failed to provide any of aniline **287**. In the sodium sulfide conditions as well as numerous others that were tried, a mixture of the anilines as well as the bis-reduced species were found. This result is likely due to the increased steric bulk of the substrate that eliminates the accessibility of the nitro group that was less hindered before coupling with the aziridine aldehyde. This approach was subsequently dropped due to the inability to selectively reduce the desired nitro moiety.



Scheme 79: Approach to the Mitomycins by use of a bis-Nitro Arene

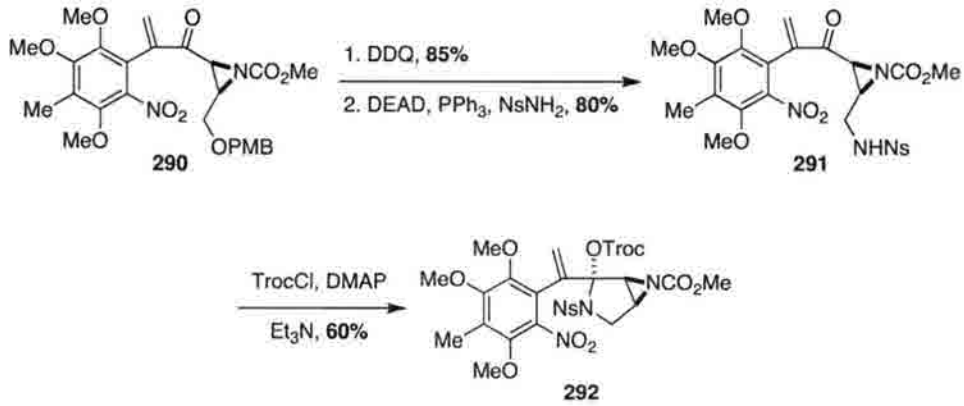
Also shown in Scheme 79 were our attempts to manipulate alcohols **285** by formation of enone **288**. This two-step sequence of oxidation with Dess-Martin periodinane followed by enone formation proceeds smoothly. As seen in other systems, hydroxymethylation conditions on the ketone derived from **285** give exclusively the enone product with none of the hydroxymethylated product being observed. This result provided evidence that the electron-rich arenes previously used were not the sole cause of enone formation, and that perhaps the real culprit might be the nitro group.

With enone **288** in hand, we wanted to explore the possibility of protecting the ketone as the dimethyl ketal as shown by Kishi in his successful synthesis of the mitomycins. As shown in Scheme 81, all attempts to form the dimethyl ketal **289** were met with failure. Even if ketal **289** could be obtained, it was presumed that selective reduction of the desired nitro group would be problematic as seen with compound **266**.

Due to these drawbacks detailed above, this approach was abandoned. However, bis-nitro arene **284** is an attractive aromatic that one could envision its effective use in the synthesis of the mitomycins in an appropriately designed system as illustrated by Jimenez.

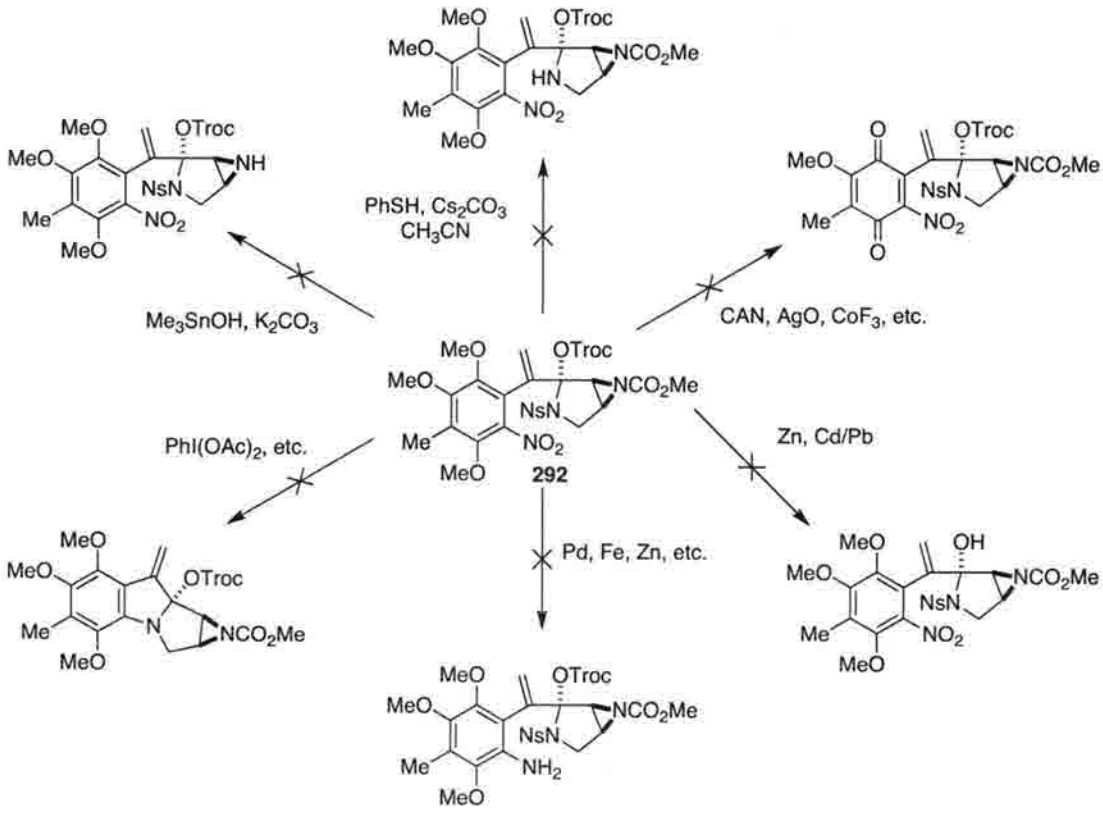
5.2.2: Formation of Terminal 5-Membered Ring

In the course of our synthetic studies toward the mitomycins, an interesting transformation was found and its potential investigated. It was found that treatment of nosyl sulfonamide **291** (obtained in two steps from **290** by PMB ether removal and Mitsunobu reaction of the alcohol with nosyl sulfonamide) provided not the expected product resulting from Troc protection of the sulfonamide, but gave Troc carbonate **292** in decent yield (Scheme 80). This transformation was explored for its generality to see if the ketone could be trapped as the TBS silyl ether or as the methyl ether present in the natural products. All of these attempts failed, as did treatment of **291** with methyl chloroformate in an attempt to form the methyl carbonate adduct of **292**. For reasons not understood, this reaction seems to be specific with formation of the Troc carbonate.



Scheme 80: Unexpected Formation of Troc Carbonate **292**

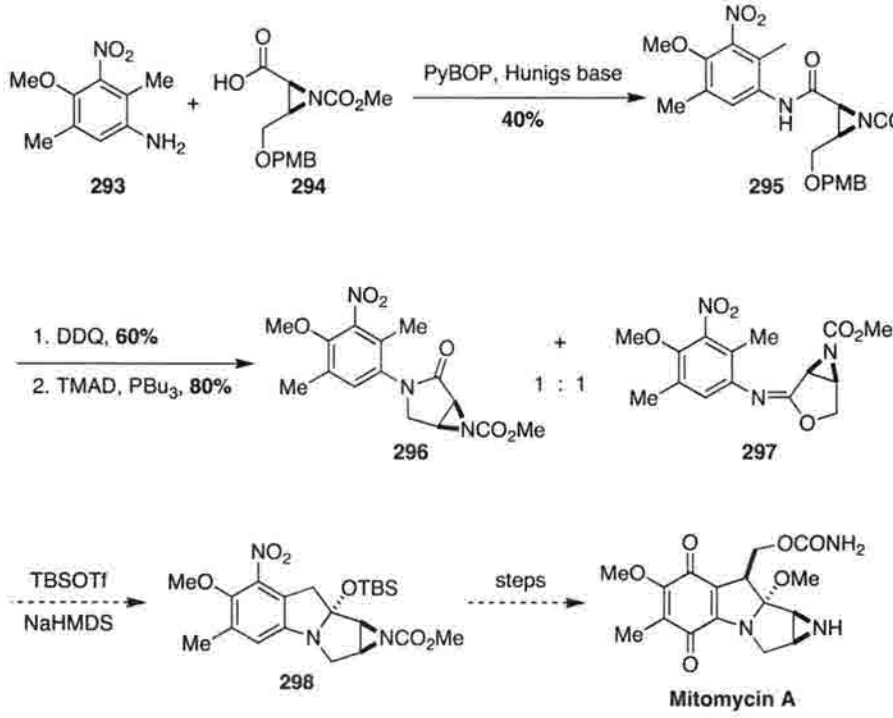
It was desired to see if Troc carbonate **292** might be a useful substrate for the synthesis of the mitomycins, and attempts in this regard are shown in Scheme 81. Compound **292** was shown in to be essentially unreactive to a variety of transformations in an attempt to access the tetracyclic core of the mitomycins. It was found that treatment of Troc carbonate with potassium carbonate in methanol did remove the Troc carbonate and gave sulfonamide **291** in 80% yield. This approach was dropped due to the inability to further manipulate **292**. Protection of the nitrogen as the sulfonamide is not a good choice for this system. The other major flaw to this approach is the presence of the nitro moiety on the aromatic ring. Although not successful in this system, this strategy showed the feasibility of trapping the enone and could be a useful transformation in an appropriately designed substrate.



Scheme 81: Attempts to Further Manipulate Troc Carbonate **292**

5.2.3: Peptide Coupling Route

Another approach to the mitomycins that was explored involved a peptide coupling of the previously published aniline **293** with acid **294** (prepared in one step from aziridine aldehyde **81** by Pinnock oxidation) to give amide **295** in 40% yield (80% based on recovered starting material, Scheme 82). Removal of the PMB ether followed by Mitsunobu cyclization did provide the desired lactam **296**, but in a 1:1 ratio with imine **297**. The formation of imine **297** demonstrated the activated nature of the amide due to the methoxy group in the para position to the amide on the aromatic ring. The yield and ratio of this transformation might be able to be improved by formation of the mesylate and treatment with base as done by Michael and coworkers in their synthesis of an aziridinomitosene (see section 2.2.3).

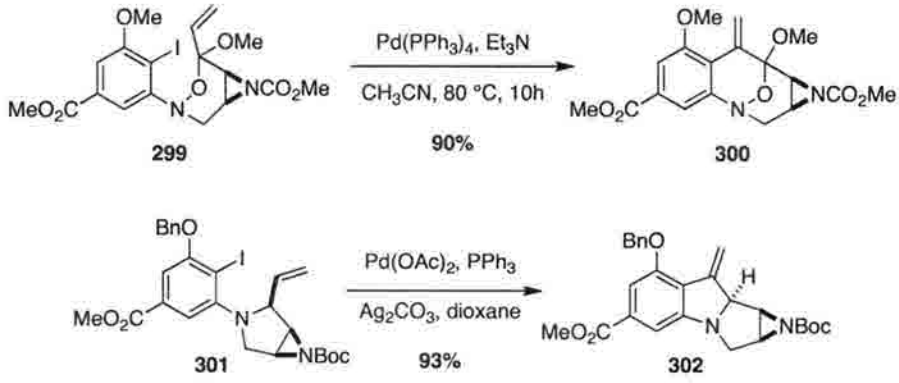


Scheme 82: Peptide Coupling Route to the Mitomycins

With amide **296** in hand, the next key step in the synthesis is formation of tetracycle **298**. It is hoped that treatment of lactam **296** with TBSOTf and base might afford cyclization with trapping of the carbonyl oxygen. This reaction will also be tried with reagent to trap the amide carbonyl as the methyl ether (MeOTf, $Me_3O{\cdot}BF_4$, etc.). If a tetracycle like **298** can be formed, the nitro group on the arene ring should allow for further alkylation in order to install the C10 carbon needed for the mitomycins. Subsequent transformations could be straightforward and provide a novel route to the mitomycins. This strategy has not been fully explored due to time constraints, yet is a promising approach to access the mitomycins.

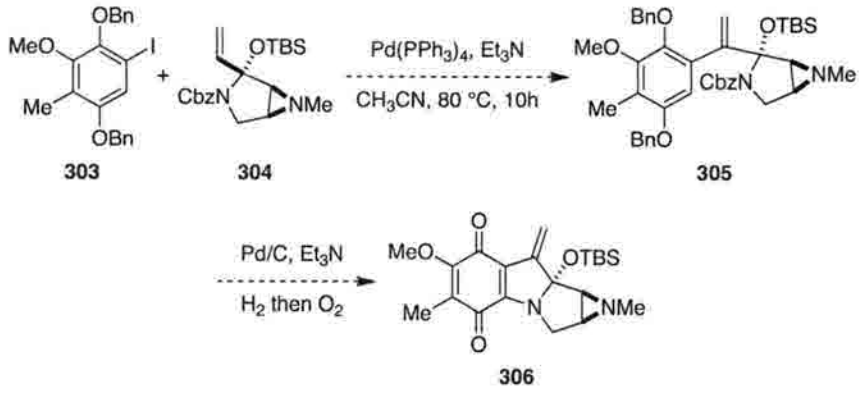
5.2.4: Intermolecular Heck Arylation Strategy:

The last approach to the mitomycins that will be discussed involves the use of an intermolecular Heck reaction as the key step in the synthesis. Literature precedent for this approach comes from Danishefsky and McClure's synthesis of FR900482 congeners, where an intramolecular Heck reaction was performed on iodoarene **299** to give the tetracycle **300** in excellent yield.[95] Trost and O'Boyle also used this strategy in their synthesis of 7-epi (+)-FR900482 in the conversion of iodoarene **301** to tetracycle **302**.[96]



Scheme 83: Precedent for Heck Arylation Strategy

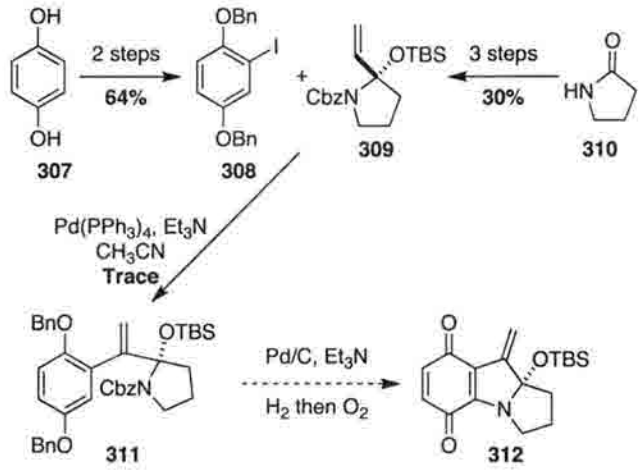
The above precedents inspired us to think of a new, highly convergent approach to the mitomycins shown in Scheme 84. The key step of the synthesis would involve Heck arylation of iodo arene **303** with the highly functionalized pyrrolidine **304** to give olefin **305**. The synthesis of iodoarene **303** is expected to be straightforward, as the trimethoxy analogue has previously been synthesized. The challenge to this route would be the synthesis of pyrrolidine **304**. It is hoped that intermediates from the synthesis of aziridine aldehyde **81** might be able to be used in the construction of **304**. Upon successful Heck arylation to obtain olefin **305**, it is hoped that removal of the protecting group and oxidation to the quinone in situ will provide quinone **306** in a similar fashion to the analogous sequence in Coleman's synthesis of desmethoxymitomycin A. Removal of the silyl group of quinone **306** would provide mitomycin H followed by methylation to afford mitomycin K.



Scheme 84: Proposed Intermolecular Heck Arylation Strategy to the Mitomycins

To check the feasibility of this type of intramolecular reaction, we designed a model system as shown in Scheme 85. This system involved the synthesis of iodoarene **308**, which was made in two steps from commercially available hydroquinone **307**. Pyrrolidine **309** was made in three steps from 2-pyrrolidinone **310**. Testing out the key Heck arylation reaction of iodoarene **308** and pyrrolidine **309** did provide olefin **311** in

trace amounts. This reaction is in development and has not been optimized. It is hopeful that optimization of this approach will provide a novel route to access the mitomycins.



Scheme 85: Model System for Heck Arylation Strategy

This section has described progress on our latest approach (third generation) to the mitomycins. We are currently three steps away from trying the key alkylation on our differentiated benzazocane. It is hopeful that enone formation will proceed with the presence of the smaller methyl ether protecting the hydroquinone oxygen in proximity to the ketone. If successful, quinone formation should provide us with the next key substrate in order to try the transannular cyclization reaction. Additionally, we have documented three other strategies that have been attempted during the course of our studies in an attempt that these might be useful for further studies in our research group on these molecules.

Chapter 6: Summary and Concluding Remarks

6.1: Summary of Progress

Despite our best efforts, the asymmetric total synthesis of any member of the mitomycins has remained elusive. Shown in Table 18 is a summary of the furthest intermediates that were made to attempt to accomplish this task, as well as the particular problem(s) that were encountered with each substrate.

In our first generation approach to the mitomycins, the farthest intermediates we obtained were enones **146**, **149**, and **150** as shown in Table 18. The major difficulties in further manipulating these substrates were twofold: 1) We were not able to remove the carbamate protecting group to allow for cyclization and tetracycle formation, and 2) Oxidation of **130**, **133**, and **134** to the corresponding quinone compounds were not successful. These first generation substrates are arguably the closest substrates to the natural products in that all of the requisite atoms and functionality at the required oxidation state (minus the quinone) are in place. It might be beneficial to go back and further investigate the oxidation reaction to the quinone with ceric ammonium nitrate. From literature precedent it stands to reason that extensive optimization of this oxidation reaction should provide at least small amounts of the desired quinone products. Even if the desired quinone was obtained in very small amounts, only 3-4 more steps would be required in order to complete the synthesis.

Benzazocane **197** was the first advanced intermediate made in our second generation approach to the mitomycins that used the bis-benzyl nitro arene. Benzazocane

197 could not be pushed further due to the inability to alkylate at C10 with all alkylating reagents tried. This reason is likely due to the addition of the bulky benzyl groups to an already demanding steric environment that prevents alkylation.

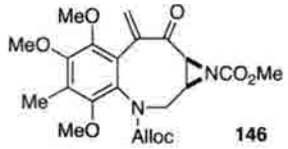
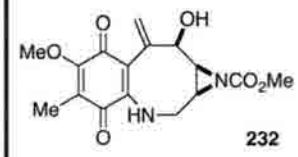
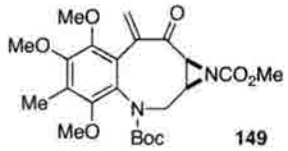
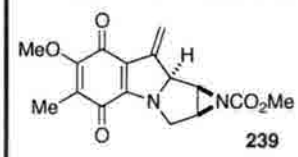
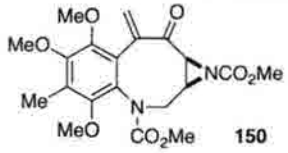
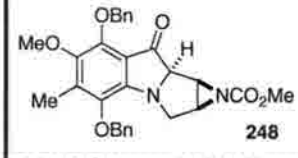
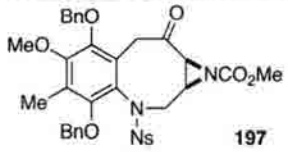
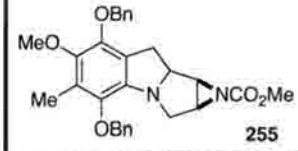
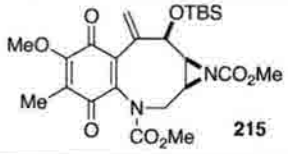
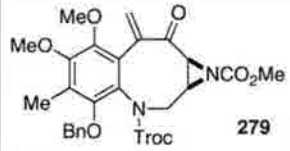
Substrate:	Problem:	Substrate:	Problem:
 **146**	Unable to: - remove Alloc carbamate - oxidize to the quinone - induce cyclization Only enone formed	 **232**	Unable to: - oxidize allylic alcohol
 **149**	Unable to: - remove Boc carbamate - oxidize to the quinone - induce cyclization Only enone formed	 **239**	Unable to: - do allylic C-H oxidation - methlyate aziridine
 **150**	Unable to: - remove Methyl carbamate - oxidize to the quinone - induce cyclization Only enone formed	 **248**	Unable to: - Install methyl aminal
 **197**	Unable to: - hydroxymethylate - methylenate - form quinone	 **255**	Unable to: - Form the indole
 **215**	Unable to: - remove silyl group - remove carbamate	 **279**	Improve methylenation Could be promising

Table 18: Summary of Farthest Substrates Towards the Mitomycins

Quinone **215** was an important system in that we demonstrated that Mitsunobu cyclization could take place with prior installation of the C10 carbon as the exocyclic olefin. From this study it was found that the Mitsunobu reaction seems to be much less affected by steric bulk than other transformations on these systems. Like benzazocane **197** mentioned above, the downfall of quinone **215** is the bulky steric nature of the system that thwarts deprotection of the TBS silyl ether.

Quinone **232** was synthesized by a reductive aminocyclization reaction of the aromatic aniline and the primary mesylate to form a benzazocine. This is the first example in the reported literature where cyclization of a similar nature takes place without the need for prior activation of the aniline. Unfortunately all attempts to further manipulate quinone **232** of the bis-benzyl protected hydroquinone precursor failed. The rationale for the above observations is not entirely understood.

The same reductive aminocyclization methodology used to form quinone **232** was also employed in the formation of mitosane **239**. This method constructs the tetracyclic core of the mitomycins in one step from an acyclic bis-mesylate precursor. All attempts to convert mitosane **239** to mitomycin K failed. Methods that were tried included attempts to do an allylic oxidation and to first make the *N*-methyl aziridine. These attempts were tried on both mitosane **239** as well as tetracycle **238**.

Ketone **248** was readily made from tetracycle **238** in two steps. It was hoped to be able to introduce oxygen by either treatment of ketone **248** with Davis oxaziridine and base, or by a Rubottom oxidation of the silyl ether of **248**. Neither of these approaches were successful and resulted mostly in decomposition. The presence of the intact aziridine might be the problem here and favors opening of the aziridine as opposed to the desired introduction of oxygen.

Indoline **255** was also constructed by the same reductive aminocyclization reaction used above in an attempt to use Jimenez's conditions for oxidation of an indole used in her total synthesis of mitomycin K. Unfortunately, all attempts for indole oxidation failed and just gave decomposition. The downfall for this route seems to be having the aziridine already in place that causes decomposition upon indole formation

and could make oxidation difficult. A suitable developed substrate without the aziridine in place could be a solution to this problem.

Enone **279** is the first advanced intermediate completed as part of our third generation approach employing the use of a differentiated aromatic piece. Alkylation was shown to be possible to afford enone **279** albeit in 5% yield. Future directions with enone **279** include optimization of the methylenation reaction, oxidation to the quinone, transannular cyclization to afford the tetracyclic core of the mitomycins. It is hopeful that this approach will successfully provide access to the natural products.

6.2: Concluding Remarks

In conclusion, multiple routes have been developed that are 3-4 steps away from the asymmetric total synthesis of mitomycin K. Several downfalls to our synthetic approach have been identified and would be changed if a totally new route to these compounds was developed. A summary of the problems that were identified and how we would fix them are shown in Figure 8. The first major downfall was the installation of the quinone-nitrogen single bond early in the synthesis while still at the hydroquinone stage. With this bond in place early on in the synthesis, oxidation to the quinone could not be performed using the bis-methoxy nitro arene. This bond also caused problems by allowing the flow of electrons through the system to open up the aziridine as well as complicating the alkylation reaction to introduce the C10 carbon.

In relation to installation of the C10 carbon, an approach where this bond was made at an early stage of the synthesis would be advantageous especially for introduction of the hydroxymethyl group. As shown throughout this work, attempted late stage

installation of the C10 carbon caused multiple headaches that could be avoided if installed earlier. The research groups of Kishi and Fukuyama both installed this bond at an early stage in their successful syntheses of the mitomycins.

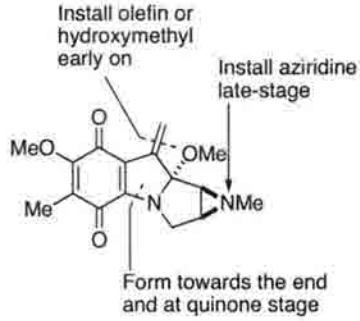


Figure 8: Modifications for a New Synthetic Route to the Mitomycins

Finally, the last modification that would be made in a revised synthesis is late-stage installment of the aziridine. Although early installation of the aziridine worked in our group for the total synthesis of FR900482, it seems to be much more difficult in the mitomycins. This is likely due to the fact that in our synthetic route we were working with protected hydroquinone intermediates that are fairly analogous to the proposed bioactive intermediates in mitomycin activation and DNA alkylation. One of the major difficulties we encountered was removal of the protecting group on the aziridine and subsequent methylation. It is no wonder that in every successful synthesis of the mitomycins to date, the aziridine moiety has been installed at a late stage in the synthesis.

In taking into account the three modifications mentioned above, a number of routes could be designed with the potential of completing the first asymmetric total synthesis of the mitomycins. It is hoped that this work will be a useful springboard and a help to those who follow in an effort to complete the asymmetric total synthesis of these intriguing and challenging molecules.

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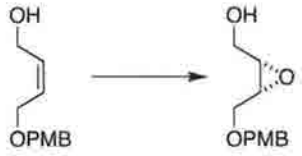
Chapter 7: Experimental

7.1: General Considerations

Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification. All air or moisture sensitive reactions were performed under a positive pressured of argon in flame-dried glassware. Tetrahydrofuran (THF), toluene, diethyl ether (Et_2O), Dichloromethane, acetonitrile, triethylamine and N,N-dimethylformamide (DMF) were obtained from a dry solvent system (activated alumina columns, positive pressure of argon). Column chromatography was performed on Merck silica gel Kieselgel 60 (230-400 mesh). Melting points were determined in open-end capillary tubes and are uncorrected. ^{1}HNMR and ^{13}CNMR spectra were recorded on Varian 300, 400 or 500 MHz spectrometers. Chemical shifts are reported in ppm relative to $CHCl_3$ at δ 7.27 (^{1}HNMR) and δ 77.23 (^{13}CNMR). Mass spectra were obtained on Fisons VG Autospec. IR spectra were obtained from thin films on a NaCl plate using a Perkin-Elmer 1600 series FT-IR spectrometer. Optical rotations were collected at 589 nm on a Rudolph Research automatic polarimeter Autopol III.

7.2: Experimental Procedures

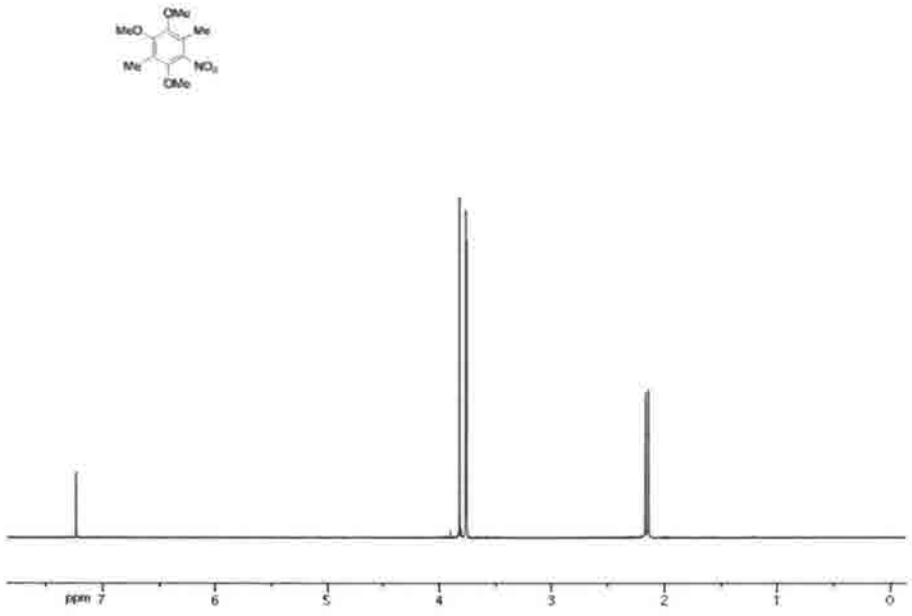
((2*S*,3*R*)-3-((4-methoxybenzyloxy)methyl)oxiran-2-yl)methanol (101)



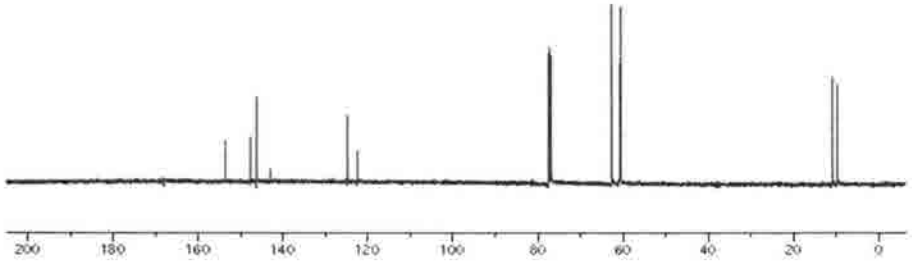
To a solution of dichloromethane (320 mL) and activated 4Å molecular sieves in a one liter flask at -24 °C was added diethyl-L-tartrate (13.1 mL, 77.6 mmol), titanium isopropoxide (19.5 mL, 65.6 mmol) and *tert*-butyl hydrogen peroxide (31 mL, 3.4 M solution in anhydrous toluene). After letting the catalyst age for 20 minutes, the allylic alcohol (12.0 g, 57.6 mmol) in a solution of dichloromethane (15 mL) was added dropwise and then stirred at -24 °C for two days. The solution was then warmed to 0 °C whereupon water (300 mL) was added and the mixture was stirred for 1 hour. A 30% aqueous sodium hydroxide solution saturated with brine (80 mL) was then added and stirring was continued at 0 °C for 1.5 hours. Over the course of time the slurry color changed from yellow to white. The slurry was then ran through a plug of celite and rinsed with dichloromethane. The layers were separated and the organic layer was washed (1 x brine), dried (Na_2SO_4), filtered and concentrated to afford epoxide **101** (12.7 g, 98%) as a white solid that was sufficiently pure to be used without further purification. All characterization data was found to be identical to those previously reported in our group (see reference 64).

1,2,4-Trimethoxy-3,6-dimethyl-5-nitrobenzene (98)

To a solution of 1,3,4-trimethoxy-2,5-dimethylbenzene (20.0 g, 102 mmol) in acetic anhydride (400 mL) at 0°C was added cupric nitrate trihydrate (47.4 g, 204 mmol). After stirring for 45 min at room temperature, the reaction mixture was passed through a short plug of silica gel, eluted with ethyl acetate and the filtrate concentrated. The residual oil was dissolved in DCM (200 mL), washed with saturated aqueous $NaHCO_3$ (150 mL) and brine (150 mL). The combined organics were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (90:10 hexanes/ethyl acetate) to afford **98** (24.0 g, 50%) as a yellow crystalline solid. ^1H NMR (400 MHz, $CDCl_3$) δ TMS: 2.14 (3H, s), 2.17 (3H, s), 3.76 (3H, s), 3.77 (3H, s), 3.82 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.5, 10.6, 60.3, 60.5, 62.5, 122.3, 124.7, 142.9, 146.1, 147.6, 153.5; IR (neat) 2997, 2942, 2854, 1571, 1532, 1471, 1370, 1253, 1094, 955 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{11}H_{15}NO_5$ 241.0950 $(MH)^+$, Found 241.0954; Mp 26-27°C.

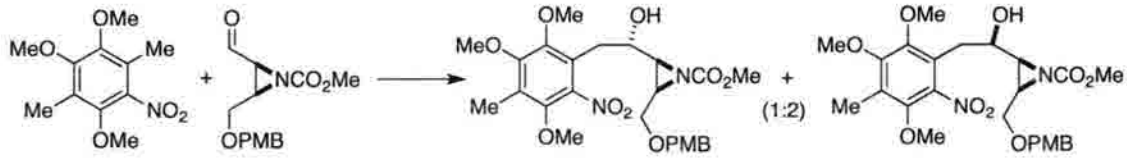


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: 00-Aromatic-HNMR



^{13}CNMR, $CDCl_3$, 100 MHz; Filename: 00-Aromatic-C13NMR

(2*R*,3*S*)-Methyl-2-((4-methoxybenzyloxy)methyl)-3-((*S*)-1-hydroxy-2-(2,3,5-trimethoxy-4-methyl-6-nitrophenyl)ethyl)aziridine-1-carboxylate (97a-b)

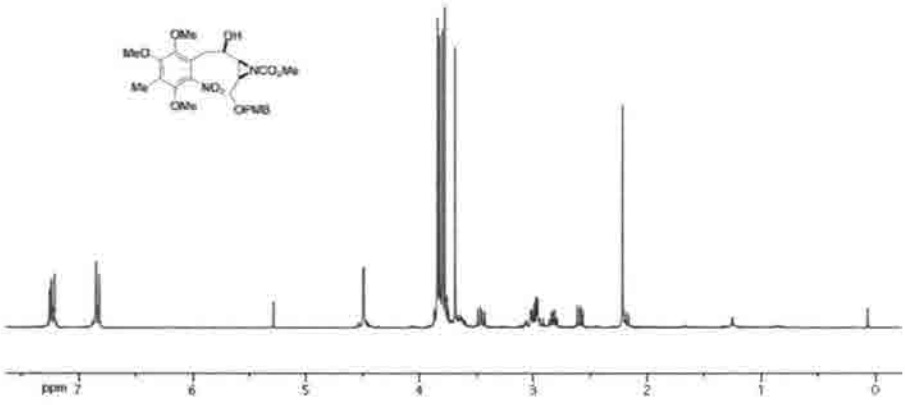


Prior to the reaction, compounds **98** (8.6 g, 36 mmol) and **81** (5.0 g, 18 mmol) were dehydrated three times by azeotropic distillation using anhydrous toluene and lastly dried under vacuum for 1h before use. A solution of **81** in dry DMF (15 mL) was then added to freshly fused zinc chloride (2.9 g, 21.5 mmol). The resulting solution was stirred under argon for 1h at rt. In a separate flask, NaHMDS (36 mL of a 1 M solution in THF, 36 mmol) was added dropwise to a solution of **98** in dry DMF (50 ml) cooled to -45°C. The mixture turned immediately deep red. To this solution was added dropwise the aziridine solution prepared above and the resulting mixture was stirred at -45°C for 3 hrs. The reaction mixture was quenched with saturated aqueous NH_4Cl (50 mL), allowed to warm to room temperature and water (50 mL) was added. The aqueous phase was extracted with DCM (5 x 150 mL), the combined organics dried over Na_2SO_4 and concentrated under reduced pressure. The crude oil was purified by flash column chromatography (60:40 hexanes/ethyl acetate) to afford the alcohols **97a** and **97b** (8.3 g, 89%) as a pale yellow oil (1:2 mixture of separable diastereomers).

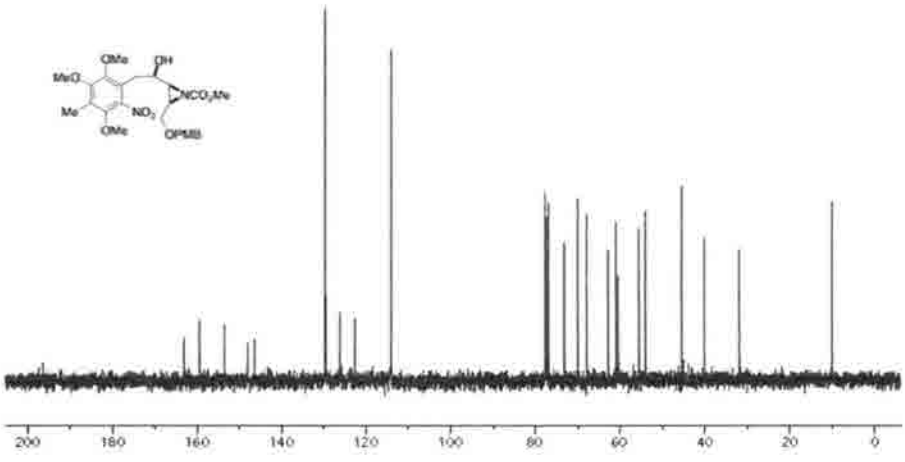
Major diastereomer: $[\alpha]_D^{20}$ -21.1 (*c*=1.0, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.22 (3H, s), 2.60 (1H, dd, *J* = 8.0, 6.0 Hz), 2.83 (1H, q, *J* = 6.0 Hz), 2.92-3.03 (3H, m), 3.46 (1H, dd, *J* = 10.5, 7.5 Hz), 3.61-3.68 (1H, m), 3.70 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 3.83 (3H, s), 3.85 (3H, s), 4.50 (2H, d, *J* = 1.5 Hz), 6.85 (2H, dt, *J* = 8.5, 2.5 Hz), 7.23

(2H, dt, J = 8.5, 2.5 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 9.7, 31.5, 39.8, 45.1, 53.7, 55.2, 60.2, 60.6, 62.5, 67.6, 69.6, 72.9, 113.8, 122.4, 125.9, 129.2, 129.5, 143.0, 146.2, 147.8, 153.3, 159.2, 162.9; IR (neat) 3479, 3002, 2944, 2856, 1730, 1612, 1533, 1466, 1370, 1299, 1115, 755 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{25}H_{32}N_2O_{10}$ 521.2135, $(MH)^+$, Found 521.2134.

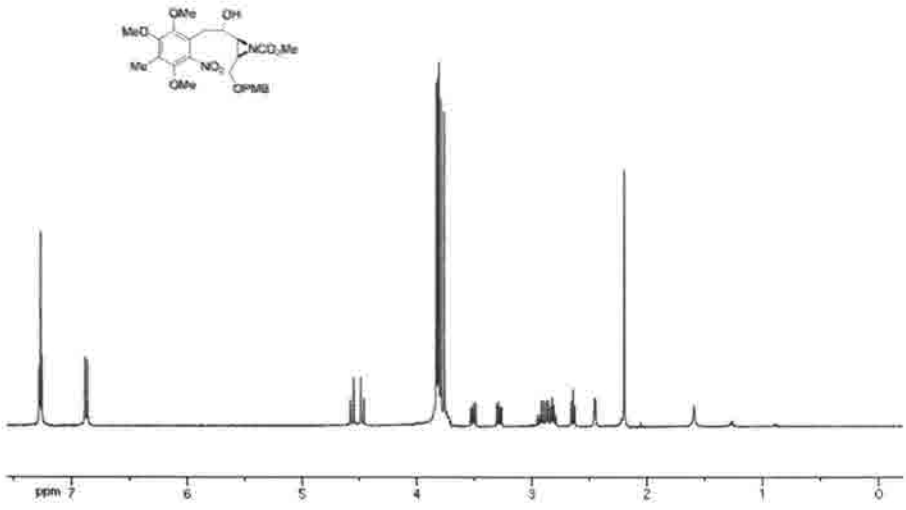
Minor diastereomer: $[\alpha]_D^{20}$ +6.1 (c=0.08, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 2.20 (3H, s), 2.45 (1H, d, J = 5.0 Hz), 2.64 (1H, t, J = 7.0 Hz), 2.78-2.95 (3H, m), 3.28 (1H, dd, J = 11.0, 5.0 Hz), 3.50 (1H, dd, J = 11.0, 7.0 Hz), 3.71-3.75 (1H, m), 3.76 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 3.82 (3H, s), 3.83 (3H, s), 4.47 (1H, d, J = 11.5 Hz), 4.56 (1H, d, J = 11.5 Hz), 6.87 (2H, dt, J = 8.5, 2.5 Hz), 7.27 (2H, dt, J = 8.5, 2.5 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 9.7, 31.3, 41.4, 45.9, 54.0, 55.3, 60.2, 60.6, 62.6, 67.0, 69.5, 72.6, 113.8, 121.5, 126.5, 129.6, 130.0, 143.1, 146.3, 147.9, 153.5, 159.3, 163.4; IR (neat) 3364, 3009, 2937, 2893, 1729, 1611, 1532, 1249, 1090, 1035 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{25}H_{32}N_2O_{10}$ 521.2135 $(MH)^+$, Found 521.2135.



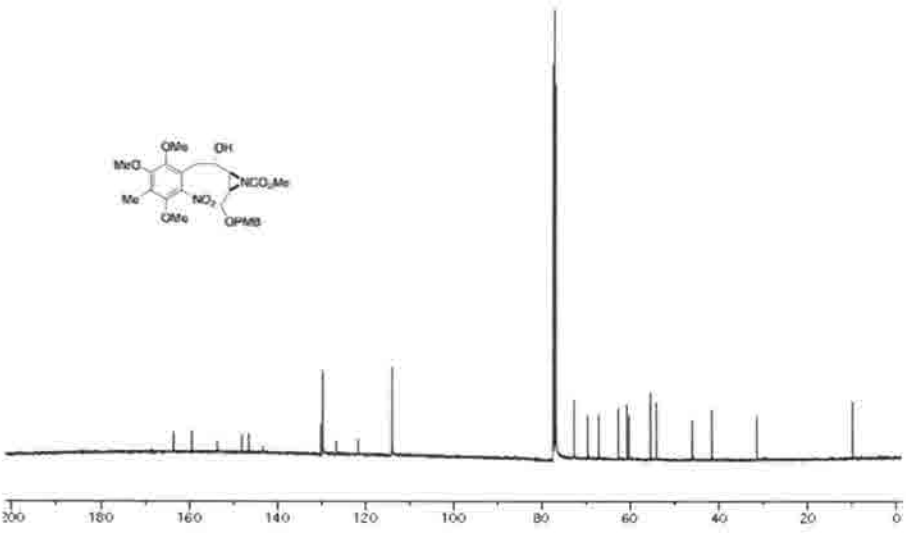
^{1}HNMR, $CDCl_3$, 400 MHz; File name: Dag-I-278-pure



^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-278-13C

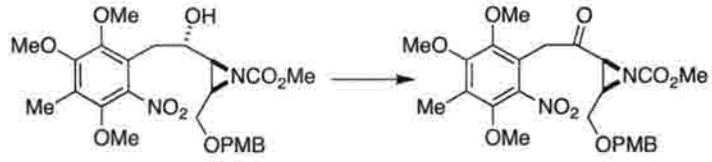


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: 01-Alcohol-1/minor-HNMR

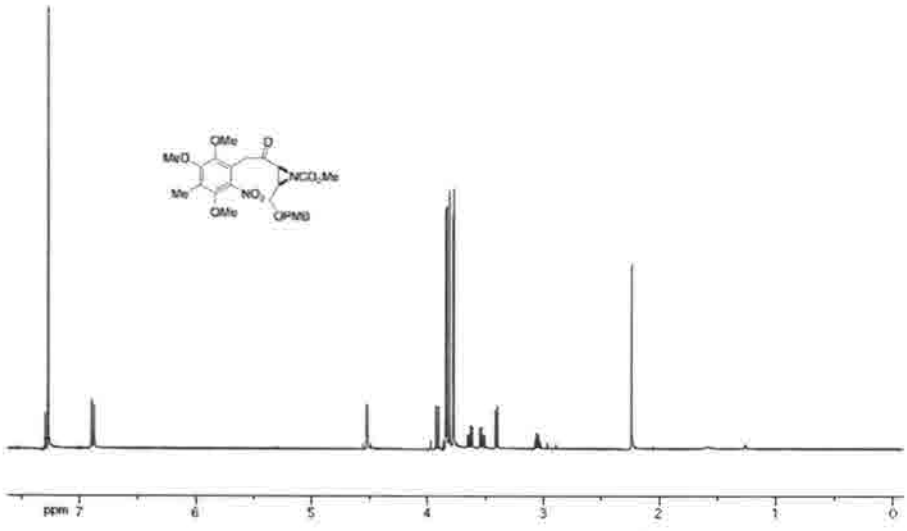


^{13}CNMR, $CDCl_3$, 100 MHz; Filename: 01-Alcohol-1/minor-C13NMR

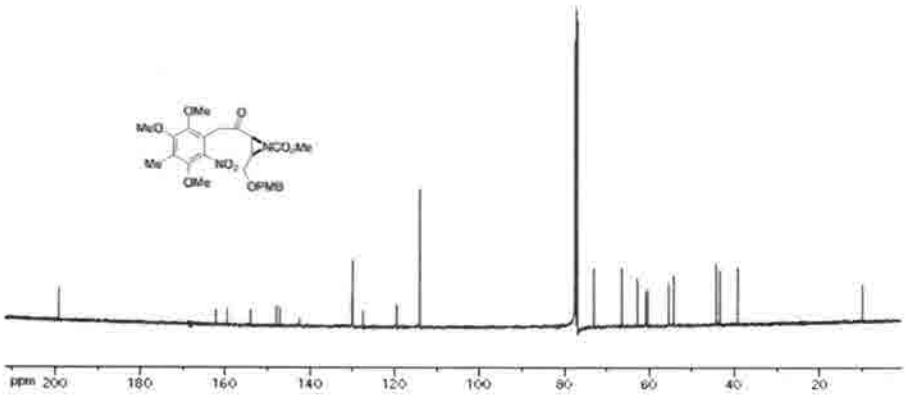
(2*R*,3*S*)-methyl 2-((4-methoxybenzyloxy)methyl)-3-(2-(2,3,5-trimethoxy-4-methyl-6-nitrophenyl)acetyl)aziridine-1-carboxylate



To alcohol **97a** (3.0 g, 5.8 mmol) in dichloromethane (200 mL) was added Dess-Martin periodinane (3.9 g, 9.3 mmol) and stirred for 2 hrs. The reaction was diluted with ether (200 mL), poured into 200 mL sat. aq. $NaHCO_3$ with 7.0 equivalents of sodium thiosulfate (10.0 g, 40.3 mmol), and stirred for 15 min. The layers were separated and the aqueous layer was backextracted with ether. The combined ether layers were washed (1 x sat. aq. $NaHCO_3$, 1 x Brine), dried (Na_2SO_4), filtered and concentrated. Purification by column chromatography (4:1 hexanes/ethyl acetate) gave the desired ketone (2.9 g, 97%) as a pale yellow oil. $[\alpha]_D^{20}$ -44.6 (*c*=1.3, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.24 (3H, s), 3.05 (1H, dd, *J* = 12.0, 6.0 Hz), 3.41 (1H, d, *J* = 6.8 Hz), 3.53 (1H, dd, *J* = 11.0, 5.4 Hz), 3.63 (1H, dd, *J* = 11.4, 5.8 Hz), 3.77 (3H, s), 3.78 (3H, s), 3.81 (3H, s), 3.83 (3H, s), 3.84 (3H, s), 3.92 (2H, dd, *J* = 26.0, 18.0 Hz), 4.52 (2H, dd, *J* = 14.6, 11.4 Hz), 6.87 (2H, d, *J* = 8.4 Hz), 7.28 (2H, d, *J* = 8.4 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 10.0, 39.2, 43.4, 44.3, 54.3, 55.5, 60.4, 60.8, 62.8, 66.5, 73.1, 114.1, 119.5, 127.4, 129.9, 130.0, 142.5, 147.0, 147.9, 154.0, 159.6, 162.2, 199.1; IR (neat) 3002, 2947, 2854, 1732, 1612, 1530, 1367, 1251 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{25}H_{30}N_2O_{10}$ 519.1979 $(MH)^+$, Found 519.1964.

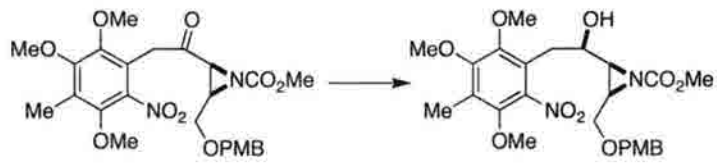


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: 02-Ketone-1-HNMR



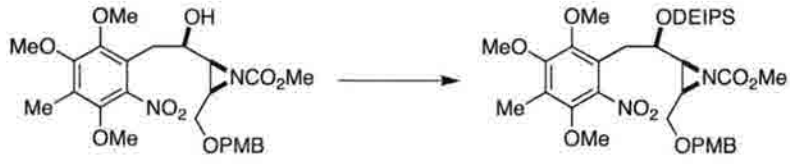
^{13}CNMR, $CDCl_3$, 300 MHz; Filename: 02-Ketone-1-C13NMR

(2*R*,3*S*)-Methyl-2-((4-methoxybenzyloxy)methyl)-3-((*S*)-1-hydroxy-2-(2,3,5-trimethoxy-4-methyl-6-nitrophenyl)ethyl)aziridine-1-carboxylate (97b)



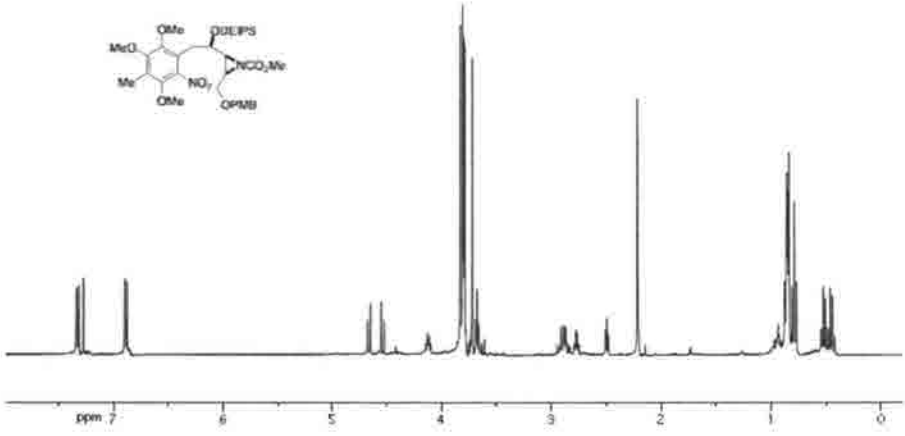
To ketone described above (1.0 g, 1.92 mmol) in 20 mL of 3:1 methanol/dichloromethane at 0 °C was added sodium borohydride (145 mg, 3.84 mmol) and stirred for 1 hr. The reaction was then quenched by the addition of brine, extracted (5 x dichloromethane), washed (brine), dried (Na_2SO_4), filtered and concentrated to give alcohol **97b** (0.95 g, 95%) as a pale yellow oil that was used without further purification. Spectral properties as physical characteristics were identical to those listed above.

(2*R*,3*S*)-Methyl-2-((4-methoxybenzyloxy)methyl)-3-((*S*)-1(diethyl(isopropyl)silyloxy) -2-(2,3,5-trimethoxy-4-methyl-6-nitrophenyl)ethyl) aziridine-1-carboxylate

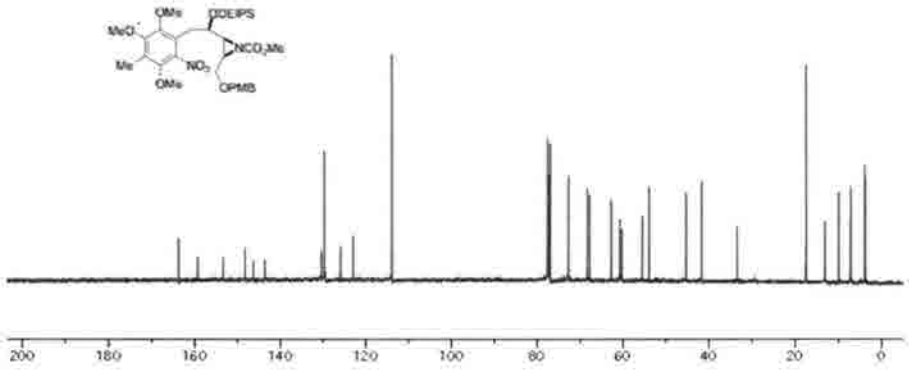


To a solution of the alcohol **97b** obtained above (5.2 g, 10.0 mmol) and imidazole (2.4 g, 35 mmol) in dry dichloromethane (250 mL) was added dropwise chlorodiethylisopropylsilane (3.3 g, 20 mmol). The mixture was stirred at room temperature for 24h. The reaction was quenched with saturated aqueous $NaHCO_3$, diluted with dichloromethane and the layers separated. The aqueous layer was extracted with ethyl acetate (2 x 50 mL). The combined organics were washed with brine, dried over

$MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (4:1 hexanes/ethyl acetate) to afford the silyl ether as a pale yellow oil (6.15 g, 89%). Major diastereomer: $[\alpha]_D^{20}$ -19.9 (c=2.0, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.42-0.65 (4H, m), 0.75-1.02 (13H, m), 2.21 (3H, s), 2.49 (1H, t, J = 6.0 Hz), 2.76 (1H, q, J = 6.0 Hz), 2.83-2.95 (2H, m), 3.61-3.77 (2H, m), 3.72 (3H, s), 3.78 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 3.83 (3H, s), 4.11 (1H, dt, J = 10.0, 5.0 Hz), 4.53 (1H, d, J = 11.5 Hz), 4.66 (1H, d, J = 11.5 Hz), 6.88 (2H, d, J = 8.5 Hz), 7.32 (2H, J = 8.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 3.4, 3.6, 6.8, 6.9, 9.6, 12.7, 17.1, 33.1, 41.4, 45.0, 53.6, 55.2, 60.0, 60.5, 62.5, 67.5, 68.5, 72.5, 113.7, 122.8, 125.7, 129.5, 130.3, 143.4, 146.2, 148.2, 153.3, 159.2, 163.7; IR (neat) 2943, 2875, 1731, 1612, 1533, 1464, 1290, 1114, 1093, 727 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{32}H_{48}N_2O_{10}Si$ 649.3156 $(MH)^+$, Found 649.3147.

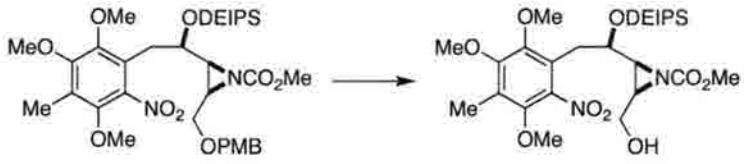


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: 03-ODEIPS-HNMR



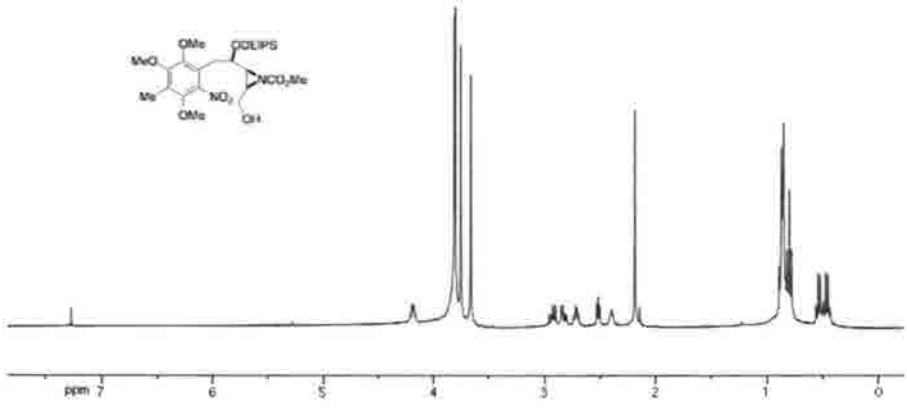
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: 03-ODEIPS-C13NMR

(2*S*,3*R*)-methyl 2-((*R*)-1-(diethyl(isopropyl)silyloxy)-2-(2,3,5-trimethoxy-4-methyl-6-nitrophenyl)ethyl)-3-(hydroxymethyl)aziridine-1-carboxylate

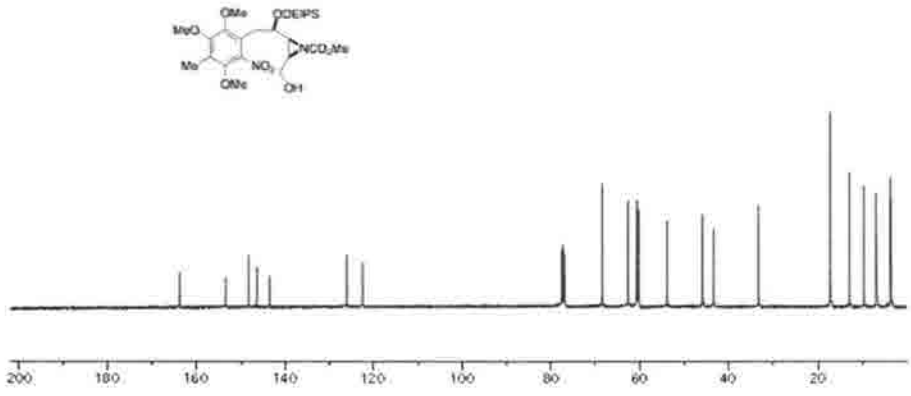


To the silyl ether described above (3.25 g, 5.0 mmol) in 100 mL of dichloromethane and 2 mL water was added DDQ (1.48 g, 6.5 mmol) and stirred for 2 hrs. The reaction mixture was then passed through a plug of neutral alumina and eluted with 10:1 dichloromethane/methanol. After concentration, the crude oil was purified by column

chromatography (3:1 hexanes/ethyl acetate to afford alcohol (2.35 g, 89%) as a pale yellow oil. $[\alpha]_D^{20}$ -36.5 (*c*=2.5, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.57-0.71 (4H, m), 0.85-1.03 (13H, m), 2.22 (3H, s), 2.54-2.63 (1H, m), 2.72 (1H, dd, *J* = 13.2, 6.6 Hz), 2.80 (2H, d, *J* = 7.4 Hz), 2.82-2.91 (1H, br), 3.10 (1H, dd, *J* = 11.4, 7.8 Hz), 3.71 (3H, s), 3.76-3.79 (1H, m), 3.78 (3H, s), 3.83 (3H, s), 3.84 (3H, s), 4.18 (1H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 3.6, 3.7, 6.9, 7.0, 9.7, 12.9, 17.2, 33.3, 43.4, 45.9, 53.8, 60.1, 60.5, 60.6, 62.6, 68.4, 122.5, 126.1, 143.5, 146.4, 148.3, 153.5, 163.8; IR (neat) 3446, 2945, 2876, 1731, 1534, 1463, 1293, 1094 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{24}H_{40}N_2O_9Si$ 529.2570 $(MH)^+$, Found 529.2581.

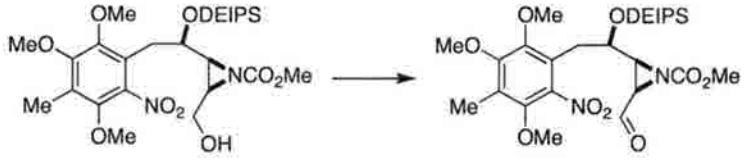


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: 04-Alcohol-2-HNMR



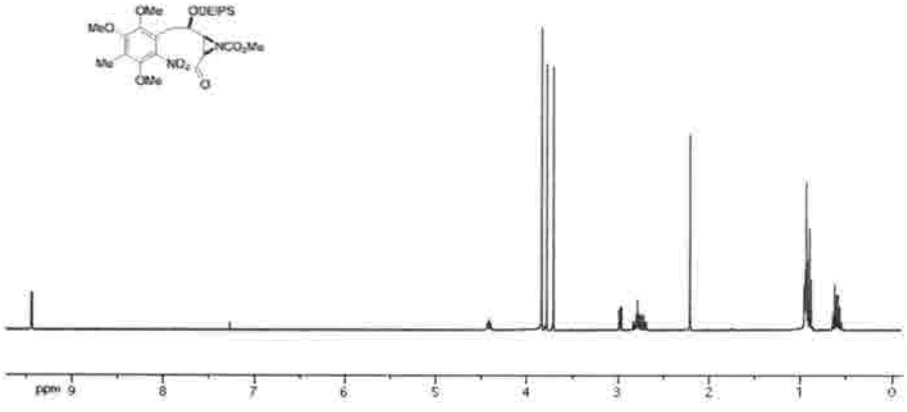
^{13}CNMR, $CDCl_3$, 300 MHz; Filename: 04-Alcohol-2-C13NMR

(2*S*,3*R*)-methyl 2-((*R*)-1-(diethyl(isopropyl)silyloxy)-2-(2,3,5-trimethoxy-4-methyl-6-nitrophenyl)ethyl)-3-formylaziridine-1-carboxylate (103)

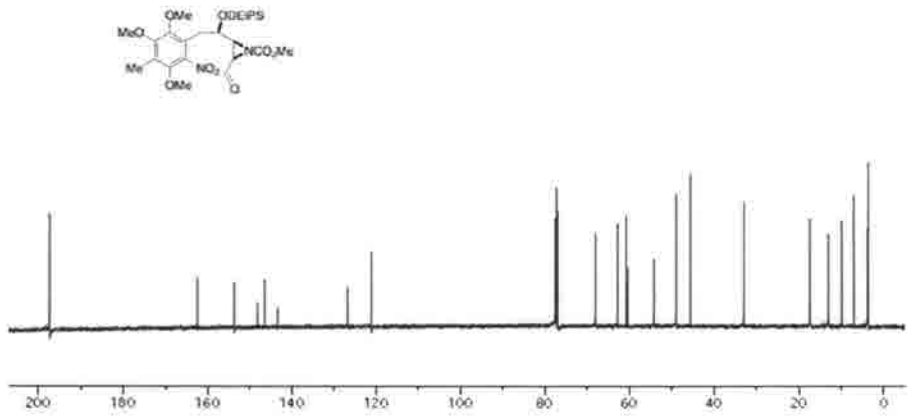


To alcohol described previously (2.64 g, 5.0 mmol) in 100 mL dichloromethane was added Dess-Martin periodinane (3.39 g, 8.0 mmol) and stirred for 2 hrs. The reaction was diluted with ether (100 mL), poured into 100 mL sat. aq. $NaHCO_3$ with 7.0 equivalents of sodium thiosulfate (8.7 g, 35 mmol), and stirred for 15 min. The layers were separated and the aqueous layer was backextracted (5 x ether). The combined ether layers were

washed (1 x sat. aq. $NaHCO_3$, 1 x Brine), dried (Na_2SO_4), filtered and concentrated. Purification by column chromatography (4:1 hexanes/ethyl acetate) gave the desired aldehyde **103** (2.29 g, 87%) as a pale yellow oil. $[\alpha]_D^{20}$ +24.3 (*c*=3.7, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.55-0.67 (4H, m), 0.89-0.98 (13H, m), 2.22 (3H, s), 2.72 (1H, dd, *J* = 13.5, 7.5 Hz), 2.78 (1H, dd, *J* = 6.6, 3.0 Hz), 2.82 (1H, dd, *J* = 13.5, 6.0 Hz), 2.99 (1H, dd, *J* = 7.2, 4.8 Hz), 3.72 (3H, s), 3.79 (3H, s), 3.85 (3H, s), 4.42 (1H, ddd, *J* = 7.5, 6.0, 3.0 Hz), 9.45 (1H, d, *J* = 4.8 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.6, 3.8, 7.0, 7.1, 9.9, 13.0, 17.4, 32.9, 45.6, 48.9, 54.2, 60.4, 60.8, 62.8, 68.0, 121.1, 126.8, 143.4, 148.2, 153.8, 162.5, 197.3; IR (neat) 2947, 2877, 1739, 1534, 1446, 1281, 1116 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{24}H_{38}N_2O_9Si$ 527.2425, $(MH)^+$, Found 527.2426.

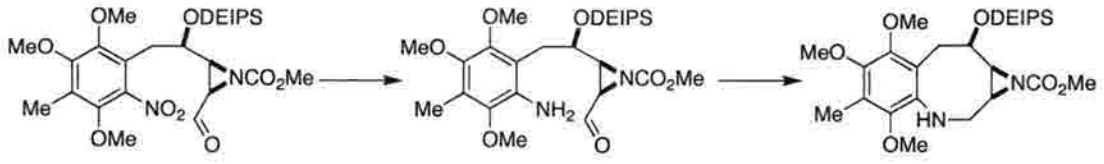


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: 05-Aldehyde-HNMR



^{13}CNMR, $CDCl_3$, 100 MHz; Filename: 05-Aldehyde-C13NMR

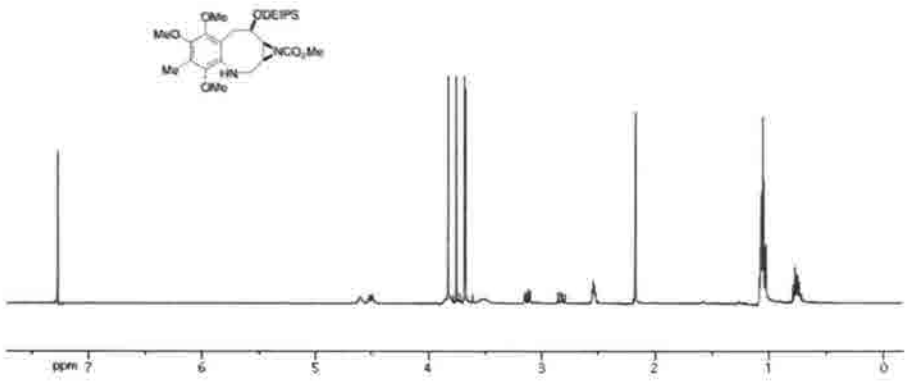
(1aS,9aS)-Methylester-1a,2,3,8,9,9a-hexahydro-4,6,7-(trimethoxy)-5-methyl-9-[[diethyl(1-methylethyl)silyl] oxy]-1H-azirino[2,3-c][1]benzazocine-1-carboxylic acid (104)



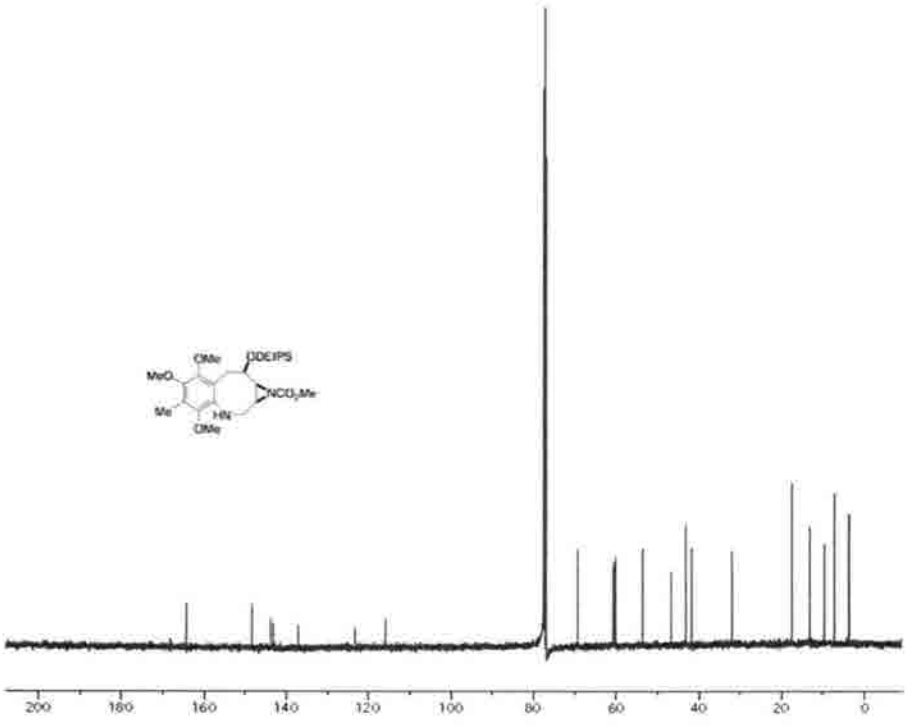
The aldehyde compound **103** obtained in the previous experiment (100 mg, 0.19 mmol) was dissolved in 100 mL anhydrous methanol in a 400 mL pressure vessel and purged with argon for 10 minutes. Ruthenium oxide (100 mg, 0.75 mmol) was added, the vessel was charged with 80 psi hydrogen gas, and then stirred at room temperature for 6 hrs. The solution was then filtered through a plug of celite (using methanol as eluant) and

concentrated to give the crude anilino aldehyde **96** (94 mg) as a colorless oil. The anilino aldehyde was found to be unstable and was taken immediately onto the next step.

The crude anilino aldehyde **96** obtained in the reduction above (94 mg, 0.19 mmol) was immediately dissolve in 300 mL of dry dichloromethane. To the reaction was added magnesium sulfate (5.0 g, 42 mmol), microwave activated molecular sieves (4 Å, 2.0 g), and then stirred under argon at reflux for 3 days. Methanol (50 mL) was added to the reaction and then cooled to 0 °C, whereupon sodium cyanoborohydride (12 mg, 0.19 mmol) and acetic acid (0.016 mL, 0.29 mmol) were added and stirred for 1 hr at 0 °C. The reaction mixture was then quenched (sat. aq. $NaHCO_3$), extracted (3 x dichloromethane), and the combined organic layers were then washed (brine), dried (Na_2SO_4), filtered and concentrated. Purification by PTLC (1:1 hexanes/ethyl acetate) gave the desired benzazocine **104** (56 mg, 62% for two steps) as a white crystalline solid. $[\alpha]_D^{20}$ -38.1 (*c*=1.2, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.72-0.79 (4H, m), 1.02-1.09 (13H, m), 2.17 (3H, s), 2.51-2.57 (2H, m), 2.82 (1H, dd, *J* = 13.5, 10.0 Hz), 3.13 (1H, dd, *J* = 14.0, 6.0 Hz), 3.44-3.64 (1H, m), 3.67 (3H, s), 3.68 (3H, s), 3.76 (3H, s), 3.83 (3H, s), 3.79-3.86 (1H, m), 4.51 (1H, dt, *J* = 10.5, 5.5 Hz), 4.60 (1H, br s); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.5, 3.6, 7.1, 9.5, 13.0, 17.3, 31.8, 41.5, 42.9, 46.5, 53.3, 59.8, 60.2, 60.5, 69.0, 115.5, 122.9, 136.7, 142.8, 143.5, 148.0, 163.8; IR (neat) 3390, 2952, 2875, 1730, 1490, 1464, 1293, 1229, 1090, 730 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{24}H_{40}N_2O_6Si$ 480.2649, $(MH)^+$, Found 480.2655; Mp 131-133 °C.

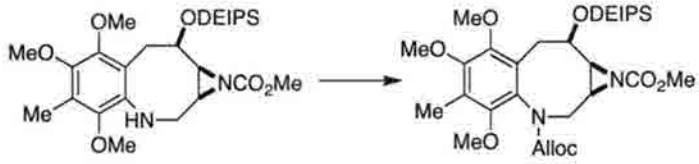


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: 06-Aniline-HNMR



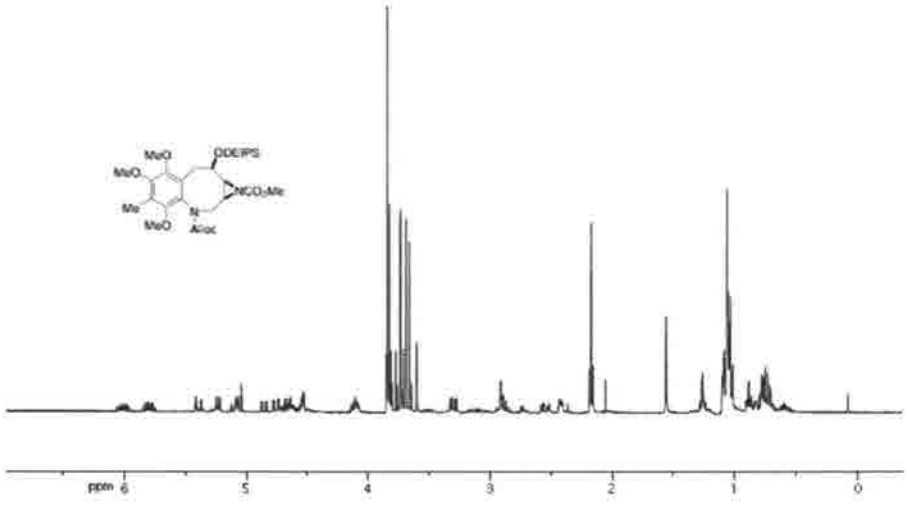
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: 06-Aniline-C13NMR

(1aS,9aS)-Methyl ester-1a,2,3,8,9,9a-hexahydro-4,6,7-(trimethoxy)-5-methyl-3-(allyloxycarbonyl)-9-[[diethyl(1-methylethyl)silyl]oxy]-1H-azirino[2,3-c][1] benzazocine-1-carboxylic acid (105)

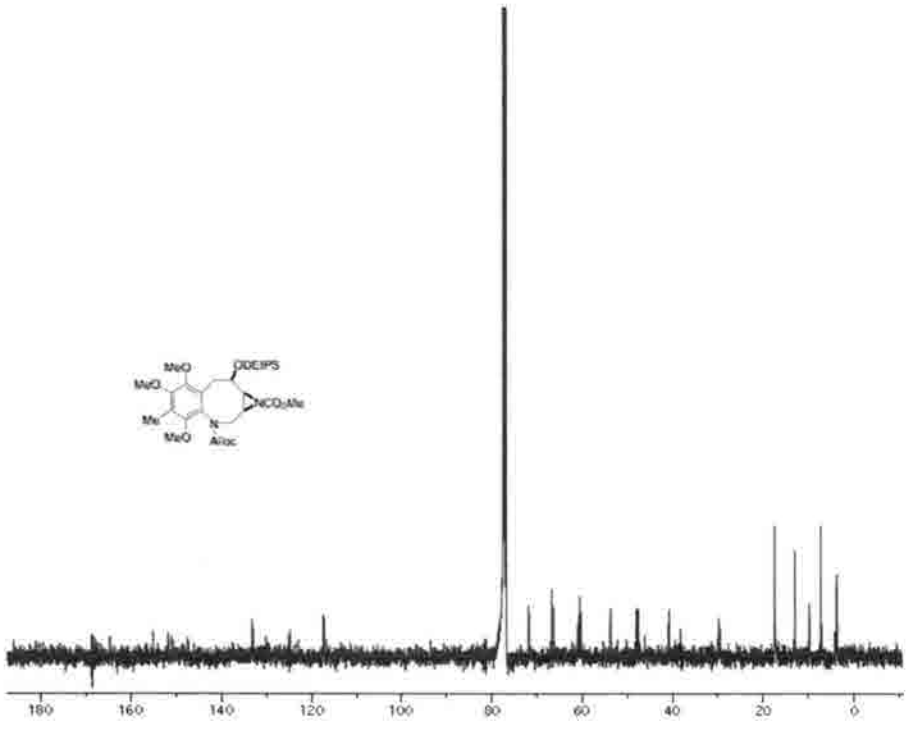


To benzazocine **104** (20 mg, 0.042 mmol) in dichloromethane (2 mL) was added sat. aq. $NaHCO_3$ (2 mL) and allyl chloroformate (22 μL, 0.21 mmol). After stirring at ambient temperature for 2 days, the reaction mixture was then extracted (5 x dichloromethane), washed (brine), dried (Na_2SO_4), filtered, and concentrated. Purification by PTLC (1:1 hexanes/ethyl acetate) gave alloc carbamate **105** (22 mg, 94 %) as a clear oil. $[\alpha]_D^{20}$ +28.9 (*c*=1.2, $CHCl_3$); Note: ^{1}HNMR and ^{13}CNMR are reported as a 1.4:1 mixture of rotamers at room temperature. At 323K onset of coalescence was observed. ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.70-0.91 (m), 1.04-1.10 (m), 1.26 (d, *J* = 3.0 Hz), 2.18 (s), 2.17 (s), 2.42 (m), 2.52, (dd, *J* = 8.5, 2.0 Hz), 2.57, (dd, *J* = 9.0, 2.0 Hz), 2.73 (dd, *J* = 9.5, 3.0 Hz), 2.89 (d, *J* = 1.5 Hz), 2.91 (d, *J* = 3.0 Hz), 3.28, (dd, *J* = 7.5, 1.5 Hz), 3.32 (dd, *J* = 7.0, 1.5 Hz), 3.60 (d, *J* = 1.5 Hz), 3.66 (s), 3.69 (s), 3.73 (d, *J* = 1.5 Hz), 3.83 (s), 3.84 (s), 3.85 (s), 4.10 (m), 4.54 (dq, *J* = 9.5, 1.5 Hz), 4.64 (dt, *J* = 8.0, 1.5 Hz), 4.68 (dt, *J* = 9.0, 1.5 Hz), 4.76 (dd, *J* = 19.0, 3.5 Hz), 4.86 (dd, *J* = 19.0, 3.5 Hz), 5.04 (t, *J* = 1.5 Hz), 5.08 (dq, *J* = 11.0, 1.5 Hz), 5.13 (q, *J* = 5.0, 1.5 Hz), 5.23 (dq, *J* = 14.5, 1.5 Hz), 5.39 (m), 5.81 (m), 6.01 (m); ^{13}CNMR (100 MHz, $CDCl_3$) 3.7, 3.9, 7.3, 9.7, 9.8, 13.0, 17.5, 29.5, 29.7, 40.7, 40.9, 47.5, 47.6, 47.9, 53.7, 53.8, 60.3, 60.4, 60.6, 60.8, 61.0, 66.3, 66.7, 71.8, 71.9, 117.2, 117.5, 122.9, 124.8 125.1, 129.7, 130.6, 132.2, 133.0, 133.3, 147.4, 151.8, 155.2,

164.6, 164.9, 168.3, 168.5, 168.7; IR (neat) 2951, 2876, 1730, 1710, 1467, 1245, 1094, 721 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{28}H_{44}N_2O_8Si$ 565.2924, $(MH)^+$, Found 565.2945.

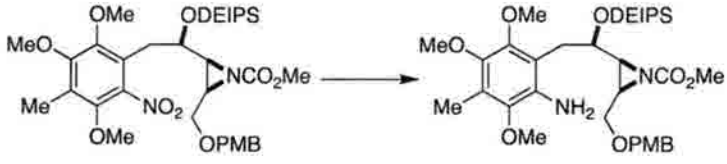


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: 07-Alloc-HNMR



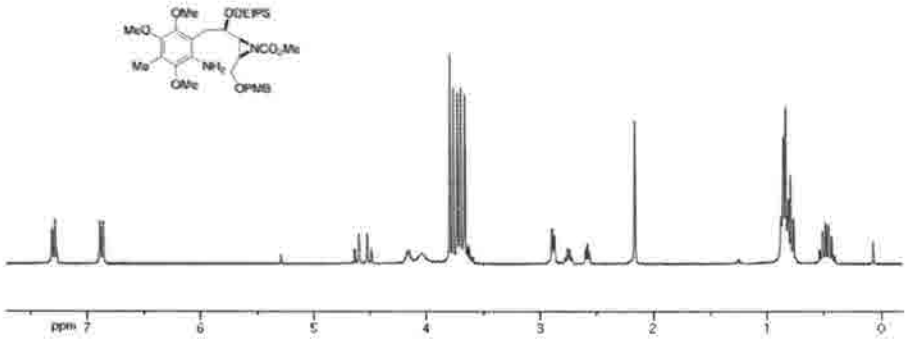
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: 07-Alloc-C13NMR

(2*R*,3*S*)-Methyl-2-((4-methoxybenzyloxy)methyl)-3-((*S*)-2-(2-amino-3,5,6-trimethoxy-4-methylphenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)aziridine-1-carboxylate

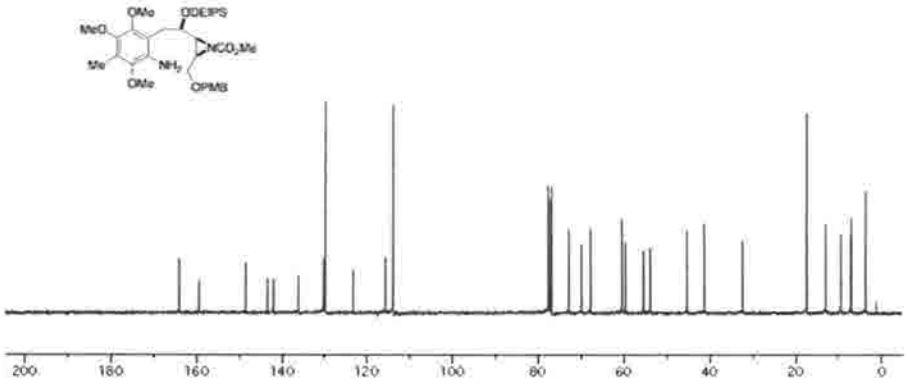


To a stirred suspension of **130** (0.72 g, 1.08 mmol) and 5% Pd-C (0.13 g) in dry methanol (6 mL) was added anhydrous ammonium formate (0.35 g, 5.57 mmol) in one portion. The mixture was stirred at room temperature for 30 min. The catalyst was then removed by filtration through a pad of celite and the filtrate was concentrated under reduced pressure.

To the residue was added H_2O (10 mL) and the aqueous phase was extracted with dichloromethane (3 x 10 mL). The combined organics were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (70:30 hexanes/ethyl acetate) to yield the aniline (0.53 g, 80%) as a pale yellow oil. $[\alpha]_D^{20}$ -7.3 (*c*=5.6, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.48 (4H, app sext, *J* = 8.0 Hz), 0.77-0.92 (13H, m), 2.17 (3H, s), 2.60 (1H, dd, *J* = 6.0, 5.0 Hz), 2.75 (1H, q, *J* = 6.0 Hz), 2.88 (2H, d, *J* = 7.0 Hz), 3.59-3.68 (2H, m), 3.67 (3H, s), 3.70 (3H, s), 3.73 (3H, s), 3.77 (3H, s), 3.80 (3H, s), 4.04 (2H, br s), 4.15 (1H, q, *J* = 6.0 Hz), 4.51 (1H, d, *J* = 11.5 Hz), 4.61 (1H, d, *J* = 11.5 Hz), 6.87 (2H, d, *J* = 8.5 Hz), 7.29 (2H, d, *J* = 8.5 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.4, 3.5, 6.7, 6.8, 9.2, 12.6, 17.1, 32.1, 41.0, 45.0, 53.5, 55.1, 59.3, 40.2, 60.2, 67.5, 69.6, 72.6, 113.6, 115.4, 123.0, 129.5, 129.9, 135.9, 141.6, 143.1, 148.2, 159.1, 163.8; IR (neat) 3436, 3361, 2952, 1731, 1613, 1513, 1464, 1248, 1090, 1014, 821, 730 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{32}H_{50}N_2O_8Si$ 618.3336, $(MH)^+$, Found 618.3361.

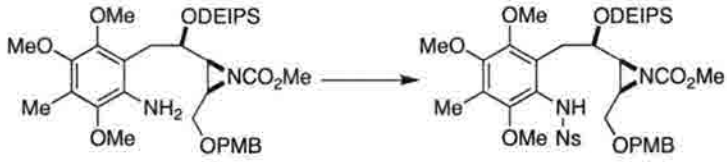


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-I-284



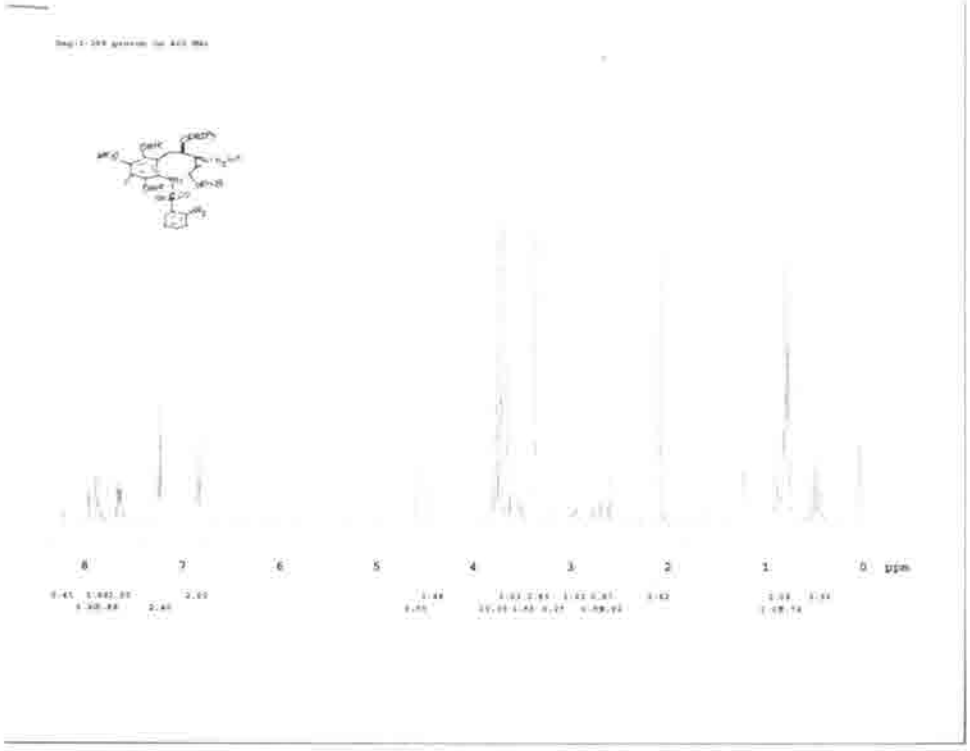
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-I-284-C13

(2*R*,3*S*)-Methyl 2-((4-methoxybenzyloxy)methyl)-3-((*S*)-1 (diethyl(isopropyl)silyloxy) -2-(2,3,5-trimethoxy-4-methyl-6-(2-nitrophenylsulfonamido)phenyl)ethyl)aziridine-1-carboxylate (131)

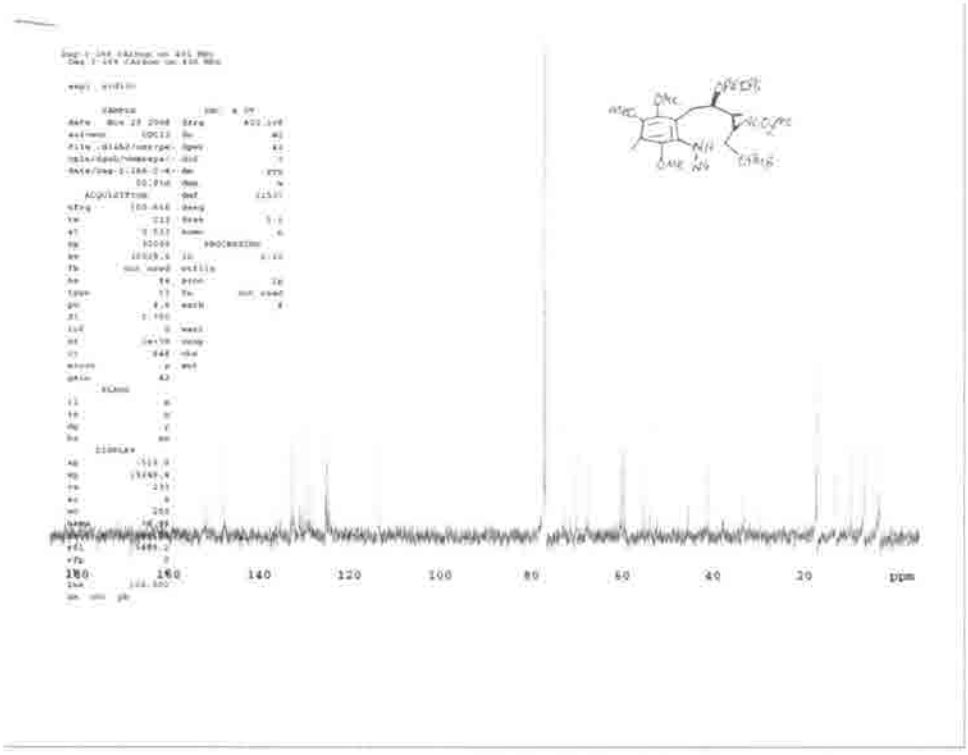


To a stirred solution of aniline (120 mg, 0.19 mmol) in dry pyridine (1 mL) was added 2-nitrobenzenesulfonyl chloride (172 mg, 0.77 mmol) portion wise over 20 min and stirred at room temperature for 1 hr. The reaction mixture was diluted with ethyl acetate and washed with brine (5 mL), 1N HCl (5 mL) and brine (5 mL). The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (70:30 hexanes/ethyl acetate) to afford nosyl sulfonamide **131** as a pale yellow oil (0.118 g, 79%). $[\alpha]_D^{20}$ +17.7 (c=1.0, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.40-0.54 (4H, m), 0.75-0.91 (13H, m), 2.09 (3H, s), 2.61 (1H, t, J = 6.5 Hz), 2.69 (1H, dt, J = 7.0, 4.5 Hz), 2.78 (1H, dd, J = 13.5, 9.0 Hz), 2.96 (1H, dd, J = 13.5, 5.0 Hz), 3.36 (3H, s), 3.48-3.55 (2H, m), 3.66 (3H, s), 3.71 (3H, s), 3.73 (3H, s) 3.77 (3H, s), 3.80-3.83 (1H, m), 4.47 (1H, d, J = 11.5 Hz), 4.59 (1H, d, J = 11.5 Hz), 6.83 (2H, d, J = 8.5 Hz), 7.24 (2H, d, J = 8.5 Hz), 7.62 (1H, dt, J = 7.5, 1.5 Hz), 7.67 (1H, dt, J = 7.5, 1.5 Hz), 7.77 (1H, s), 7.89 (1H, dd, J = 9.0, 1.5 Hz), 7.97 (1H, dd, J = 9.0, 1.5 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.3, 6.7, 9.5, 12.8, 17.0, 17.3, 29.6, 33.0, 40.9, 45.2, 53.6, 55.1, 59.4, 59.7, 60.1, 67.5, 69.8, 72.5, 113.6, 124.3, 124.8, 125.0, 125.2, 128.5, 129.4, 129.9, 130.3, 131.0, 132.3, 132.8, 135.4, 136.4, 147.6, 151.3, 151.9,

159.1, 163.5; IR (neat) 3319, 2930, 1728, 1542, 1464, 1409, 1251, 1172, 1082, 732 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{38}H_{53}N_3O_{12}SSi$ 804.3197, $(MH)^+$, Found 804.3191.

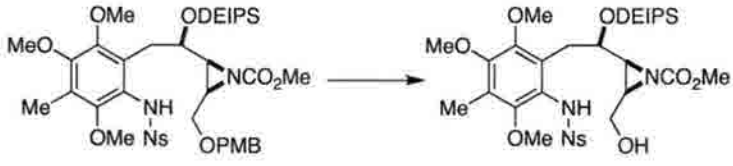


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-266



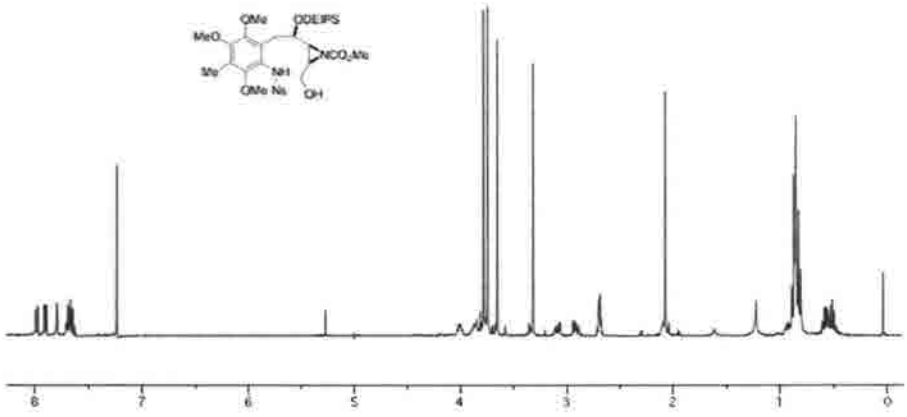
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-266-Carbon

(2*S*,3*R*)-Methyl 2-((*S*)-1-(diethyl(isopropyl)silyloxy)-2-(2,3,5-trimethoxy-4-methyl-6-(2-nitrophenyl sulfonamido)phenyl)ethyl)-3-(hydroxymethyl) aziridine-1-carboxylate (132)

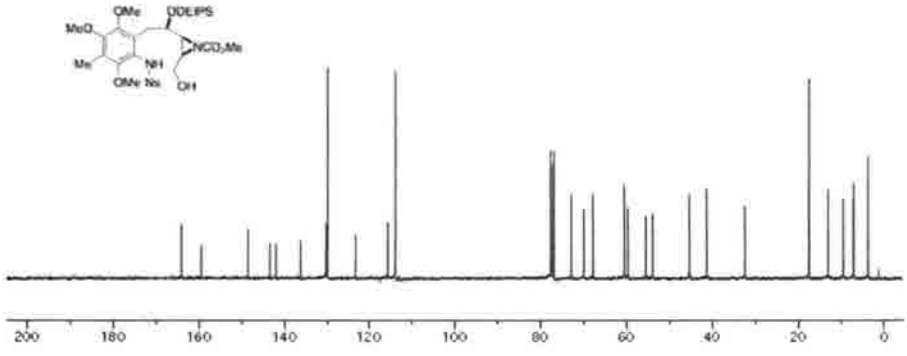


To a stirred solution of sulfonamide **131** (52 mg, 0.084 mmol) in 15:1 solution of dichloromethane/H_2O (1 mL) was added DDQ (20 mg, 0.084 mmol) in one portion. The reaction mixture immediately turned green and over the course of 1.5 h the mixture turned brown-orange. After stirring for 2 hours, the reaction mixture was passed through a short pad of activated alumina using 10:1 dichloromethane/methanol as eluant. The

filtrate was concentrated under reduced pressure and the crude product was purified by flash column chromatography (1:1 hexanes/ethyl acetate) to afford the alcohol **132** (39 mg, 87%) as a colorless oil. $[\alpha]_D^{20}$ +24.2 (*c*=1.6, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.45-0.63 (4H, m), 0.81-0.95 (13H, m), 2.07 (3H, s), 2.69 (2H, m), 2.91 (1H, dd, *J* = 13.0, 8.5 Hz), 3.08 (1H, dd, *J* = 13.5, 5.5 Hz), 3.32 (3H, s), 3.65 (3H, s), 3.74 (3H, s), 3.78 (3H, s), 3.83-3.89 (1H, m), 4.00 (1H, m), 7.64 (1H, td, *J* = 7.5, 1.5 Hz), 7.68 (1H, td, *J* = 7.5, 1.5 Hz), 7.79, (1H, s), 7.90 (1H, dd, *J* = 9.0, 1.5 Hz), 7.98 (1H, dd, *J* = 9.0, 1.5 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) 3.6, 3.7, 6.8, 9.5, 13.0, 17.1, 33.0, 42.4, 45.8, 53.7, 59.4, 59.8, 60.3, 70.3, 124.4, 124.6, 124.9, 128.5, 131.1, 132.4, 132.8, 135.4, 147.5, 147.7, 151.3, 151.8, 163.5; IR (neat) 3319, 3099, 2940, 2877, 1727, 1694, 1514, 1464, 1382, 1296, 1079, 1016, 733, 653 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{30}H_{45}N_3O_{11}SSi$ 684.2622, $(MH)^+$, Found 684.2615.

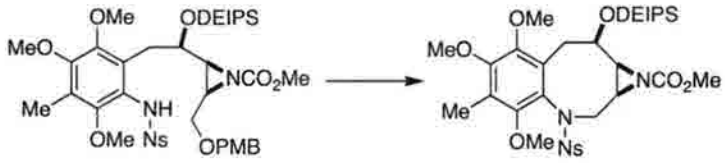


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-285-400

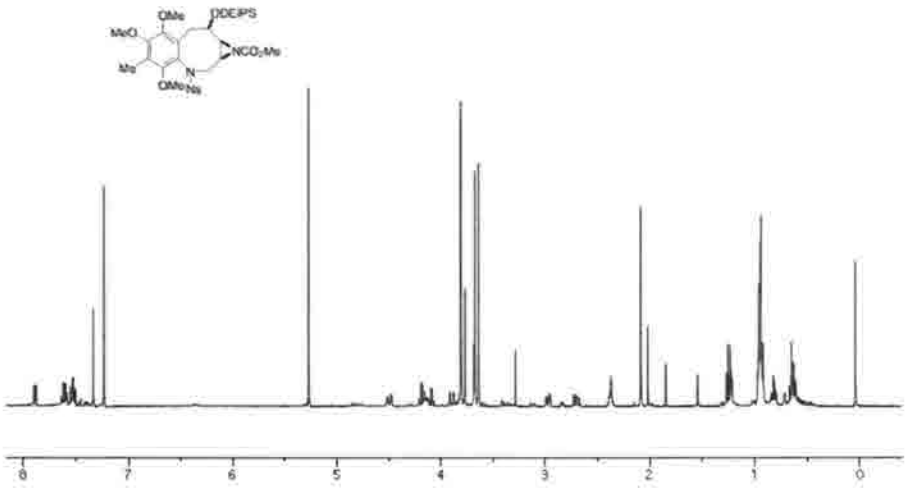


^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-284-C13

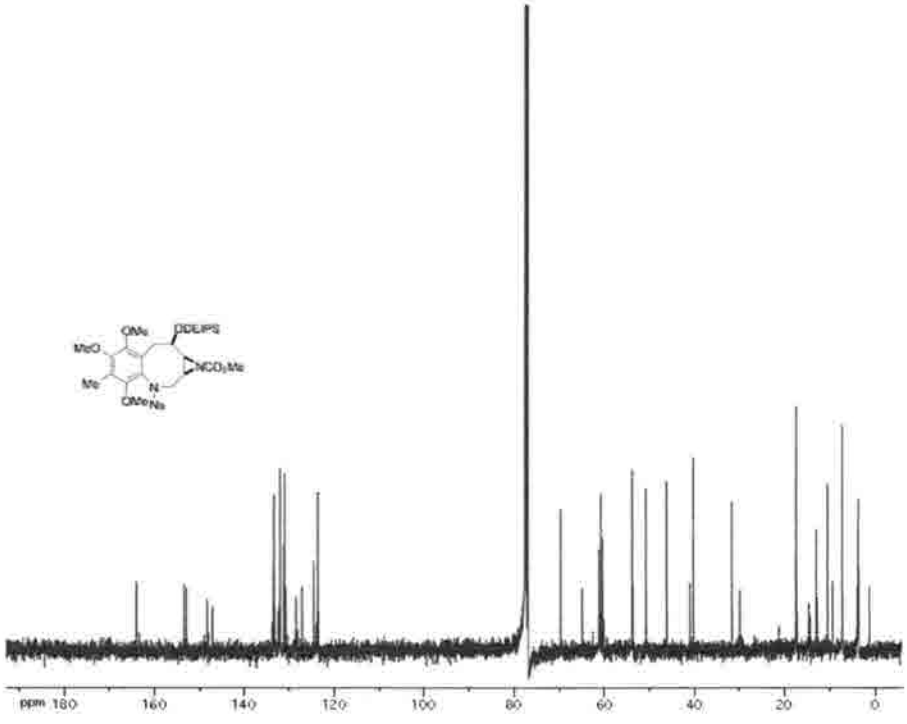
(1a*S*,9a*S*)-methyl ester-1a,2,3,8,9,9a-hexahydro-4,6,7-(trimethoxy)-5-methyl-3-(2-nitrobenzylsulfonyl amino)-9-[[diethyl(1-methylethyl)silyl]oxy]-1H-azirino[2,3-c][1]benzazocine-1-carboxylic acid (133)



To a stirred solution of **132** (40 mg, 0.057 mmol) and triphenylphosphine (27 mg, 0.10 mmol) in dry toluene (2.5 mL) was added DEAD (14 μL of a 40% solution in toluene, 0.086 mmol). The mixture was stirred at room temperature and monitored by TLC until complete consumption of the starting material (30 min). The reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (4:1 hexanes/ethyl acetate) to afford the benzazocine **133** (26 mg, 70%) as a colorless oil. $[\alpha]_D^{20}$ +4.8 (*c*=1.0, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.65 (4H, m), 0.79-0.85 (2H, m), 0.92-0.97 (17H, m), 2.09 (3H, s), 2.35-2.40 (2H, m), 2.70 (1H, dd, *J* = 13.5, 9.5 Hz), 2.97 (1H, dd, *J* = 13.5, 4.5 Hz), 3.29 (1H, s), 3.64 (3H, s), 3.68 (3H, s), 3.81 (6H, s), 3.88 (1H, d, *J* = 2.5 Hz), 3.91 (1H, s), 4.06-4.16 (2H, m), 4.49 (2H, dd, *J* = 14.5, 5.0 Hz), 7.50-7.55 (2H, m), 7.58-7.64 (2H, m), 7.89 (1H, dd, *J* = 9.0, 1.5 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) 3.4, 3.5, 7.0, 10.2, 12.7, 14.4, 17.2, 31.4, 40.0, 45.9, 50.4, 53.5, 60.1, 60.5, 60.8, 69.4, 123.5, 124.4, 127.0, 128.3, 130.9, 131.0, 132.1, 133.2, 133.3, 147.0, 148.1, 152.7, 153.2, 153.9; IR (neat) 3450, 3314, 2926, 1731, 1545, 1463, 1372, 1260, 942, 731 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{30}H_{43}N_3O_{10}SSi$ 666.2516, $(MH)^+$, Found 666.2498.

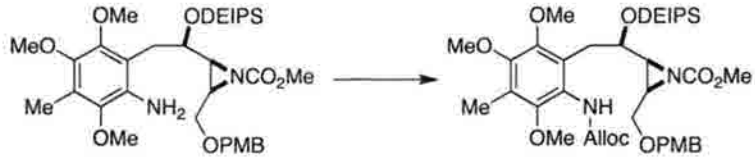


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-296-400-h



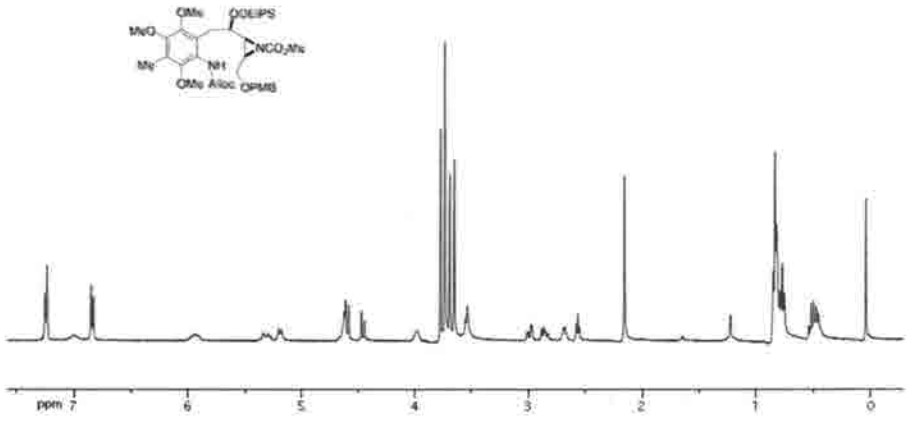
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-296-400-c13

(2*R*,3*S*)-Methyl-2-((4-methoxybenzyloxy)methyl)-3-((*S*)-2-(2-(allyloxycarbonyl)-3,5,6-trimethoxy-4-methylphenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)aziridine-1-carboxylate (137a)

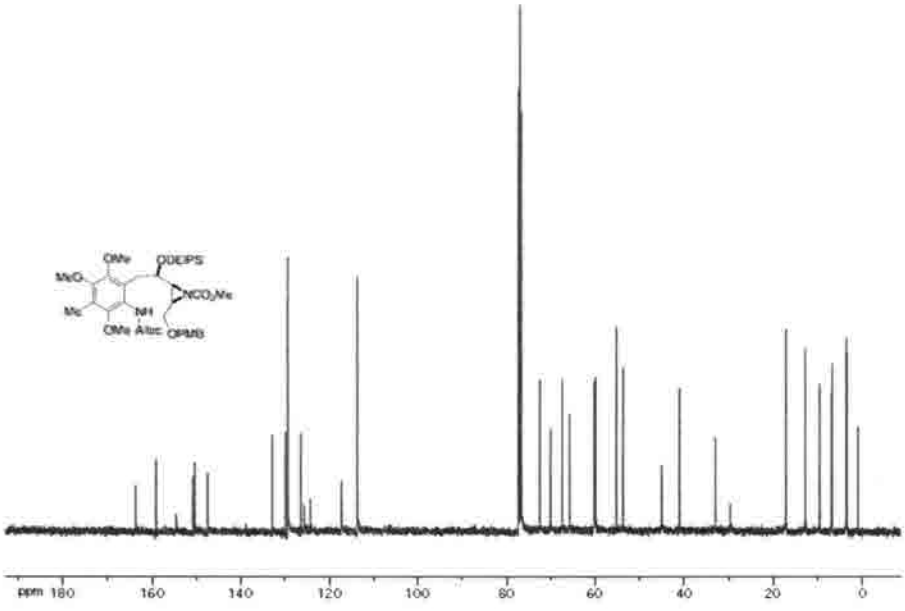


To a stirred solution of aniline **134** (250 mg, 0.40 mmol) and allyl chloroformate (68 μL, 0.80 mmol) in Dichloromethane (2 mL) was added sat. aq $NaHCO_3$ (0.8 mL). The reaction was vigorously stirred at room temperature overnight (12 hrs). The reaction mixture was diluted with EtOAc and brine (10 mL) was added. The phases were separated and the aqueous phase was extracted with ethyl acetate (3 x 10 mL). The combined organics were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (7:3 hexanes/ethyl acetate) to afford allyl carbamate **137a** (210 mg, 75%) as a colorless oil. $[\alpha]_D^{20}$ -3.1 (*c*=3.0, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.49 (4H, app sext, *J* = 8.0 Hz), 0.75-0.94 (13H, m), 2.15 (3H, s), 2.56 (1H, t, *J* = 6.0 Hz), 2.68 (1H, q, *J* = 6.0 Hz), 2.85 (1H, dd, *J* = 13.0, 9.0 Hz), 2.98 (1H, dd, *J* = 13.5, 4.5 Hz), 3.52-3.57 (2H, m), 3.65 (3H, s), 3.69 (3H, s), 3.72 (3H, s), 3.73 (3H, s), 3.77 (3H, s), 3.98 (1H, br s), 4.45 (1H, d, *J* = 11.5 Hz), 4.59 (1H, d, *J* = 11.5 Hz), 4.62-4.68 (2H, m), 5.18 (1H, d, *J* = 10.0 Hz), 5.31 (1H, d, *J* = 16.5 Hz), 5.93 (1H, m), 6.83 (2H, d, *J* = 7.0 Hz), 6.99 (1H, br s), 7.24 (2H, d, *J* = 6.5 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.4, 3.5, 6.7, 6.8, 12.7, 17.1, 32.9, 41.0, 53.7, 55.2, 59.9, 60.2, 65.8, 67.4, 70.0, 72.5, 113.7, 117.2, 124.3, 126.5, 129.4,129.9, 133.0, 147.6, 150.4, 150.9,

154.6, 159.2, 163.8; IR (neat) 3330, 2939, 1724, 1613, 1513, 1465, 1228, 1090, 757, 729 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{36}H_{54}N_2O_{10}Si$ 703.3626, $(MH)^+$, Found 703.3613.

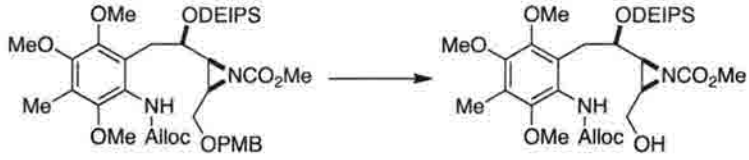


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-286-h-400



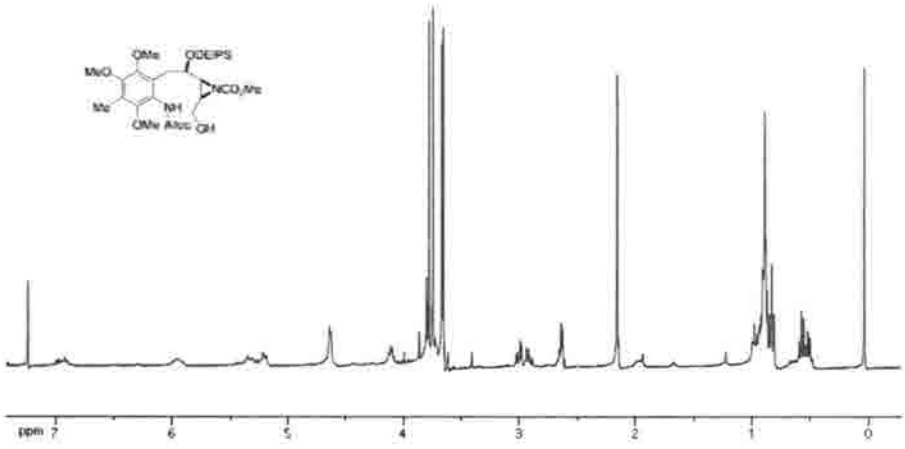
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-286-400-C13

(2*S*,3*R*)-Methyl-2-((*S*)-2-(2-(allyloxycarbonyl)-3,5,6-trimethoxy-4-methylphenyl)-1-(diethyl(isopropyl)silyl oxy)ethyl)-3-(hydroxymethyl)aziridine-1-carboxylate (138a)

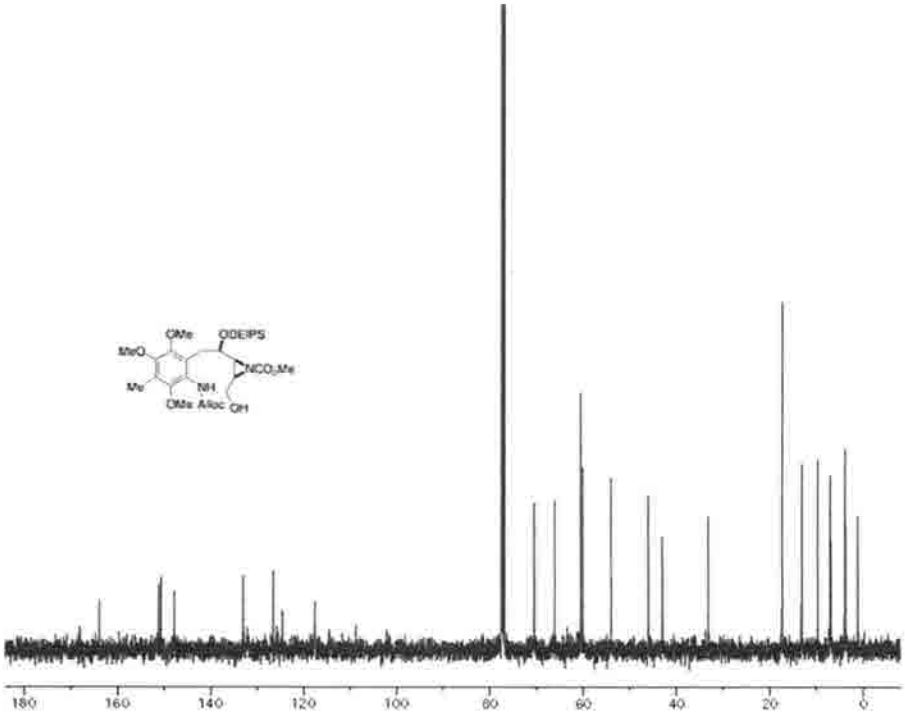


To a stirred solution of ally carbamate **137a** (190 mg, 0.26 mmol) in 15:1 solution of dichloromethane/H_2O (3 mL) was added DDQ (800 mg, 0.34 mmol) in one portion. The reaction mixture immediately turned green and over the course of 1.5 h the mixture turned brown-orange. After 2 h, the reaction mixture was passed through a short pad of activated alumina using 10:1 dichloromethane/methanol as eluant. The filtrate was

concentrated under reduced pressure and the crude product was purified by flash column chromatography (1:1 hexanes/ethyl acetate) to afford the alcohol **138a** (130 mg, 87%) as a colorless oil. $[\alpha]_D^{20}$ -13.6 (*c*=1.0, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.50 (2H, q, *J* = 7.5 Hz), 0.56 (2H, q, *J* = 7.5 Hz), 0.81-0.98 (13H, m) 1.97 (1H, br s), 2.15 (3H, s), 2.61-2.67 (2H, m), 2.91 (2H, dd, *J* = 13.5, 8.0 Hz), 3.00 (1H, dd, *J* = 13.5, 5.5 Hz), 3.65 (3H, s), 3.67 (3H, s), 3.74 (3H, s), 3.78 (3H, s), 3.80 (1H, d, *J* = 2.0 Hz), 4.08-4.13 (1H, m), 4.63 (2H, d, *J* = 2.0 Hz), 5.20 (1H, d, *J* = 10.0 Hz), 5.34 (1H, d, *J* = 17.0 Hz), 5.95 (1H, m), 6.91 (1H, br s); ^{13}CNMR (100 MHz, $CDCl_3$) 3.8, 3.9, 4.1, 6.9, 7.0, 9.7, 13.1, 13.3, 17.4, 33.2, 43.1, 46.0, 54.0, 60.1, 60.5, 63.4, 66.1, 70.5, 94.6, 108.8, 117.7, 124.7, 126.6, 133.1, 147.9 150.8, 151.2, 163.9; IR (neat) 3346, 2942, 2877, 1728, 1602, 1504, 1464, 1230, 1090, 1045, 728 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{28}H_{46}N_2O_9Si$ 583.3050, $(MH)^+$, Found 583.3034.

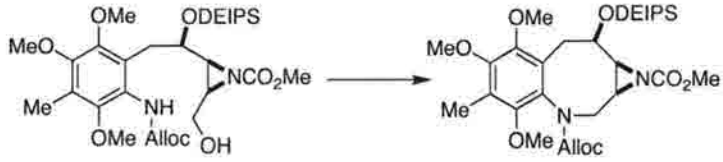


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-273-H-400



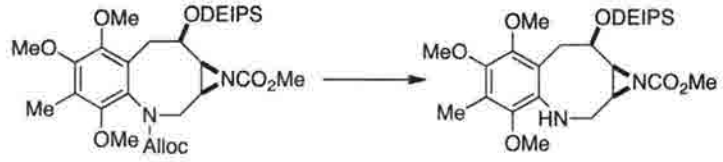
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-273-C-400

(1aS,9aS)-Methylester-1a,2,3,8,9,9a-hexahydro-4,6,7-(trimethoxy)-5-methyl-3-(allyloxycarbonyl)-9-[[diethyl(1-methylethyl)silyl]oxy]-1H-azirino[2,3-c][1] benzazocine-1-carboxylic acid (139a)



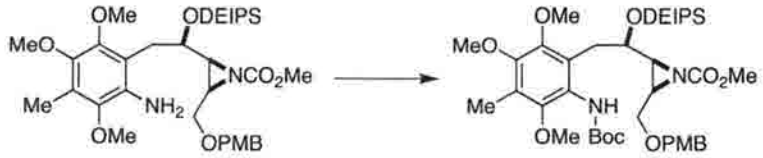
To a solution of **138a** (180 mg, 0.31 mmol) and tributylphosphine (140 μL, 0.56 mmol) in dry toluene (12 mL) was added TMAD (81 mg, 0.56 mmol). The solution turned immediately deep purple and turned to a light brown color as the reaction progressed. The mixture was stirred under argon at room temperature and monitored by TLC until complete consumption of the starting material (8h). The reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (80:20 hexanes/ethyl acetate) to afford the benzazocine **139a** (142 mg, 81%) as a colorless oil. All spectral and characterization data were identical with that of **105** prepared from the reductive amination route (see above).

(1aS,9aS)-Methylester-1a,2,3,8,9,9a-hexahydro-4,6,7-(trimethoxy)-5-methyl-9-[[diethyl(1-methylethyl)silyl] oxy]-1H-azirino[2,3-c][1]benzazocine-1-carboxylic acid (104)



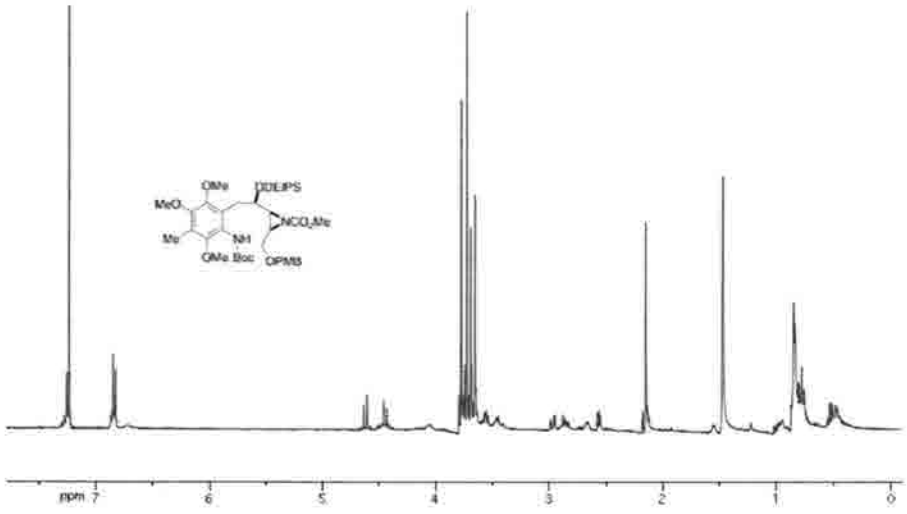
To a solution of **139a** (25 mg, 0.035 mmol) in dry THF (0.3 mL) was successively added acetic acid (6 mL, 0.14 mmol) and tetrakis(triphenylphosphine)palladium (0.02 mg, 1.8 mmol). The reaction mixture was stirred at room temperature for 2h. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (7:3 hexanes/ethyl acetate) to afford the benzazocine **104** (13 mg, 72%) as a white solid. All spectral and characterization data were identical with that of **104** prepared from the reductive amination route. $[\alpha]_D^{20}$ -38.1 (c=1.2, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.72-0.79 (4H, m), 1.02-1.09 (13H, m), 2.17 (3H, s), 2.51-2.57 (2H, m), 2.82 (1H, dd, J = 13.5, 10.0 Hz), 3.13 (1H, dd, J = 14.0, 6.0 Hz), 3.44-3.64 (1H, m), 3.67 (3H, s), 3.68 (3H, s), 3.76 (3H, s), 3.83 (3H, s), 3.79-3.86 (1H, m), 4.51 (1H, dt, J = 10.5, 5.5 Hz), 4.60 (1H, br s); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.5, 3.6, 7.1, 9.5, 13.0, 17.3, 31.8, 41.5, 42.9, 46.5, 53.3, 59.8, 60.2, 60.5, 69.0, 115.5, 122.9, 136.7, 142.8, 143.5, 148.0, 163.8; IR (neat) 3390, 2952, 2875, 1730, 1490, 1464, 1293, 1229, 1090, 730 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{24}H_{40}N_2O_6Si$ 480.2649, $(MH)^+$, Found 480.2655; Mp 131-133 °C.

(2*S*,3*R*)-methyl-2-((*R*)-2-(2-(*tert*-butoxycarbonylamino)-3,5,6-trimethoxy-4-methylphenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-((4-methoxybenzyloxy) methyl)aziridine-1-carboxylate (137b)

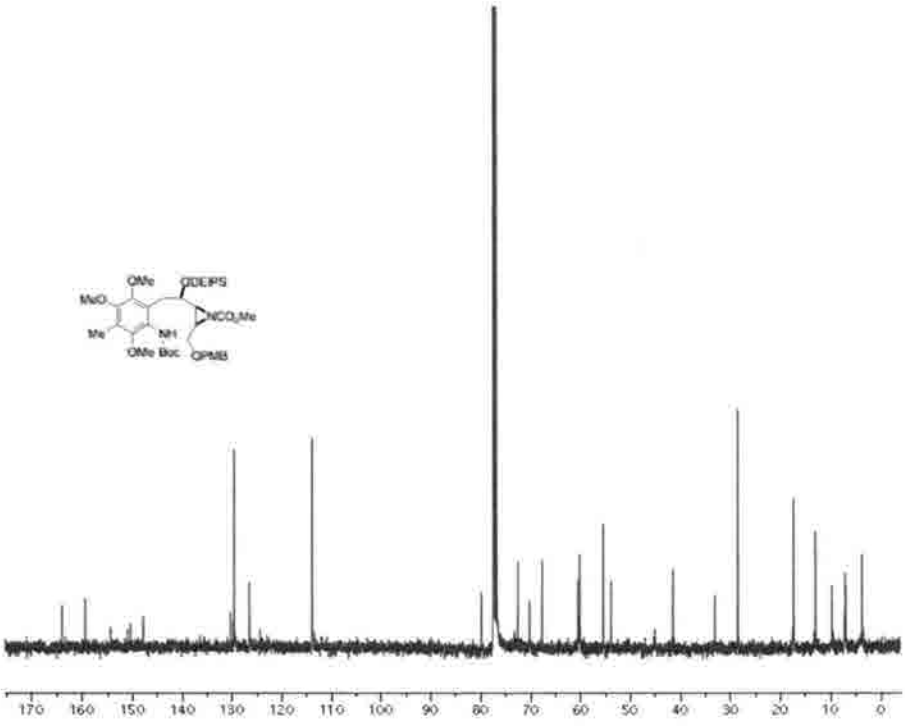


To a solution of aniline **134** (780 mg, 1.26 mmol) in THF (9 mL) was added Boc anhydride (330 mg, 1.51 mmol) and heated at reflux for 36 h. The reaction was allowed

to cool to room temperature and the solvent was evaporated. Column chromatography (5:1 hexanes/ethyl acetate) gave Boc-carbamate **137b** (848 mg, 94%) as a red oil. $[\alpha]_D^{20}$ -4.0 (c=0.6, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.44-0.55 (4H, m), 0.76-1.02 (13H, m), 1.47 (9H, s) 2.15 (3H, s), 2.56 (t, J = 6.0 Hz), 2.66 (1H, m), 2.85 (1H, dd, J = 13.6, 5.6 Hz), 2.97 (1H, dd, J = 13.6, 4.4 Hz), 3.45 (1H, m), 3.56 (1H, m)3.65 (3H, s), 3.69 (3H, s), 3.73 (3H, s), 3.78 (3H, s) 4.06 (1H, m), 4.45 (1H, d, J = 11.2 Hz), 4.62 (1H, d, J = 11.6 Hz), 6.7 (1H, s), 6.84 (2H, d, J = 8.8 Hz), 7.24 (2H, d, J = 8.5 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.7, 3.8, 7.0, 7.1, 7.3, 9.8, 13.0, 17.4, 17.5, 28.5, 33.1, 41.5, 53.9, 55.5, 60.2, 60.5, 67.7, 70.3, 72.6, 79.9, 113.9, 126.6, 129.6, 130.3, 147.9, 150.4, 151.0, 154.4, 159.4, 164.0; IR (neat) 3356, 2938, 2875, 2245, 1728, 1612, 1513, 1247, 1091 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{37}H_{58}N_2O_{10}Si$ 719.3939 $(MH)^+$, Found 719.3906.

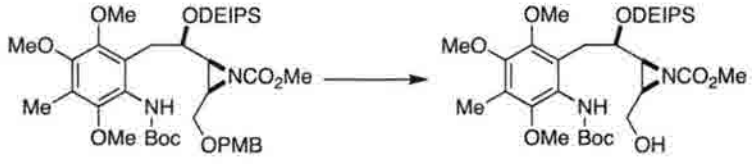


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-455-400-H



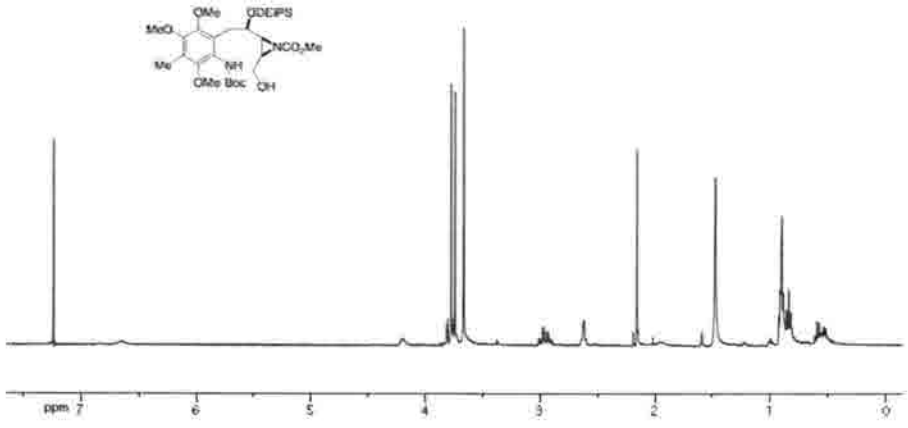
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-455-400MHz-13C

(2*S*,3*R*)-methyl-2-((*R*)-2-(2-(*tert*-butoxycarbonylamino)-3,5,6-trimethoxy-4-methyl-phenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-(hydroxymethyl)aziridine-1-carboxylate (138b)

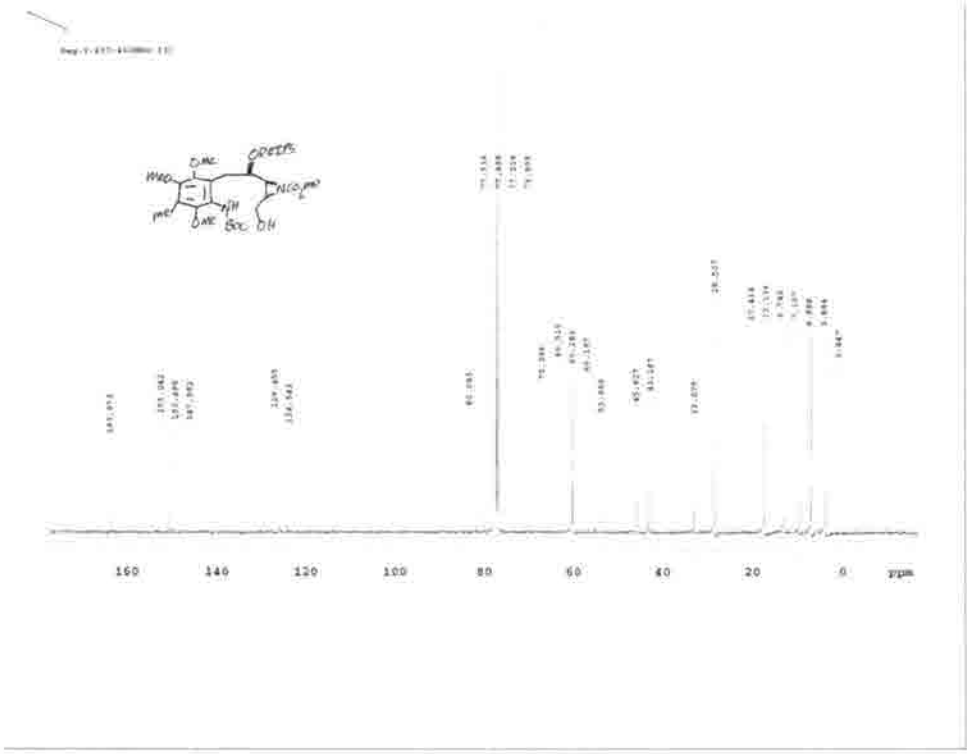


To a stirred solution of the carbamate **137b** (820 mg, 1.14 mmol) in 15:1 solution of dichloromethane/H_2O (12 mL) was added DDQ (336 mg, 1.48 mmol) in one portion. The reaction mixture immediately turned black and over the course of 1.5 h the mixture turned dark blue. After 2 h, the reaction mixture was passed through a short pad of activated alumina using 10:1 dichloromethane/methanol as eluant. The filtrate was

concentrated under reduced pressure and the crude product was purified by flash column chromatography (3:2 hexanes/ethyl acetate) to afford the alcohol **138b** (475 mg, 70%) as a colorless oil. $[\alpha]_D^{20}$ -27.5 (*c*=0.4, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.44-0.61 (4H, m), 0.80-1.02 (13H, m), 1.48 (9H, s), 2.16 (3H, s), 2.62 (2H, m), 2.95 (2H, m), 3.66 (3H, s), 3.74 (3H, s), 3.77 (3H, s), 4.20 (1H, m), 6.65 (1H, s); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.8, 6.9, 7.1, 9.7, 13.1, 17.4, 28.5, 33.1, 43.2, 45.8, 53.9, 60.2, 60.3, 60.5, 70.4, 80.1, 124.5, 126.4, 147.9, 150.5, 151.0, 163.9; IR (neat) 3389, 2939, 2876, 1728, 1465, 1263, 1090 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{29}H_{50}N_2O_9Si$ 599.3363, $(MH)^+$, Found 599.3351.

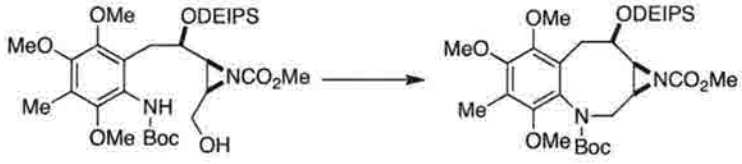


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-457-400MHz-1H



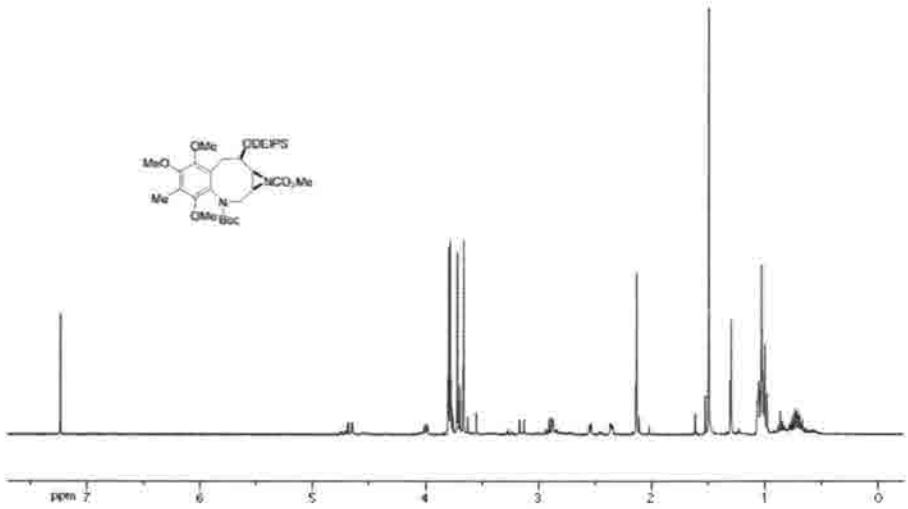
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-457-400MHz-13C

(1a*S*,9*R*,9a*S*)-3-*tert*-butyl 1-methyl 9-(diethyl(isopropyl)silyloxy)-4,6,7-trimethoxy-5-methyl-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (139b)

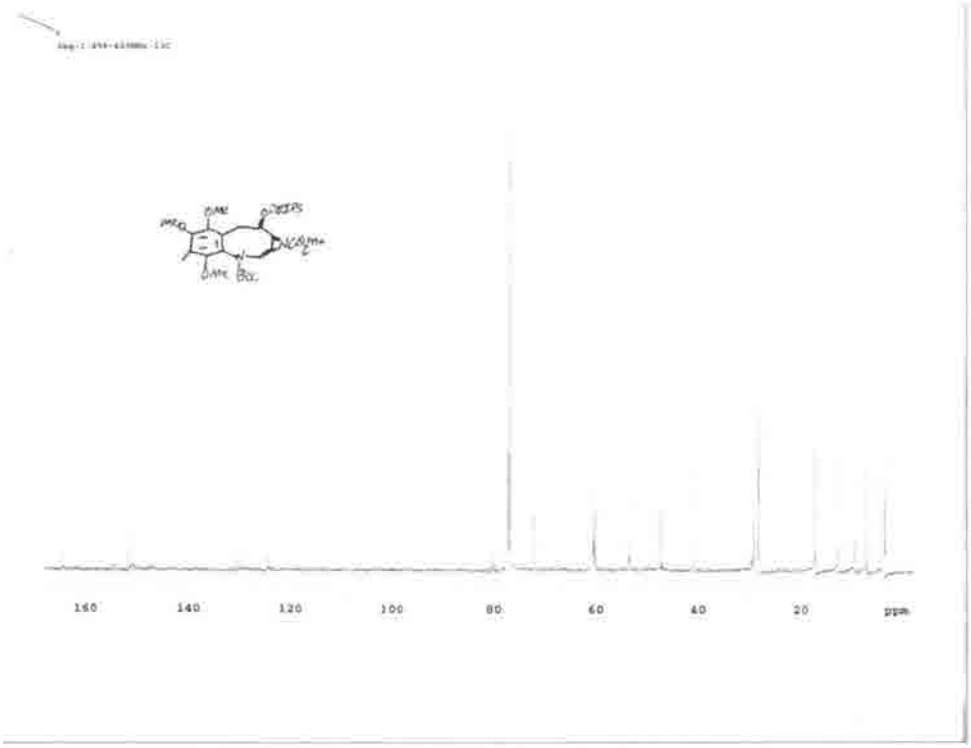


To a solution of **138b** (475 mg, 0.79 mmol) and tributylphosphine (356 μL, 1.43 mmol) in dry toluene (35 mL) was added TMAD (205 mg, 1.19 mmol). The solution immediately turned black and slowly changed to a red cloudy color as the reaction progressed. The mixture was stirred under argon at room temperature and monitored by TLC until complete consumption of the starting material (24 h). The reaction mixture was

concentrated under reduced pressure and the residue was purified by flash column chromatography (4:1 hexanes/ethyl acetate) to afford benzazocine **139b** (270 mg, 60%) as a colorless oil. $[\alpha]_D^{20}$ +30.7 (c=1.7, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.52-0.78 (4H, m), 0.98-1.06 (13H, m), 1.30 (3H, s), 1.49 (6H, s), 2.13 (3H, s), 2.35 (1H, dd, J = 6.8, 2.8 Hz), 2.53 (1H, dd, J = 6.8, 0.8 Hz), 2.86-2.91 (2H, m), 3.14 (1H, dd, J = 16.0, 0.8 Hz), 3.66 (3H, s), 3.72 (3H, s), 3.78 (3H, s), 3.79 (3H, s), 3.99 (1H, dt, J = 8.8, 4.8, 2.4), 4.67 (1H, dd, J = 15.6, 4.0 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) 3.7, 3.9, 7.3, 9.7, 13.0, 17.4, 17.5, 28.4, 28.5, 29.2, 40.9, 47.7, 53.6, 60.2, 60.5, 60.7, 72.3, 80.5, 124.8, 129.6, 130.1, 130.9, 147.5, 151.1, 151.7, 154.4, 155.0, 164.7; IR (neat) 2953, 2876, 1731, 1699, 1470, 1245, 1094 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{29}H_{48}N_2O_8Si$ 580.3179 $(MH)^+$, Found 580.3155.

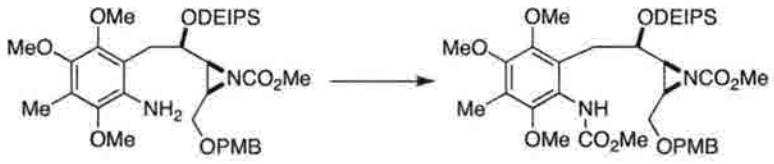


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-458-400MHz-1H



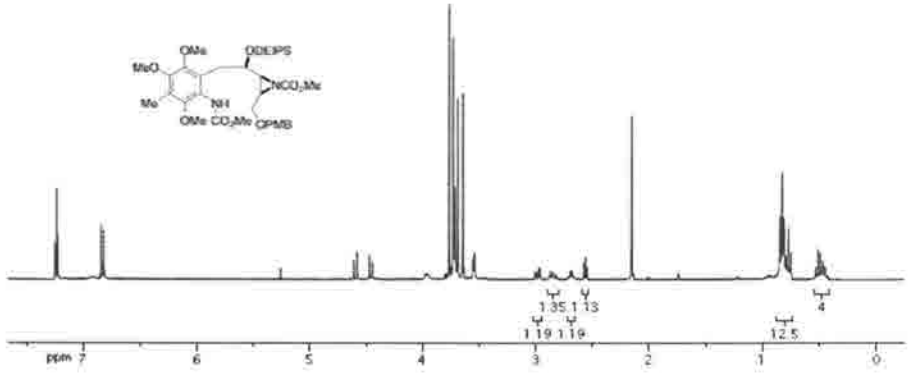
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-458-400MHz-13C

(2*S*,3*R*)-methyl2-((*R*)-1-(diethyl(isopropyl)silyloxy)-2-(2,3,5-trimethoxy-6-(methoxycarbonylamino)-4-methylphenyl)ethyl)-3-((4-methoxybenzyloxy)methyl) aziridine-1-carboxylate (137c)

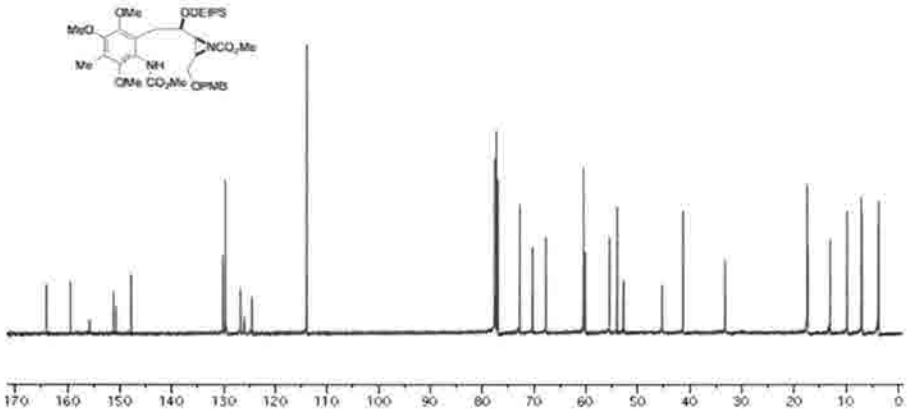


To aniline **134** (330 mg, 0.53 mmol) in dichloromethane (10 mL) was added $NaHCO_3$ (90 mg, 1.06 mmol) and methyl chloroformate (90 μL, 0.80 mmol). After stirring at room temperature for 17 hrs, the reaction mixture was diluted with water and extracted with dichloromethane. The combined organics were then washed (brine), dried (Na_2SO_4), filtered and concentrated. Purification of the crude product by flash column

chromatography (7:3 hexanes/ethyl acetate) gave the desired carbamate **137c** (210 mg, 75%) as a colorless oil. $[\alpha]_D^{20}$ +2.0 (c=0.65, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.42-0.53 (4H, m), 0.75-0.85 (13H, m), 2.15 (3H, s), 2.56 (1H, t, J = 5.6 Hz), 2.68 (1H, q, J = 6.0 Hz), 2.84 (1H, dd, J = 17.6, 8.8 Hz), 2.98 (1H, dd, J = 13.6, 4.8 Hz), 3.54 (1H, d, J = 5.6 Hz), 3.64 (3H, s), 3.69 (3H, s), 3.72 (3H, s), 3.73 (3H, s), 3.76 (3H, s), 3.95 (1H, m), 4.45 (1H, d, J = 11.6 Hz), 4.59 (1H, d, J = 11.6 Hz), 6.83 (2H, d, J = 8.8 Hz), 6.91 (1H, s), 7.23 (2H, d, J = 8.4 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.7, 3.8, 6.9, 7.1, 9.8, 13.0, 17.3, 17.4, 33.2, 41.3, 45.3, 52.7, 54.0, 55.4, 60.1, 60.5, 67.7, 70.2, 72.8, 113.9, 124.5, 126.0, 126.8, 129.7, 130.2, 147.8, 150.7, 151.2, 155.7, 159.4, 164.0; IR (neat) 3326, 2953, 2875, 1731, 1513, 1464, 1233, 1090 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{34}H_{52}N_2O_{10}Si$ 699.3283, $(MH)^+$, Found 699.3267.

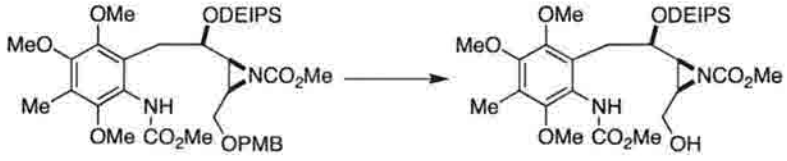


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-III-035-400-1H



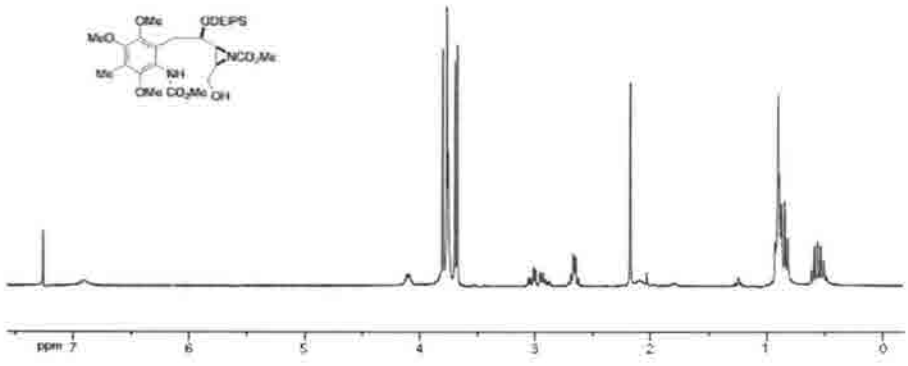
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-III-035-400-13C

(2*S*,3*R*)-methyl2-((*R*)-1-(diethyl(isopropyl)silyloxy)-2-(2,3,5-trimethoxy-6-(methoxy carbonylamino)-4-methylphenyl)ethyl)-3-(hydroxymethyl)aziridine-1-carboxylate (138c)

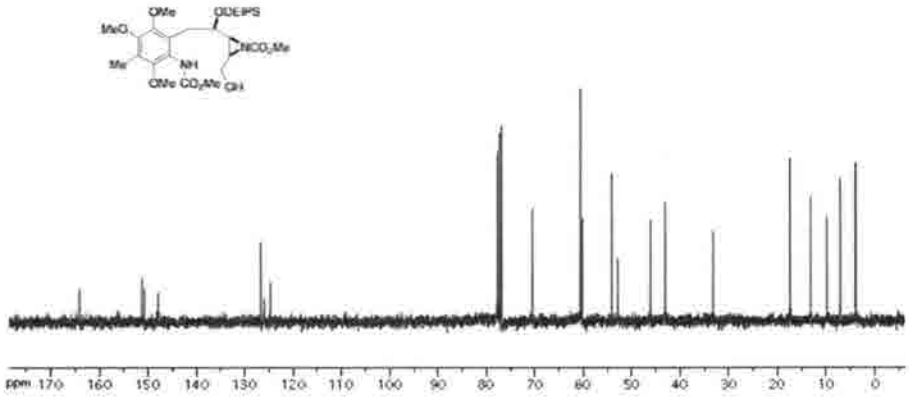


To a stirred solution of the carbamate **137c** (820 mg, 1.21 mmol) in 15:1 solution of dichloromethane/H_2O (26 mL) was added DDQ (350 mg, 1.57 mmol) in one portion. The reaction mixture immediately turned green and over the course of 1.5 h the mixture turned brown-orange. After 2 h, the reaction mixture was passed through a short pad of activated alumina using 10:1 dichloromethane/methanol as eluant. The filtrate was

concentrated under reduced pressure and the crude product was purified by flash column chromatography (1:1 hexanes/ethyl acetate) to afford the alcohol **138c** (500 mg, 73%) as a colorless oil. $[\alpha]_D^{20}$ -13.7 (*c*=1.7, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.48-0.62 (4H, m), 0.82-0.93 (13H, m), 2.09 (1H, s), 2.18 (3H, s), 2.67 (2H, m), 2.91 (1H, m), 3.02 (1H, dd, *J* = 18.0, 7.2 Hz), 3.67 (3H, s), 3.69 (3H, s), 3.75 (3H, s), 3.76 (3H, s), 3.80 (3H, s), 4.09 (1H, m), 6.90 (1H, s); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.8, 3.9, 7.0, 7.1, 9.8, 13.2, 17.4, 33.2, 43.1, 46.1, 52.8, 54.1, 60.2, 60.6, 70.5, 124.7, 125.9, 126.7, 147.9, 150.8, 151.3, 164.1; IR (neat) 3379, 2953, 2876, 1731, 1505, 1464, 1233, 1091 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{26}H_{44}N_2O_9Si$ 557.2885, $(MH)^+$, Found 557.2888.

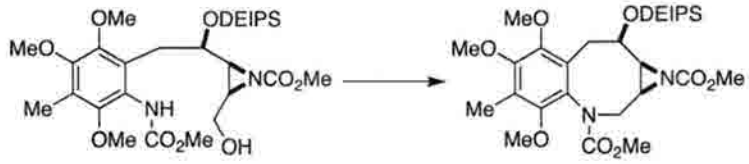


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-III-037



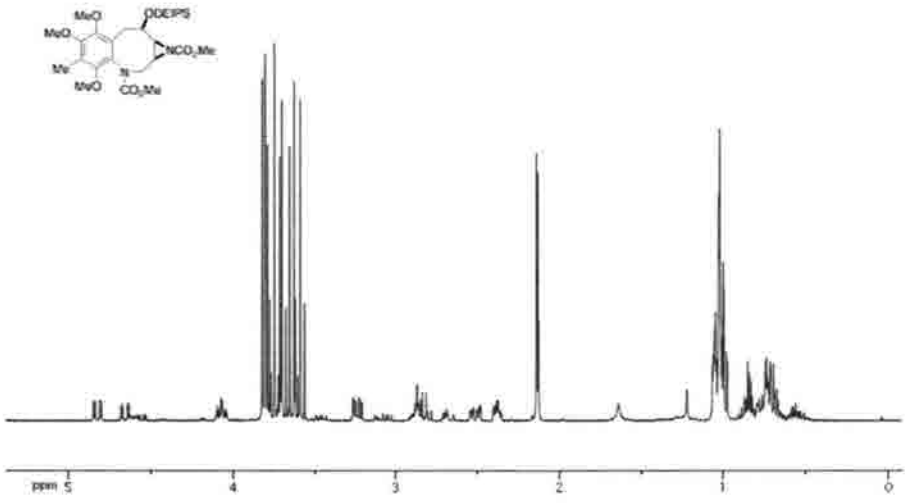
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-III-037-13C

(1a*S*,9*R*,9a*S*)-dimethyl-9-(diethyl(isopropyl)silyloxy)-4,6,7-trimethoxy-5-methyl-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (139c)

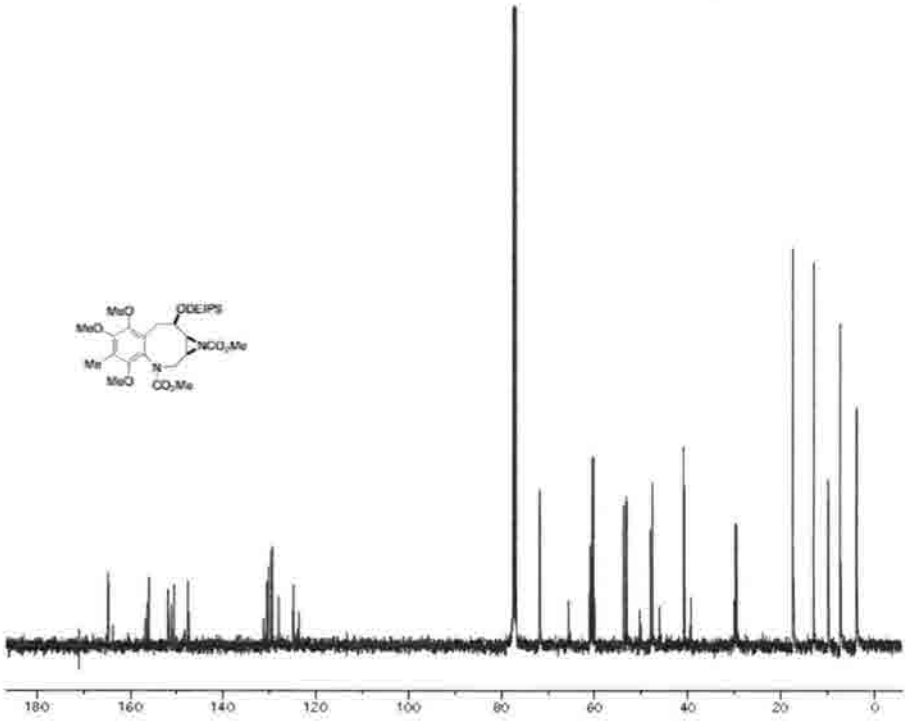


To a solution of **138c** (350 mg, 0.63 mmol) and tributylphosphine (282 μL, 1.13 mmol) in dry toluene (25 mL) was added TMAD (161 mg, 0.94 mmol). The solution immediately turned yellow and slowly changed to a white cloudy color as the reaction progressed. The mixture was stirred under argon at room temperature and monitored by TLC until complete consumption of the starting material (24 h). The reaction mixture was

concentrated under reduced pressure and the residue was purified by flash column chromatography (3:2 hexanes/ethyl acetate) to afford the benzazocine **139c** (324 mg, 96%) as a colorless oil. $[\alpha]_D^{20}$ +41.3 (c=0.8, $CHCl_3$); ^{1}HNMR and ^{13}CNMR exist as a 1:1 mixture of rotamers that did not coalesce at 323 K. ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.65-0.79 (m), 0.81-0.89 (m), 2.13 (s), 2.14 (s), 2.35-2.42 (m), 2.49 (ddd, J = 6.8, 2.4, 0.8 Hz), 2.54 (ddd, J = 6.8, 2.4, 0.8 Hz), 2.68-2.71 (m), 2.78-2.93 (m), 3.22 (dd, J = 6.8, 1.6 Hz), 3.25 (dd, J = 6.8, 1.6 Hz), 3.58 (s), 3.62 (s), 3.65 (s), 3.70 (s), 3.71 (s), 3.75 (s), 3.79 (s), 3.80 (s), 3.81 (s), 3.82 (s), 4.04-4.10 (m), 4.65 (dd, J = 15.2, 4.0 Hz), 4.83 (dd, J = 15.2, 4.0 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 3.6, 3.8, 7.3, 9.7, 9.8, 12.9, 17.5, 29.4, 29.8, 39.3, 40.7, 40.9, 47.5, 47.9, 48.0, 53.0, 53.1, 53.7, 53.8, 60.2, 60.5, 60.7, 60.9, 61.2, 65.6, 71.7, 71.8, 123.7, 124.9, 125.0, 128.0, 129.4, 129.7, 130.3, 130.7, 131.3, 147.4, 147.6, 150.6, 151.1, 151.2, 151.8, 151.9, 156.0, 156.3, 156.8, 163.7, 164.6, 164.8; IR (neat) 2945, 2868, 1731, 1460, 1244, 1091 cm^{-1}.

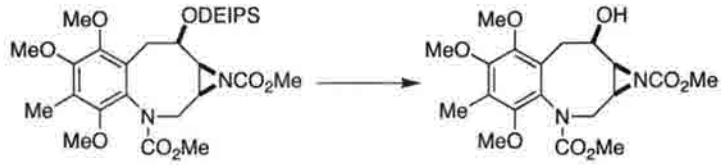


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-III-039-1H-400

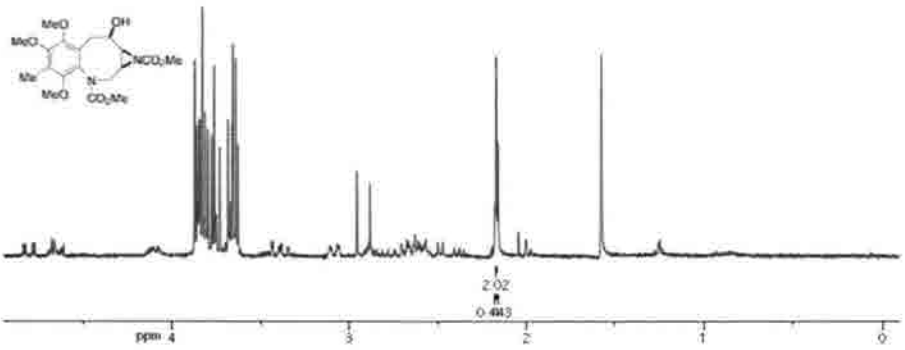


^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-III-039-13C-400

(1a*S*,9*R*,9a*S*)-dimethyl 9-hydroxy-4,6,7-trimethoxy-5-methyl-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate

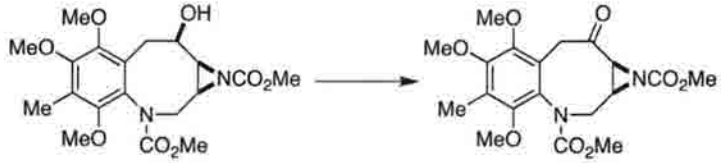


To a solution of benzazocine **139c** (400 mg, 0.74 mmol) in 10:1 DMF/H_2O (15 mL) was added TASF (306 mg, 1.11 mmol) and stirred overnight. The solution was quenched by addition of sat. $NaHCO_3$ and extracted 3 times with dichloromethane. The organic portions were combined, dried with Na_2SO_4 and concentrated. The crude product was purified by column chromatography (7:3 ethyl acetate/hexanes) to give the alcohol (300 mg, 90%) and a colorless oil. ^{1}HNMR exists as a 2:1:0.4 mixture of rotamers that did not coalesce at 393 K. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.16, (s), 2.17 (s), 2.18 (s), 2.35-2.41 (m), 2.47 (d, J = 0.8 Hz), 2.49 (d, J = 0.8 Hz), 2.54-2.59 (m), 2.60-2.62 (m), 2.63-2.64 (m), 2.65-2.67 (m), 2.69-2.71 (m), 2.73-2.75 (m), 3.05-3.08 (m), 3.09-3.12 (m), 3.34-3.35 (m), 3.38-3.41 (m), 3.43-3.44 (m), 3.63 (s), 3.64 (s), 3.66 (s), 3.67 (s), 3.69 (s), 3.73 (s), 3.77 (s), 3.78 (s), 3.80 (s), 3.82 (s), 3.83 (s), 3.84 (s), 3.85 (s), 3.86 (s), 3.87 (s), 4.07-4.13 (m), 4.61-4.69 (m), 4.78 (d, J = 3.6 Hz), 4.83 (d, J = 3.6 Hz).



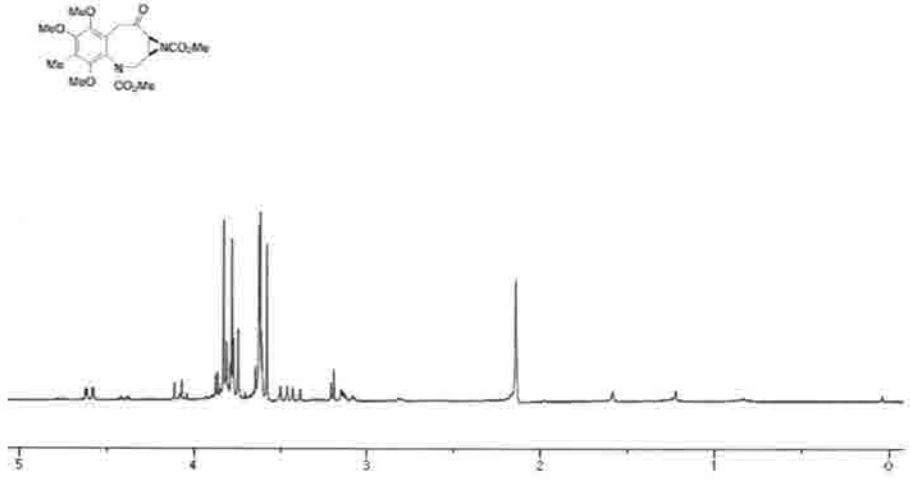
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-II-239

(1a*S*,9a*S*)-dimethyl-4,6,7-trimethoxy-5-methyl-9-oxo-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (144c)

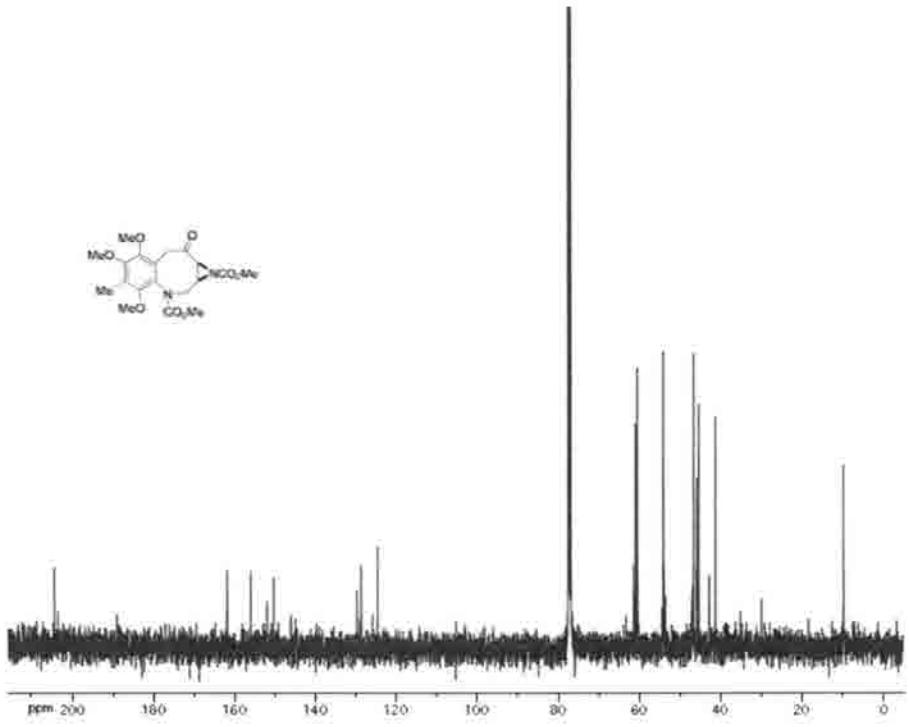


To a solution of the secondary alcohol obtained above (300 mg, 0.73 mmol) in dichloromethane (35 mL) with 4Å molecular sieves (350 mg) was added *N*-methylmorpholine *N*-oxide (NMO) (130 mg, 1.09 mmol), tetrapropylammonium perruthenate (TPAP) (13 mg, 0.036 mmol) and stirred for 1 hr at room temperature. The solution was filtered through a silica plug using ethyl acetate as eluant and concentrated to give pure **144c** (300 mg, 100%) as a white foam. $[\alpha]_D^{20}$ -24.5 (c=0.9, $CHCl_3$); ^{1}HNMR

(400 MHz, $CDCl_3$) δ TMS: 2.14 (3H, s), 3.06-3.15 (1H, m), 3.20 (1H, d, J = 6.8 Hz), 3.38-3.50 (2H, m), 3.58 (3H, s), 3.62 (3H, s), 3.63 (3H, s), 3.78 (3H, s), 3.83 (3H, s), 4.04-4.11 (1H, m), 4.59 (1H, dd, J = 15.2, 3.6 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 9.8, 41.2, 45.3, 45.9, 46.7, 54.1, 60.3, 60.4, 60.8, 124.5, 128.6, 129.7, 150.3, 152.0, 156.0, 161.9; IR (neat) 3000, 2953, 2948, 1721, 1467, 1276, 1251, 1081, 1030 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{19}H_{24}N_2O_8$ 409.1610, $(MH)^+$, Found 409.1605.

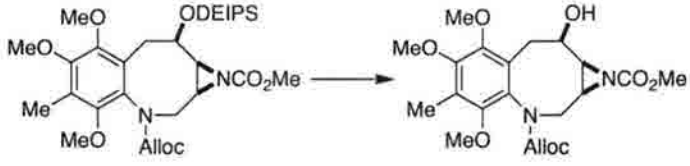


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-II-241-400-1H



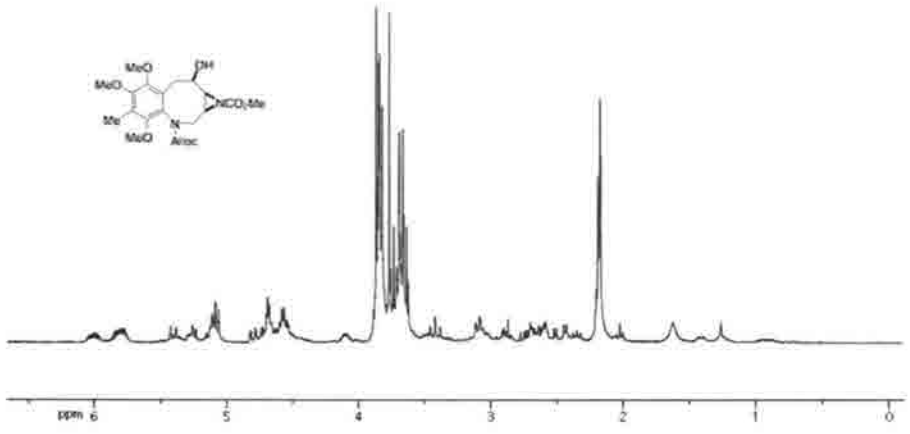
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-II-241-400-13C

(1a*S*,9*R*,9a*S*)-3-allyl 1-methyl 9-hydroxy-4,6,7-trimethoxy-5-methyl-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate

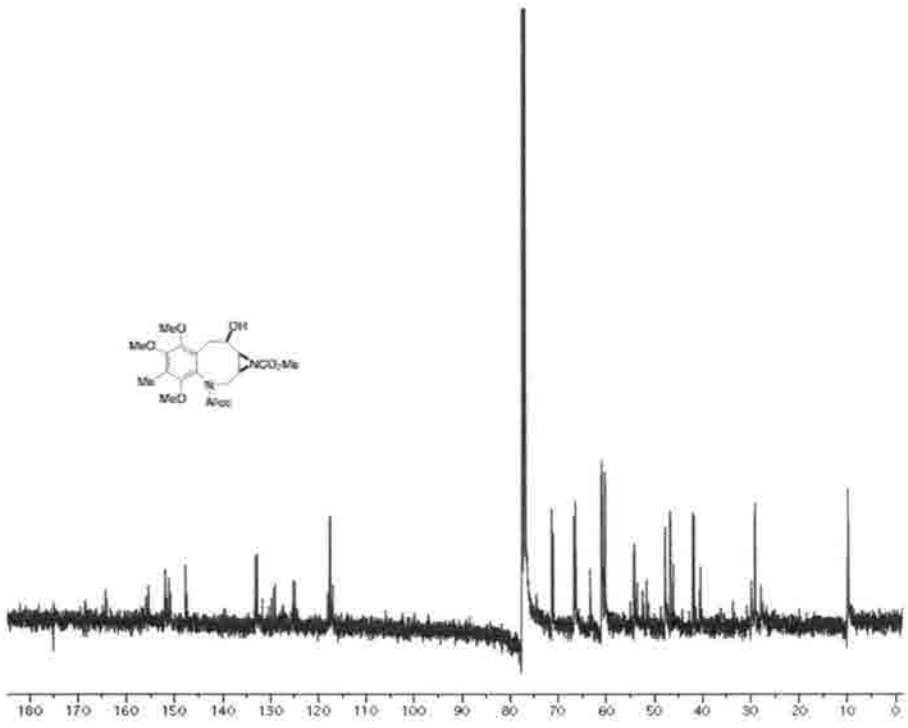


To the alloc carbamate described above (200 mg, 0.35 mmol) in 7 mL of 10:1 DMF/water was added tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) (150 mg, 0.53 mmol) and stirred for 12 hrs. The reaction was quenched with sat. aq. NH_4Cl, extracted (4 x dichloromethane), combined organics washed (brine), dried (Na_2SO_4), filtered, and concentrated. Purification by column chromatography (3:1 ethyl acetate/hexanes) afforded the desired alcohol (130 mg, 85%) as a colorless oil. $[\alpha]_D^{20}$ -0.3

(c=1.9, $CHCl_3$); Note: ^{1}HNMR and ^{13}CNMR spectra (shown on the next page) are reported as a 1.4:1 mixture of rotamers at room temperature. At 323K no onset of coalescence was observed; HRMS (FAB+) m/z Calc. for $C_{21}H_{28}N_2O_8$ 437.1918, $(MH)^+$, Found 437.1926.

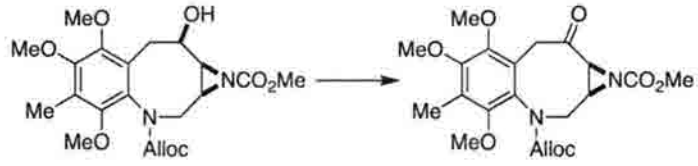


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-366



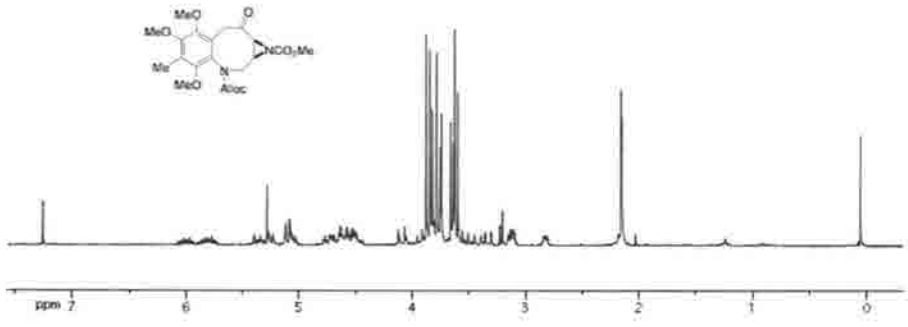
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: gz-II-040204-C13

(1a*S*,9a*S*)-3-allyl 1-methyl 4,6,7-trimethoxy-5-methyl-9-oxo-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (144a)



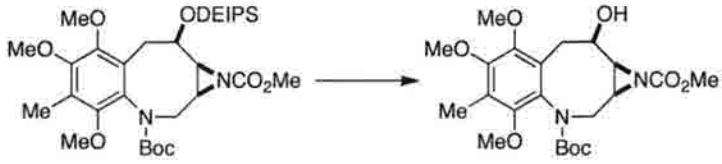
To alcohol from the previous step (130 mg, 0.31 mmol) in dichloromethane (10 mL) was added Dess-Martin periodinane (254 mg, 0.53 mmol) and stirred for 2 hrs. The reaction was diluted with ether (20 mL), poured into 20 mL sat. aq. $NaHCO_3$ with 7.0 equivalents of sodium thiosulfate (0.55 g, 2.17 mmol), and stirred for 15 min. The layers were separated and the aqueous layer was backextracted (5 x ether). The combined ether layers

were washed (1 x sat. aq. $NaHCO_3$, 1 x Brine), dried (Na_2SO_4), filtered and concentrated. Purification by column chromatography (3:2 hexanes/ethyl acetate) gave the desired ketone **144a** (98 mg, 75%) as a colorless oil. ^{1}HNMR (below) is reported as a 1:1 mixture of rotamers that failed to coalesce during VTNMR experiments at 323 K. HRMS (FAB+) m/z Calc. for $C_{21}H_{26}N_2O_8$ 435.1762, $(MH)^+$, Found 435.1764.

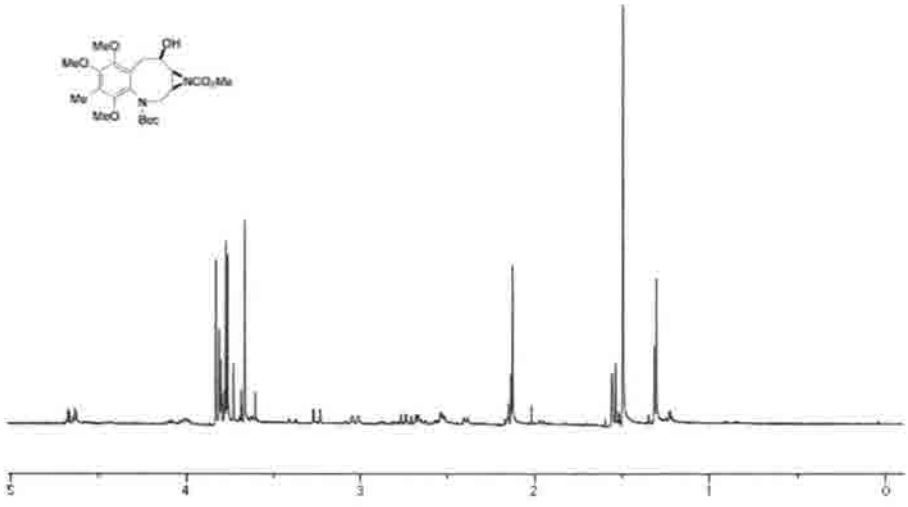


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-I-367

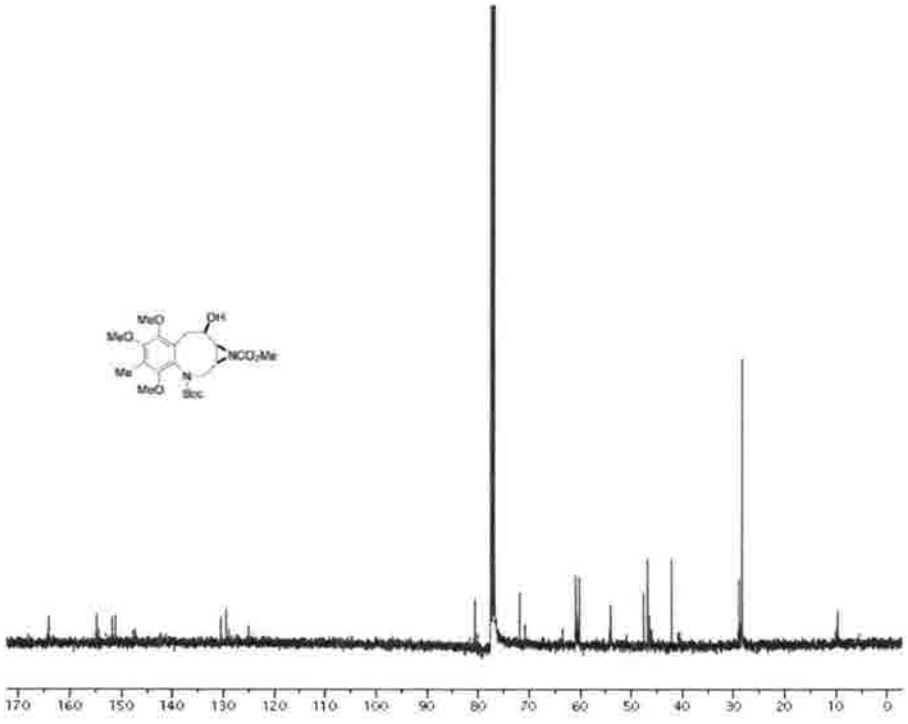
(1a*S*,9*R*,9a*S*)-3-*tert*-butyl 1-methyl 9-hydroxy-4,6,7-trimethoxy-5-methyl-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate



To a solution of benzazocine **139b** (258 mg, 0.44 mmol) in 10:1 DMF/H_2O (10 mL) was added TASF (183 mg, 0.66 mmol) and stirred overnight. The solution was quenched by addition of sat. $NaHCO_3$ and extracted 3 times with dichloromethane. The organic portions were combined, dried with Na_2SO_4 and concentrated. The crude product was purified by column chromatography (1:1 hexanes/ethyl acetate) to give the secondary alcohol (194 mg, 97%) and a colorless oil. $[\alpha]_D^{20}$ -31.0 (c=0.3, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 1.30 (s 3H), 1.49 (6H, s), 2.13 (3H, s), 2.48-2.59 (1H, m), 2.62-2.83 (2H, m), 3.01-3.09 (1H, m), 3.25 (1H, dd, J = 11.6, 1.2 Hz), 3.78 (1H, dd, J = 15.2, 2.4 Hz), 3.67 (3H, s), 3.76 (3H, s), 3.77 (3H, s), 3.83 (3H, s) 3.98-4.12 (1H, m); ^{13}CNMR (100 MHz, $CDCl_3$) δ 9.7, 28.4, 42.1, 46.5, 46.8, 47.6, 54.1, 60.2, 60.8, 60.9, 71.9, 80.6, 125.1, 129.4, 130.6, 141.7, 151.2, 151.8, 154.8, 164.2; IR (neat) 3379, 2917, 1698, 1469, 1244 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{22}H_{32}N_2O_8$ 453.2236, $(MH)^+$, Found 453.2221.

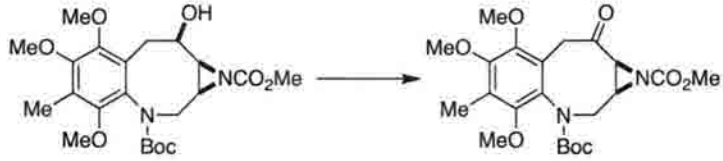


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-459-400MHz-1H



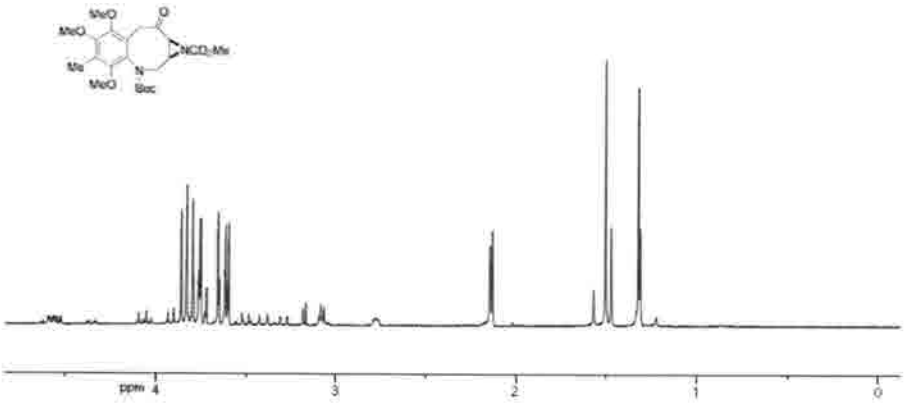
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-459-400MHz-13C

(1a*S*,9a*S*)-3-*tert*-butyl-1-methyl-4,6,7-trimethoxy-5-methyl-9-oxo-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (144b)

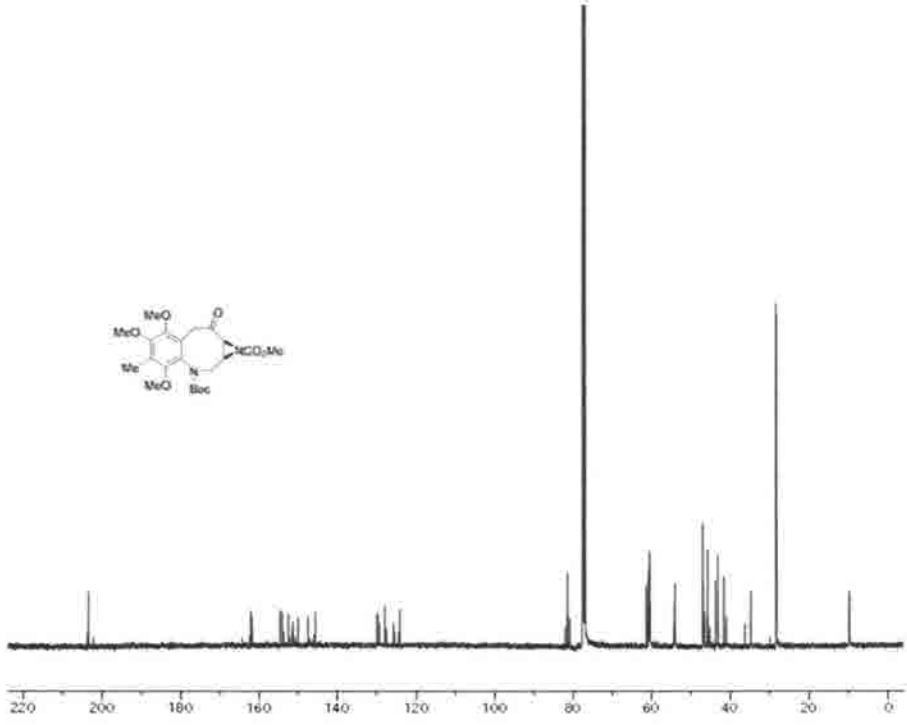


To a solution of the alcohol mentioned above (194 mg, 0.43 mmol) in dichloromethane (15 mL) was added Dess-Martin periodinane (350 mg, 0.73 mmol) and stirred for 2 hrs. The reaction was diluted with ether (15 mL) and poured into a solution of sat. $NaHCO_3$ (15 mL) with seven-fold excess of sodium thiosulfate (610 mg) and stirred for 15 minutes. The layers were separated and the aqueous layer was extracted 3 x ether. The organic layers were combined, dried over Na_2SO_4 and concentrated. Pure product was obtained by column chromatography (3:2 hexanes/ethyl acetate) to give **144b** (150 mg, 78%) as a white foam. $[\alpha]_D^{20}$ -31.0 (c=0.3, $CHCl_3$); ^{1}HNMR and ^{13}CNMR are reported as a 1:1 mixture of conformational isomers. ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 1.31 (s), 1.32 (s), 1.47 (s), 1.50 (s), 2.13 (s), 2.14 (s), 2.76-2.79 (m), 3.05-3.10 (m), 3.17 (d, J = 6.8 Hz), 3.26-3.27 (m), 3.30-3.31 (m), 3.38 (s), 3.42 (s), 3.48 (s), 3.52 (s), 3.59 (d, J = 0.8 Hz), 3.60 (d, J = 0.8 Hz), 3.61 (d, J = 0.8 Hz), 3.65 (d, J = 0.8 Hz), 3.66 (d, J = 0.8 Hz), 3.72 (d, J = 0.8 Hz), 3.74 (d, J = 0.8 Hz), 3.75 (d, J = 0.8 Hz), 3.76 (d, J = 0.8 Hz), 3.79 (d, J = 0.8 Hz), 3.83 (d, J = 0.8 Hz), 3.86 (d, J = 0.8 Hz), 3.90 (s), 3.93 (s), 4.03-4.09 (m), 4.54-4.62 (m); ^{13}CNMR (100 MHz, $CDCl_3$) δ 9.6, 9.7, 9.8, 28.2, 28.3, 28.4, 34.7, 36.2, 40.9, 41.6, 43.0, 43.1, 43.7, 45.1, 45.7, 45.8, 45.9, 46.5, 46.8, 46.8, 46.9, 53.9, 54.1, 54.2, 60.2, 60.3, 60.4, 60.5, 60.6, 60.7, 60.8, 61.2, 61.3, 80.7, 81.3, 81.5, 81.9, 124.1, 125.7, 127.6, 127.9, 129.3, 129.7, 129.8, 129.9, 145.7, 147.7, 150.2, 151.5, 152.7, 154.2, 154.8,

162.0, 162.3, 203.5; IR (neat) 2938, 2847, 1735, 1700, 1469, 1273, 1028; HRMS (FAB+) m/z Calc. for $C_{22}H_{30}N_2O_8$ 450.2002, $(MH)^+$, Found 450.2013.

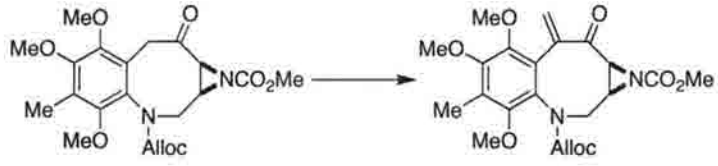


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-I-461-400MHz-1H



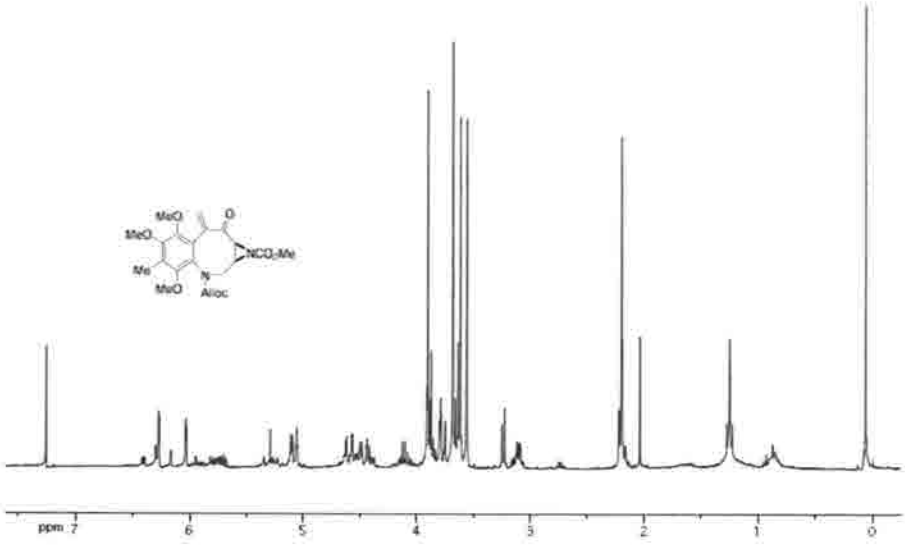
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-I-461-400MHz-13C

(1a*S*,9a*S*)-3-allyl 1-methyl 4,6,7-trimethoxy-5-methyl-8-methylene-9-oxo-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (146)



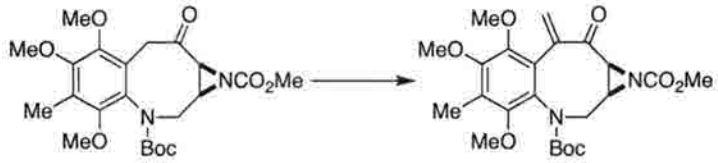
To benzazocane **144a** (77 mg, 0.18 mmol) and 2-propanol (1.5 mL) in a small vial was added dimethylamine HCl salt (17 mg, 0.21 mmol), formalin (70 μL, 0.87 mmol), triethylamine (8 μL, 0.054 mmol), water (115 μL) and heated at 90 °C for 12 hrs. After cooling to room temperature, the solution was diluted with chloroform and washed successively with 10% citric acid, sat. aq. Sodium bicarbonate, and brine. The solution was then dried (magnesium sulfate), filtered and concentrated. Purification of the crude

by PTLC (1:1 ethyl acetate/hexanes) gave enone **146** (64 mg, 82%) as a yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.20 (3H, s), 3.06-3.15 (1H, m), 3.24 (1H, d, J = 6.6 Hz), 3.56 (3H, s), 3.61 (3H, s), 3.68 (3H, s), 3.90 (3H, s), 4.37-4.64 (3H, m), 5.05-5.11 (1H, m), 5.23-5.35 (1H, s), 5.67-6.01 (1H, m), 6.03 (1H, d, J = 1.8 Hz), 6.27 (1H, d, J = 2.1 Hz).

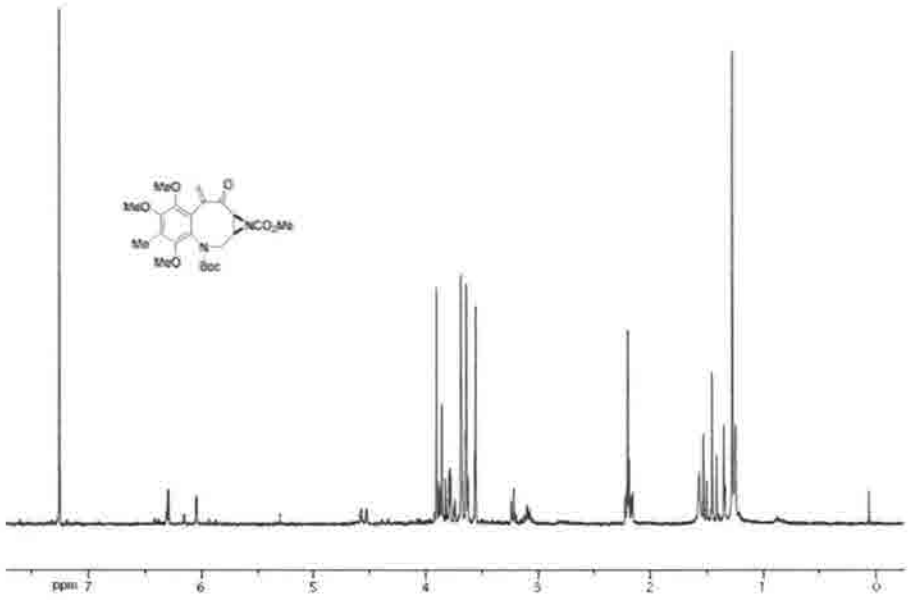


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-I-373

(1a*S*,9a*S*)-3-*tert*-butyl-1-methyl-4,6,7-trimethoxy-5-methyl-8-methylene-9-oxo-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (149)

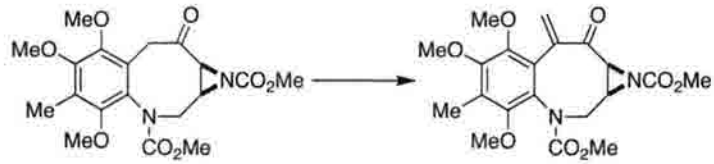


To a solution of DMSO (200 μL) in a small vial was added paraformaldehyde (1 mg, 0.036 mmol) and triton B (5 μL, 40% wt. solution in MeOH/DMSO, 0.0044 mmol) and stirred for 15 minutes. Benzazocane **144b** (10 mg, 0.022 mmol) in DMSO (800 μL) was then added and the vial was capped and heated at 75 °C for 20 hrs. The solution was allowed to cool to room temperature then quenched with sat. aq. $NaHCO_3$. Ethyl acetate was added and the layers were separated and the aqueous layer was extracted 3 x ethyl acetate. The organic portions were combined, washed with brine, dried over Na_2SO_4 and purified by PTLC (1:1 EtOAc/Hex) to give **149** (9.2 mg, 92%) as a white foam. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 1.27-1.54 (9H, s, Boc rotamers), 2.20, (3H, s), 3.07-3.10 (1H, m), 3.22 (1H, d, J = 4.5 Hz), 3.56 (3H, s), 3.64 (3H, s), 3.69 (3H, s), 3.77-3.80 (2H, m), 3.90 (3H, s), 4.55 (1H, dd, J = 15.6, 3.0 Hz), 6.04 (1H, d, J = 2.1 Hz), 6.30 (1H, d, 2.1 Hz).



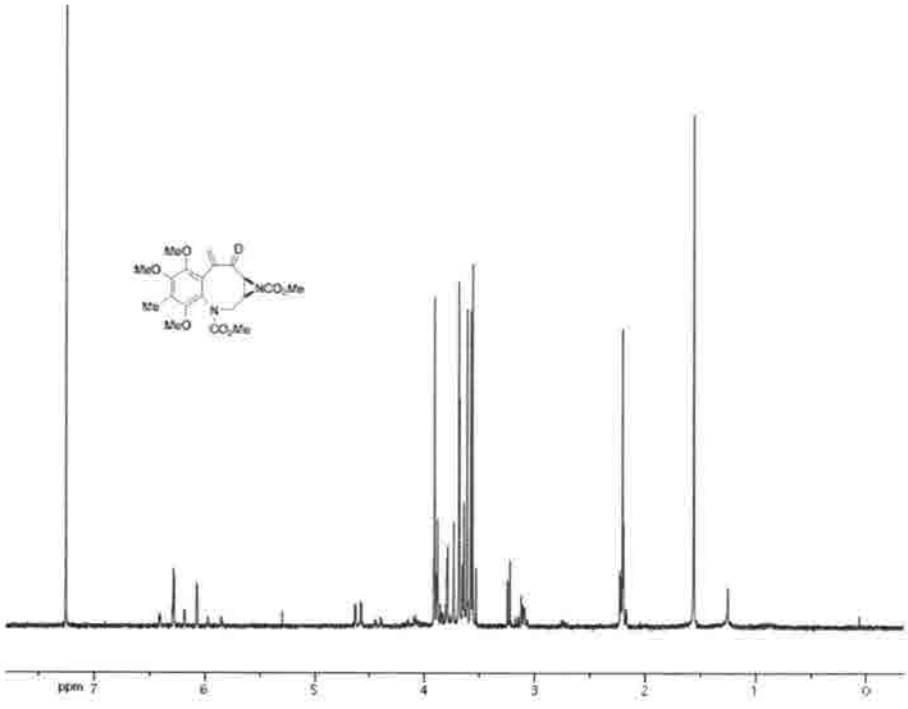
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-II-203

(1a*S*,9a*S*)-dimethyl-4,6,7-trimethoxy-5-methyl-8-methylene-9-oxo-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (150)



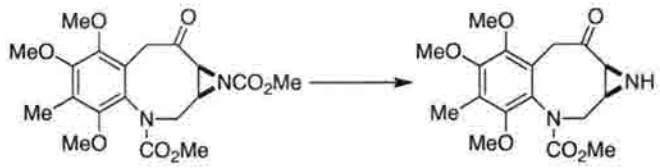
To a solution of DMSO (200 μL) in a small vial was added paraformaldehyde (1 mg, 0.036 mmol) and triton B (5 μL, 40% wt. solution in MeOH/DMSO, 0.0048 mmol) and stirred for 15 minutes. Benzazocane **144c** (10 mg, 0.024 mmol) in DMSO (800 μL) was then added and the vial was capped and heated at 75 °C for 20 hrs. The solution was allowed to cool to room temperature then quenched with sat. aq. $NaHCO_3$. Ethyl acetate was added and the layers were separated and the aqueous layer was extracted 3 x ethyl

acetate. The organic portions were combined, washed with brine, dried over Na_2SO_4 and purified by PTLC (1:1 ethyl acetate/hexanes) to give **150** (6.0 mg, 60%) as a white foam. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.20 (3H, s), 3.08-3.12 (1H, m), 3.23 (1H, d, J = 6.6 Hz), 3.56 (3H, s), 3.58 (3H, s), 3.62 (3H, s), 3.69 (3H, s), 3.79 (1H, d, J = 2.1 Hz), 3.91 (3H, s), 4.60 (1H, dd, J = 15.3, 3.0 Hz), 6.07 (1H, d, J = 2.1 Hz), 6.28 (1H, d, J = 2.1 Hz).

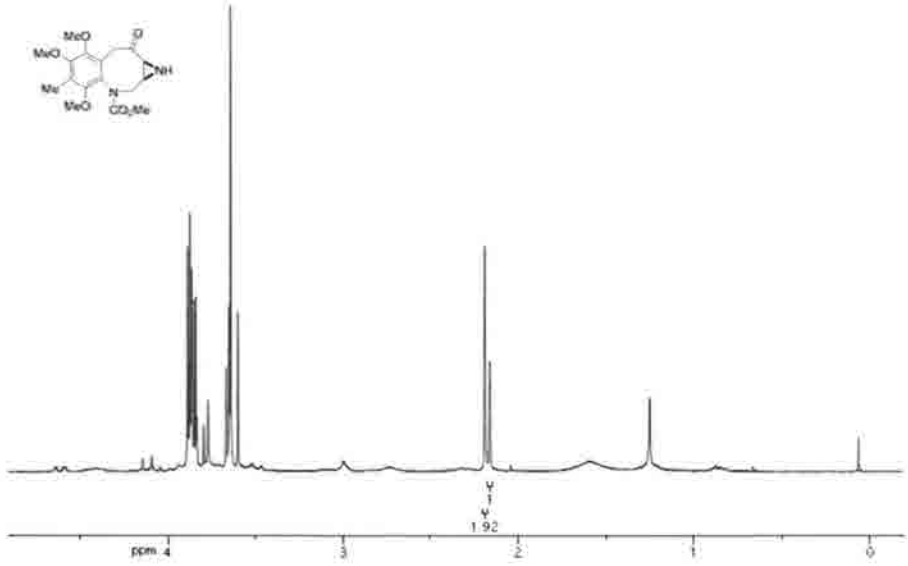


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-II-173

(1a*S*,9a*S*)-methyl-4,6,7-trimethoxy-5-methyl-9-oxo-1a,2,9,9a-tetrahydro-1*H*-azirino [2,3-*f*]benzo[*b*]azocine-3(8*H*)-carboxylate (151)

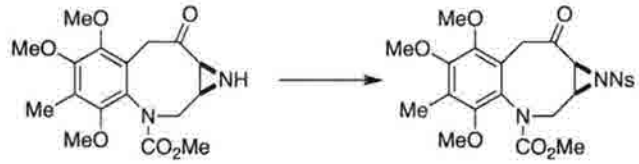


To benzazocane **144c** (10 mg, 0.025 mmol) in dichloroethane (1 mL) was added trimethyltin hydroxide (22 mg, 0.12 mmol) and heated at 80 °C for 2 hrs. After cooling to room temperature, the solution was concentrated and the resulting crude purified by PTLC (7:3 ethyl acetate/hexanes) to give free aziridine **151** (7.3 mg, 85%) as a white foam. ^{1}HNMR spectra was obtained as a 2:1 mixture of rotamers. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 1.59 (s, br), 2.16 (s), 2.19 (s), 2.73 (s, br), 2.99 (m), 3.60 (s), 3.65 (s), 3.66 (s), 3.84 (s), 3.85 (s), 3.86 (s), 3.88 (s), 3.89 (s), 4.09 (s), 4.15 (s), 4.41 (m), 4.59 (m), 4.64 (m).



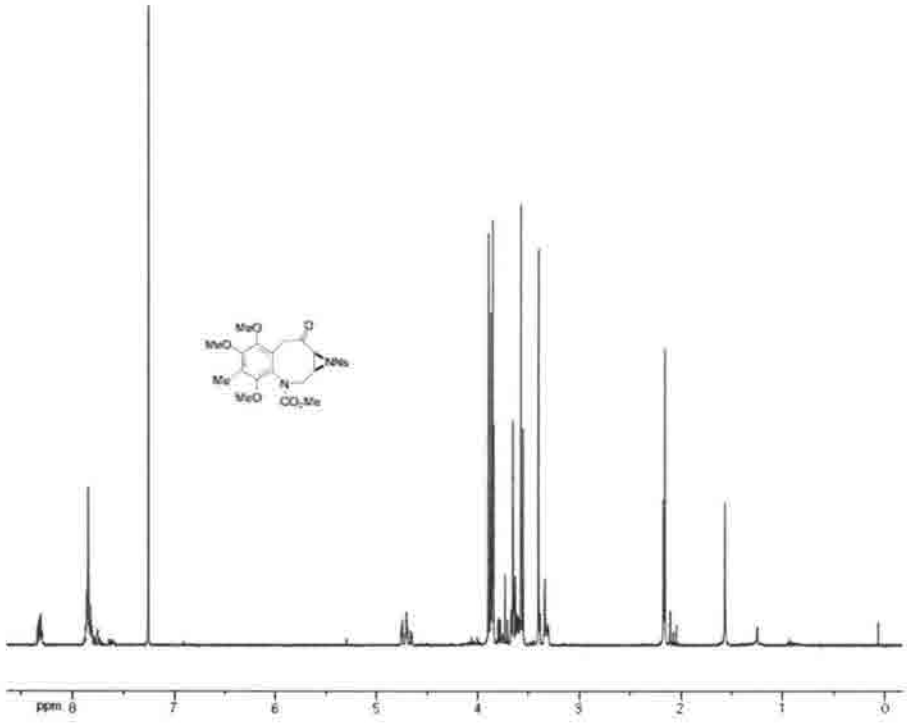
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-II-170

(1a*S*,9a*S*)-methyl-4,6,7-trimethoxy-5-methyl-1-(2-nitrophenylsulfonyl)-9-oxo-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-3(8*H*)-carboxylate (152)



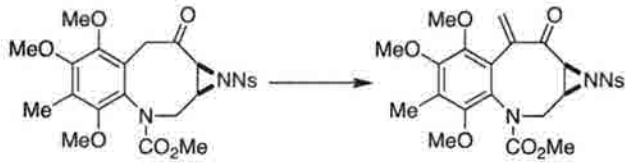
Free aziridine **151** (13 mg, 0.038 mmol) in dichloromethane (2mL) was added pyridine (10 μL, 0.12 mmol) and 2-nitrobenzenesulfonyl chloride (17 mg, 0.076 mmol) and stirred at room temperature for 15 hrs. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ and then extracted 4 x dichloromethane. The organic layers were combined, washed (brine), dried (Na_2SO_4) and concentrated. Purification of the crude material by PTLC (7:3 ethyl acetate/hexanes) provided the desired sulfonamide **152** (10 mg, 60%) as

a white foam. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.16 (3H, s), 3.30-3.35 (1H, m), 3.40 (3H, s), 3.58 (3H, s), 3.67 (3H, s), methoxy group rotamers at: 3.84 (s), 3.85 (s), 3.87 (s), and 3.89 (s). 4.65-4.76 (1H, m), 7.80-7.87 (3H, m), 8.30-8.35 (2H, m).



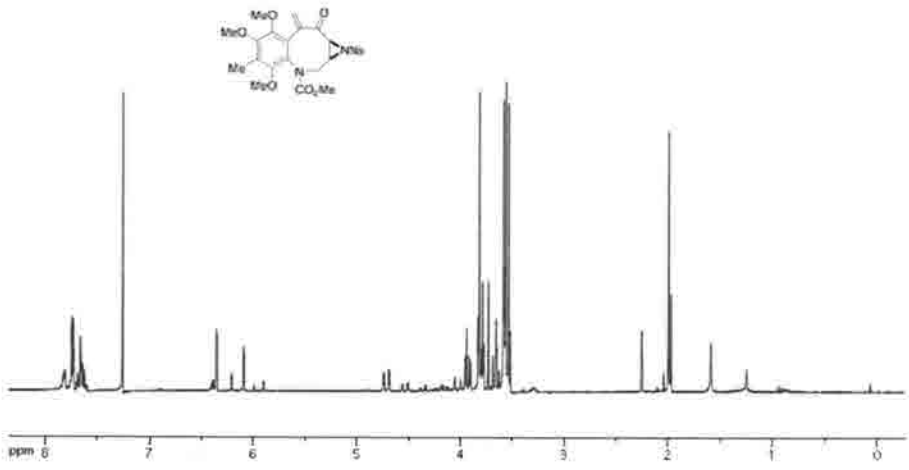
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-II-233

(1a*S*,9a*S*)-methyl 4,6,7-trimethoxy-5-methyl-8-methylene-1-(2-nitrophenylsulfonyl)-9-oxo-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-3(8*H*)-carboxylate (153)



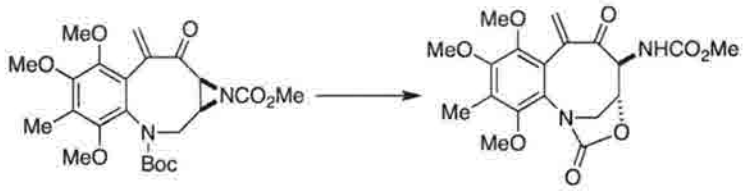
To sulfonamide **152** (24 mg, 0.045 mmol) in 20:3 THF/water (4 mL) was added formalin (255 μL, 5.01 mmol), LiOH (18 μL, 1M solution in water, 0.018 mmol) and stirred for 24

hrs. The reaction was then quenched with brine, extracted (4 x ethyl acetate), dried (Na_2SO_4), filtered and concentrated. Purification of the crude by PTLC (7:3 ethyl acetate/hexanes) provided enone **153** (15 mg, 60%) as a white foam. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.00 (3H, s), 3.54 (3H, s), 3.57 (3H, s), 3.59 (3H, s), 3.65 (1H, d, J = 2.1 Hz), 3.82 (3H, s), 3.93, (1H, dd, J = 11.4, 4.2 Hz), 4.71 (1H, dd, J = 15.6, 3.0 Hz), 6.09 (1H, d, J = 1.8 Hz, 6.35 (1H, d, J = 2.1 Hz).

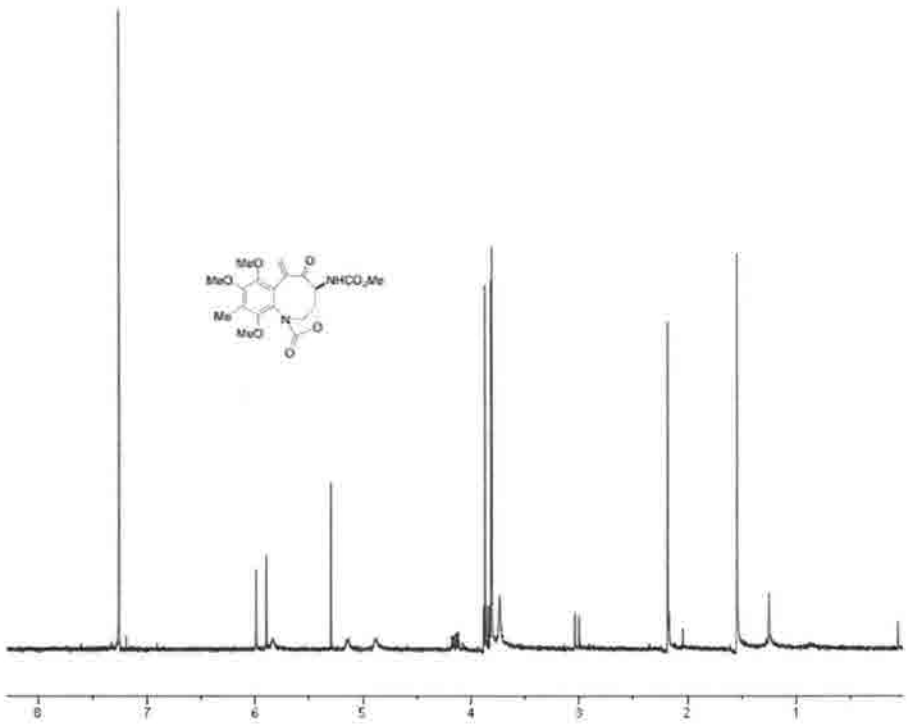


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-II-278

Undesired cyclic carbamate formation (164)

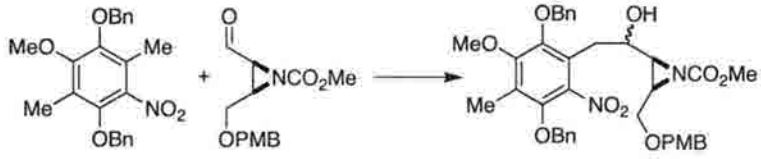


To enone **149** (9 mg, 0.019 mmol) in dichloromethane was added 4Å molecular sieves (30 mg) and cooled to 0 °C. Boron trifluoride diethyl etherate (12 μL, 0.097 mmol) in 200 μL of dichloromethane was added dropwise to the solution over 30 minutes and then stirred for an additional hour. The reaction was filtered through a pad of celite using ethyl acetate as the eluant and concentrated. Purification of the crude material using PTLC (1:1 hexanes/ethyl acetate) gave cyclic carbamate **164** (7 mg, 87%) as a colorless oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.18 (3H, s), 3.02 (1H, d, J = 11.7 Hz), 3.75 (3H, s), 3.81 (3H, s), 3.82 (3H, s), 3.87 (3H, s), 4.15 (1H, dddd, J = 17.7, 11.7, 3.9, 0.9 Hz), 4.88 (1H, m), 5.14 (1H, m), 5.83 (1H, s, br), 5.90 (1H, s), 5.99 (1H, s); IR (neat) 3334, 2923, 2851, 1772, 1728, 1695, 1506, 1470, 1119; HRMS (FAB+) m/z Calc. 406.1376, $(MH)^+$, Found 406.1362.



^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-II-201

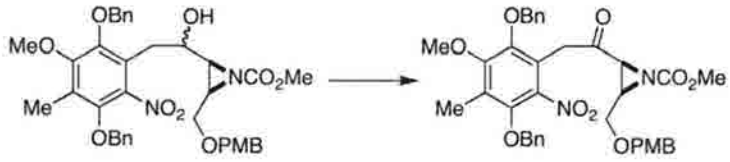
(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-hydroxyethyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (192)



Prior to the reaction, compounds **184** (15.07 g, 0.038 mol) and **81** (4.28 g, 0.015 mol) were dehydrated three times by azeotropic distillation using anhydrous toluene and lastly dried under vacuum for 1h before use. A solution of **81** in dry DMF (70 mL) was then added to freshly fused zinc chloride (3.06 g, 0.023 mol). The resulting solution was stirred under argon for 1h at room temperature. In a separate flask, NaHMDS (38 mL of a 1 M solution in THF, 0.038 mol) was added dropwise to a solution of **184** in dry DMF

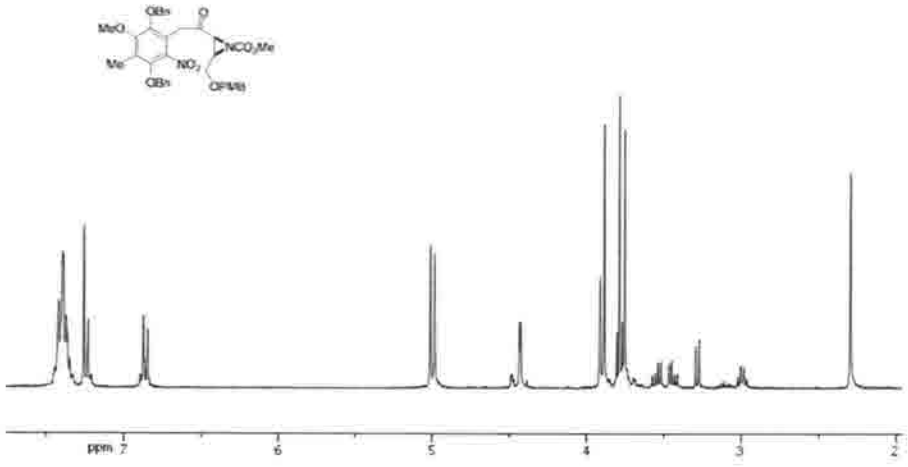
(140 ml) cooled to -45°C. The mixture turned immediately deep red. To this solution was added dropwise the aziridine solution prepared above and the resulting mixture was stirred at -45°C for 3 hrs. The reaction mixture was quenched with saturated aqueous NH_4Cl (80 mL), allowed to warm to room temperature and water (80 mL) was added. The aqueous phase was extracted with DCM (5x150 mL), the combined organics dried over Na_2SO_4 and concentrated under reduced pressure. The crude oil was purified by flash column chromatography (3:2 hexanes/ethyl acetate) to afford the alcohol **192** (8.5 g, 85%) as a pale yellow oil (1:1 mixture of separable diastereomers). To aid in ease of purification, the mixture of alcohols was taken immediately oxidized to the ketone.

(2*S*,3*R*)-methyl-2-(2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)acetyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate

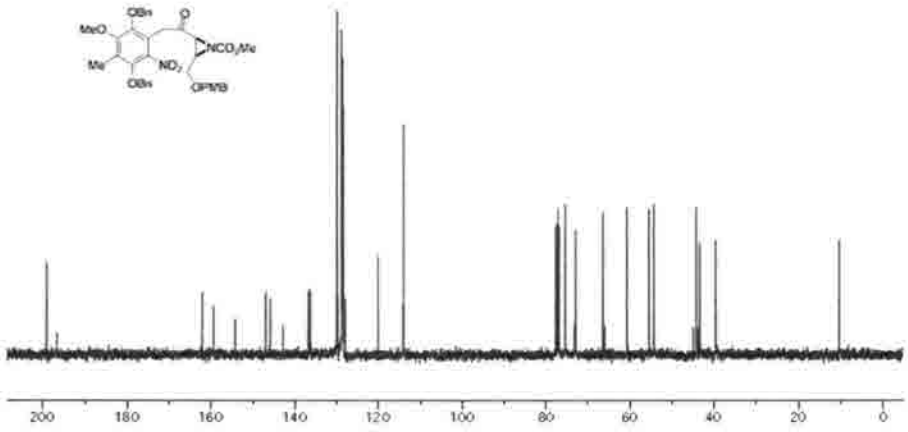


To alcohols **192** (4.2 g, 6.24 mmol) in CH_2Cl_2 (85 mL) was added Dess-Martin periodinane (4.23 g, 9.98 mmol) and stirred for 2 hrs. The reaction was diluted with ether (140 mL), poured into 100 mL sat. aq. $NaHCO_3$ with 7.0 equivalents of sodium thiosulfate (10.8 g, 43.7 mmol), and stirred for 15 min. The layers were separated and the aqueous layer was backextracted with ether. The combined ether layers were washed (1 x sat. aq. $NaHCO_3$, 1 x Brine), dried (Na_2SO_4), filtered and concentrated. Purification by column chromatography (7:3 hexanes/ethyl acetate) gave the desired ketone (4.15 g, 98%) as a pale yellow oil. $[\alpha]_D^{20}$ -31.7 (c=1.45, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.29 (3H, s), 2.98 (1H, q, J = 6.0 Hz), 3.28 (1H, d, J = 6.9 Hz), 3.43 (1H, dd, J =

11.1, 5.1 Hz), 3.54 (1H, dd, J = 11.4, 6.0 Hz), 3.75 (3H, s), 3.79 (3H, s), 3.88 (3H, s), 4.43 (2H, d, J = 2.7 Hz), 5.00 (4H, d, J = 8.4 Hz), 6.85 (2H, d, J = 8.7 Hz), 7.24 (2H, d, J = 8.5 Hz), 7.39 (10H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 10.3, 39.6, 43.4, 44.2, 54.3, 55.5, 60.7, 66.5, 72.9, 75.5, 76.9, 77.1, 114.0, 120.1, 121.9, 128.4, 128.6, 128.7, 128.8, 128.9, 129.8, 129.9, 130.0, 136.3, 136.8, 142.9, 145.9, 147.0, 154.3, 159.5, 162.2, 199.1; IR (neat) 2953, 1735, 1611, 1530, 1455, 1249, 1089 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{37}H_{38}N_2O_{10}$ 693.2418, $(MH)^+$, Found 693.2371.

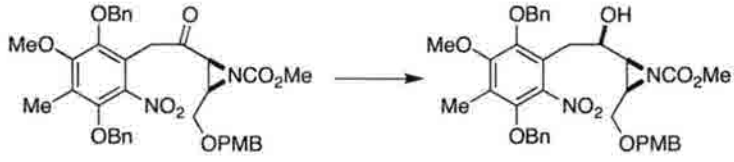


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-155-1H



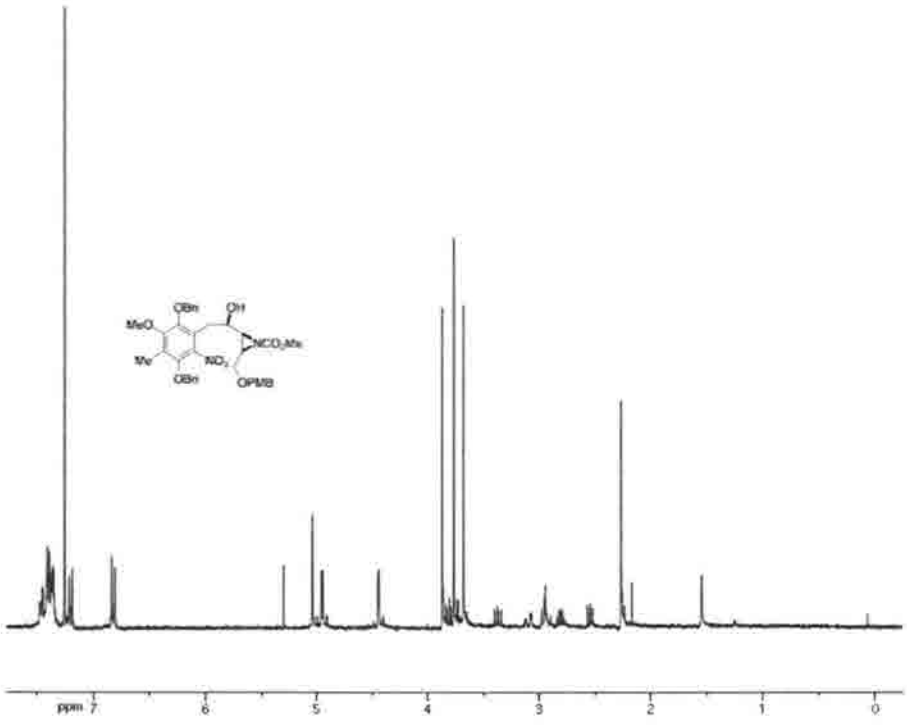
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-III-155-13C

(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-hydroxyethyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (193)



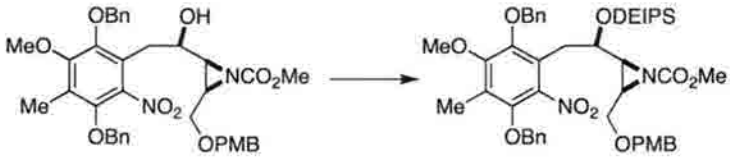
To ketone described above (1.3 g, 1.92 mmol) in 40 mL of 3:1 methanol/dichloromethane at 0 °C was added sodium borohydride (146 mg, 3.87 mmol) and stirred for 1 hr. The reaction was then quenched by the addition of brine, extracted (5 x dichloromethane), washed (brine), dried (Na_2SO_4), filtered and concentrated to give alcohol **193** (1.3 g, 99%) as a pale yellow oil that was used without further purification. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.27 (3H, s), 2.55, (1H, dd, J = 8.4, 6.3 Hz), 2.81 (1H, m), 2.93-2.98 (2H,

m), 3.07-3.13 (1H, m), 3.35 (1H, dd, J = 10.5, 7.5 Hz), 3.68 (3H, s), 3.77 (3H, s), 3.87 (3H, s), 4.44 (2H, d, J = 2.4 Hz), 4.95 (2H, d, J = 2.7 Hz), 5.04 (2H, s), 6.83 (2H, d, J = 8.7 Hz), 7.21 (2H, d, J = 8.7 Hz), 7.35-7.48 (10H, m).



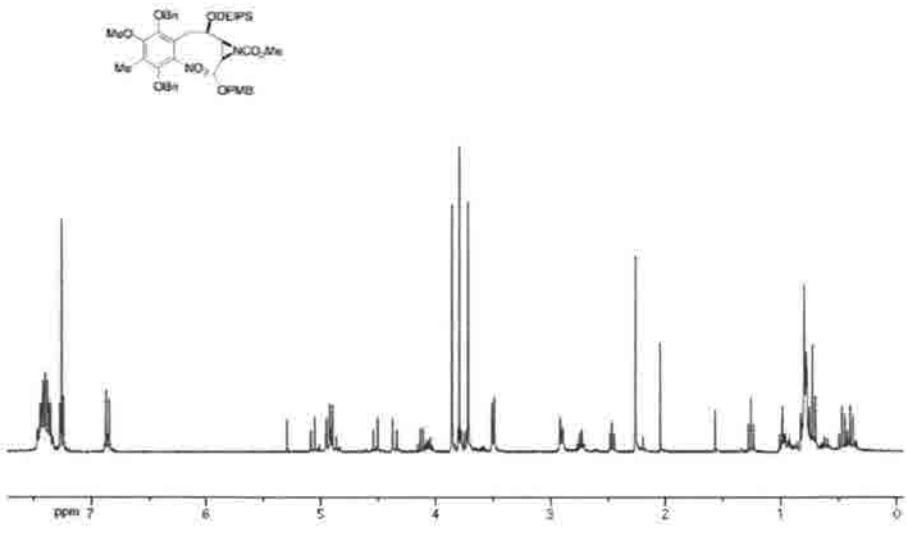
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-111

(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate



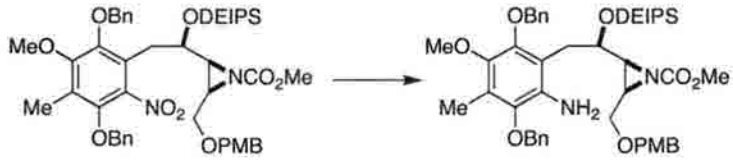
To a solution of alcohol **193** obtained above (1.15 g, 1.71 mmol) and imidazole (410 mg, 6.0 mmol) in dry dichloromethane (25 mL) was added dropwise

chlorodiethylisopropylsilane (630 μL, 3.42 mmol). The mixture was stirred at room temperature for 24h. The reaction was quenched with saturated aqueous $NaHCO_3$, diluted with dichloromethane and the layers separated. The aqueous layer was extracted with dichloromethane (2 x 50 mL). The combined organics were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (4:1 hexanes/ethyl acetate) to afford the desired silyl ether as a pale yellow oil (1.20 g, 85%). ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.34-0.50 (4H, m), 0.70-0.83 (13H, m), 2.26 (3H, s), 2.47 (1H, t, J = 6.0 Hz), 2.73 (1H, q, J = 6.3 Hz), 3.50 (2H, d, J = 5. 7Hz), 3.72 (3H, s), 3.79 (3H, s), 3.86 (3H, s), 4.03-4.08 (1H, m), 4.35 (1H, d, J = 11.4 Hz), 4.52 (1H, d, J = 11.4 Hz), 4.86-4.96 (3H, m), 5.07 (1H, d, J = 10.8 Hz), 6.85 (2H, d, J = 8.4 Hz), 7.25 (2H, d, J = 8.4 Hz), 7.33-7.47 (10H, m).

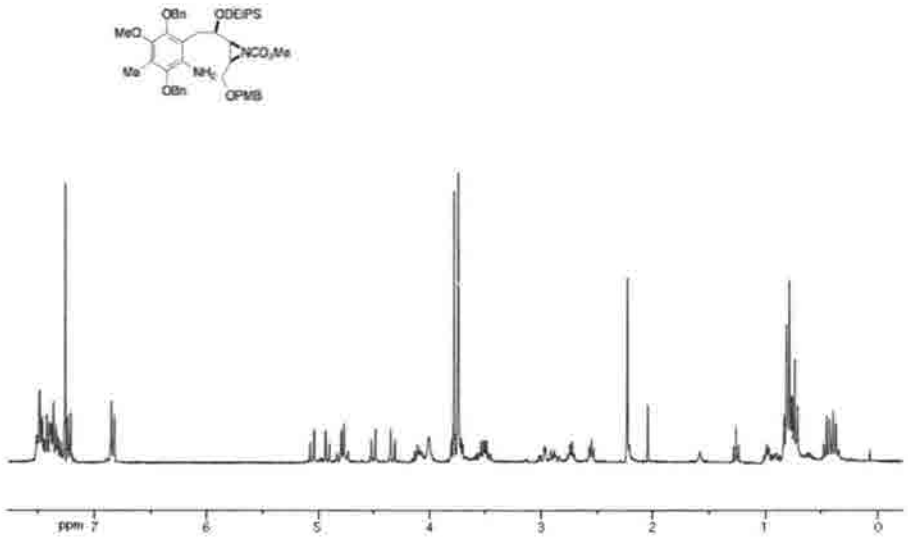


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-115

(2*S*,3*R*)-methyl 2-((*R*)-2-(2-amino-3,6-bis(benzyloxy)-5-methoxy-4-methylphenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate

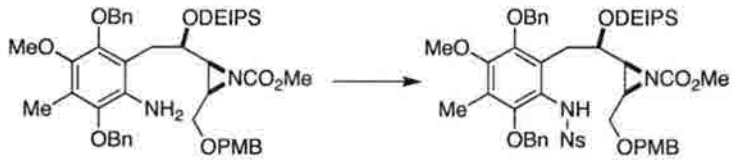


To DEIPS ether made above (1.13 g, 1.41 mmol) in 4:1 acetone/water (38 mL) was added zinc dust (462 mg, 7.05 mmol), ammonium chloride (755 mg, 14.11 mmol,), and stirred for 1 hr. The solution was then concentrated, diluted (ethyl acetate), washed (1 x water, 1 x brine), dried (Na_2SO_4) and concentrated to give the desired aniline (1.07 g, 98% yield) as a yellow oil that was taken on without further purification. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.34-0.48 (4H, m), 0.70-0.83 (13H, m), 2.23 (3H, s), 2.55 (1H, t, J = 6.0 Hz), 2.73 (1H, q, 6.0 Hz), 2.83-3.03 (2H, m), 3.45-3.55 (2H, m), 3.73 (3H, s), 3.74 (3H, s), 3.78 (3H, s), 4.00 (2H, s, br), 4.05-4.12 (1H, m), 4.32 (1H, d, J = 11.4 Hz), 4.50 (1H, d, J = 11.4 Hz), 4.77 (2H, q, J = 11.1 Hz), 4.91 (1H, d, J = 10.8 Hz), 5.05 (1H, d, J = 10.8 Hz), 6.83 (2H, d, J = 8.4 Hz), 7.23 (2H, d, J = 8.4 Hz), 7.30-7.52 (10H, m).



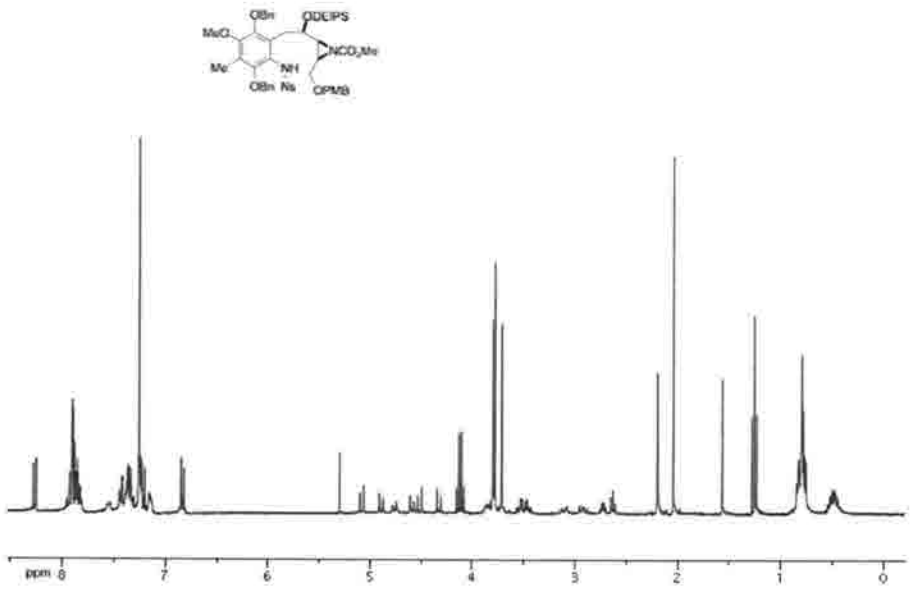
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-119

(2*S*,3*R*)-methyl2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-(2-nitrophenyl sulfonamido)phenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-((4-methoxybenzyloxy) methyl)aziridine-1-carboxylate



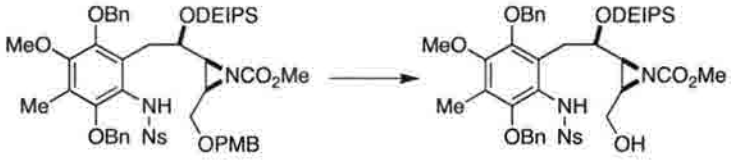
To the aniline described above (1.07 g, 1.39 mmol) in dichloromethane (50 mL), was added pyridine (560 μL, 6.94 mmol), nosyl chloride (920 mg, 4.17 mmol), and stirred for 48 hrs. The reaction mixture was then diluted (water), extracted (4 x dichloromethane), combined organics washed (brine), dried (Na_2SO_4), filtered and concentrated. Purification of the crude material by column chromatography (4:1 hexanes/ethyl acetate)

gave the desired sulfonamide (1.10 g, 83%) as a white foam. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.42-0.54 (4H, m), 0.76-0.85 (13H, m), 2.20 (3H, s), 2.63 (1H, t, J = 6.6 Hz), 2.70-2.76 (1H, m), 2.88-2.96 (1H, m), 3.07-3.14 (1H, m), 3.43-3.57 (2H, m), 3.72 (3H, s), 3.78 (3H, s), 3.80 (3H, s), 4.12 (2H, q, J = 7.2 Hz), 4.32 (1H, d, J = 11.4 Hz), 4.51 (1H, d, J = 11.4 Hz), 4.59 (1H, d, J = 11.1 Hz), 4.75 (1H, d, J = 10.8 Hz), 4.89 (1H, d, J = 11.7 Hz), 5.08 (1H, d, J = 11.1 Hz), 6.83 (2H, d, J = 8.7 Hz), 7.13-7.16 (1H, m), 7.25 (2H, d, J = 8.7 Hz), 7.33-7.45 (7H, m), 7.53-7.57 (1H, m), 7.83-7.94 (9H, m), 8.26 (2H, dd, J = 7.8 1.5 Hz).

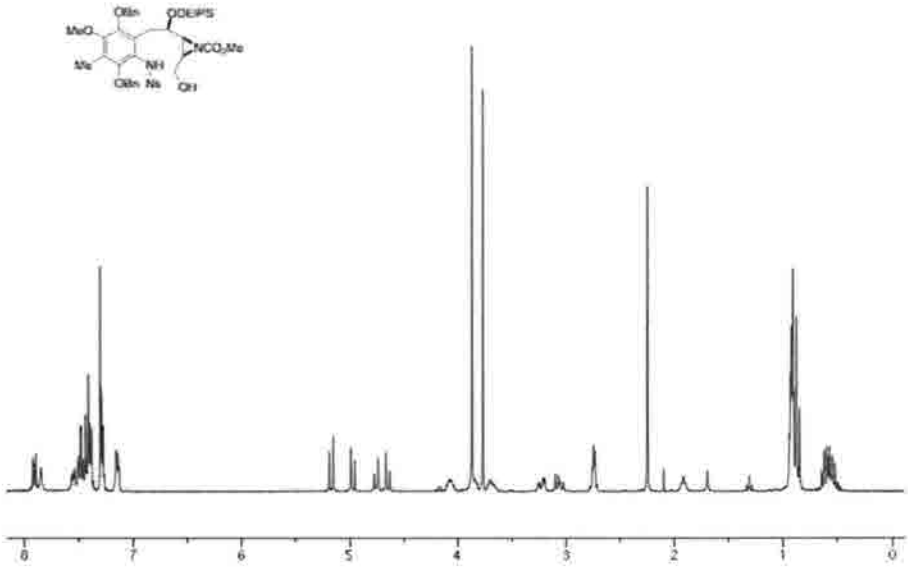


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-120

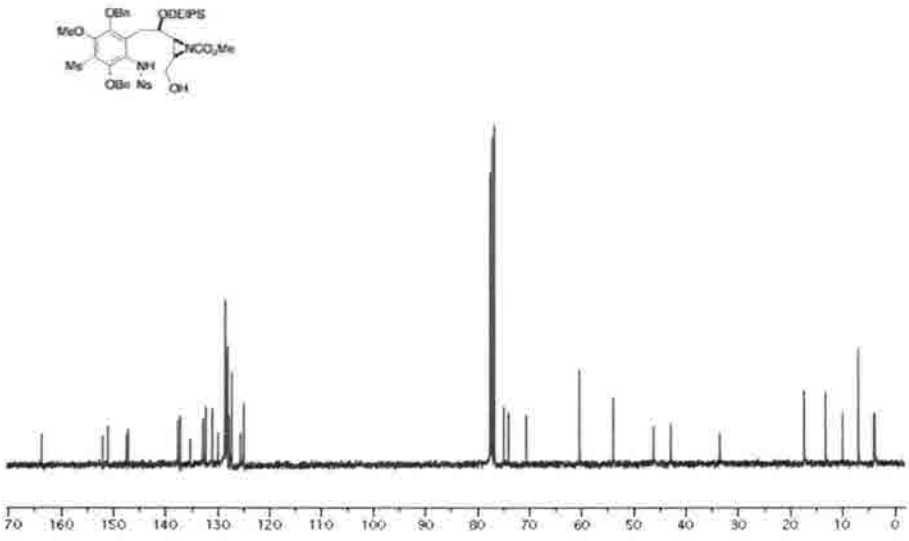
(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-(2-nitrophenyl sulfonamido)phenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-(hydroxymethyl) aziridine-1-carboxylate (194)



To the PMB ether (1.10 g, 1.15 mmol) in 20 mL of dichloromethane and 1 mL water was added DDQ (340 mg, 1.5 mmol) and stirred for 2 hrs. The reaction mixture was then passed through a plug of neutral alumina and eluted with 10:1 dichloromethane/methanol. After concentration, the crude oil was purified by column chromatography (3:1 hexanes/ethyl acetate to afford alcohol **194** (470 mg, 49%) as a brown foam. $[\alpha]_D^{20}$ +33.5 (*c*=0.8, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.50-0.65 (4H, m), 0.84-0.95 (13H, m), 1.92 (1H, s, br), 2.25 (3H, s), 2.72-2.75 (2H, m), 3.05 (1H, dd, *J* = 13.8, 9.0 Hz), 3.22 (1H, dd, *J* = 13.5, 4.8 Hz), 3.62-3.74 (1H, m), 3.77 (3H, s), 3.87 (3H, s), 4.03-4.11 (1H, m), 4.64 (1H, d, *J* = 8.1 Hz), 4.75 (1H, d, *J* = 8.1 Hz), 4.97 (1H, d, *J* = 11.1 Hz), 5.17 (1H, d, *J* = 11.1 Hz), 7.13-7.16 (2H, m), 7.27-7.29 (2H, m), 7.37-7.57 (8H, m), 7.85 (1H, s, br), 7.89-7.92 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 3.8, 4.0, 7.0, 10.0, 13.3, 17.4, 33.5, 42.9, 46.2, 53.9, 60.5, 70.6, 74.1, 75.0, 124.9, 125.0, 125.7, 127.3, 127.8, 128.2, 128.3, 128.6, 128.8, 130.0, 131.1, 132.3, 132.9, 133.3, 137.2, 137.6, 147.3, 147.5, 151.1, 152.1, 163.7; IR (neat) 3543, 3302, 2953, 2875, 1729, 1540, 1460, 1370, 1068 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{42}H_{53}N_3O_{11}SSi$ 836.3242 $(MH)^+$, Found 836.3235.

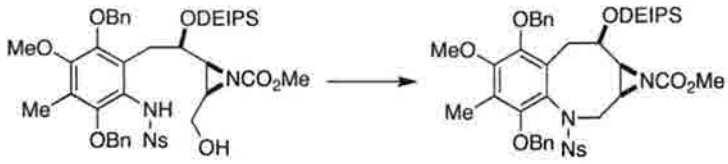


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-126-1H



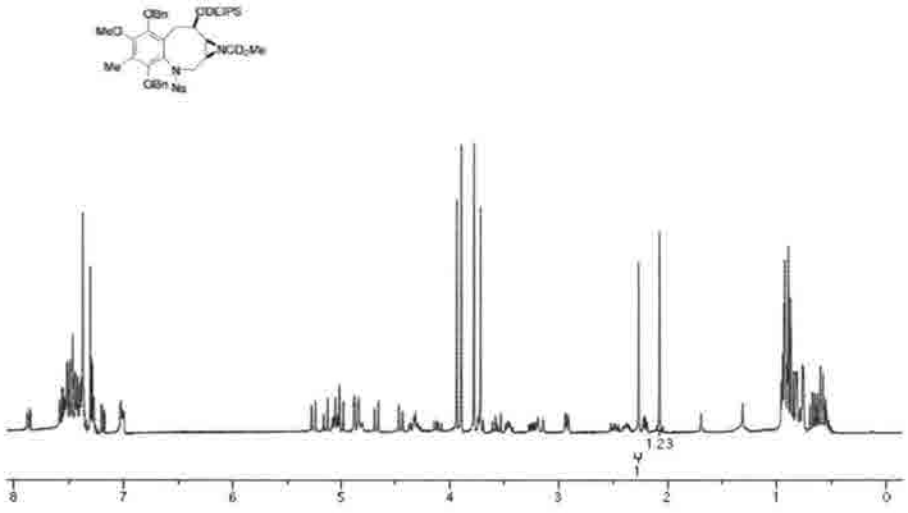
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-III-126-13C

(1a*S*,9*R*,9a*S*)-methyl-4,7-bis(benzyloxy)-9-(diethyl(isopropyl)silyloxy)-6-methoxy-5-methyl-3-(2-nitrophenylsulfonyl)-1a,2,3,8,9,9a-hexahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1-carboxylate (195)

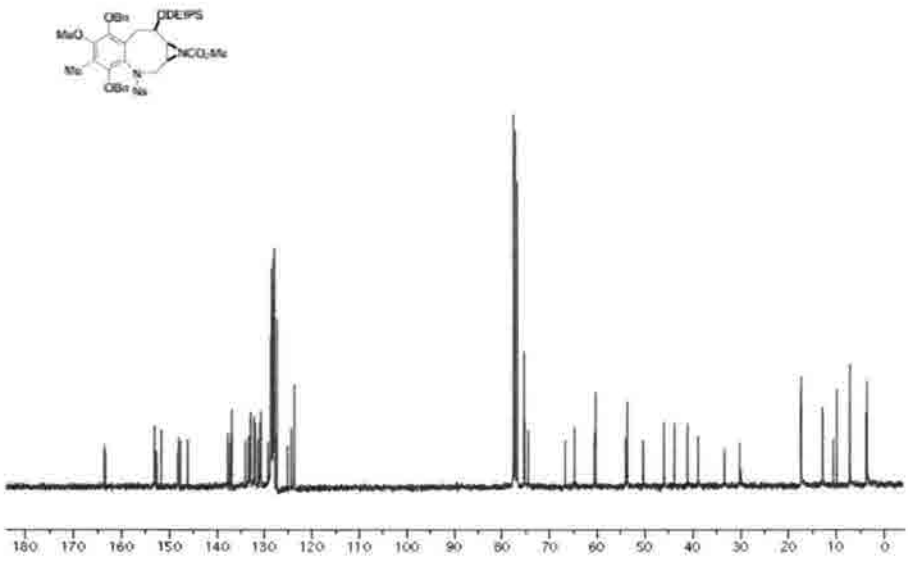


To alcohol **194** (780 mg, 0.93 mmol) in benzene (43 mL) was added triphenylphosphine (442 mg, 1.68 mmol) and diethylazodicarboxylate (DEAD) (333 μL, 40% wt. solution in toluene, 1.40 mmol). After stirring at room temperature for 4 days the reaction was concentrated and the crude material purified by column chromatography (4:1 hexanes/ethyl acetate) to give benzazocine **195** (700 mg, 92%) as a white foam. $[\alpha]_D^{20}$ +54.0 (*c*=1.0, $CHCl_3$); ^{1}HNMR and ^{13}CNMR showed that **195** exists as a 1.2:1 mixture of rotamers that did not coalesce at 393 K. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.51-0.70 (m), 0.76-0.97 (m), 2.07 (s), 2.20 (dd, *J* = 6.0, 4.5 Hz), 2.27 (s), 2.35-2.41 (m), 2.48 (dd, *J* = 13.5, 8.4 Hz), 2.92 (dd, *J* = 6.9, 3.9 Hz), 3.16 (dd, *J* = 13.8, 1.2 Hz), 3.23 (dd, *J* = 14.1, 6.9 Hz), 3.41-3.48 (m), 3.56 (dd, *J* = 14.1, 9.0 Hz), 3.72 (s), 3.78 (s), 3.89 (s), 3.94 (s), 4.11 (dd, *J* = 12.6, 8.4 Hz), 4.27-4.37 (m), 4.44 (d, *J* = 11.1 Hz), 4.67 (d, *J* = 10.8 Hz), 4.79-4.81 (m), 4.86 (dd, *J* = 10.8, 1.8 Hz), 4.98-5.08 (m), 5.14 (d, *J* = 10.8 Hz), 5.25 (d, *J* = 10.8 Hz), 7.00 (d, *J* = 3.6 Hz), 7.03 (d, *J* = 2.1 Hz), 7.19 (dd, *J* = 7.8, 1.2 Hz), 7.27-7.30 (m), 7.34-7.59 (m), 7.86 (dd, *J* = 7.8, 1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 3.5, 3.6, 3.8, 7.1, 7.2, 9.9, 10.6, 12.8, 12.9, 17.3, 17.36, 17.4, 30.2, 33.6, 38.9, 41.1, 43.8, 46.0, 50.4, 53.7, 54.1, 60.4, 60.6, 64.8, 66.7, 74.5, 75.4, 75.4, 123.7, 124.4, 125.1, 127.4, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 128.8, 129.2, 130.8, 131.0, 131.4, 131.9,l 132.2, 132.9, 133.0, 133.2, 133.4, 134.0, 136.9, 137.1, 137.4, 137.8, 146.2, 147.7,

148.0, 148.3, 151.7, 152.7, 152.9, 153.2, 163.4, 163.7; IR (neat) 2953, 2875, 1729, 1545, 1440, 1370, 1294, 1064 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{42}H_{51}N_3NaO_{10}SSi$ 840.2956 $(M + Na)^+$, Found 840.2953.

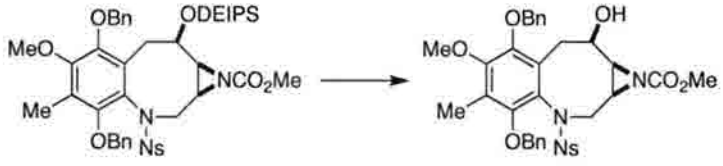


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-114-1H



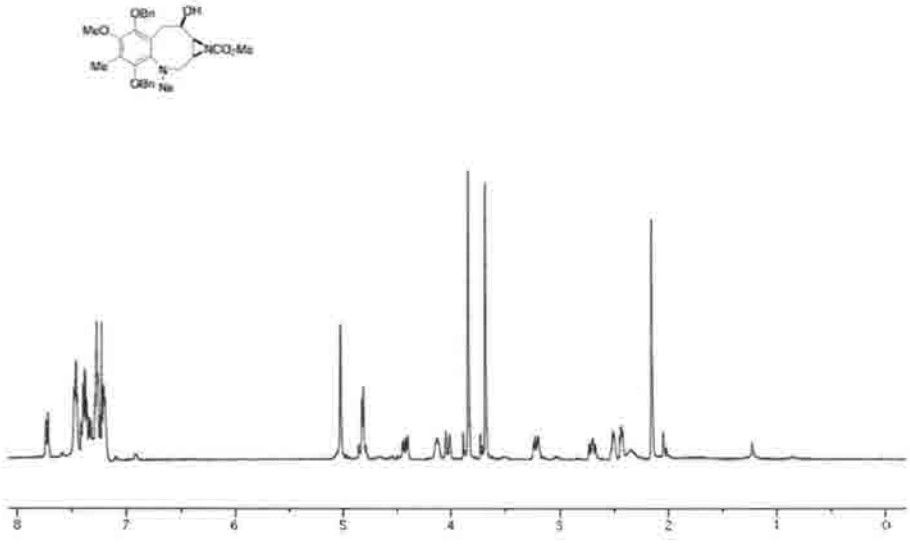
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-III-114-13C

(1a*S*,9*R*,9a*S*)-methyl-4,7-bis(benzyloxy)-9-hydroxy-6-methoxy-5-methyl-3-(2-nitro phenylsulfonyl)-1a,2,3,8,9,9a-hexahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1-carboxylate (196)

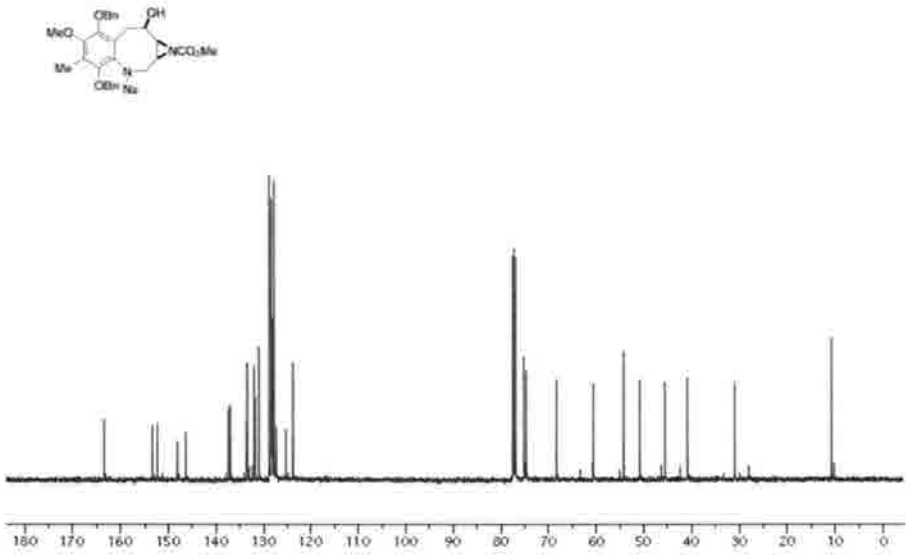


To benzazocine **195** (470 mg, 0.57 mmol), was added 15.5 mL of a 1.5 M of pyridinium hydrofluoride solution (prepared by addition of 1.0 g HF·Pyr to 20 mL of dry THF containing 5 mL pyridine) and stirred at room temperature for 16 hrs. The solution was then diluted with ethyl acetate, washed (1 x sat. aq. $NaHCO_3$, 1 x brine), dried (Na_2SO_4), filtered and concentrated. Purification of the crude by column chromatography (1:1

hexanes/ethyl acetate) gave alcohol **196** (200 mg, 51%) as a white foam. $[\alpha]_D^{20}$ -30.8 (c=0.43, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 2.16 (3H, s), 2.34 (1H, s, br), 2.43 (1H, dd, J = 4.5, 3.0 Hz), 2.50-2.53 (1H, m), 3.22 (1H, dd, J = 10.2, 3.6 Hz), 3.69 (3H, s), 3.85 (3H, s), 4.04 (1H, dd, J = 10.8, 2.1 Hz), 4.10-4.15 (1H, m), 4.43 (1H, dd, J = 10.8, 4.5 Hz), 4.82 (2H, dd, 11.7, 8.4 Hz), 5.03 (2H, s), 7.20-7,26 (2H, m), 7.24-7.30 (3H, m), 7.33-7.43 (4H, m), 7.45-7.50 (3H, m), 7.72-7.75 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.7, 30.9, 40.9, 45.6, 50.8, 54.2, 60.6, 68.3, 74.7, 75.2, 123.8, 125.3, 127.3, 127.8, 128.2, 128.3, 128.5, 128.8, 131.0, 131.8, 132.0, 133.4, 133.6, 137.0, 137.4, 146.4, 148.1, 152.4, 153.4, 163.5; IR (neat) 3476, 2927, 1727, 1544, 1440, 1370, 1252, 1078 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{35}H_{35}N_3O_{10}S$ 690.2113 $(MH)^+$, Found 690.2115.

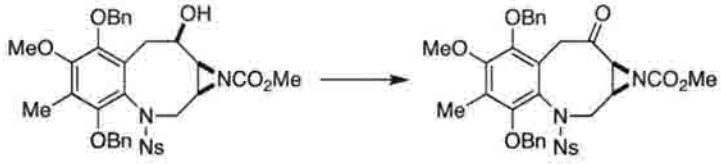


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-III-169-400-1H



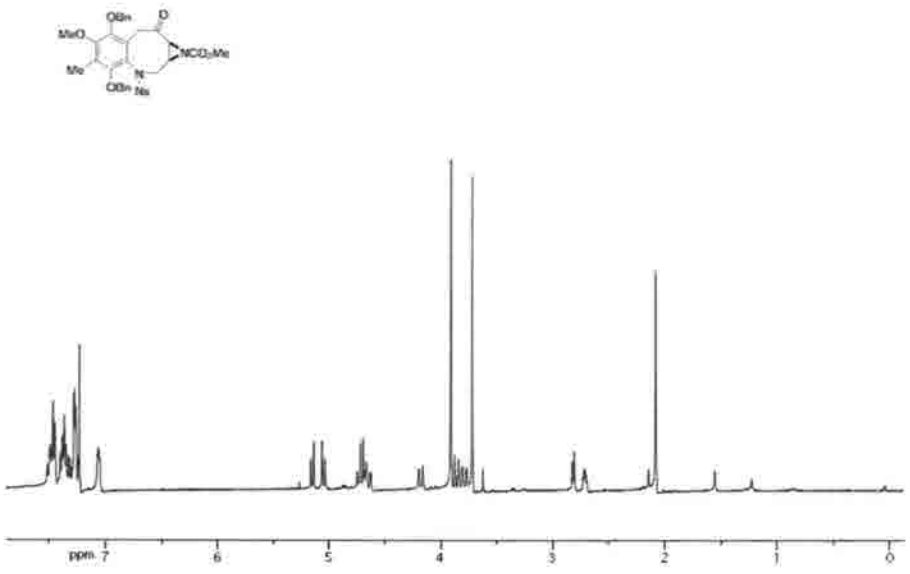
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-III-169-400-13C

(1a*S*,9a*S*)-methyl-4,7-bis(benzyloxy)-6-methoxy-5-methyl-3-(2-nitrophenylsulfonyl)-9-oxo-1a,2,3,8,9,9a-hexahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1-carboxylate (197)

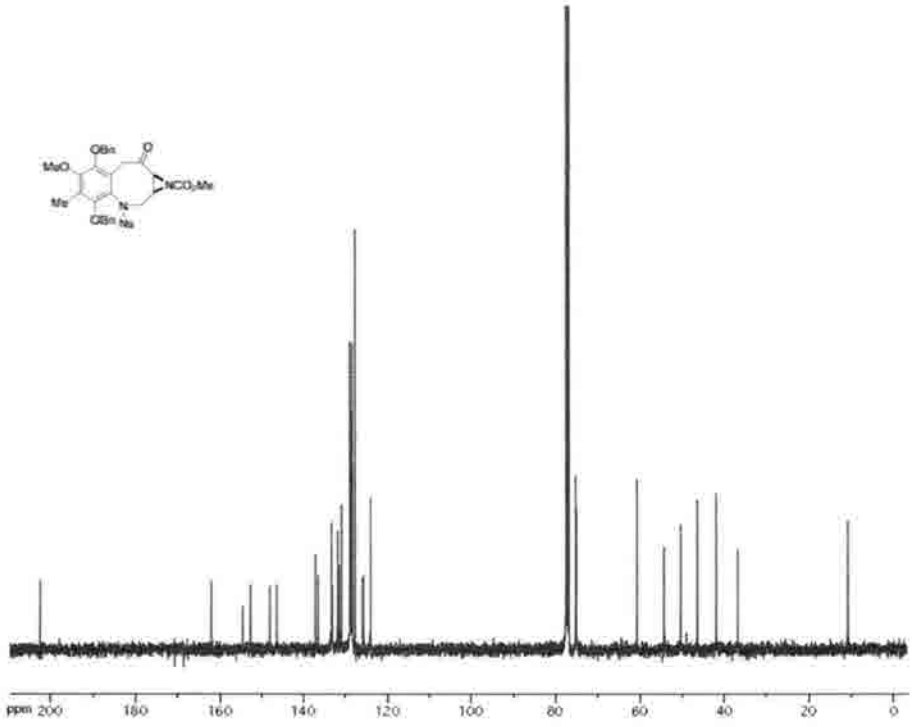


To alcohol **196** (310 mg, 0.45 mmol) in dichloromethane (6.5 mL) and molecular sieves (4Å, 930 mg) was added pyridinium dichromate (507 mg, 1.35 mmol) and stirred at room temperature for 16 hrs. The reaction mixture was run through a pad of silica using ethyl acetate as eluant and then concentrated. Further purification of the crude material by column chromatography (3:2 hexanes/ethyl acetate) gave benzazocane **197** (260 mg, 85%) as a white foam. $[\alpha]_D^{20}$ -118.0 (c=1.2, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ

TMS: 2.08 (3H, s), 2.68-2.74 (1H, m), 2.81 (1H, d, J = 7.2 Hz), 3.72 (3H, s), 3.76-3.87 (2H, m), 3.91 (3H, s), 4.17 (1H, d, J = 14.0 Hz), 4.62-4.75 (2H, m), 5.04 (1H, d, J = 10.8 Hz), 5.15 (1H, d, J = 10.8 Hz), 7.05-7.07 (1H, m), 7.26-7.29 (2H, m), 7.31-7.40 (3H, m), 7.45-7.52 (3H, m); ^{13}CNMR (100 MHz, $CDCl_3$) δ 10.8, 36.8, 41.9, 46.4, 50.3, 54.2, 60.7, 75.1, 75.2, 124.0, 125.7, 125.9, 127.7, 128.4, 129.0, 133.4, 136.5, 137.2, 146.4, 148.1, 152.7, 154.5, 162.0, 202.5; IR (neat) 3030, 2955, 1734, 1544, 1370, 1280, 1168, 1017 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{35}H_{33}N_3O_{10}S$ 688.1956 $(MH)^+$, Found 688.1959.

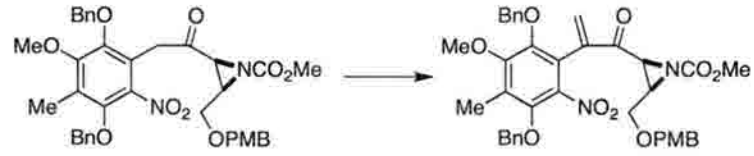


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-III-148-400-1H



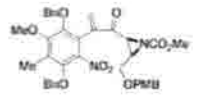
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-III-148-400-13C

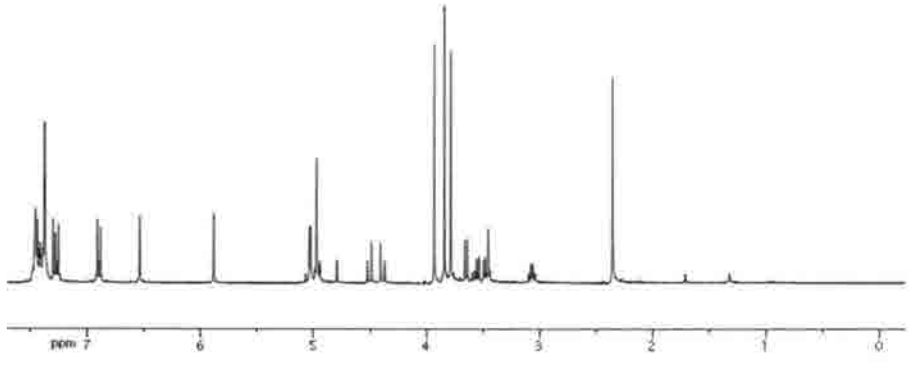
(2*S*,3*R*)-methyl2-(2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)acryloyl) -3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (205)



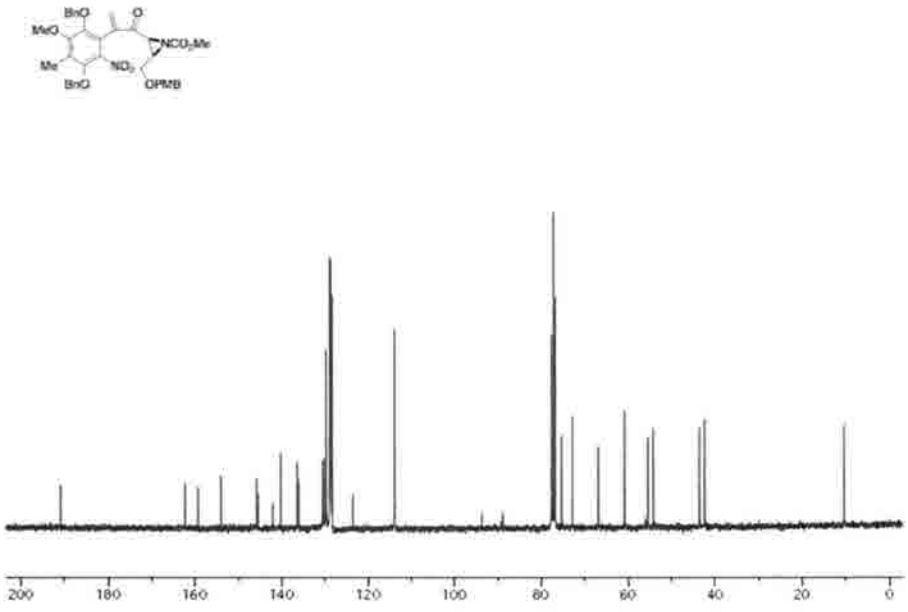
To ketone **204** (1.2 g, 1.79 mmol), in 20:3 THF/water (75 mL) at 0 °C was added formalin (15 mL, 37% wt. solution in water, 200 mmol), lithium hydroxide (716 μL, 1.0 M solution in water, 0.72 mmol), and then warmed to room temperature and stirred. After 20 hrs of stirring at room temperature, the reaction was diluted with brine and extracted 4 x dichloromethane. The combined organic layer was then washed (brine), dried (Na_2SO_4), filtered and concentrated. Purification of the crude by column chromatography

(3:2 hexanes/ethyl acetate) gave enone **205** (1.2 g, 99%) as a yellow oil. $[\alpha]_D^{20}$ -35.3 (c=0.85, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.35 (3H, s), 3.06 (1H, q, J = 6.0 Hz), 3.46 (1H, m), 3.55 (1H, dd, J = 11.1, 6.3 Hz), 3.64 (1H, d, J = 6.9 Hz), 3.78 (3H, s), 3.84 (3H, s), 3.93 (3H, s), 4.38 (1H, d, J = 11.1 Hz), 4.50 (1H, d, J = 11.3 Hz), 4.94-5.04 (4H, m), 5.89 (1H, s), 6.54 (1H, s), 6.89 (2H, d, J = 8.7 Hz), 7.26 (2H, d, J = 8.7 Hz), 7.36-7.48 (10H, m); ^{13}CNMR (100 MHz, $CDCl_3$) δ 10.4, 42.4, 43.6, 54.2, 55.4, 60.8, 66.9, 72.9, 75.3, 113.9, 123.5, 128.3, 128.6, 128.7, 128.8, 129.0, 129.8, 130.2, 130.6, 136.1, 136.4, 140.3, 142.1, 145.5, 145.8, 154.0, 159.4, 162.4, 190.9; IR (neat) 2953, 1735, 1611, 1531, 1363, 1246, 1117, 984 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{38}H_{38}N_2O_{10}$ 682.2526 $(MH)^+$, Found 682.2508.



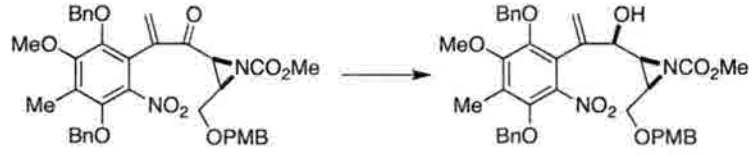


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-249-300-1H



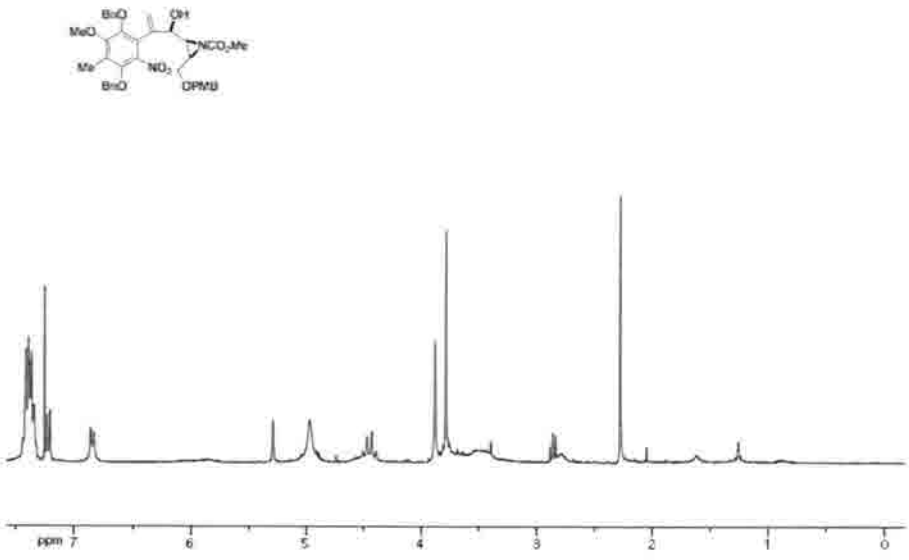
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-III-249-300-13C

(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-hydroxyallyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (206)



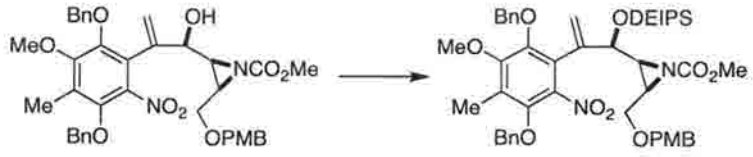
Enone **205** (450 mg, 0.65 mmol), in 3:1 methanol/dichloromethane (25 mL) at 0 °C was added cerium trichloride (1.2 g, 3.27 mmol), and sodium borohydride (73 mg, 1.95 mmol). After stirring at 0 °C for 45 minutes the reaction was quenched with water, extracted (4 x ethyl acetate), washed (brine), dried (Na_2SO_4) filtered and concentrated to afford alcohol **206** (450 mg, 99%) as a yellow oil that was taken on without further purification. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.28 (3H, s), 2.79 (1H, s, br), 2.86 (1H,

t, J = 7.8 Hz), 3.39-3.56 (4H, m), 3.78 (3H, 3), 3.88 (3H, s), 4.44 (2H, q, J = 11.4 Hz), 4.96 (4H, m), 5.28 (1H, s), 6.85 (2H, d, J = 8.1 Hz), 7.23 (2H, d, J = 8.4 Hz), 7.33-7.45 (10H, m).



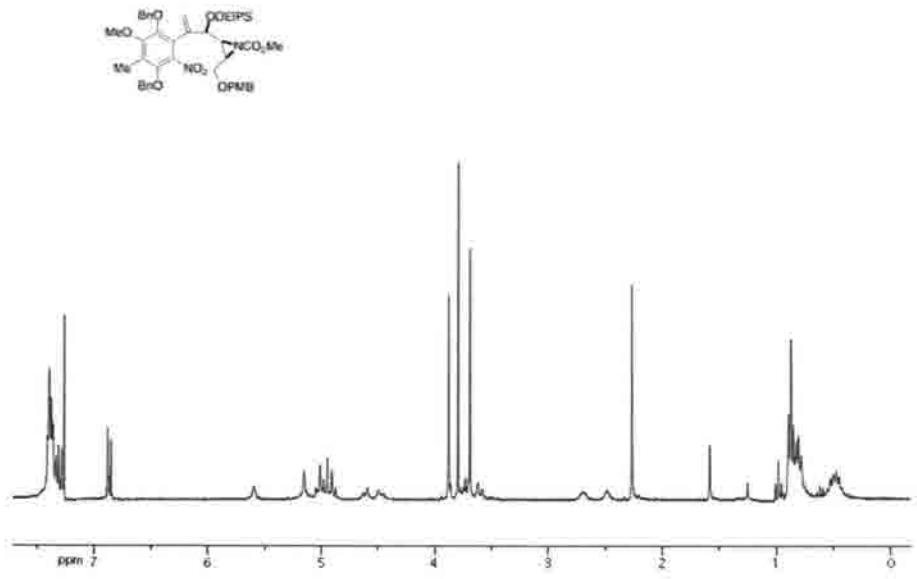
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-276

(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-(diethyl(isopropyl)silyloxy)allyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate



To alcohol **206** (26 mg, 0.038 mmol), in dichloromethane (1 mL) was added imidazole (9 mg, 0.13 mmol), diethylisopropylchloro silane (14 μL, 0.076 mmol) and then stirred for

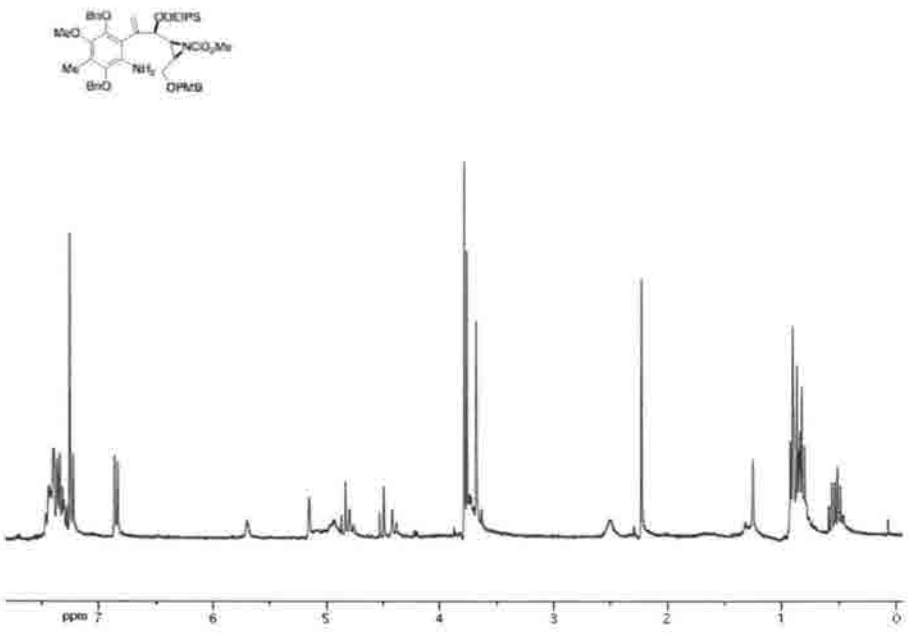
24 hrs. The reaction mixture was then diluted with ethyl acetate, washed (1 x water, 1 x brine), dried (Na_2SO_4), filtered and concentrated. Purification of the crude residue by PTLC (3:2 hexanes/ethyl acetate) gave the desired silyl ether (13 mg, 43%) as a yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.42-0.55 (4H, m), 0.75-0.89 (13H, m), 2.27 (3H, s), 2.47-2.50 (1H, m), 2.67-2.71 (1H, m), 3.57-3.62 (1H, m), 3.69 (3H, s), 3.80 (3H, s), 3.88 (3H, s), 4.46-4.64 (2H, m), 4.87-5.05 (4H, m), 5.15 (1H, s), 5.59 (1H, s), 6.86 (2H, d, J = 8.7 Hz), 7.29 (2H, d, J = 8.7 Hz), 7.33-7.43 (10H, m).



^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-273

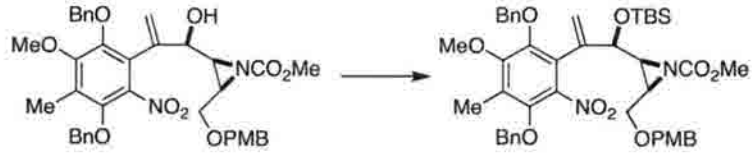
(2*S*,3*R*)-methyl-2-((*R*)-2-(2-amino-3,6-bis(benzyloxy)-5-methoxy-4-methylphenyl)-1-(diethyl(isopropyl)silyloxy)allyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (207)

To silyl ether above (13 mg, 0.016 mmol) in 4:1 acetone/water (500 μL) was added zinc dust (5 mg, 0.080 mmol), ammonium chloride (9 mg, 0.16 mmol), and stirred at room temperature for 40 minutes. The reaction mixture was concentrated, diluted with ethyl acetate, washed (1 x water, 1 x brine), dried (Na_2SO_4), filtered and concentrated to give aniline **207** (11 mg, 90%) that was used without further purification. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.45-0.59 (4H, m), 0.76-0.92 (13H, m), 2.23 (3H, s), 2.51 (2H, s, br), 3.68 (3H, s), 3.76 (3H, s), 3.79 (3H, s), 4.40 (1H, d, J = 10.8 Hz), 4.51 (1H, d, J = 10.8 Hz), 4.82 (2H, q, J = 10.8 Hz), 4.90-4.98 (2H, m), 5.16 (1H, s), 5.72 (1H, s), 6.85 (2H, d, J = 8.7 Hz), 7.24 (2H, d, J = 8.7 Hz), 7.28-7.47 (10H, m).



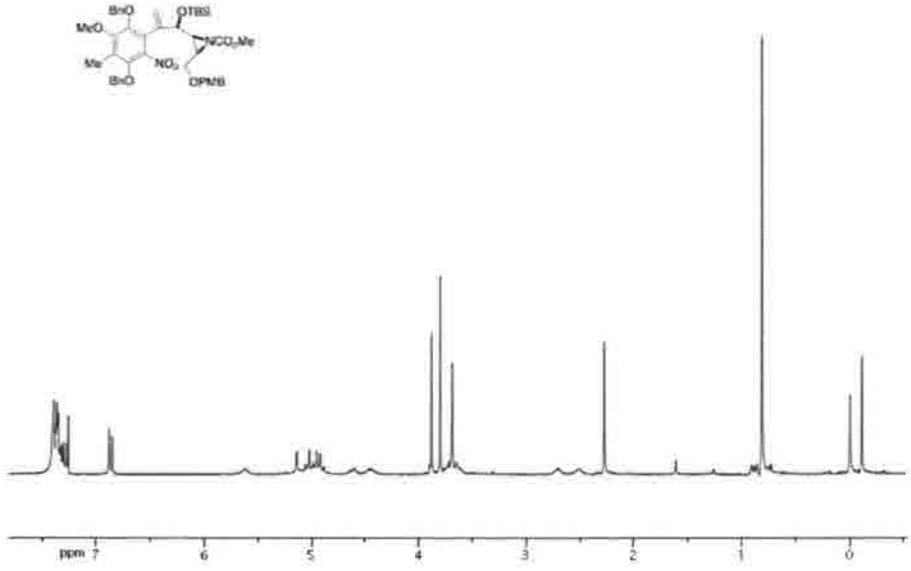
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-274

(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-(*tert*-butyldimethylsilyloxy)allyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (210)



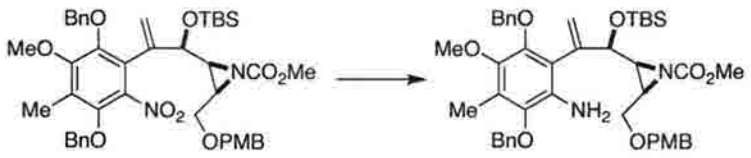
Alcohol **206** (450 mg, 0.65 mmol) in dichloromethane (17 mL) was cooled to -78 °C. Lutidine (300 μL, 2.60 mmol) was added followed by dropwise addition of TBSOTf (300 μL, 1.31 mmol) and warmed to room temperature. After stirring at room temperature for 45 minutes the reaction was diluted with ethyl acetate and washed (1 x sat. aq. $NaHCO_3$, 1 x brine), dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by

column chromatography (4:1 hexanes/ethyl acetate) to give TBS ether **210** (240 mg, 46%) as a colorless oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: -0.11 (3H, s), -0.01 (3H, s), 0.81 (9H, s), 2.27 (3H, s), 2.49-2.53 (1H, m), 2.68-2.73 (1H, m), 3.68 (3H, s), 3.80 (3H, s), 3.88 (3H, s), 4.39-4.47 (1H, m), 4.58-4.63 (1H, m), 4.88-5.14 (6H, m), 5.61 (1H, s), 6.86 (2H, d, J = 8.7 Hz), 7.29 (2H, d, 8.4 Hz), 7.33-7.44 (10H, m).

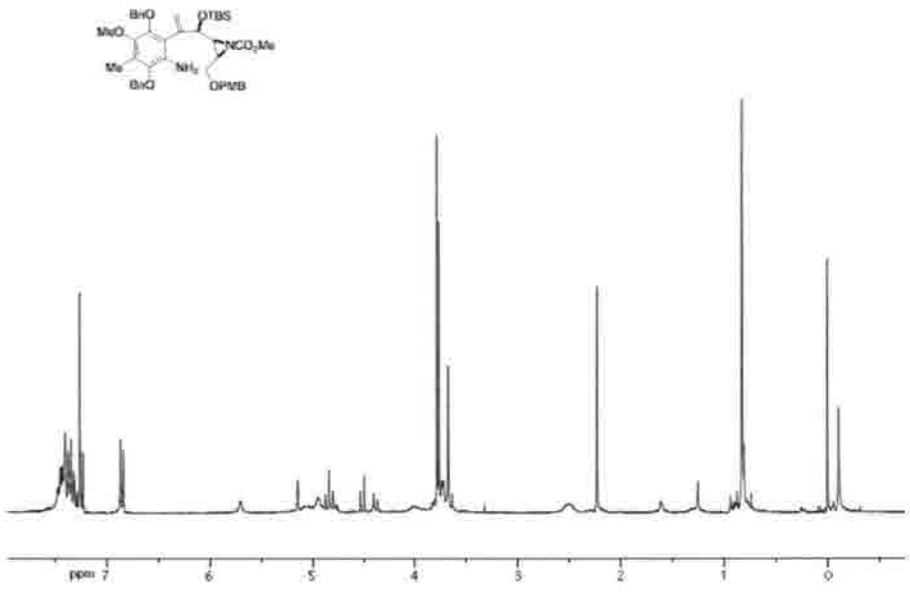


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-286

(2*S*,3*R*)-methyl-2-((*R*)-2-(2-amino-3,6-bis(benzyloxy)-5-methoxy-4-methylphenyl)-1-(*tert*-butyldimethylsilyloxy)allyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate

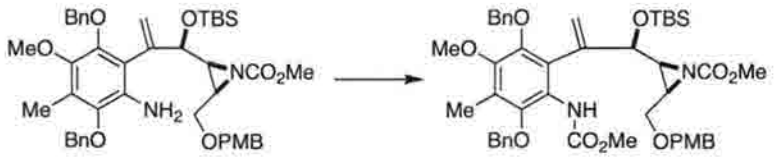


To silyl ether **210** (240 mg, 0.30 mmol) in 4:1 acetone/water (10 mL) was added zinc dust (98 mg, 1.50 mmol), ammonium chloride (160 mg, 3.00 mmol), and stirred at room temperature for 40 minutes. The reaction mixture was concentrated, diluted with ethyl acetate, washed (1 x water, 1 x brine), dried (Na_2SO_4), filtered and concentrated to give the desired aniline (219 mg, 95%) that was used without further purification. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: -0.11 (3H, s), 0.01 (3H, s), 0.83 (9H, s), 2.23 (3H, s), 2.46-2.55 (2H, m), 3.68 (3H, s), 3.73 (1H, m), 3.77 (3H, s), 3.79 (3H, s), 3.95-4.05 (1H, m), 4.38 (1H, d, J = 11.1 Hz), 4.51 (1H, d, J = 11.1 Hz), 4.78 (1H, d, J = 7.8 Hz), 4.85 (1H, d, J = 7.8 Hz), 4.94-5.08 (2H, m), 5.15 (1H, t, J = 1.8 Hz), 5.70 (1H, s), 6.85 (2H, d, J = 8.7 Hz), 7.23 (2H, d, J = 8.7 Hz), 7.29-7.47 (10H, m).



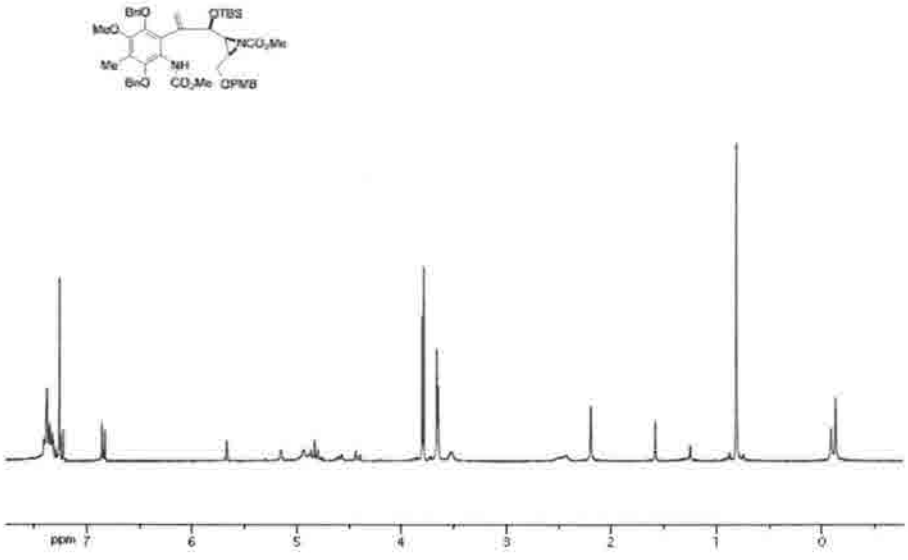
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-281

(2*S*,3*R*)-methyl 2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-6-(methoxycarbonylamino)-4-methylphenyl)-1-(*tert*-butyldimethylsilyloxy)allyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate



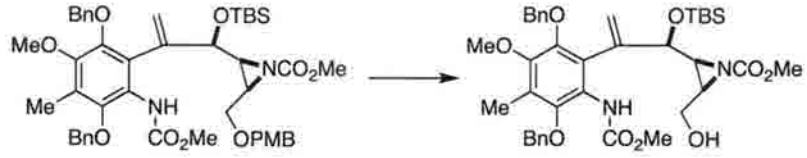
To the aniline obtained in the previous step (190 mg, 0.25 mmol), in dichloromethane (4 mL) was added sodium bicarbonate (42 mg, 0.50 mmol), methyl chloroformate (28 μL, 0.37 mmol) and stirred for 22 hrs at room temperature. The reaction was then quenched (water), extracted (4 x ethyl acetate), washed (brine), dried (Na_2SO_4), filtered and

concentrated. Purification of the crude by column chromatography (3:2 hexanes/ethyl acetate) provided the desired carbamate (82 mg, 40%) as a yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: -0.13 (3H, s), -0.09 (3H, s), 2.20 (3H, s), 2.40-2.53 (1H, m), 3.51-3.54 (1H, m), 3.65 (3H, s), 3.66 (3H, s), 3.79 (3H, s), 3.80 (3H, s), 4.40 (1H, d, J = 11.4 Hz), 4.60 (1H, d, J = 11.4 Hz), 4.80-4.96 (5H, m), 5.15 (1H, s), 5.67 (1H, t, J = 1.8 Hz), 6.83 (2H, d, J = 8.7 Hz), 7.23 (2H, d, J = 8.4 Hz), 7.30-7.41 (10H, m).

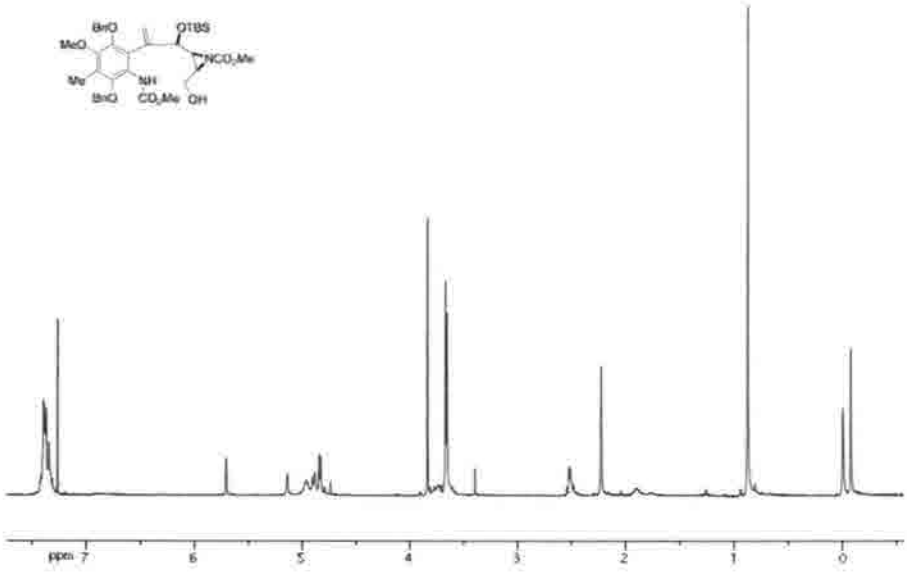


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-282

(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-6-(methoxycarbonylamino)-4-methylphenyl)-1-(*tert*-butyldimethylsilyloxy)allyl)-3-(hydroxymethyl)aziridine-1-carboxylate (211)



To a stirred solution of the carbamate obtained previously (90 mg, 0.11 mmol) in 15:1 solution of dichloromethane/H_2O (6 mL) was added DDQ (30 mg, 0.13 mmol) in one portion. The reaction mixture immediately turned green and over the course of 1.5 h the mixture turned brown-orange. After 2 h, the reaction mixture was passed through a short pad of activated alumina using 10:1 dichloromethane/methanol as eluant. The filtrate was concentrated under reduced pressure and the crude product was purified by PTLC (1:1 hexanes/ethyl acetate) to afford the alcohol **211** (62 mg, 80%) as a colorless oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: -0.08 (3H, s), -0.01 (3H, s), 0.87 (9H, s), 1.90 (1H, s, br), 2.23 (3H, s), 2.48-2.55 (2H, m), 3.65 (3H, s), 3.67 (3H, s), 3.83 (3H, s), 4.73-4.91 (4H, m), 4.95 (1H, s), 5.14 (1H, s), 5.70 (1H, t, J = 1.8 Hz), 7.31-7.42 (10H, m).

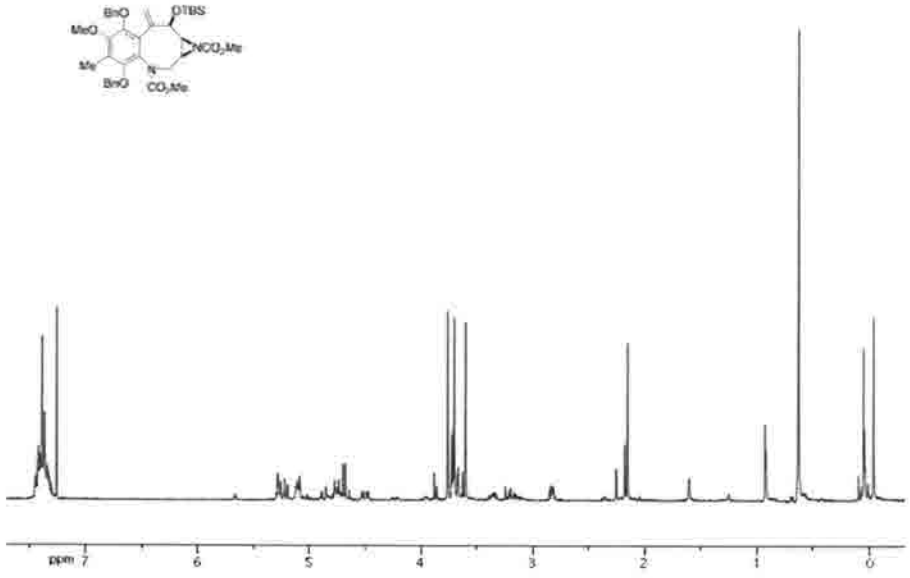


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-290

(1a*S*,9*R*,9a*S*)-dimethyl 4,7-bis(benzyloxy)-9-(*tert*-butyldimethylsilyloxy)-6-methoxy-5-methyl-8-methylene-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (212)

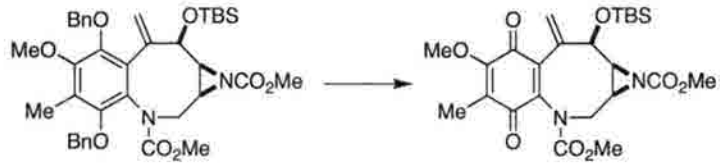
To a solution of **211** (55 mg, 0.078 mmol) and tributylphosphine (35 μL, 0.14 mmol) in dry toluene (5 mL) was added TMAD (20 mg, 0.12 mmol). The solution immediately turned yellow and slowly changed to a white cloudy color as the reaction progressed. The mixture was stirred under argon at room temperature and monitored by TLC until complete consumption of the starting material (5 h). The reaction mixture was

concentrated under reduced pressure and the residue was purified by PTLC (3:2 hexanes/ethyl acetate) to afford the benzazocine **212** (45 mg, 85%) as a colorless oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: -0.03 (3H, s), 0.05 (3H, s), 0.63 (9H, s), 2.16 (3H, s), 2.80-2.85 (1H, m), 3.07-3.25 (2H, m), 3.32-3.40 (1H, m), 3.60 (3H, s), 3.70 (3H, s), 3.76 (3H, s), 3.86 (1H, d, J = 6.9 Hz), 4.50 (1H, dd, J = 13.2, 4.8 Hz), 4.64-4.89 (3H, m), 5.09-5.30 (4H, m), 7.31-7.45 (10H, m).

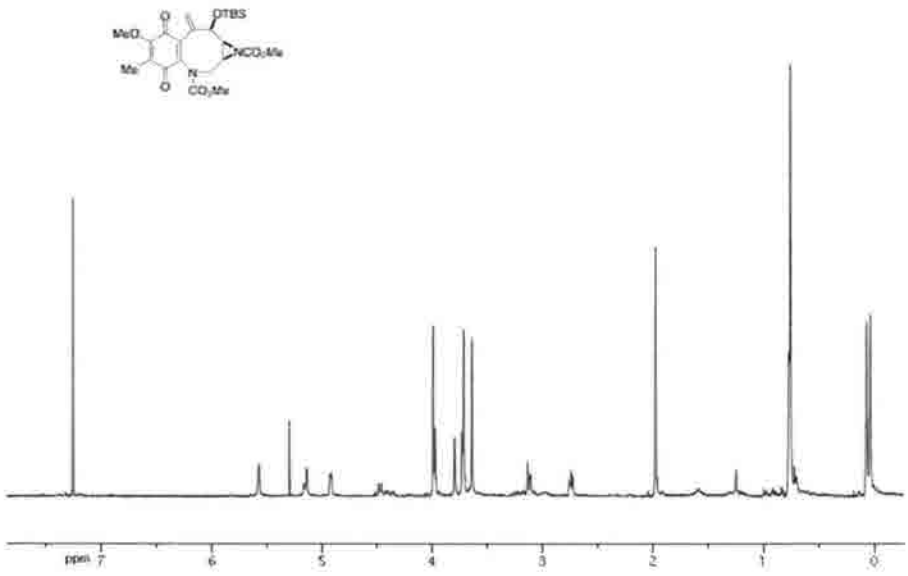


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-291

(1a*S*,9*R*,9a*S*)-dimethyl-9-(*tert*-butyldimethylsilyloxy)-6-methoxy-5-methyl-8-methylene-4,7-dioxo-1a,2,7,8,9,9a-hexahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(4*H*)-dicarboxylate (215)

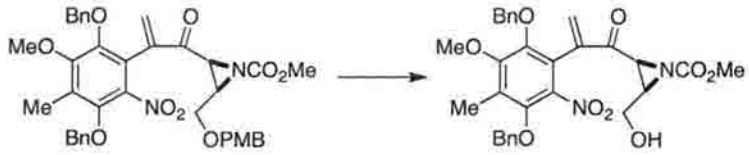


To benzazocine **212** (16 mg, 0.023 mmol) in ethyl acetate (2 mL) was added 10% Pd/C (25 mg), triethylamine (20 μL, 0.14 mmol), stirred under a hydrogen balloon for 3 hrs and then stirred under an oxygen balloon for 30 minutes. The solution was then ran through a plug of celite using ethyl acetate as eluant and concentrated. Purification of the crude material by PTLC (3:2 hexanes/ethyl acetate, plate was deactivated prior to use with 4% triethylamine/hexanes) to give quinone **215** (11.2 mg, 96%) as a dark orange oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.04 (3H, s), 0.07, (3H, s), 0.76 (9H, s), 1.98 (3H, s), 2.74 (1H, t, J = 4.5 Hz), 3.07-3.26 (2H, m), 3.64 (3H, s), 3.71 (3H, s), 3.99 (3H, s), 4.34-4.52 (1H, m), 4.92 (1H, d, J = 1.8 Hz), 5.15 (1H, d, J = 7.5 Hz), 5.58 (1H, s).



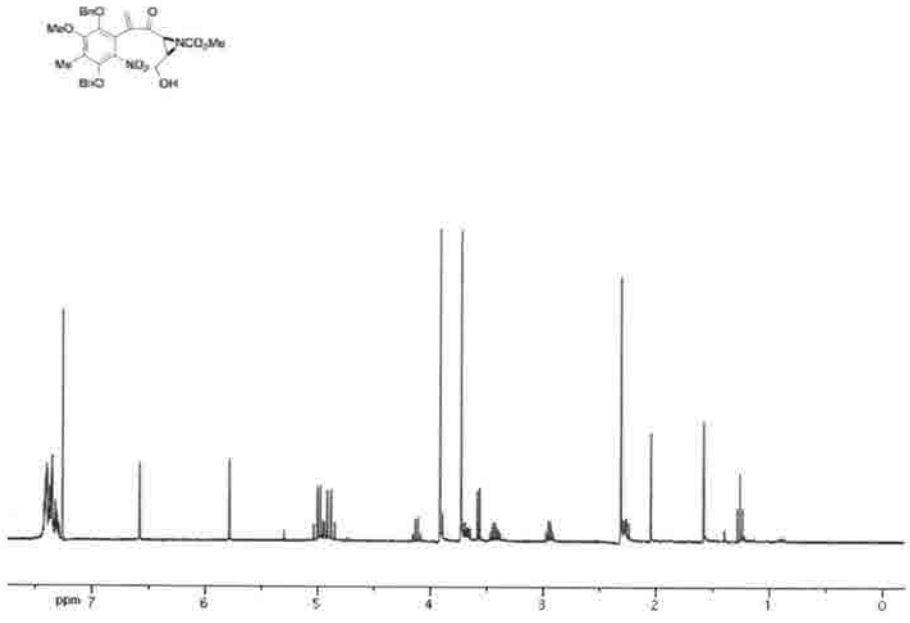
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-308

(2*S*,3*R*)-methyl 2-(2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)acryloyl) -3-(hydroxymethyl)aziridine-1-carboxylate (225)

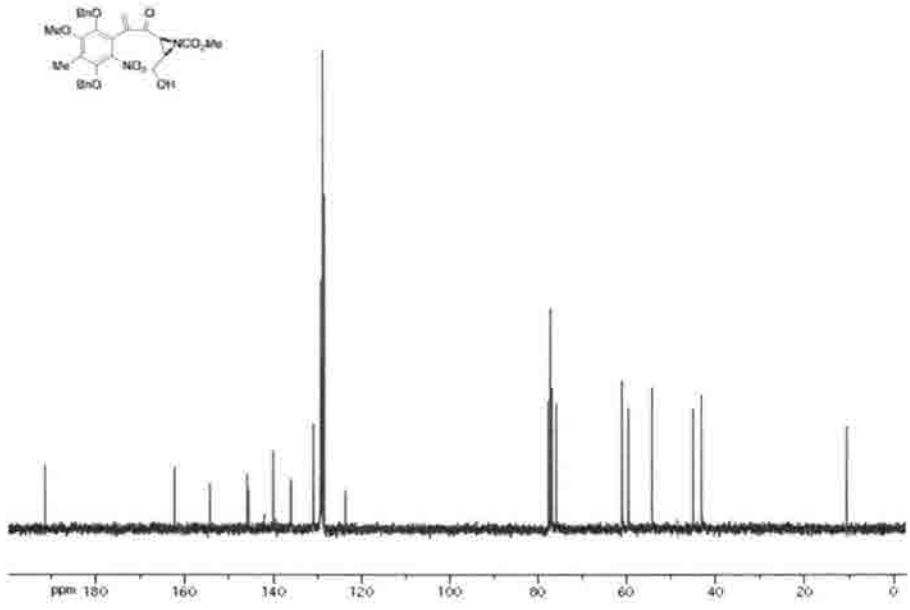


To a stirred solution of the enone **205** (400 mg, 0.58 mmol) in 15:1 solution of dichloromethane/water (13 mL) was added DDQ (172 mg, 0.76 mmol) in one portion. The reaction mixture immediately turned green and over the course of 1.5 h the mixture turned orange. After 2 h, the reaction mixture was passed through a short pad of neutral alumina using 10:1 dichloromethane/methanol as eluant. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography

(1:1 hexanes/ethyl acetate) to afford the alcohol **225** (280 mg, 86%) as a yellow oil. $[\alpha]_D^{20}$ -43.3 (*c*=0.8, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.25 (1H, dd, *J* = 7.5, 6.0 Hz), 2.31 (3H, s), 2.94 (1H, dd, *J* = 12.0, 6.6 Hz), 3.42 (1H, q, *J* = 6.0 Hz), 3.56 (1H, d, *J* = 6.6 Hz), 3.66 (1H, dd, *J* = 8.1, 5.4 Hz), 3.72 (3H, s), 3.91 (3H, s), 4.86 (1H, d, *J* = 10.5 Hz), 4.92 (1H, d, *J* = 7.5 Hz), 5.79 (1H, s), 6.58 (1H, s), 7.29-7.43 (10H, m); ^{13}CNMR (100 MHz, $CDCl_3$) δ 10.5, 43.1, 45.0, 54.3, 59.6, 61.0, 75.9, 123.6, 128.4, 128.8, 129.0, 129.2, 129.3, 130.9, 135.9, 136.0, 140.0, 145.9, 154.4, 162.4, 191.4; IR (neat) 3491, 2953, 1734, 1532, 1442, 1362, 1291, 1118, 980 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{30}H_{30}N_2O_9$ 562.1951 $(MH)^+$, Found 562.1947.

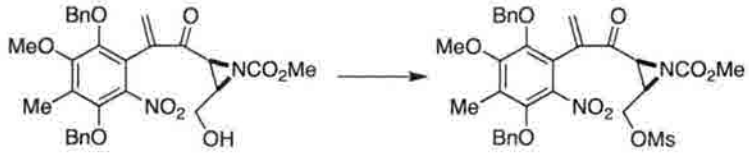


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-046



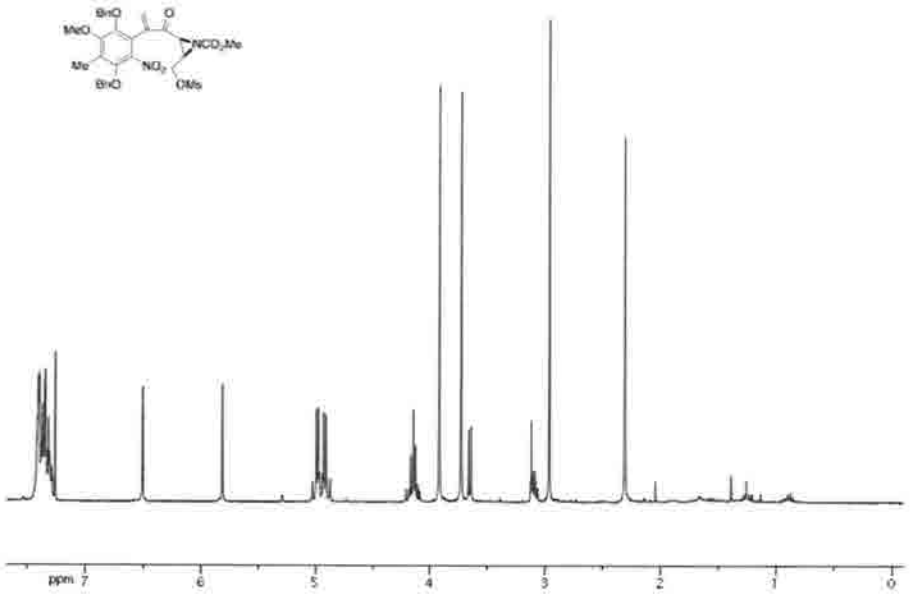
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-IV-046-13C

(2*S*,3*R*)-methyl-2-(2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl) acryloyl)-3-((methylsulfonyloxy)methyl)aziridine-1-carboxylate (226)

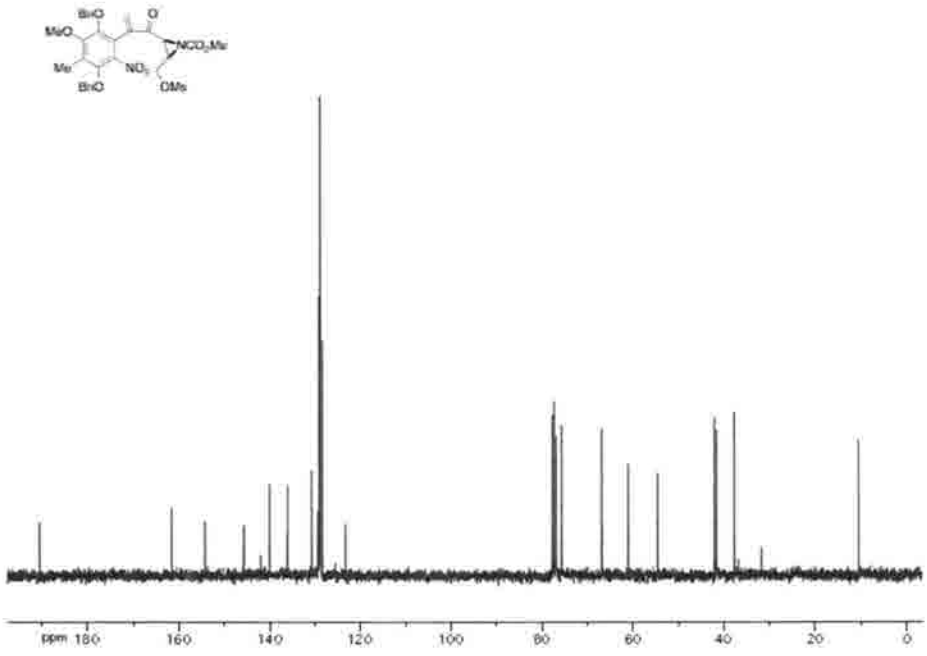


To alcohol **225** (560 mg, 1.0 mmol) and triethylamine (420 μL, 3.0 mmol) in dichloromethane (25 mL) at 0 °C was added methanesulfonyl chloride (116 μL, 1.5 mmol,) slowly dropwise and stirred at this temperature for 30 minutes. The reaction was then quenched (brine), extracted (4x dichloromethane), washed (brine), dried (Na_2SO_4) and concentrated. Purification by column chromatography gave mesylate **226** (580 mg, 91%) as a yellow foam. $[\alpha]_D^{20}$ -40.3 (*c*=0.8, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS:

2.31 (3H, s), 2.97 (3H, s), 3.06-3.12 (1H, m), 3.65 (1H, d, J = 6.6 Hz), 3.73 (3H, s), 3.92 (3H, s), 4.08-4.21 (2H, app. septet, J = 7.2, 4.8 Hz), 4.91 (2H, dd, J = 10.8, 7.5 Hz), 4.98 (2H, dd, J = 10.8, 7.5 Hz), 5.81 (1H, s), 6.50 (1H, s), 7.29-7.43 (10H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 10.5, 31.8, 37.7, 41.6, 42.1, 54.5, 61.0, 68.8, 75.7, 123.3, 128.4, 128.8, 129.2, 129.5, 130.8, 136.0, 136.1, 140.1, 145.7, 145.8, 154.3, 161.7, 190.5; IR (neat) 2939, 1738, 1532, 1442, 1361, 1176, 963 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{31}H_{32}N_2O_{11}S$ 640.1727, $(MH)^+$, Found 640.1729.

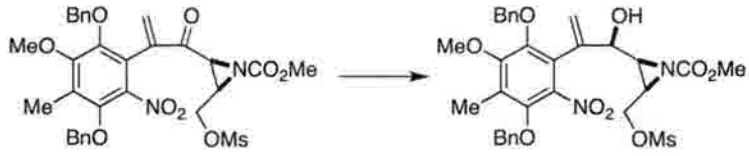


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-047-1H-300



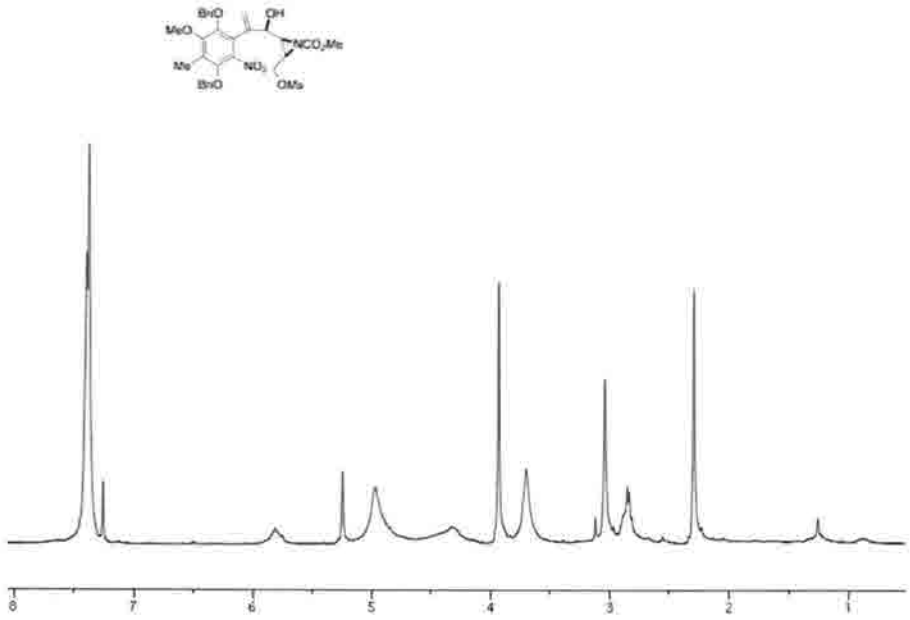
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-047-300-13C

(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-hydroxyallyl)-3-((methylsulfonyloxy)methyl)aziridine-1-carboxylate (227)

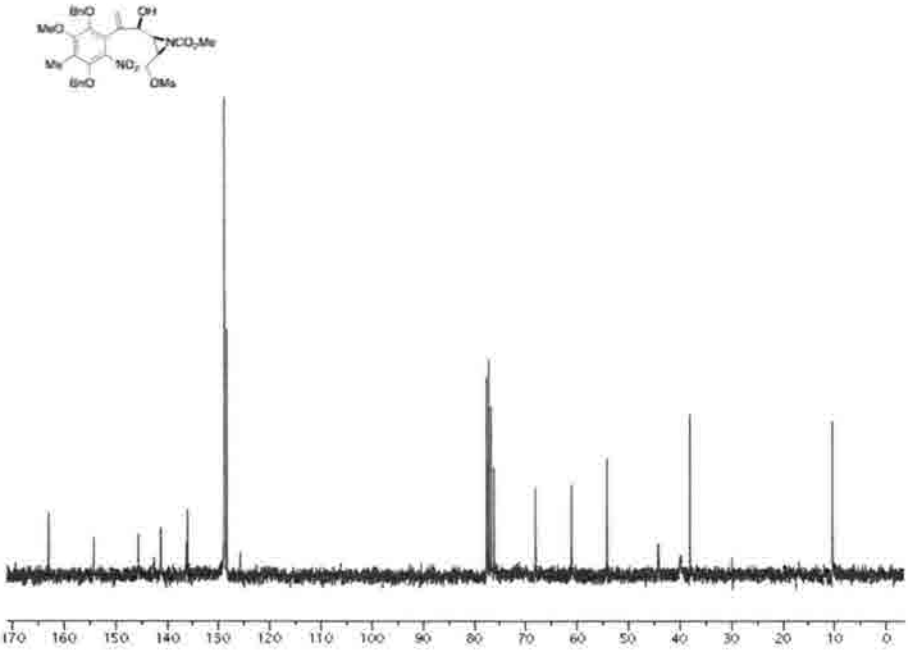


To mesylate **226** (580 mg, 0.91 mmol) in 3:1 methanol/dichloromethane 30 mL) at 0 °C was added ceriumtrichloride heptahydrate (1.68 g, 4.53 mmol) and sodium borohydride (103 mg, 2.73 mmol) and stirred for 30 minutes at the same temperature. The methanol was then evaporated followed by addition of ethyl acetate. Then reaction was then washed (1 x water, 1 x brine), dried (Na_2SO_4), and concentrated to afford pure alcohol **227** (560 mg, 99% yield) as a white foam, which was taken directly on without further

purification. $[\alpha]_D^{20}$ -16.5 (c=0.95, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.30 (3H, s), 2.83-2.88 (2H, m), 3.04 (3H, s), 3.71 (3H, s), 3.94 (3H, s), 4.20-4.60 (2H, m), 4.80-5.11 (4H, m), 5.25 (1H, s), 5.75-5.86 (1H, m), 7.31-7.40 (10H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 10.4, 38.1, 44.2, 54.2, 61.1, 68.1, 76.2, 125.8, 128.4, 128.7, 128.8, 128.84, 128.9, 136.1, 136.2, 141.3, 145.6, 154.2, 163.0; IR (neat) 3506, 3031, 2939, 1732, 1534, 1362, 1175, 958 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{31}H_{34}N_2O_{11}S$ 642.1883, $(MH)^+$, Found 642.1874.

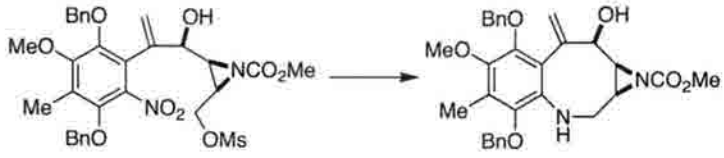


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-048-300-1H



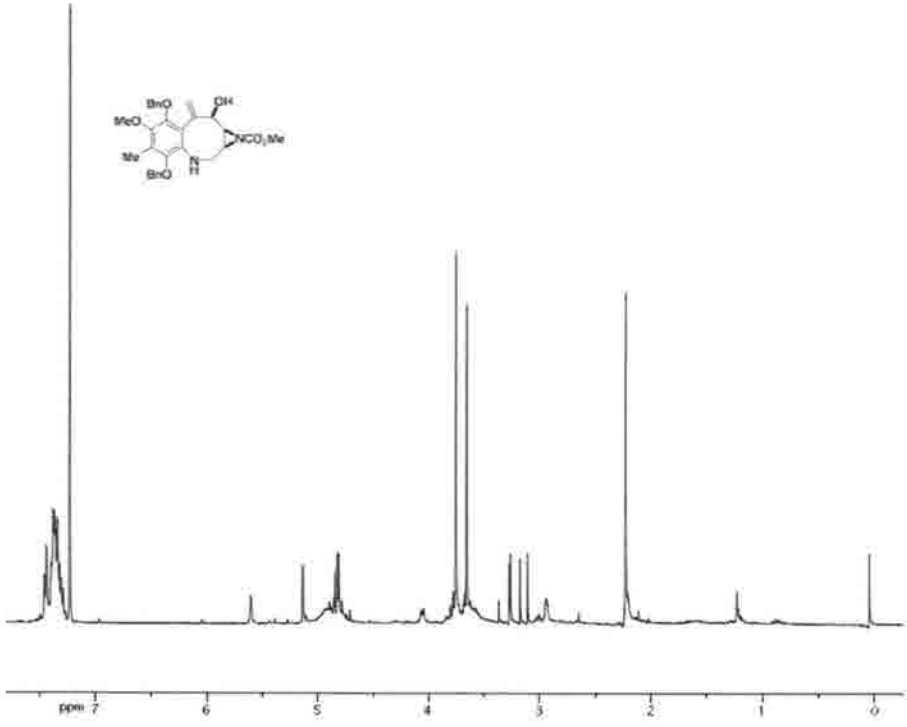
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-048-300-13C

(1a*S*,9*R*,9a*S*)-methyl 4,7-bis(benzyloxy)-9-hydroxy-6-methoxy-5-methyl-8-methylene -1a,2,3,8,9,9a-hexahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1-carboxylate (229)

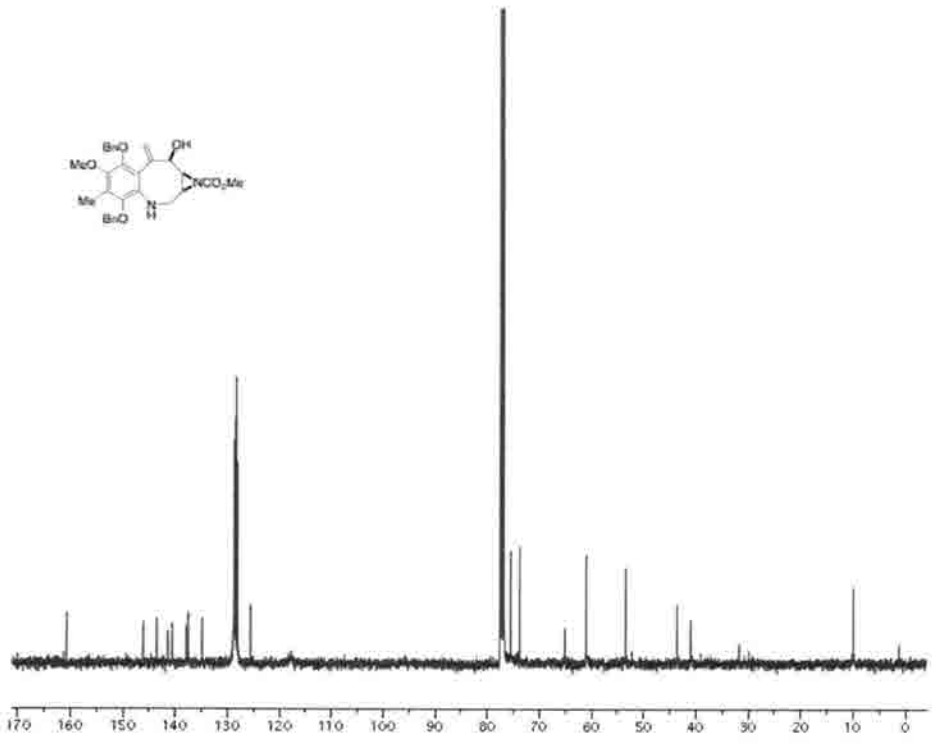


To alcohol **227** (560 mg, 0.87 mmol) in 4:1 acetone/water (21 mL) was added zinc (284 mg, 4.35 mmol) and ammonium chloride (465 mg, 8.7 mmol) and then stirred for 3 hours. The reaction was then concentrated, diluted (ethyl acetate), washed (1 x water, 1 x brine), dried (Na_2SO_4), and concentrated to give benzazocine **229** (440 mg, 99% yield) as a yellow foam that was used directly without further purification. $[\alpha]_D^{20}$ +35.5 (*c*=1.1, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 2.23 (3H, s), 2.93-2.95 (1H, m), 3.11 (1H,

s), 3.18 (1H, s), 3.26 (1H, d, J = 4.4 Hz), 3.66 (3H, s), 3.76 (3H, s), 4.05 (1H, d, J = 8.4 Hz), 4.80 (1H, d, J = 6.8 Hz), 4.84 (1H, d, 8.4 Hz), 4.87-4.96 (2H, m), 5.14 (1H, s), 5.61 (1H, s), 7.29-7.46 (10H, m); ^{13}CNMR (100 MHz, $CDCl_3$) δ 9.9, 41.0, 43.6, 53.5, 61.0, 65.1, 73.8, 75.5, 125.6, 128.1, 128.4, 128.6, 128.8, 134.9, 137.6, 137.9, 140.6, 141.5, 143.6, 146.1, 160.7; IR (neat) 3412, 3366, 2934, 1725, 1607, 1456, 1178, 1070 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{30}H_{32}N_2O_6$ 517.2333, $(MH)^+$, Found 517.2348.

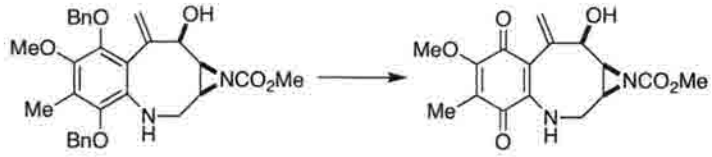


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-017-400-1H



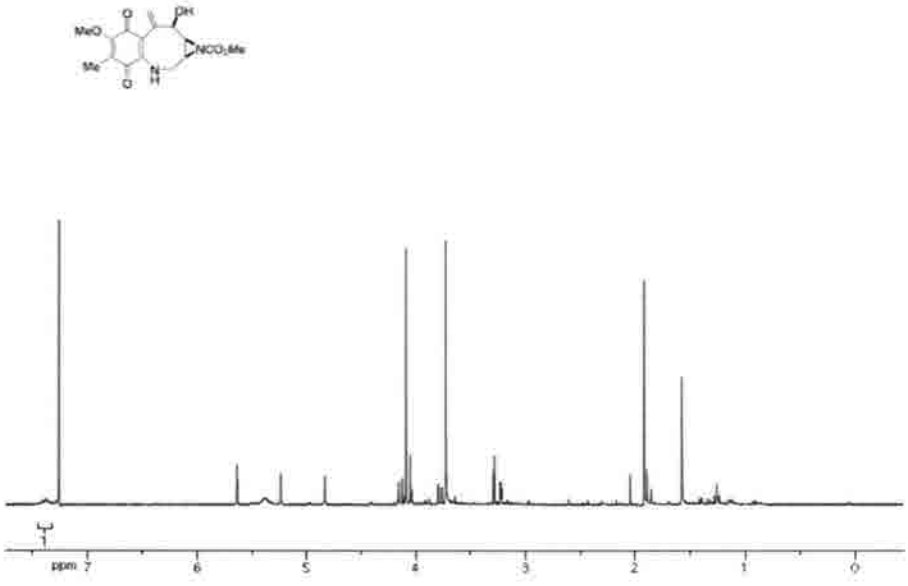
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-017-400-13C

(1a*S*,9*R*,9a*S*)-methyl9-hydroxy-6-methoxy-5-methyl-8-methylene-4,7-dioxo-1a,2,3,4, 7,8,9,9a-octahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1-carboxylate (232)



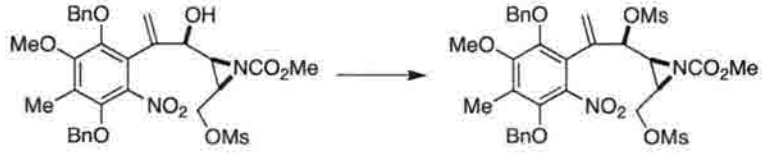
Benzazocine **229** (15 mg, 0.029 mmol, 1.0 equivalent), triethylamine (24 μL, 0.17 mmol, 6.0 equivalents), 10% Pd/C (58 mg), and dry ethyl acetate (2 mL) were stirred under a hydrogen balloon (1 hour) and then an oxygen balloon (30 minutes). The reaction was then filtered through a plug of celite (eluted with ethyl acetate) and concentrated. Pure quinone **232** (6 mg, 62% yield) was obtained by PTLC (70% ethyl acetate/hexanes).

^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 1.92 (3H, s), 3.21 (1H, dd, J = 3.6, 1.5 Hz), 3.73 (3H, s), 4.09 (3H, s), 4.84 (1H, s), 5.24 (1H, s), 5.38 (1H, s, br), 5.64 (1H, s), 7.38 (1H, s)



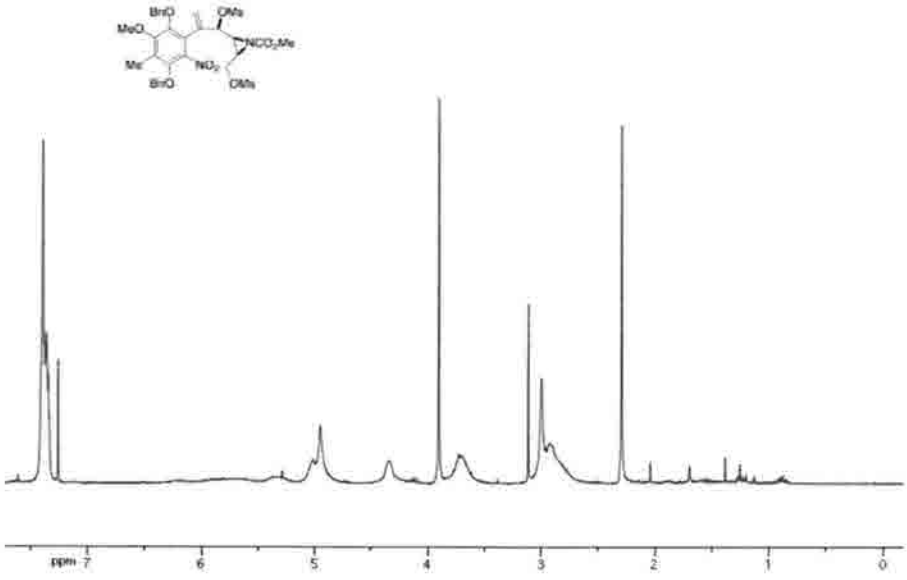
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-388

(2*S*,3*R*)-methyl 2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-(methylsulfonyloxy)allyl)-3-((methylsulfonyloxy)methyl)aziridine-1-carboxylate (237)

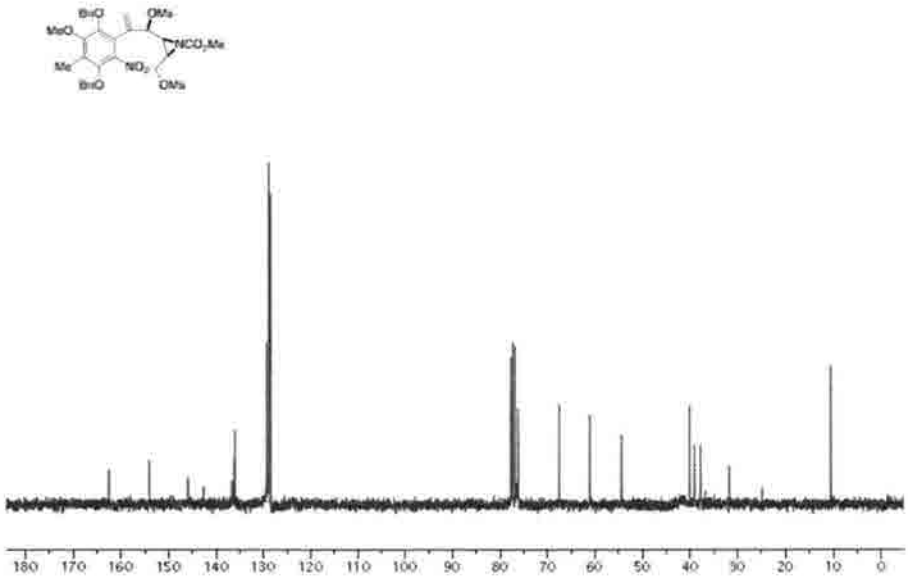


To alcohol **227** (259 mg, 0.40 mmol, 1.0 equivalent) in dichloromethane (12 mL) at 0 °C was added triethyl amine (170 μl, 1.2 mmol, 3.0 equivalents), methanesulfonyl chloride (46 μl, 0.60 mmol, 1.5 equivalents) dropwise and stirred for 30 minutes. The reaction was

then quenched (water), extracted (4 x CH_2Cl_2), washed (brine), dried (Na_2SO_4), and concentrated. Column chromatography (60% hexanes/ethyl acetate) provided bis-mesylate **237** (239 mg, 83% yield) as a yellow oil. $[\alpha]_D^{20}$ -23.2 (*c*=1.6, $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 2.29 (3H, s), 2.76-2.93 (4H, m), 3.00 (3H, s), 3.11 (1H, s), 3.64-3.77 (3H, m), 3.90 (3H, s), 4.32-4.35 (2H, m), 4.95 (3H, m), 4.98-5.05 (2H, m), 5.32-5.35 (1H, m), 7.34-7.39 (10H, m); ^{13}CNMR (100 MHz, $CDCl_3$) δ 10.5, 24.9, 31.8, 37.8, 39.1, 40.1, 54.4, 61.2, 67.6, 76.2, 76.9, 128.5, 128.7, 128.8, 129.3, 136.0, 136.2, 142.7, 146.0, 154.1, 162.5; IR (neat) 3032, 2940, 1735, 1536, 1454, 1362, 1176, 960 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{32}H_{36}N_2O_{13}S_2$ 720.1658, $(M+Na)^+$, Found 720.1663.

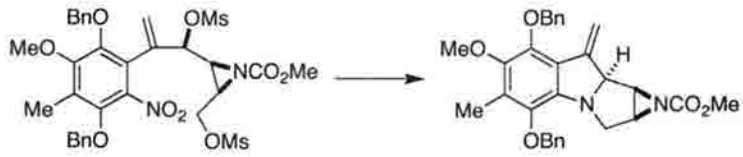


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-049-300-1H



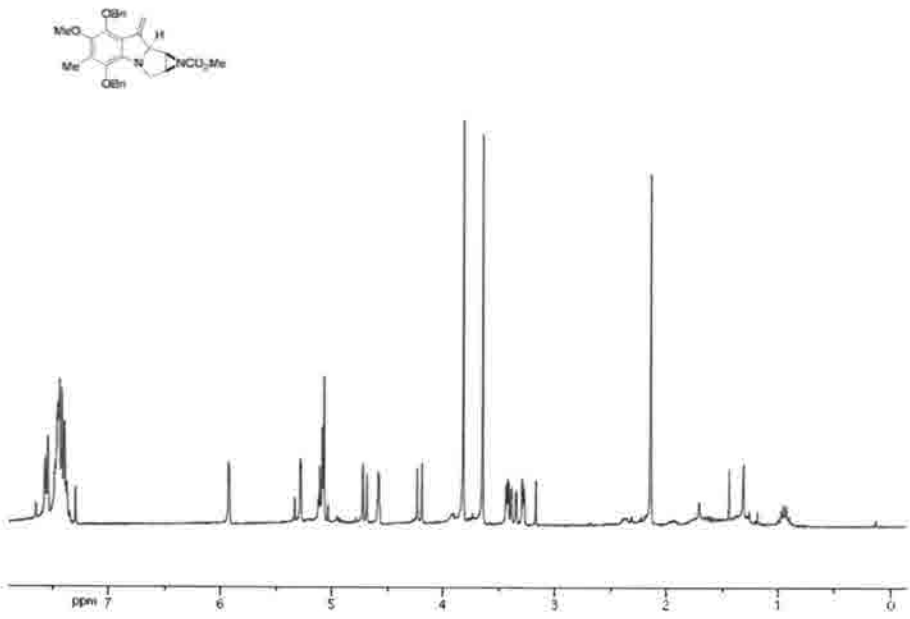
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-049-300-13C

Tetracycle (238) formation *via* reductive aminocyclization

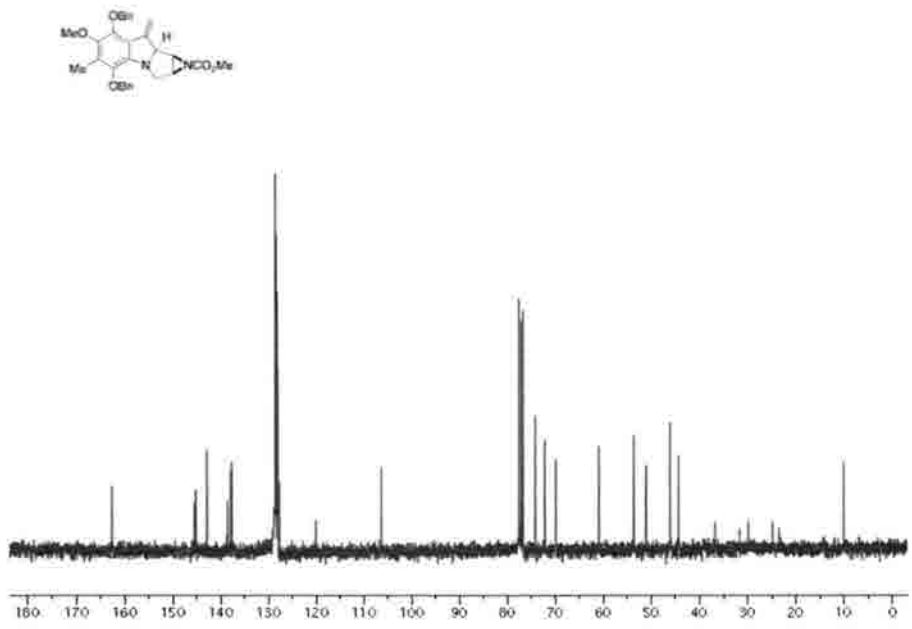


To bis-mesylate **227** (230 mg, 0.32 mmol) in 4:1 acetone/water (8.5 mL) was added zinc (104 mg, 1.6 mmol) and ammonium chloride (171 mg, 3.2 mmol) and then stirred for 4 days. The reaction was then concentrated, diluted (ethyl acetate), washed (1 x water, 1 x brine), dried (Na_2SO_4), and concentrated. The reaction was then purified using column chromatography (3:1 hexanes/ethyl acetate) to provide pure tetracycle **238** (72 mg, 45% yield) as a white foam. $[\alpha]_D^{20}$ +14.6 (c=2.5, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.14 (3H, s), 3.28 (1H, dd, J = 4.5, 1.8 Hz), 3.70 (1H, dd, J = 12.6, 1.8 Hz), 3.65 (3H, s),

3.82 (3H, s), 4.21 (1H, d, J = 12.3 Hz), 4.58 (1H, m), 4.70 (1H, d, J = 14.4 Hz), 5.04-5.13 (3H, m), 5.28 (1H, d, J = 2.1 Hz), 5.92 (1H, d, J = 2.4 Hz), 7.37-7.49 (9H, M), 7.55-7.57 (2H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 10.0, 24.8, 29.9, 36.8, 44.4, 46.1, 51.1, 53.7, 60.9, 69.9, 72.2, 74.2, 106.4, 120.1, 127.7, 128.0, 128.1, 128.2, 128.4, 128.6137.7, 138.0, 138.6, 142.9, 145.2, 145.3, 145.5, 162.6; IR (neat) 3031, 2929, 1726, 1472, 1279, 1099, 1001 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{30}H_{30}N_2O_5$ 498.2154, $(MH)^+$, Found 498.2150.

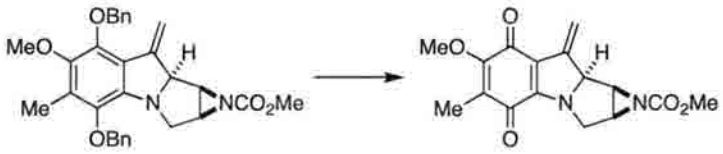


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-050-300-1H



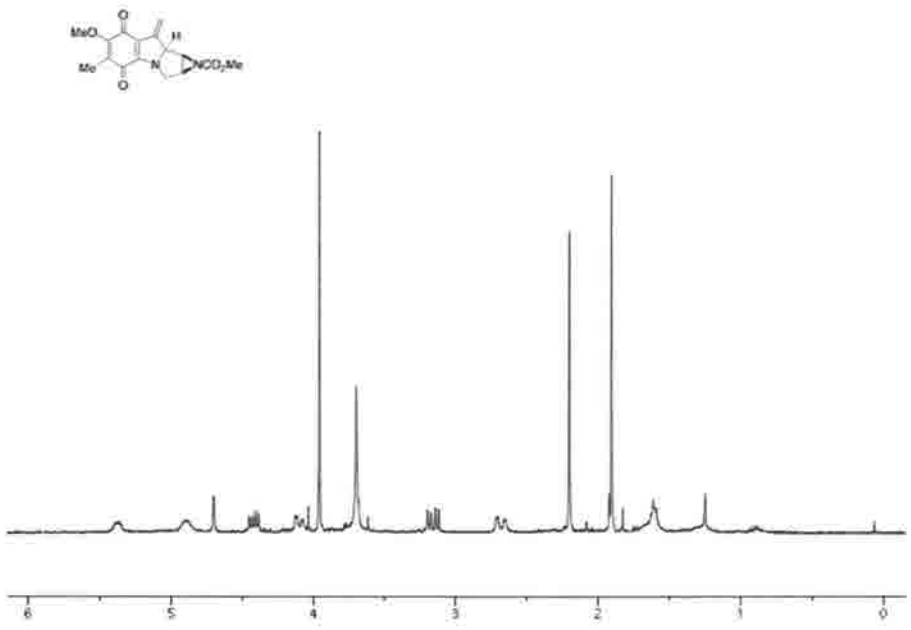
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-050-300-13C

_N_-methylcarbamoyl-9a-desmethoxymitomycin K (Mitosane 239)



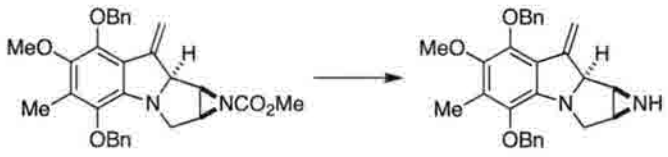
Tetracycle **238** (5 mg, 0.010 mmol, 1.0 equivalent), triethylamine (8 μL, 0.06 mmol, 6.0 equivalents), 10% Pd/C (11 mg), and dry ethyl acetate (500 μL) was stirred under a hydrogen balloon (1 hour) and then under an oxygen balloon (30 minutes). The reaction was then filtered through a plug of celite (eluted with ethyl acetate) and concentrated. Pure mitosane **239** (1.8 mg, 55% yield) was obtained by PTLC (1:1 ethyl acetate/hexanes). ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 1.91 (3H, s), 2.20 (3H, s), 2.64-

2.72 (1H, m), 3.15 (1H, dd, J = 16.5, 7.5 Hz), 3.70 (3H, s), 3.96 (3H, s), 4.07-4.13 (1H, m), 4.41 (1H, dd, J = 13.2, 7.2 Hz), 4.70 (1H, s), 4.87-4.92 (1H, m), 5.36-5.40 (1H, m).



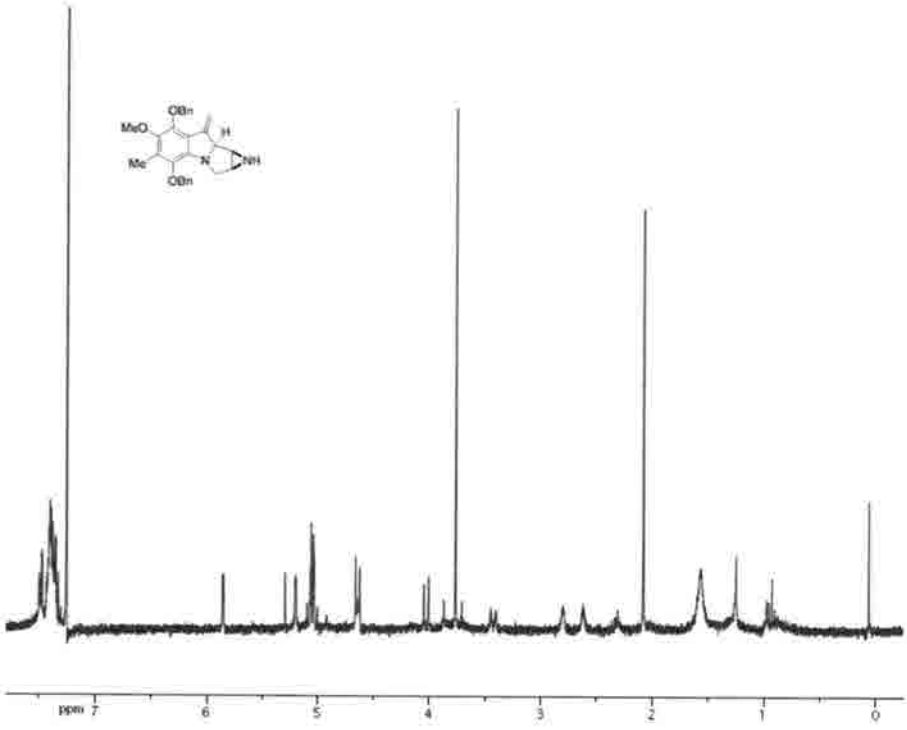
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-III-468-quinone

Free aziridine tetracycle (245)



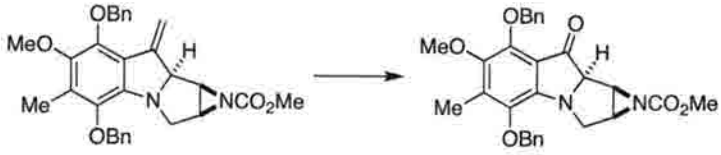
To a solution of tetracycle **238** (10 mg, 0.20 mmol) in THF (1 mL) was added L-Selectride (100 μL, 1.0 M solution in THF, 0.1 mmol), warmed to 0 °C and stirred for 20 minutes. The reaction was then quenched (sat. aq. NH_4Cl), extracted (4 x CH_2Cl_2), washed (brine), dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by PTLC (3:2 ethyl acetate/hexanes) to give **245** (2.4 mg, 27%) as a colorless oil.

^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.08 (3H, s), 2.62 (1H, s), 2.80 (1H, s), 3.40-3.46 (1H, m), 3.77 (3H, s), 4.02 (1H, d, J = 12.6 Hz), 4.62-4.66 (2H, m), 5.01-5.10 (3H, m), 5.21 (1H, d, J = 2.4 Hz), 5.85 (1H, d, J = 2.4 Hz), 7.22-7.45 (8H, m), 7.47-7.50 (2H, m).



^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-057

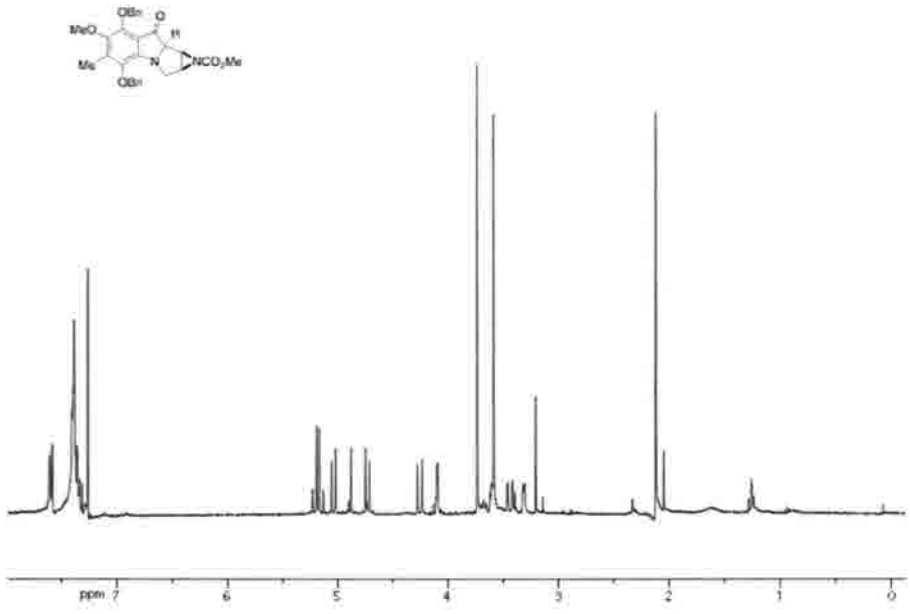
Tetracyclic ketone (248)



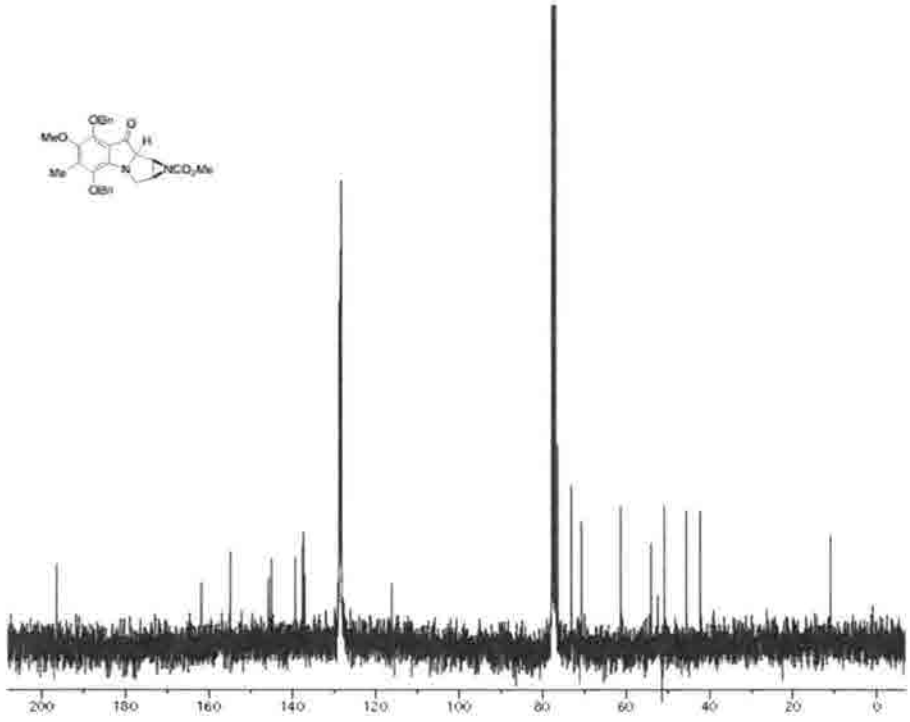
To tetracycle **238** (12 mg, 0.024 mmol, 1.0 equivalent) and NMO (5.6 mg, 0.048 mmol, 2.0 equivalents) in THF/acetone/water (3:1:1, 1 mL) was added OsO_4 (1% solution in water, 30 μL) and stirred for 90 minutes. The reaction was then quenched (water/brine),

extracted (4 x ethyl acetate), washed (brine), dried (Na_2SO_4), and concentrated to afford the crude diol (12 mg) that was taken directly onto the next step without purification.

To the crude diol (12 mg, 0.022 mmol, 1.0 equivalent) in THF/water (4:1) was added sodium periodate (38mg, 0.18 mmol, 8.0 equivalents) and stirred for 4 hours. The reaction was then diluted (ethyl acetate), ran through a celite plug (eluted with ethyl acetate), washed (water, brine) and concentrated. Pure ketone **248** (5.5 mg, 50% yield over two steps) was obtained by PTLC (3:2 ethyl acetate /hexanes). $[\alpha]_D^{20}$ +115.0 (*c*=0.6, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.12 (3H, s), 3.20 (1H, s), 3.30-3.32 (1H, m), 3.44 (1H, dd, J = 12.9, 1.2 Hz), 3.59 (3H, s), 3.73 (3H, s), 4.10 (1H, d, J = 4.5 Hz), 4,25 (1H, d, J = 12.9 Hz), 4.72 (1H, d, J = 11.1 Hz), 4.8 (1H, d, J = 7.2 Hz), 5.03 (1H, d, J = 11.1 Hz), 5.18 (2H, q, J = 10.5 Hz), 7.31-7.46 (8H, m), 7.59 (2H, d, J = 7.5 Hz); ^{13}CNMR (75 MHz, $CDCl_3$) δ 11.0, 42.2, 45.5, 50.8, 53.9, 61.2, 70.6, 73.0, 116.0, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8, 137.1, 137.4, 145.1, 145.8, 155.0, 161.9, 196.5; IR (neat) 3344, 3031, 2935, 1715, 1709, 1478, 1273, 1069, 1000 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{29}H_{28}N_2O_6$ 501.2022, $(MH)^+$, Found 501.2022.

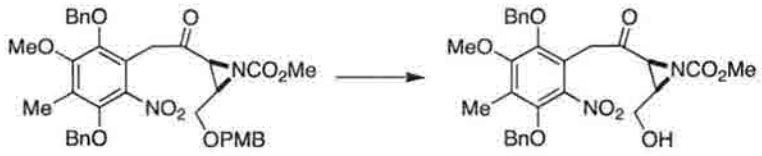


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-243

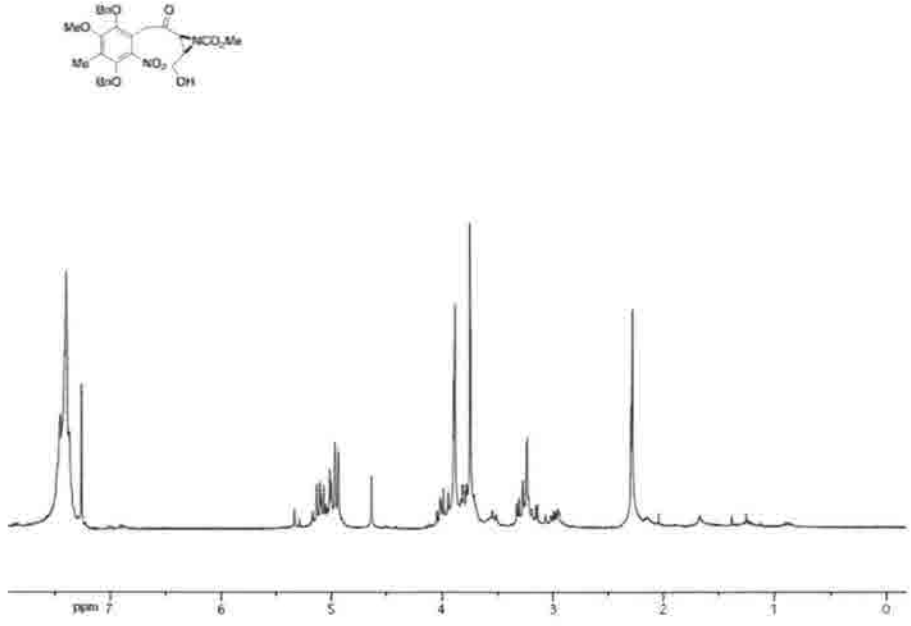


^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-243

(2*S*,3*R*)-methyl 2-(2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)acetyl)-3-(hydroxymethyl)aziridine-1-carboxylate (251)

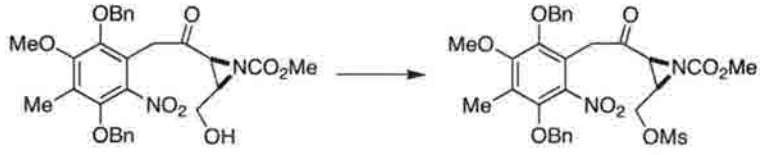


To a stirred solution of the ketone **204** (300 mg, 0.44 mmol) in 15:1 solution of dichloromethane/water (10 mL) was added DDQ (132 mg, 0.58 mmol) in one portion. The reaction mixture immediately turned green and over the course of 1.5 h the mixture turned orange. After 2 h, the reaction mixture was passed through a short pad of neutral alumina using 10:1 dichloromethane/methanol as eluant. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography (1:1 hexanes/ethyl acetate) to afford the alcohol **251** (207 mg, 85%) as a yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.28 (3H, s), 2.94-3.07 (1H, m), 3.13-3.33 (3H, m), 2.50-3.60 (1H, m), 3.75 (3H, s), 3.89 (3H, s), 4.94-5.14 (4H, m), 7.35-7.48 (10H, m).



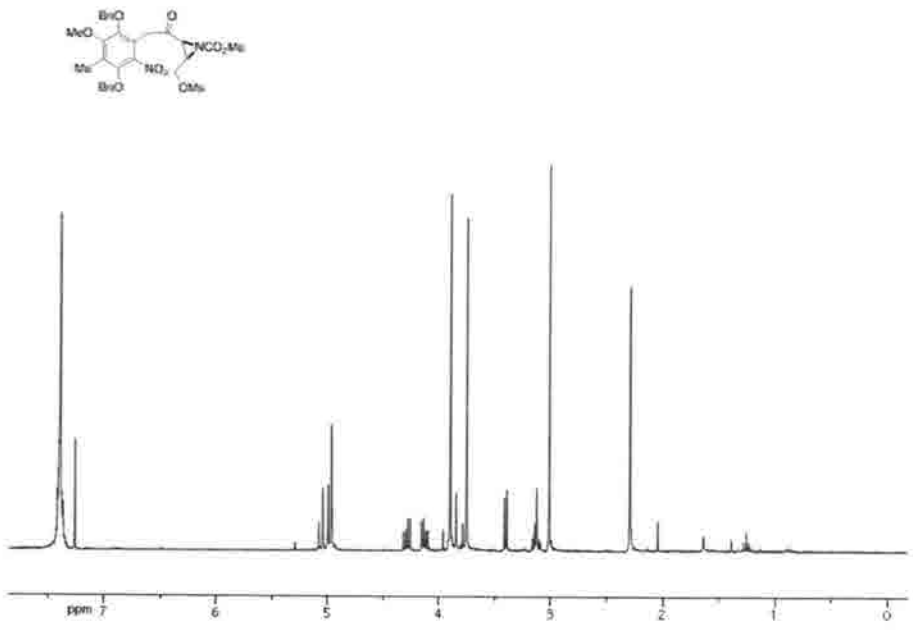
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-006

(2*S*,3*R*)-methyl-2-(2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)acetyl)-3-((methylsulfonyloxy)methyl)aziridine-1-carboxylate (252)



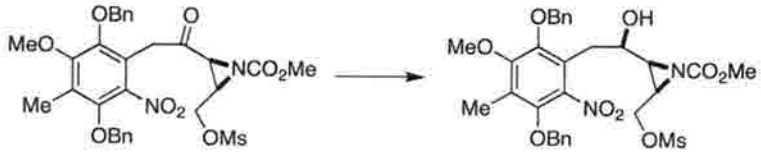
To alcohol **251** (205 mg, 0.37 mmol) and triethylamine (156 μL, 1.11 mmol) in dichloromethane (10 mL) at 0 °C was added methanesulfonyl chloride (43 μL, 0.56 mmol,) slowly dropwise and stirred at this temperature for 30 minutes. The reaction was then quenched (brine), extracted (4 x dichloromethane), washed (1 x brine), dried (Na_2SO_4) and concentrated. Purification by column chromatography (1:1 hexanes/ethyl acetate) gave mesylate **252** (124 mg, 53%) as a yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ

TMS: 2.30 (3H, s), 3.02 (3H, s), 3.10-3.17 (2H, m), 3.40 (1H, d, J = 6.0 Hz), 3.75 (3H, s), 3.90 (3H, s), 4.13 (1H, dd, J = 11.7, 6.0 Hz), 4.19 (1H, dd, J = 12.0, 6.9 Hz), 4.96-5.08 (4H, m), 7.35-7.43 (10H, m).



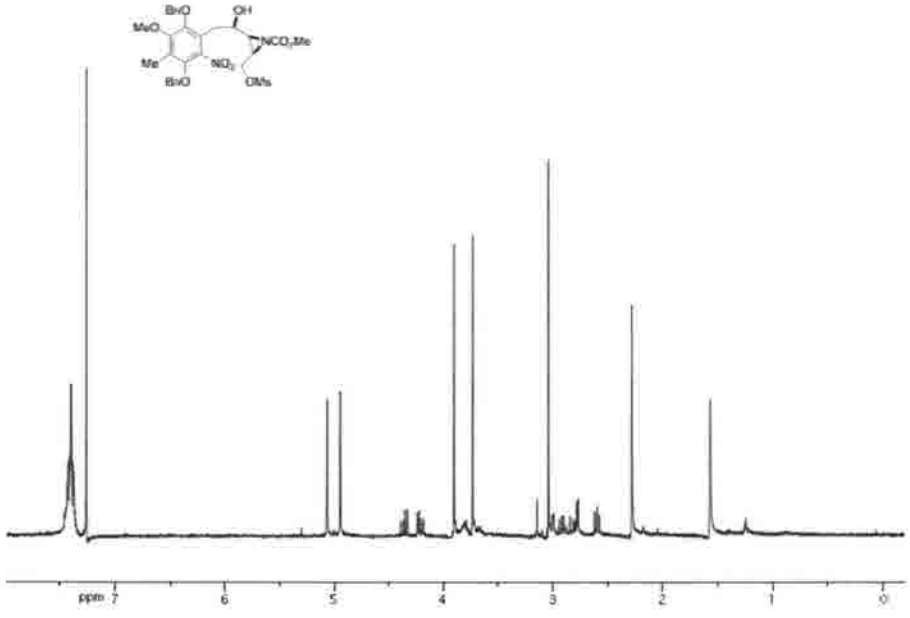
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-007

(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-hydroxyethyl)-3-((methylsulfonyloxy)methyl)aziridine-1-carboxylate (253)



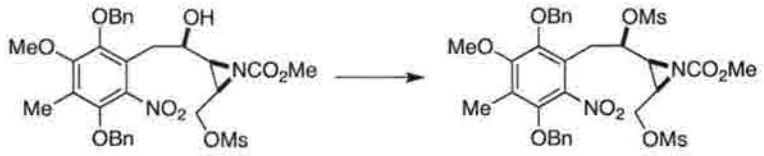
To mesylate **252** (120 mg, 0.19 mmol) in 3:1 methanol/dichloromethane (10 mL) at 0 °C was added ceriumtrichloride heptahydrate (355 mg, 0.95 mmol) and sodium borohydride (21 mg, 0.57 mmol) and stirred for 30 minutes at the same temperature. The methanol

was then evaporated followed by addition of ethyl acetate. Then reaction was then washed (1 x water, 1 x brine), dried (Na_2SO_4), and concentrated to afford pure alcohol **253** (111 mg, 93% yield) as a white foam, which was taken directly on without further purification. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.28 (3H, s), 2.60 (1H, t, J = 6.3 Hz), 2.78 (1H, dd, J = 8.7, 4.5 Hz), 2.88-2.94 (2H, m), 3.00 (1H, d, J = 3.0 Hz), 3.04 (3H, s), 3.73 (3H, s), 3.81 (1H, m), 3.90 (3H, s), 4.20 (1H, dd, J = 12.0, 5.7 Hz), 4.36 (1H, dd, J = 12.0, 6.9 Hz), 4.95 (2H, s), 5.06 (2H, m), 7.36-7.46 (10H, m).

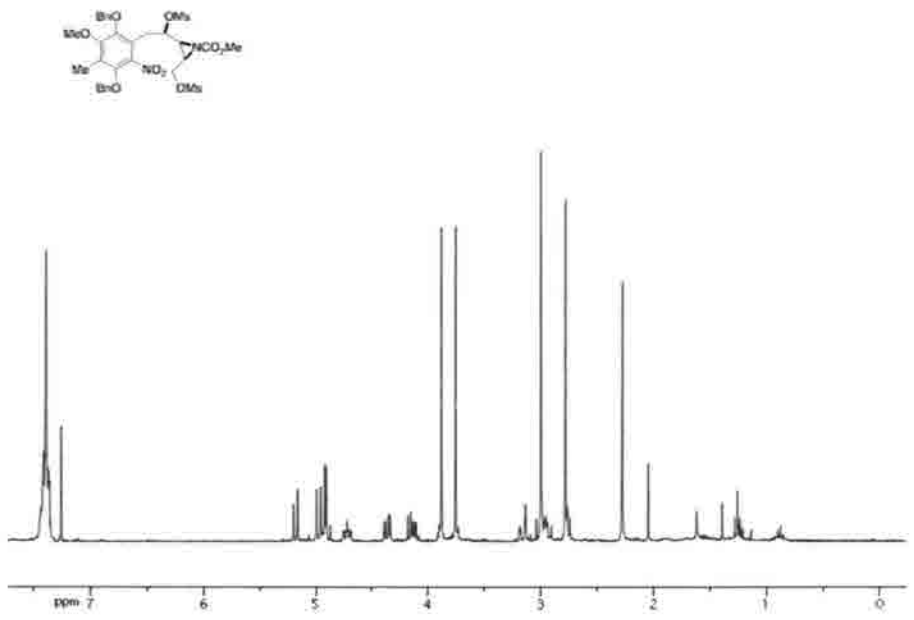


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-008

(2*S*,3*R*)-methyl-2-((*R*)-2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-(methylsulfonyloxy)ethyl)-3-((methylsulfonyloxy)methyl)aziridine-1-carboxylate (254)

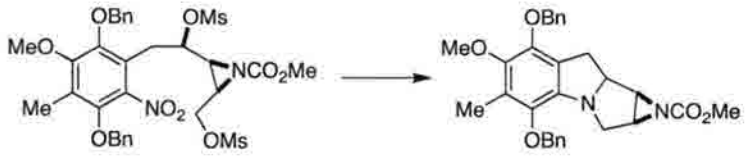


To alcohol **253** (110 mg, 0.17 mmol) and triethylamine (73 μL, 0.52 mmol) in dichloromethane (6 mL) at 0 °C was added methanesulfonyl chloride (20 μL, 0.26 mmol,) slowly dropwise and stirred at this temperature for 30 minutes. The reaction was then quenched (brine), extracted (4 x dichloromethane), washed (1 x brine), dried (Na_2SO_4) and concentrated. Purification by column chromatography (1:1 hexanes/ethyl acetate) gave mesylate **254**(112 mg, 93%) as a yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.27 (3H, s), 2.76 (1H, dd, J = 8.1, 6.3 Hz), 2.78 (3H, s), 2.94-2.97 (1H, m), 3.00 (3H, s), 3.13-3.19 (1H, m), 3.76 (3H, s), 3.88 (3H, s), 4.11 (1H, dd, J = 7.2, 3.6 Hz), 4.17 (1H, d, J = 7.8 Hz), 4.69-4.76 (1H, m), 4.92 (2H, dd, J = 18.0, 10.5 Hz), 4.97 (1H, d, J = 11.1 Hz), 5.18 (1H, d, J = 11.1 Hz), 7.36-7.45 (10H, m).



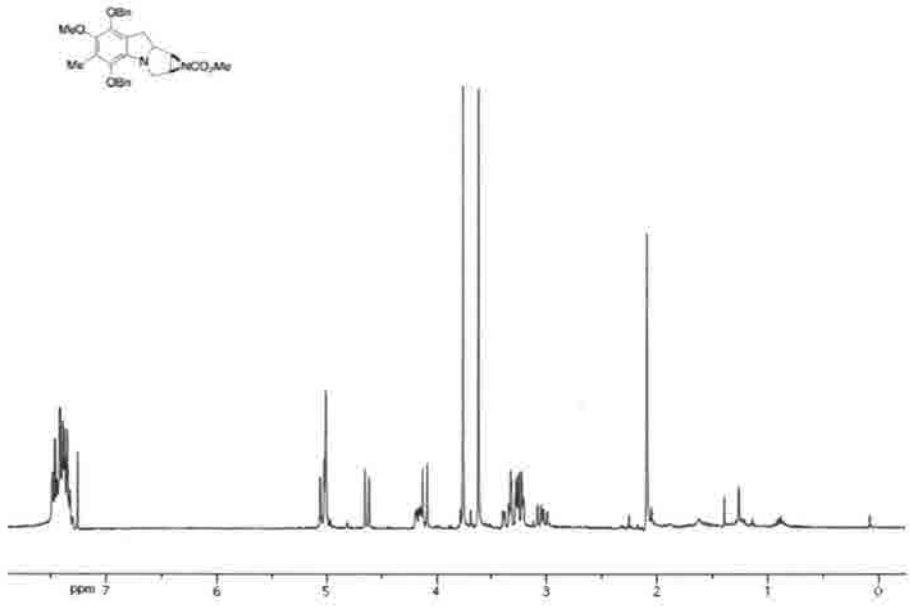
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-009

Indoline (255)



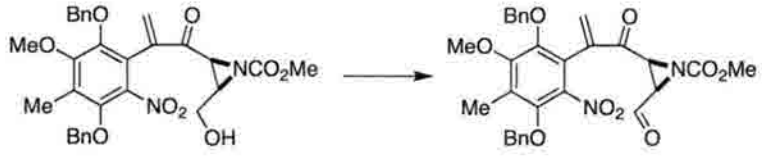
To bis-mesylate **254** (110 mg, 0.15 mmol) in 4:1 acetone/water (4 mL) was added zinc (50 mg, 0.78 mmol) and ammonium chloride (80 mg, 1.5 mmol) and then stirred for 5 days. The reaction was then concentrated, diluted (ethyl acetate), washed (1 x water, 1 x brine), dried (Na_2SO_4), and concentrated. The reaction was then purified using column chromatography (3:1 hexanes/ethyl acetate) to provide pure indoline **255** (38 mg, 53% yield) as a white foam. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.09 (3H, s), 3.04 (1H, dd, J = 16.2, 10.2 Hz), 3.22 (1H, dd, J = 4.5, 1.8 Hz), 3.27 (1H, dd, J = 4.5, 2.1 Hz), 3.32-3.40

(1H, m), 3.62 (3H, s), 3.76 (3H, s), 4.10 (1H, d, J = 12.9 Hz), 4.16 (1H, dq, J = 6.6, 4.5, 2.1 Hz), 4.63 (1H, d, J = 11.1 Hz), 4.97-5.06 (3H, m), 7.31-7.49 (10H, m).



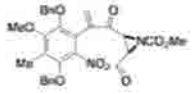
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-010

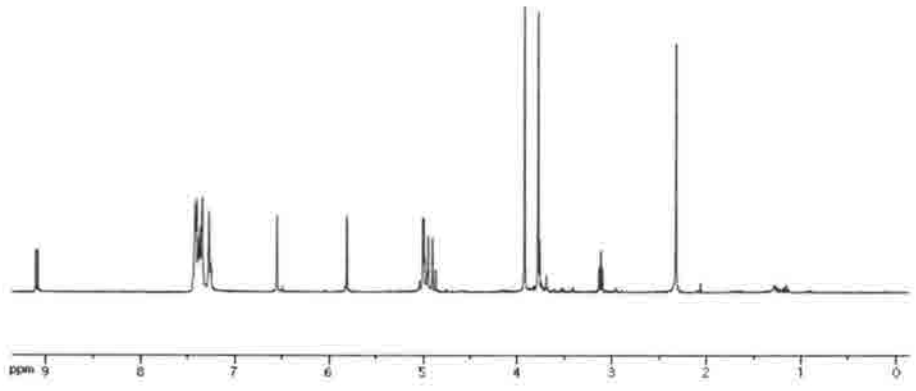
(2*S*,3*R*)-methyl 2-(2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)acryloyl) -3-formylaziridine-1-carboxylate (260)



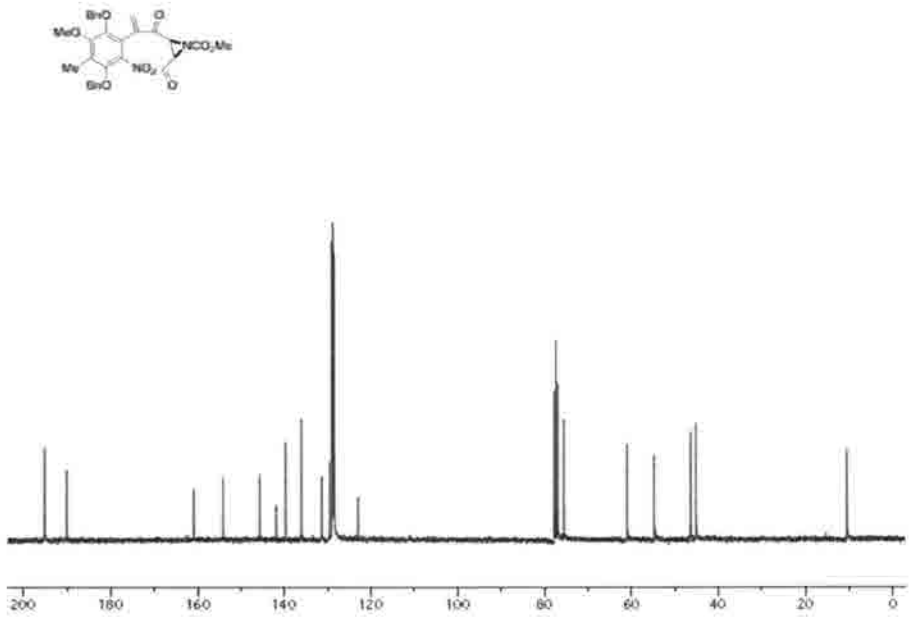
To alcohol **225** (205 mg, 0.36 mmol) in CH_2Cl_2 (10 mL) was added Dess-Martin periodinane (247 mg, 0.58 mmol) and stirred for 2 hrs. The reaction was diluted with ether (10 mL), poured into 10 mL sat. aq. $NaHCO_3$ with 7.0 equivalents of sodium thiosulfate (625 mg, 2.52 mmol), and stirred for 15 min. The layers were separated and

the aqueous layer was backextracted with ether. The combined ether layers were washed (1 x sat. aq. $NaHCO_3$, 1 x Brine), dried (Na_2SO_4), filtered and concentrated. Purification by column chromatography (3:2 hexanes/ethyl acetate) gave aldehyde **260** (176 mg, 88%) as a pale yellow oil. $[\alpha]_D^{20}$ -14.6 (c=0.75, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.32 (3H, s), 3.12 (1H, t, J = 6.0 Hz), 3.78 (3H, s), 3.92 (3H, s), 4.76-5.04 (4H, m), 5.81 (1H, s), 6.55 (1H, s), 7.25-7.28 (2H, m), 7.33-7.42 (8H, m), 9.1 (1H, d, J = 5.7 Hz); ^{13}CNMR (75 MHz, $CDCl_3$) δ 10.4, 45.1, 46.4, 54.7, 61.0, 75.5, 123.0 128.5, 128.7, 128.8, 129.0, 129.5, 131.4, 136.1, 139.7, 141.9, 145.7, 145.8, 154.3, 161.0, 190.1, 195.1; IR (neat) 3032, 2954, 2884, 1741, 1728, 1532, 1362, 1276, 1119, 958 cm^{-1}; HRMS (FAB+) m/z Calc. 560.1795, $(MH)^+$, Found 560.1799.



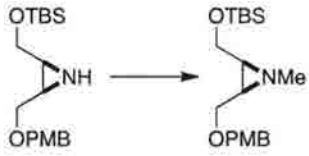


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-099-300-1H



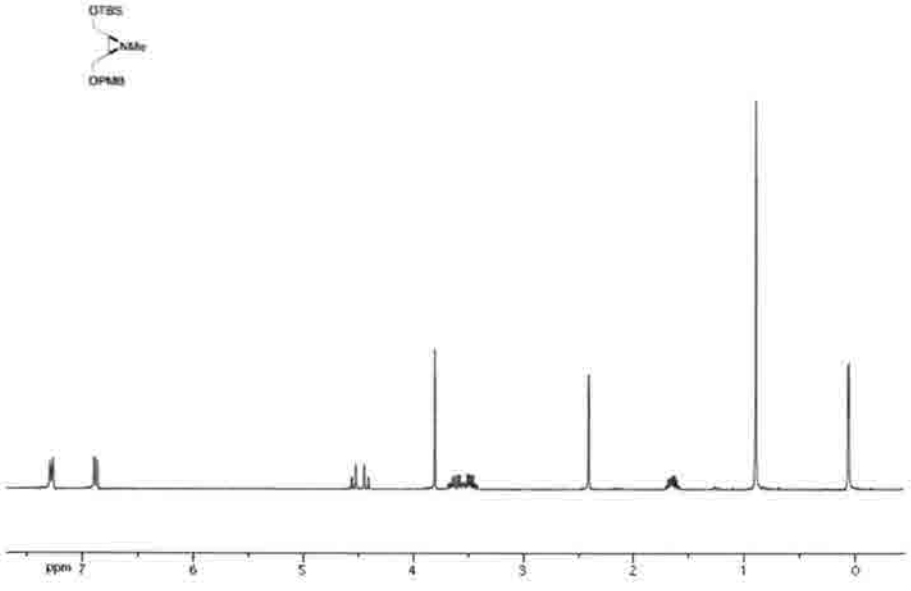
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-099-300-13C

(2*S*,3*R*)-2-((*tert*-butyldimethylsilyloxy)methyl)-3-((4-methoxybenzyloxy)methyl)-1-methylaziridine (264)

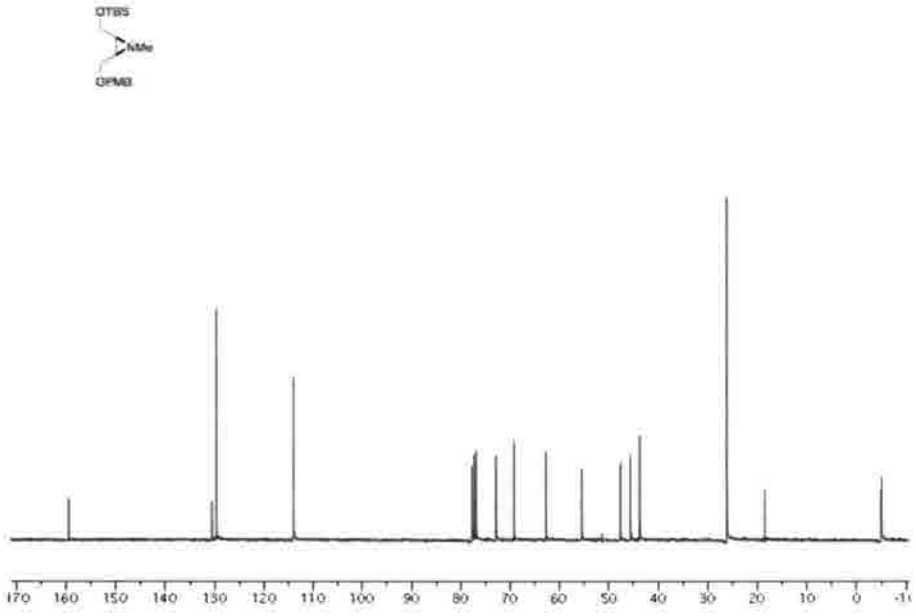


To aziridine **263** (5.46 g, 0.016 mol, 1.0 equiv) in THF (160 mL) at -78 °C was added *n*-BuLi (11.6 mL, 0.0186 mol, 1.15 equiv.) dropwise and then continued stirring at -78 °C for 1 hour. The reaction was then quenched (water), extracted (4 x EtOAc), washed (brine), dried (Na_2SO_4), filtered and concentrated. The crude product was purified by column chromatography (3:1 hexanes/ethyl acetate) to afford product **264** (1.04 g, 19%) as a colorless oil. $[\alpha]_D^{20}$ +7.4 (*c*=2.5, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.06

(6H, d, J = 2.1 Hz), 0.90 (9H, s), 1.59-1.71 (2H, m), 2.42 (3H, s), 3.42-3.54 (2H, m), 3.54-3.68 (2H, m), 3.80 (3H, s), 4.42 (2H, d, J = 11.4 Hz), 4.53 (2H, d, J = 11.4 Hz), 6.87 (2H, d, J = 8.4 Hz), 7.27 (2H, d, J = 8.1 Hz); ^{13}CNMR (75 MHz, $CDCl_3$) δ -5.1, -4.9, 18.5, 26.1, 43.8, 45.6, 47.7, 55.5, 62.7, 69.2, 72.9, 114.0, 129.7, 130.6, 159.4; IR (neat) 2953, 2856, 1613, 1513, 1463, 1249, 1087 cm^{-1}; HRMS (FAB+) m/z Calc. for $C_{19}H_{33}NO_3Si$ 351.2130, $(MH)^+$, Found 351.2127.

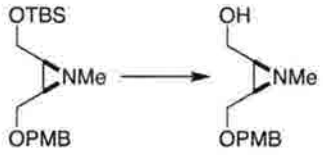


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-123-300-1H



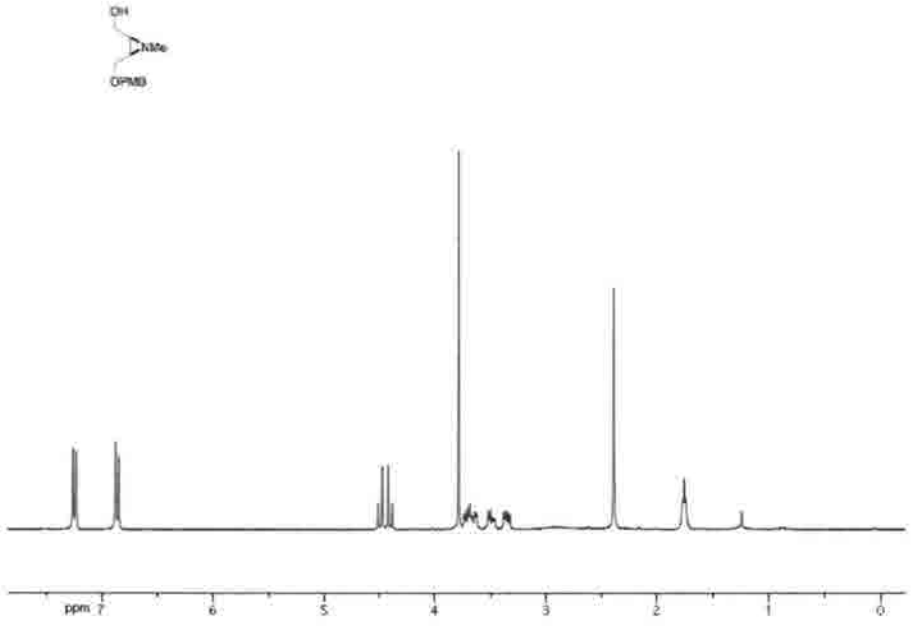
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-123-300-13C

((2*S*,3*R*)-3-((4-methoxybenzyloxy)methyl)-1-methylaziridin-2-yl)methanol (265)



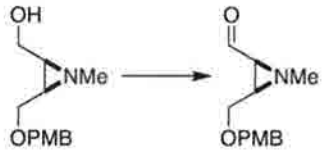
To **264** (1.04 g, 2.96 mmol, 1.0 equiv.) in THF (30 mL) at 0 °C was added TBAF (3.55 mL, 1M in THF solution, 3.55 mmol, 1.2 equiv.) slowly dropwise and then stirred at the same temperature for 1.5 hours. The solution was then quenched, (sat. aq. NH_4Cl), extracted (4 x EtOAc), washed (brine), dried (Na_2SO_4), filtered and concentrated. Purification of the crude material by column chromatography (3:2 chloroform/acetone) provided alcohol **265** (540 mg, 77%) as a colorless oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 1.74-1.77 (2H, m), 2.39 (3H, s), 3.34 (1H, dd, J = 10.5, 6.6 Hz), 3.49 (1H, dd, J =

12.0, 6.3 Hz), 3.62-3.74 (2H, m), 3.79 (3H, s), 4.39 (1H, d, J = 11.4 Hz), 4.79 (1H, d, J = 11.4 Hz), 6.86 (2H, d, J = 8.7 Hz), 7.24 (2H, d, J = 8.7 Hz).



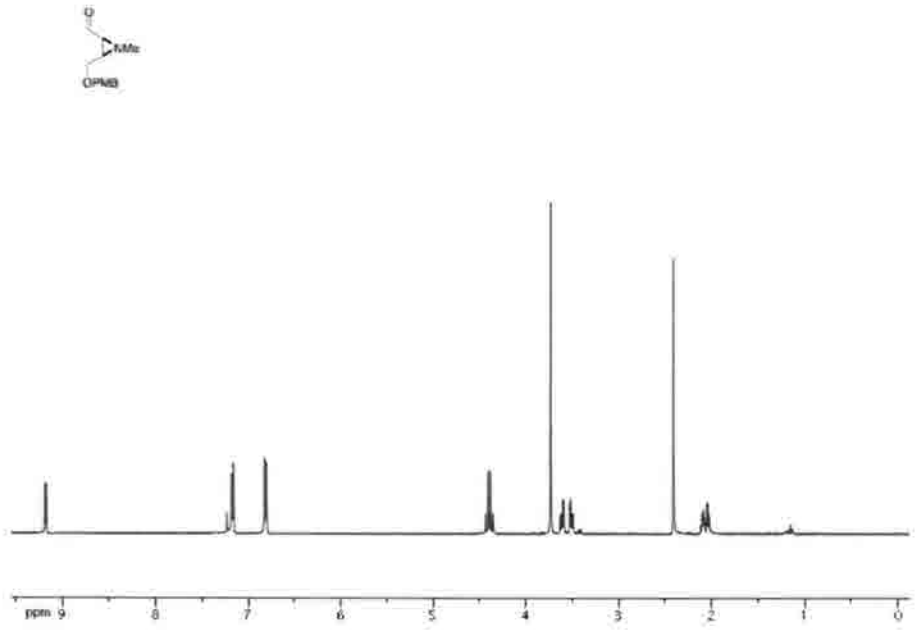
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-131

(2*S*,3*R*)-3-((4-methoxybenzyloxy)methyl)-1-methylaziridine-2-carbaldehyde (266)

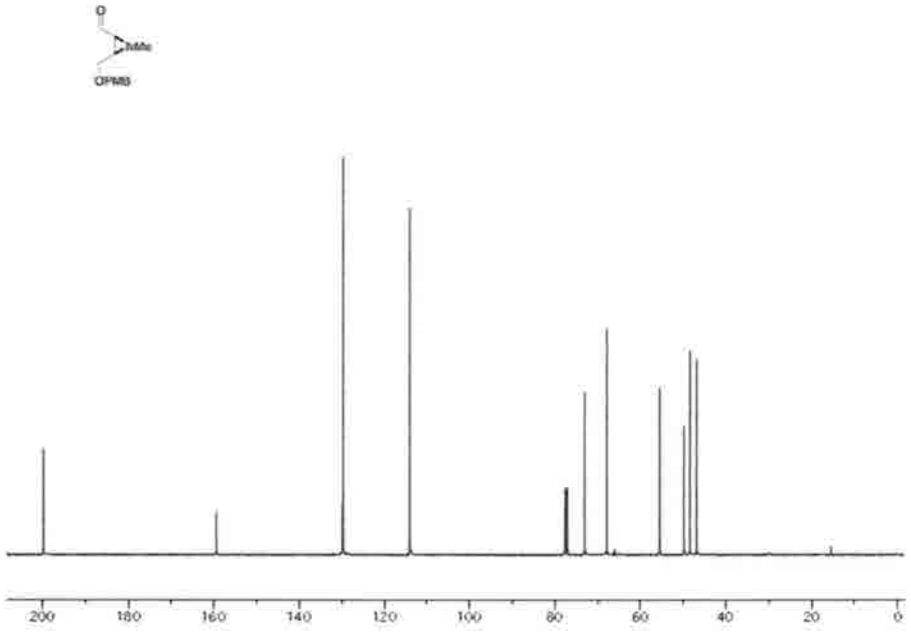


To **265** (540 mg, 2.28 mmol) in CH_2Cl_2 (22 mL) was added Dess-Martin periodinane (1.5 g, 3.64 mmol,) and stirred for 2 hours. The solution was the diluted (ether, 25 mL) and poured in sat. aq. $NaHCO_3$ with 7 equivalents of $Na_2S_2O_3 \cdot 5H_2O$ (3.95 g) and stirred until the cloudy solution became clear (~15 min). The layers were separated and the aqueous layer was backextracted (5 x ether). The organic layers were then combined, washed

(water, brine), dried (Na_2SO_4), filtered and concentrated. Purification of the crude material by column chromatography (3:2 Hexanes/ethyl acetate) provided alcohol **266** (405 mg, 77%) as a colorless oil. ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 2.03 (1H, dd, J = 5.1, 3.9 Hz), 2.09 (1H, dd, J = 6.6, 3.9 Hz), 2.41 (3H, s), 3.50 (1H, dd, J = 8.1, 3.6 Hz), 3.61 (1H, dd, J = 7.8, 4.5 Hz), 3.74 (3H, s), 4.39 (2H, q, J = 8.1 Hz), 6.81 (2H, d, J = 6.3 Hz), 7.17 (2H, d, J = 5.7 Hz), 9.17 (1H, d, J = 3.9 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 46.8, 48.4, 49.8, 55.4, 67.8, 73.0, 114.0, 129.6, 129.8, 159.5, 199.8; IR (neat) 2954, 2908, 2837, 1715, 1612, 1513, 1248, 1099 cm^{-1}.

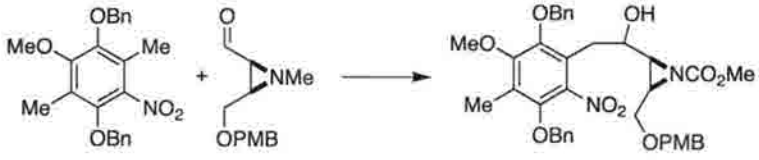


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-IV-178-400-1H



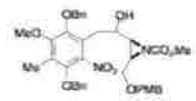
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-IV-178-400-13C

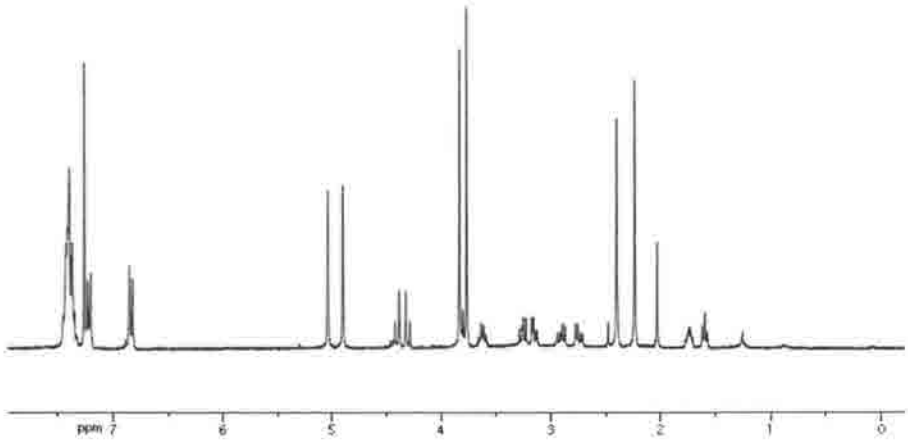
(2*S*,3*R*)-methyl2-(2-(2,5-bis(benzyloxy)-3-methoxy-4-methyl-6-nitrophenyl)-1-hydroxyethyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (267)



Prior to the reaction, compounds **184**(1.75 g, 4.46 mmol) and **266** (420 mg, 1.78 mmol) were dehydrated three times by azeotropic distillation using anhydrous toluene and lastly dried under vacuum for 1h before use. A solution of **266** in dry DMF (8 mL) was then added to freshly fused zinc chloride (363 mg, 2.67 mmol). The resulting solution was stirred under argon for 1h at rt. In a separate flask, NaHMDS (4.46 mL of a 1 M solution in THF, 4.46 mmol) was added dropwise to a solution of **184** in dry DMF (15 ml) cooled

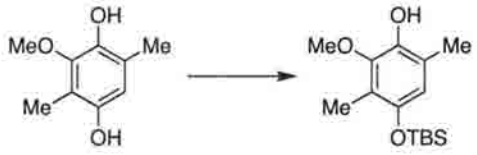
to -45°C. The mixture turned immediately deep red. To this solution was added dropwise the aziridine solution prepared above and the resulting mixture was stirred at -45°C for 3 hrs. The reaction mixture was quenched with saturated aqueous NH_4Cl (10 mL), allowed to warm to room temperature and water (10 mL) was added. The aqueous phase was extracted with DCM (5 x 15 mL), the combined organics dried over Na_2SO_4 and concentrated under reduced pressure. The crude oil was purified by flash column chromatography (7:3 hexanes: ethyl acetate) to afford the alcohol **267** (278 mg, 25%) as a pale yellow oil (a single diastereomer!). ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 1.59 (1H, t, J = 6.9 Hz), 1.71-1.76 (1H, m), 2.24 (3H, s), 2.40 (1H, s), 2.74 (1H, dd, J = 13.2, 6.0 Hz), 2.90 (1H, dd, J = 13.8, 7.8 Hz), 3.15 (1H, dd, J = 10.8, 4.2 Hz), 3.26 (1H, dd, J = 10.8, 7.8 Hz), 3.59-3.66 (1H, m), 3.77 (3H, s), 3.83 (3H, s), 4.30 (1H, d, J = 11.4 Hz), 4.40 (1H, d, J = 11.7 Hz), 4.90 (2H, s), 5.04 (2H, s), 6.83 (2H, d, J = 8.4 Hz), 7.22 (2H, d, J = 8.7 Hz), 7.33-7.46 (10H, m).





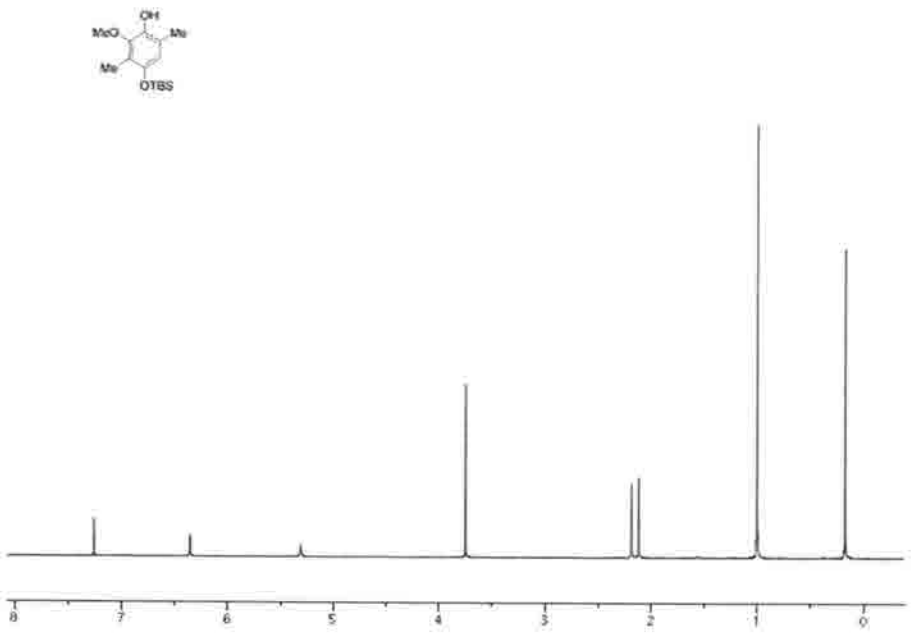
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-194

4-(*tert*-butyldimethylsilyloxy)-2-methoxy-3,6-dimethylphenol (268)

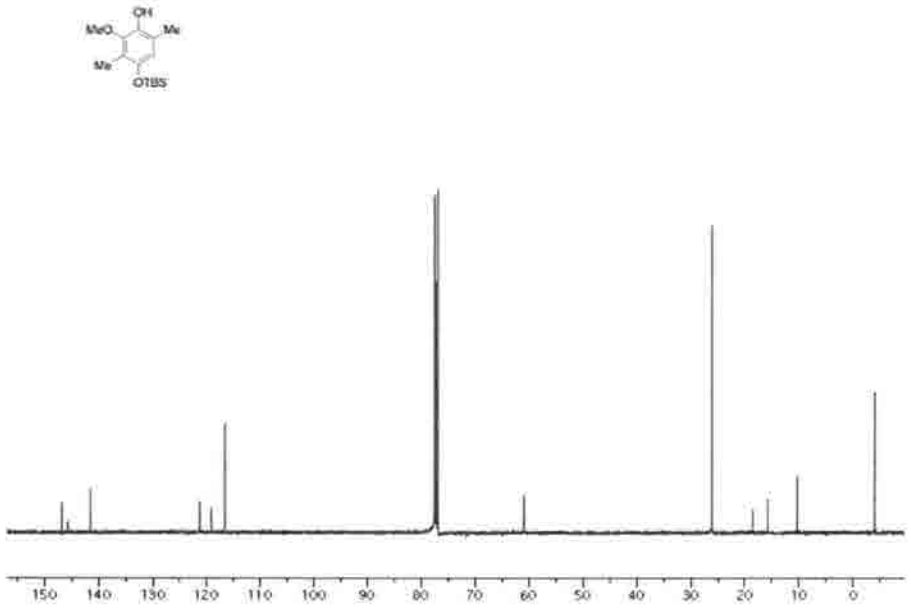


To hydroquinone **173** (11.0 g, 65 mmol) in DMF (130 mL) and cooled to -45 °C, was added imidazole (18 g, 27 mmol) followed by dropwise addition of TBSCl (12 g, 78 mmol) in 60 mL of DMF. The reaction was warmed to room temperature and stirred for 2 hours. The reaction mixture was then quenched with water, extracted (4 x ethyl acetate), washed (2 x water, 1 x brine), dried ($MgSO_4$), filtered, and concentrated. Purification of the crude by column chromatography (95:5 hexanes/ethyl acetate) gave phenol **268** (10 g, 56%) as a yellow oil. ^{1}HNMR (400 MHz, $CDCl_3$) δ TMS: 0.18 (6H, s), 1.01 (9H, s), 2.12

(3H, s), 2.19 (3H, s), 3.75 (3H, s), 5.31 (1H, s), 6.36 (1H, s); ^{13}CNMR (100 MHz, $CDCl_3$) δ -4.0, 10.4, 15.8, 18.5, 26.1, 60.9, 116.6, 119.1, 121.3, 141.5, 145.7, 146.8; IR (neat) 3546, 3450, 2956, 2930, 2858, 1593, 1489 cm^{-1}.

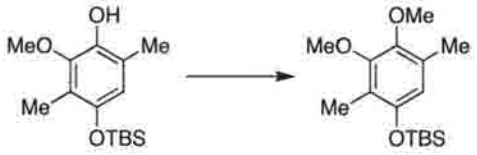


^{1}HNMR, $CDCl_3$, 400 MHz; Filename: Dag-IV-253



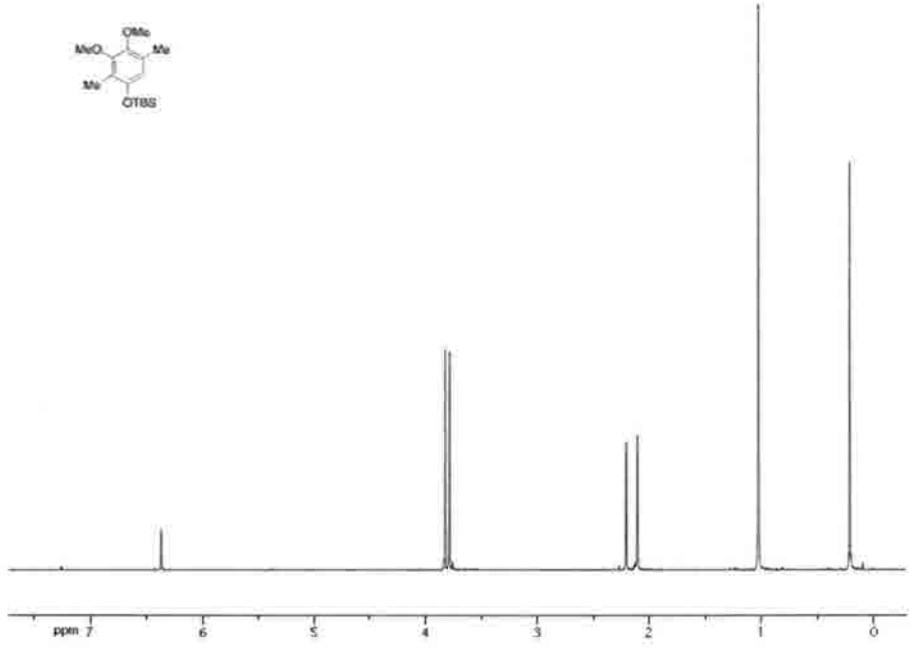
^{13}CNMR, $CDCl_3$, 100 MHz; Filename: Dag-IV-253-400-13C

***tert*-butyl(3,4-dimethoxy-2,5-dimethylphenoxy)dimethylsilane (269)**

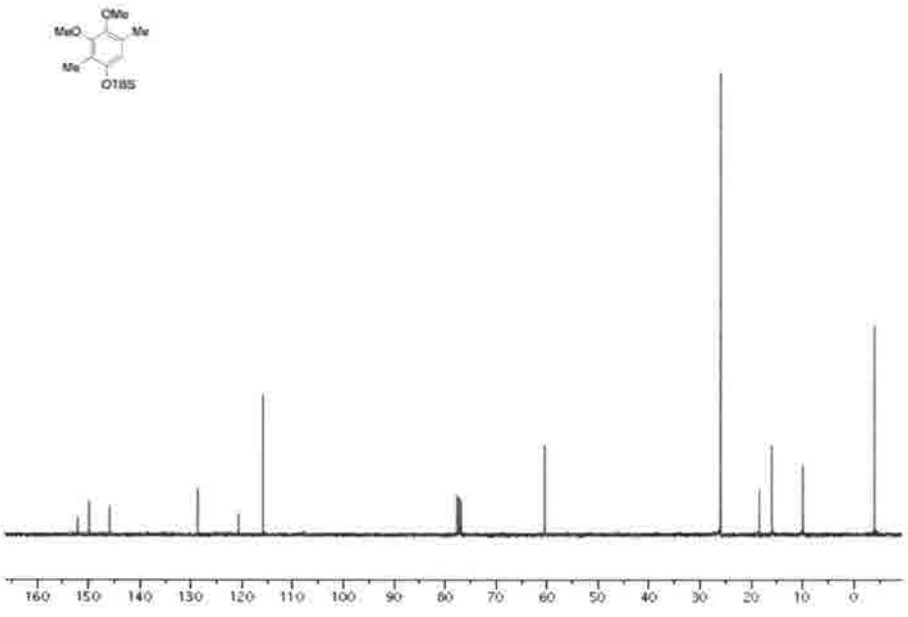


To phenol **268** (9.7 g, 34 mmol) in DMF (300 mL) at 0 °C was added cesium carbonate (13.4 g, 41 mmol) and then methyl iodide (3.3 mL, 51 mmol) dropwise. Then solution was then warmed and stirred at room temperature for 10 hrs. The reaction mixture was poured in water (200 mL), extracted (4 x dichloromethane), washed (2 x water, 1 x brine), dried (Na_2SO_4), filtered and concentrated. Purification of the crude material by column chromatography (97:3 hexanes/ ethyl acetate) gave arene **269** (6.8 g, 68%) as a light yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.21 (6H, s), 1.02 (9H, s), 2.10 (3H,

s), 2.20 (3H, s), 3.78 (3H, s), 3.82 (3H, s), 6.37 (1H, s); ^{13}CNMR (75 MHz, $CDCl_3$) δ - 4.0, 9.9, 16.0, 18.5, 26.0, 60.4, 60.5, 115.9, 120.7, 128.7, 145.9, 149.9, 152.1; IR (neat) 2956, 2931, 2858, 1608, 1578, 1483, 1403, 1123, 903 cm^{-1}.

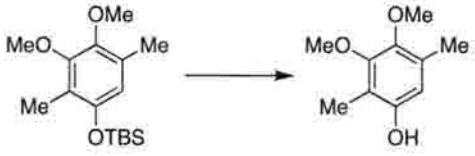


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-256



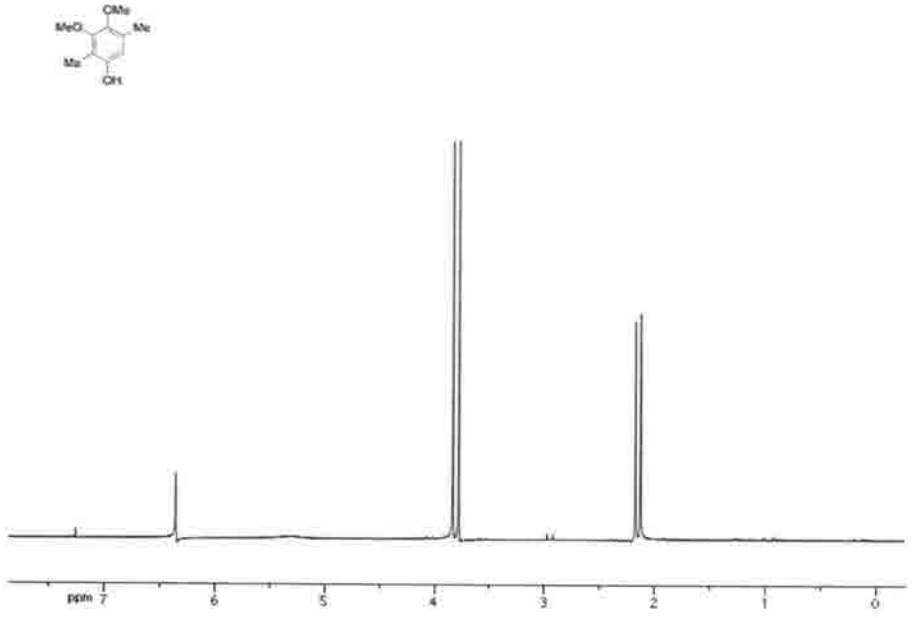
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-256-13C

3,4-dimethoxy-2,5-dimethylphenol (270)

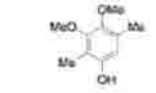


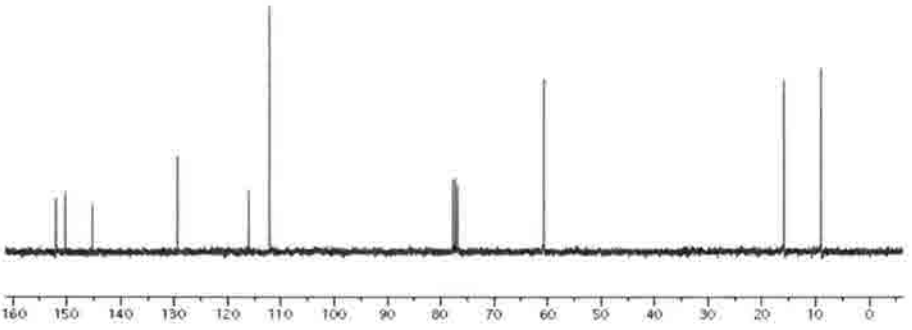
To phenol **269** (6.8 g, 23 mmol) in THF (150 mL) was added tetrabutylammonium fluoride (34 mL, 1M solution in THF, 34 mmol) dropwise and then stirred for 30 minutes. The reaction mixture was the quenched (water), extracted (4 x ethyl acetate), washed (1 x brine), dried (Na_2SO_4), filtered, and concentrated. Purification of the crude material by column chromatography (9:1 hexanes/ethyl acetate) gave phenol **270** (5.8 g, 99%) as a colorless oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.13 (3H, s), 2.18 (3H, s), 3.78 (3H, s), 3.83 (3H, s), 5.31 (1H, s, br), 6.36 (3H, s); ^{13}CNMR (75 MHz, $CDCl_3$) δ 9.0,

15.9, 60.7, 112.2, 116.1, 129.4, 145.2, 150.3, 152.0; IR (neat) 3394, 2933, 2862, 2829, 1616, 1592, 1465, 1406, 1187 cm^{-1}.



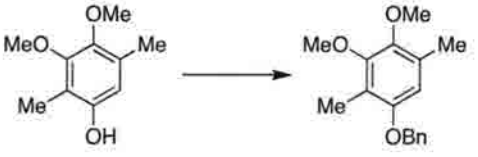
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-260





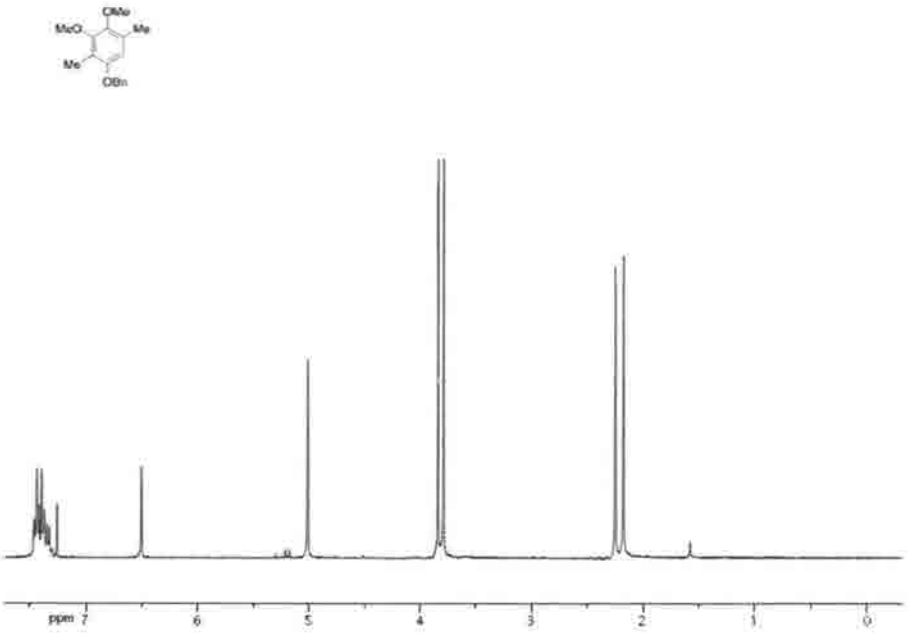
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-260-13C

1-(benzyloxy)-3,4-dimethoxy-2,5-dimethylbenzene (271)



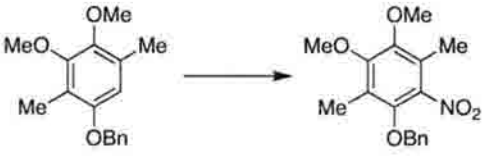
To the previously described phenol **270** (4.2 g, 23 mmol) in DMF (200 mL) at 0 °C was added cesium carbonate (9.0 g, 28 mmol), benzyl bromide (4.1 mL, 35 mmol) dropwise, and then stirred at room temperature for 17 hrs. The reaction mixture was then poured into water (200 mL), extracted (4 x dichloromethane), washed (2 x water, 1 x brine), dried (Na_2SO_4), filtered, and concentrated. Purification of the crude material by column chromatography (95:5 hexanes/ethyl acetate) gave aromatic **271** (5.85 g, 98%) as a

colorless oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.17 (3H, s), 2.25 (3H, s), 3.79 (3H, s), 3.84 (3H, s), 5.01 (2H, s), 6.51 (1H, s), 7.32-7.46 (5H, m).



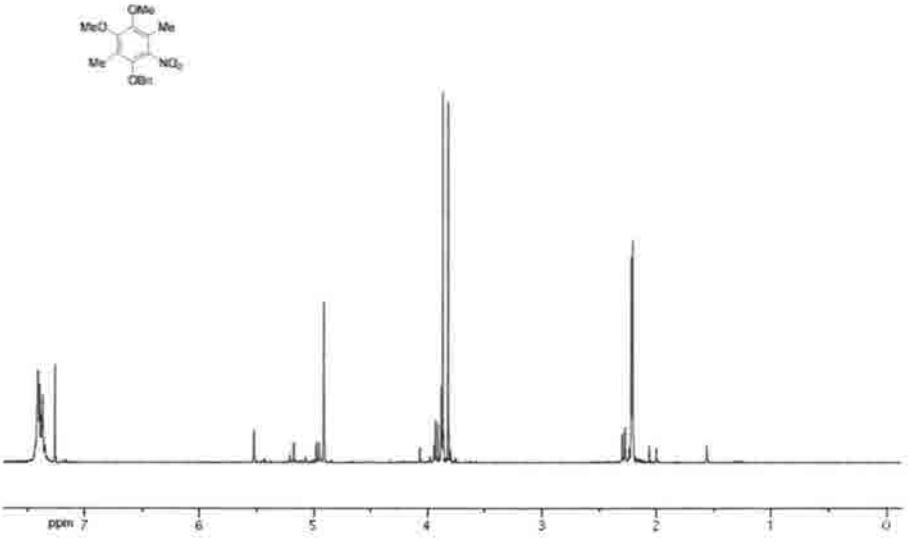
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-276

1-(benzyloxy)-3,4-dimethoxy-2,5-dimethyl-6-nitrobenzene (272)



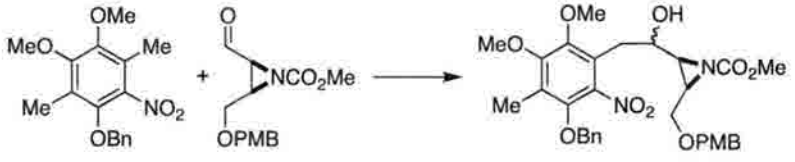
To arene **271** (6.2 g, 24 mmol) in acetic anhydride (120 mL) at 0 °C was added cupric nitrate trihydrate (11.2 g, 48 mmol) and stirred at 0 °C for 30 minutes. The reaction mixture was poured into ice water (75 mL), extracted (4 x benzene), washed (1 x 5% sodium bicarbonate, 1 x brine), dried (Na_2SO_4), filtered, and concentrated. Purification of the crude material by column chromatography (benzene) gave the desired nitro arene **272**

(4.45 g, 58%) as a yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.21 (3H, s), 2.22 (3H, s), 3.82 (3H, s), 3.87 (3H, s), 4.91 (2H, s), 7.34-7.43 (5H, m).



^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-283

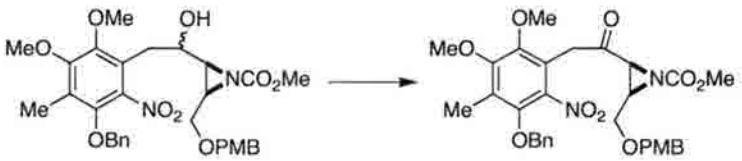
(2*S*,3*R*)-methyl-2-((*R*)-2-(3-(benzyloxy)-5,6-dimethoxy-4-methyl-2-nitrophenyl)-1-hydroxyethyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (273)



Prior to the reaction, compounds **272** (4.45 g, 14 mmol) and **81** (1.56 g, 5.6 mmol) were dehydrated three times by azeotropic distillation using anhydrous toluene and lastly dried under vacuum for 1h before use. A solution of **81** in dry DMF (25 mL) was then added to freshly fused zinc chloride (1.14 g, 8.4 mmol). The resulting solution was stirred under

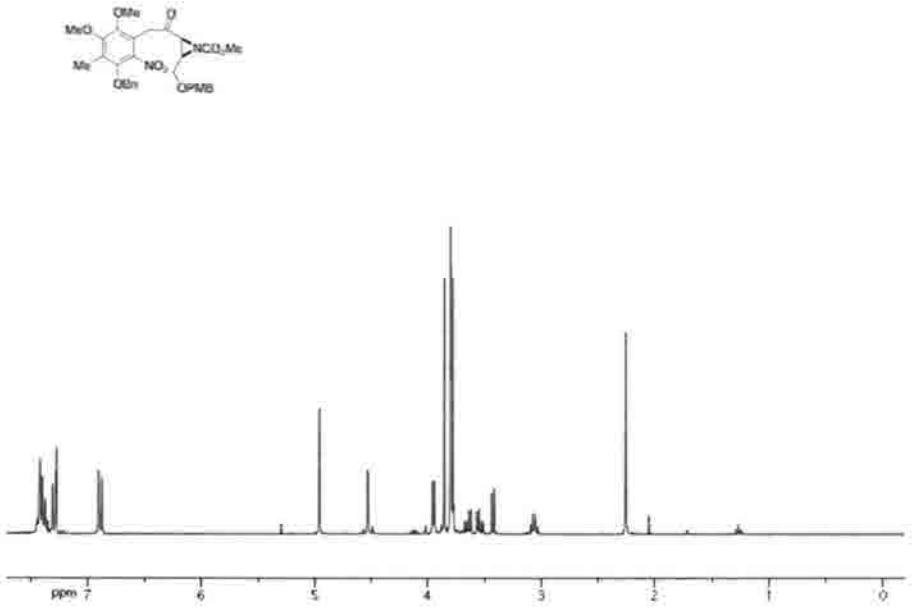
argon for 1h at room temperature. In a separate flask, NaHMDS (14 mL of a 1 M solution in THF, 14 mmol) was added dropwise to a solution of **272** in dry DMF (50 ml) cooled to -45°C. The mixture turned immediately deep red. To this solution was added dropwise the aziridine solution prepared above and the resulting mixture was stirred at -45°C for 3 hrs. The reaction mixture was quenched with saturated aqueous NH_4Cl (25 mL), allowed to warm to room temperature and water (25 mL) was added. The aqueous phase was extracted with DCM (5 x 30 mL), the combined organics dried over Na_2SO_4 and concentrated under reduced pressure. The crude oil was purified by flash column chromatography (3:2 hexanes/ethyl acetate) to afford the alcohol **273** (2.47 g, 74%) as a pale yellow oil (1:1 mixture of separable diastereomers). To aid in ease of purification, the mixture of alcohols was immediately oxidized to the ketone.

(2*S*,3*R*)-methyl 2-(2-(3-(benzyloxy)-5,6-dimethoxy-4-methyl-2-nitrophenyl)acetyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (274)

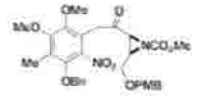


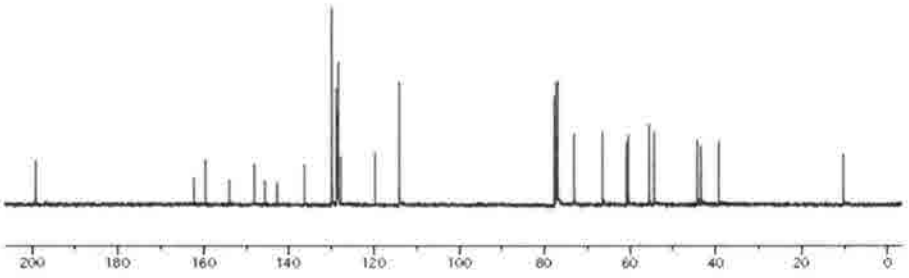
To alcohols **273** (2.5 g, 4.1 mmol) in dichloromethane (55 mL) was added Dess-Martin periodinane (2.8 g, 6.6 mmol) and stirred for 2 hrs. The reaction was diluted with ether (50 mL), poured into 50 mL sat. aq. $NaHCO_3$ with 7.0 equivalents of sodium thiosulfate (10.8 g, 43.7 mmol), and stirred for 15 min. The layers were separated and the aqueous layer was backextracted with ether. The combined ether layers were washed (1 x sat. aq. $NaHCO_3$, 1 x Brine), dried (Na_2SO_4), filtered and concentrated. Purification by column chromatography (3:2 hexanes/ethyl acetate) gave the desired ketone **274** (2.31 g, 95%) as

a pale yellow oil. $[\alpha]_D^{20}$ -23.2 (c=2.0, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.26 (3H, s), 3.06 (1H, q, J = 5.7 Hz), 3.42 (1H, d, J = 6.9 Hz), 3.54 (1H, dd, J = 11.1, 5.4 Hz), 3.65 (1H, dd, J = 11.1, 5.7 Hz), 3.78 (3H, s), 3.79 (3H, s), 3.80 (3H, s), 3.86 (3H, s), 3.95 (1H, d, J = 5.4 Hz), 4.53 (2H, d, 1.8 Hz,), 4.96 (2H, s), 6.89 (2H, d, J = 8.7 Hz), 7.28 (2H, d, J = 8.4 Hz), 7.35-7.45 (5H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 10.3, 39.2, 43.4, 44.3, 54.3, 55.5, 60.4, 60.9, 66.5, 73.1, 114.1, 119.7, 127.8, 128.4, 128.7, 128.8, 129.9, 136.3, 142.8, 145.6, 148.0, 154.0, 159.6, 162.2, 199.1; IR (neat) 3004, 2951, 1735, 1611, 1519, 1460, 1251, 1091 cm^{-1}.



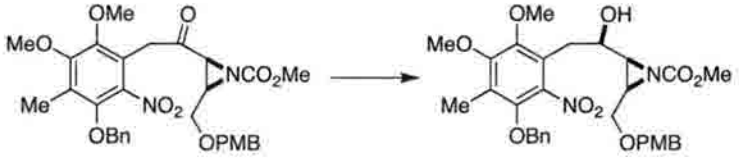
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-285-300-1H





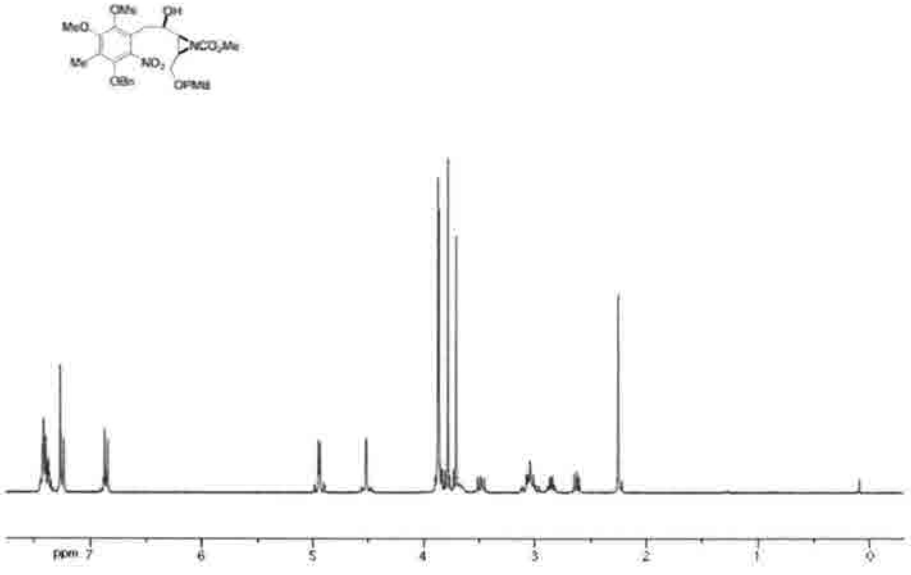
^{13}CNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-285-300-13C

(2*S*,3*R*)-methyl-2-((*R*)-2-(3-(benzyloxy)-5,6-dimethoxy-4-methyl-2-nitrophenyl)-1-hydroxyethyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (275)

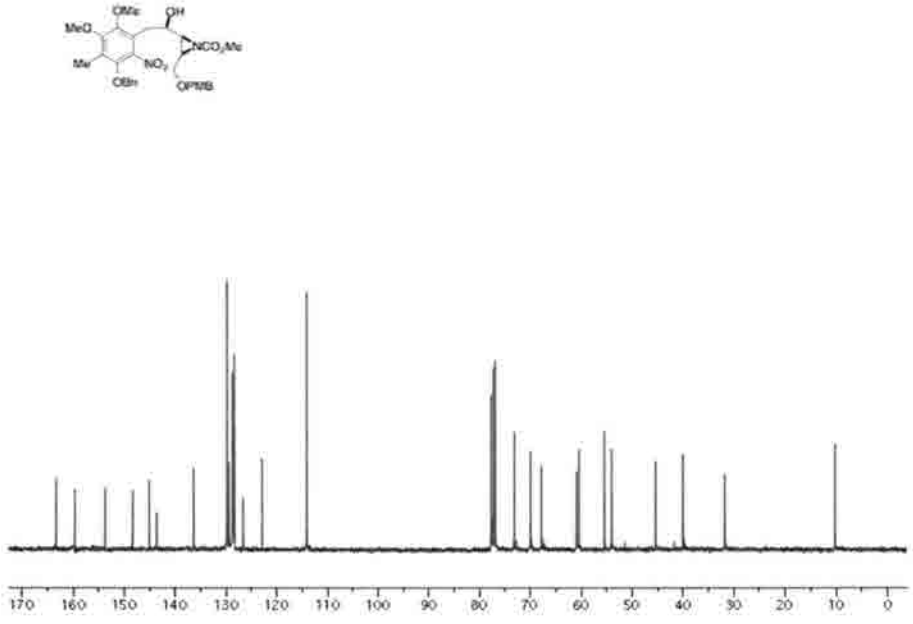


To ketone **274** (1.0 g, 1.68 mmol) in 20 mL of 3:1 methanol/dichloromethane at 0 °C was added sodium borohydride (127 mg, 3.36 mmol) and stirred for 1 hr. The reaction was then quenched by the addition of brine, extracted (5 x dichloromethane), washed (brine), dried (Na_2SO_4), filtered and concentrated to give alcohol **275** (950 mg, 95%) as a pale yellow oil that was used without further purification. $[\alpha]_D^{20}$ -4.7 (c=0.85, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.26 (3H, s), 2.62 (1H, dd, J = 8.4, 6.3 Hz), 2.85

(1H, q, J = 6.3 Hz), 2.97-3.13 (2H, m), 3.48 (1H, dd, J = 10.8, 7.2 Hz), 3.71 (3H, s), 3.79 (3H, s), 3.87 (3H, s), 3.88 (3H, s), 4.52 (d, J = 2.4 Hz), 4.95 (2H, d, J = 2.7 Hz), 6.85 (2H, d, J = 8.7 Hz), 7.25 (2H, d, J = 8.7 Hz), 7.35-7.45 (5H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 10.2, 31.8, 40.0, 45.3, 54.0, 55.5, 60.4, 60.9, 67.8, 70.0, 73.2, 76.9, 114.1, 122.9, 126.7, 128.4, 128.6, 128.8, 129.5, 129.8, 129.9, 136.4, 143.7, 145.2, 148.4, 153.7, 156.6, 163.3; IR (neat) 3472, 2941, 1729, 1612, 1532, 1249, 1091 cm^{-1}.

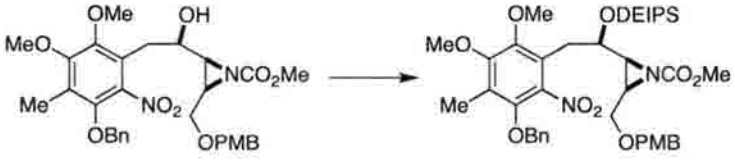


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-286-300-1H



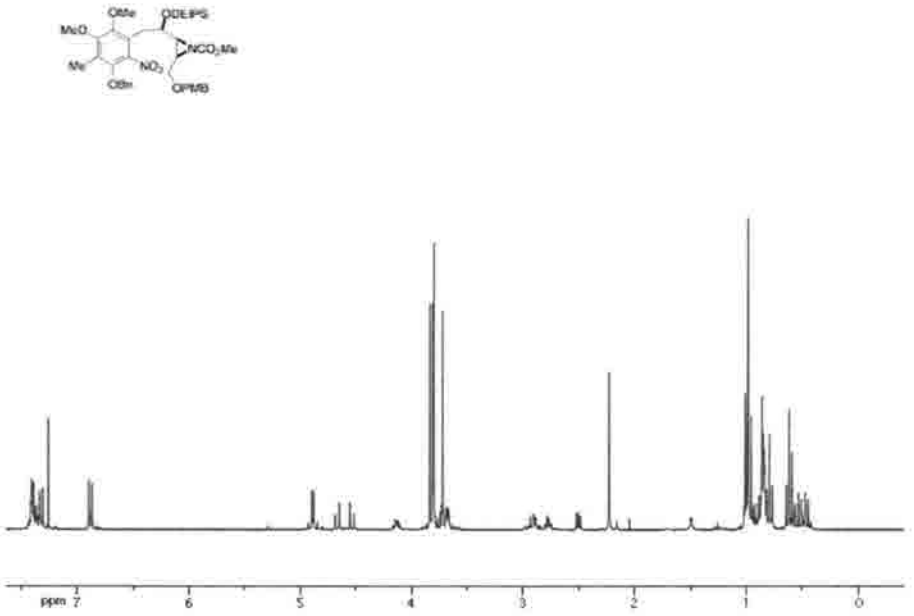
^{13}CNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-286-300-13C

(2*S*,3*R*)-methyl-2-((*R*)-2-(3-(benzyloxy)-5,6-dimethoxy-4-methyl-2-nitrophenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate (274)

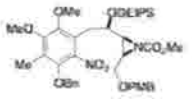


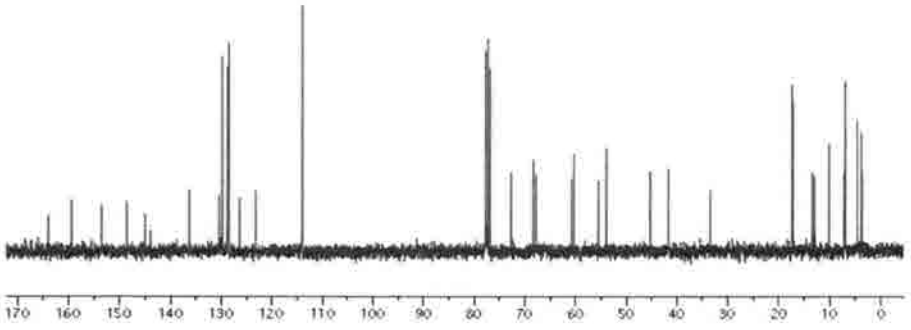
To a solution of the alcohol obtained above (950 mg, 1.59 mmol) and imidazole (379 mg, 5.57 mmol) in dry DCM (20 mL) was added dropwise chlorodiethylisopropylsilane (587 μL, 3.18 mmol). The mixture was stirred at room temperature for 24h. The reaction was quenched with saturated aqueous $NaHCO_3$, diluted with dichloromethane and the layers separated. The aqueous layer was extracted with dichloromethane (3 x 50 mL). The

combined organics were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (4:1 hexanes/ethyl acetate) to afford silyl ether **274** as a pale yellow oil (1.18 g, 99%). $[\alpha]_D^{20}$ -8.7 (*c*=1.0, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.42-0.56 (4H, m), 0.76-0.90 (13H, m), 2.23 (3H, s), 2.50 (1H, dd, *J* = 6.6, 5.4 Hz), 2.74-2.81 (1H, m), 2.84-2.98 (2H, m), 3.72 (3H, s), 3.80 (3H, s), 3.81 (3H, s), 3.84 (3H, s), 4.10-4.17 (1H, m), 4.53 (1H, d, *J* = 11.4 Hz), 4.66 (1H, d, *J* = 11.4 Hz), 4.88 (2H, dd, *J* = 15.0, 10.5 Hz), 6.88 (2H, d, *J* = 8.7 Hz), 7.34 (2H, d, *J* = 8.7 Hz), 7.36-7.42 (5H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 3.7, 3.8, 4.6, 7.0, 7.2, 10.1, 13.0, 13.4, 17.2, 17.4, 33.5, 41.7, 45.2, 53.8, 55.5, 60.3, 60.7, 67.8, 68.3, 72.7, 113.9, 123.2, 126.4, 128.5, 128.7, 128.8, 129.9, 130.5, 136.3, 144.0, 145.0, 148.6, 153.5, 158.4, 164.0; IR (neat) 2951, 2875, 1731, 1534, 1249, 1094 cm^{-1}.



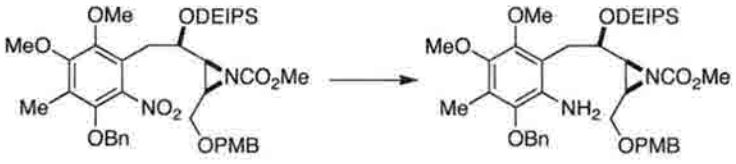
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-287-300-1H





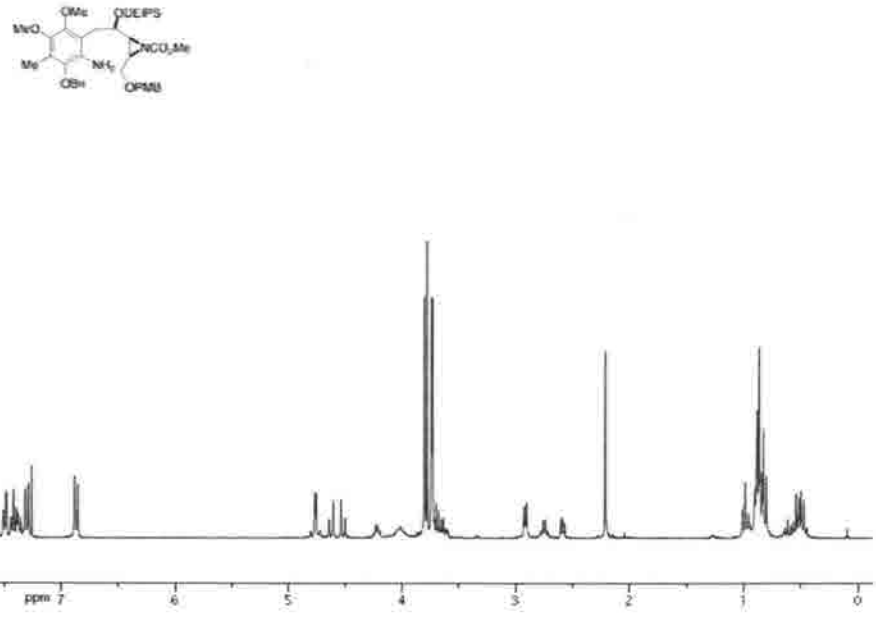
^{13}CNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-287-300-13C

(2*S*,3*R*)-methyl-2-((*R*)-2-(2-amino-3-(benzyloxy)-5,6-dimethoxy-4-methylphenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-((4-methoxybenzyloxy)methyl)aziridine-1-carboxylate

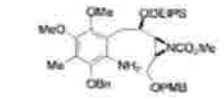


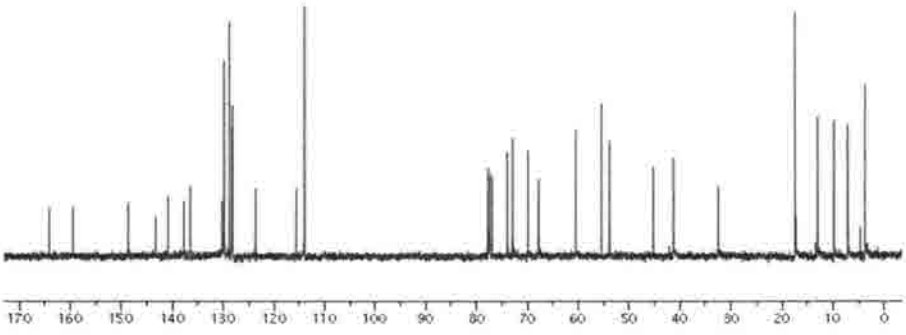
To DEIPS ether **274** (970 mg, 1.34 mmol) in 4:1 acetone/water (15 mL) was added zinc dust (437 mg, 6.69 mmol), ammonium chloride (716 mg, 13.40 mmol,), and stirred for 1 hr. The solution was then concentrated, diluted (EtOAc), washed (water, brine), dried (Na_2SO_4) and concentrated to give aniline **277** (640 mg, 71% yield) as a yellow oil that was taken on without further purification. $[\alpha]_D^{20}$ -3.8 (c=0.95, $CHCl_3$); ^{1}HNMR (300

MHz, $CDCl_3$) δ TMS: 0.45-0.59 (4H, m), 0.80-0.91 (13H, m), 2.21 (3H, s), 2.58 (1H, dd, J = 6.3, 4.8 Hz), 2.75 (1H, q, J = 5.7 Hz), 2.91 (1H, d, J = 6.6 Hz), 3.73 (3H, s), 3.74 (3H, s), 3.78 (3H, s), 3.80 (3H, s), 3.989-4.04 (1H, m), 4.22 (1H, q, J = 5.7 Hz), 4.51 (1H, d, J = 11.4, Hz), 4.62 (1H, d, J = 11.4 Hz), 4.76 (2H, dd, J = 14.7, 11.1 Hz), 6.86 (2H, d, J = 8.7 Hz), 7.29 (2H, d, J = 8.7 Hz), 7.36-7.45 (3H, m), 7.48-7.51 (2H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 3.7, 7.0,7.1, 7.2, 9.8, 13.0, 17.3, 17.5, 32.5, 41.3, 45.2, 53.9, 55.4, 60.5, 67.8, 69.9, 72.9, 74.0, 114.0, 155.6, 123.6, 128.3, 128.3, 128.8, 129.9, 130.2, 136.5, 137.8, 140.9, 143.3, 148.7, 159.5, 164.1; IR (neat) 3436, 3364, 2953, 2875, 1730, 1613, 1513, 1464, 1293, 1248, 1090 cm^{-1}.



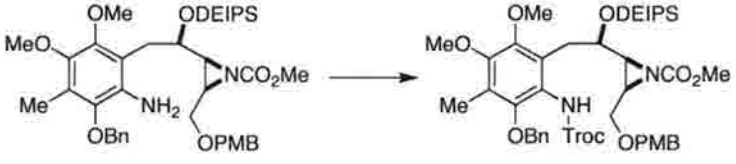
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-292-300-1H





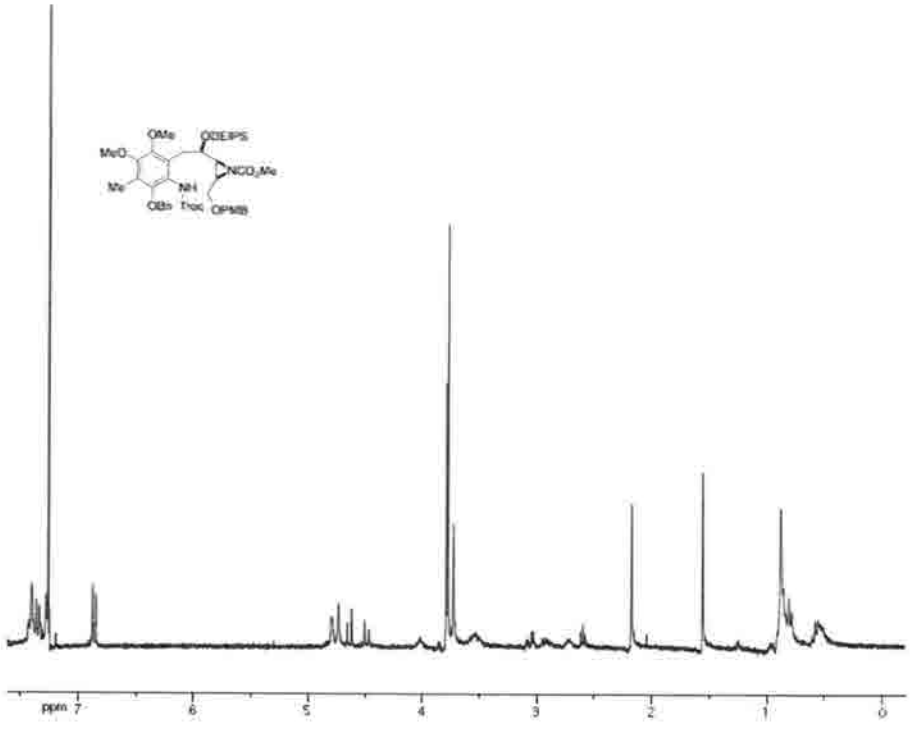
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-292-300-13C

(2*S*,3*R*)-methyl-2-((*R*)-2-(3-(benzyloxy)-5,6-dimethoxy-4-methyl-2-((2,2,2-trichloro-ethoxy)carbonylamino)phenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-((4-methoxy-benzyloxy)methyl)aziridine-1-carboxylate



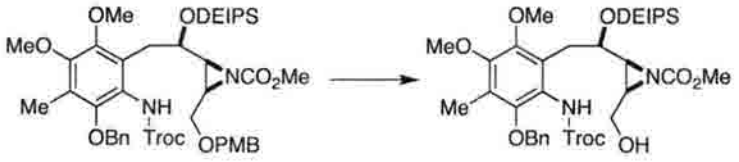
The aniline obtained from the previous experiment (640 mg, 0.92 mmol) in dichloromethane (15 mL) was added pyridine (375 μL, 4.6 mmol) followed by dropwise addition of 2,2,2-trichloroethyl carbamate (186 μL, 1.38 mmol). After stirring for 20 hours at room temperatur the reaction was then quenched (water), extracted (4 x dichloromethane), washed (brine), dried (Na_2SO_4), filtered and concentrated. Purification

by column chromatography (3:2 hexanes/ethyl acetate) gave the desired Troc carbamate (470 mg, 60%) as a pale yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.46-0.58 (4H, m), 0.74-0.91 (13H, m), 2.18 (3H, s), 2.60 (1H, t, J = 5.7 Hz), 2.68-2.75 (1H, m), 2.87-2.96 (1H, m), 3.03-3.09 (1H, m), 3.49-3.58 (3H, m), 3.73 (3H, s), 3.78 (3H, s), 3.80 (3H, s), 3.98-4.04 (1H, m), 4.48 (1H, d, J = 11.7 Hz), 4.63 (1H, d, J = 11.4 Hz), 4.71-4.73 (2H, m), 4.78-4.80 (2H, m), 6.85 (2H, d, J = 9.3 Hz), 7.27 (2H, d, J = 9.0 Hz), 7.32-7.44 (5H, m).

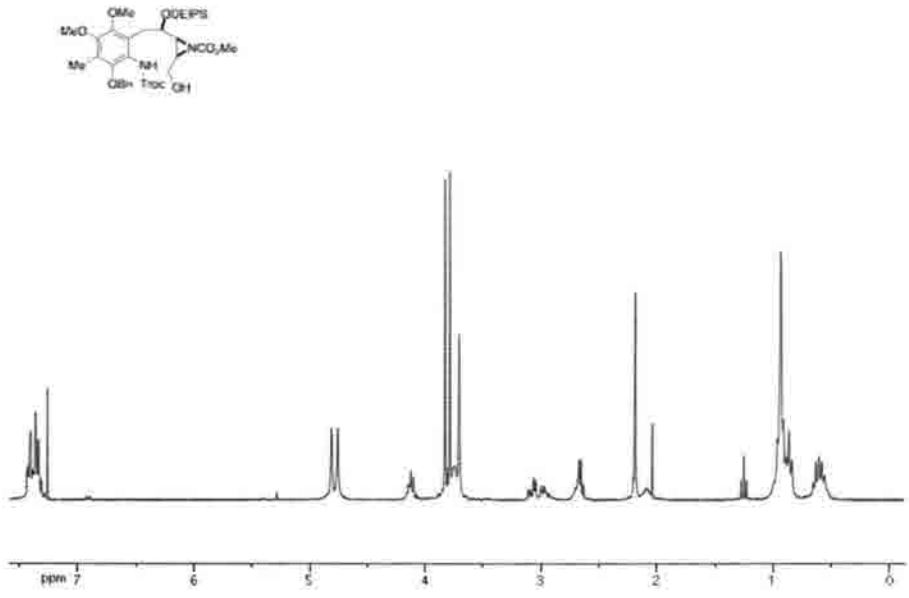


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-295

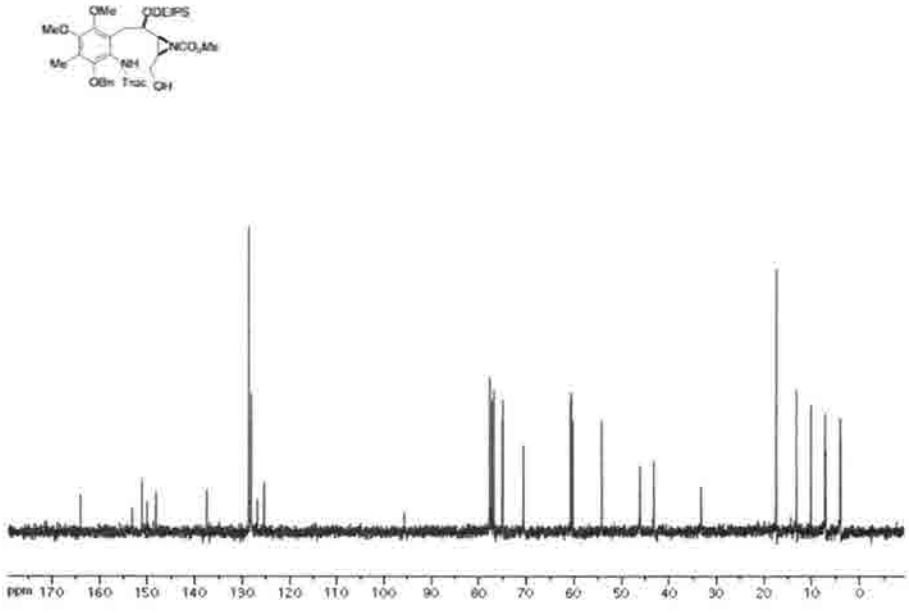
(2*S*,3*R*)-methyl-2-((*R*)-2-(3-(benzyloxy)-5,6-dimethoxy-4-methyl-2-((2,2,2-trichloro-ethoxy)carbonylamino)phenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-(hydroxy-methyl)aziridine-1-carboxylate (275)



To a stirred solution of the Troc carbamate obtained above (465 mg, 0.53 mmol) in 15:1 solution of dichloromethane/water (10 mL) was added DDQ (158 mg, 0.69 mmol) in one portion. The reaction mixture immediately turned green and over the course of 1.5 h the mixture turned orange. After 2 h, the reaction mixture was passed through a short pad of neutral alumina using 10:1 dichloromethane/methanol as eluant. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography (3:2 hexanes/ethyl acetate) to afford the alcohol **275** (465 mg, 75%) as a white foam. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.53-0.66 (4H, m), 0.83-0.96 (13H, m), 2.18 (3H, s), 2.63-2.72 (2H, m), 2.95 (1H, dd, J = 13.5, 7.8 Hz), 3.07 (1H, dd, J = 13.5, 5.4 Hz), 3.70 (3H, s), 3.78 (3H, s), 3.83 (3H, s), 4.12-4.16 (1H, m), 4.76 (2H, s), 4.81 (2H, s), 7.31-7.43 (5H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 3.9, 4.0, 7.0, 7.2, 10.2, 13.2, 17.4, 33.3, 43.2, 46.1, 54.1, 60.2, 60.6, 60.7, 70.6, 74.9, 75.1, 95.8, 125.4, 126.8, 128.1, 128.2, 128.7, 137.5, 148.2, 150.1, 151.2, 153.2, 164.0.

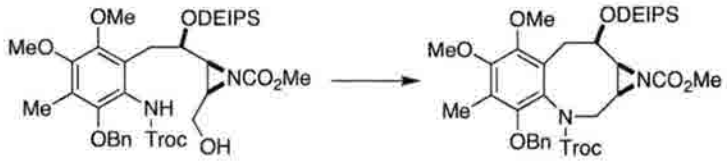


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-296

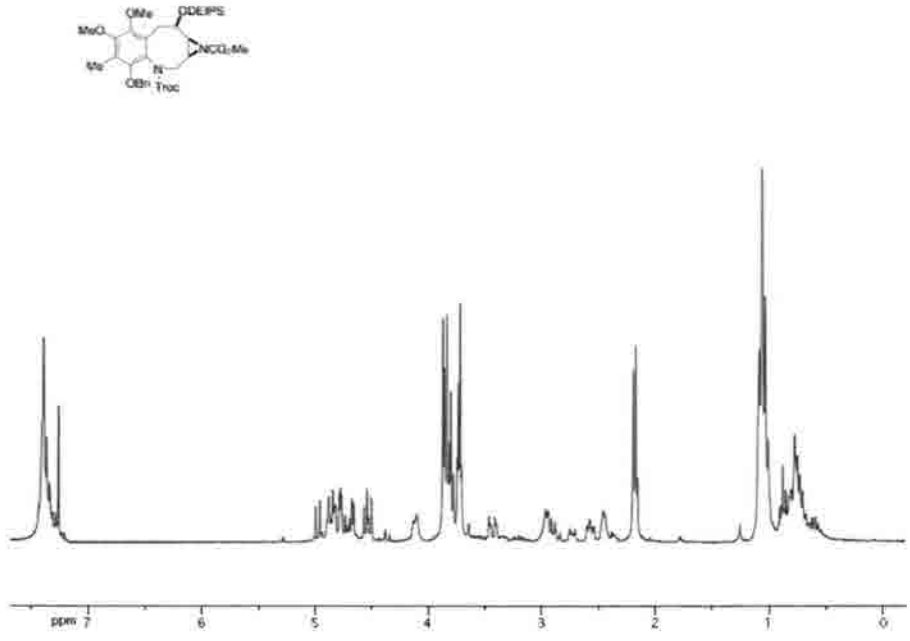


^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-296-13C

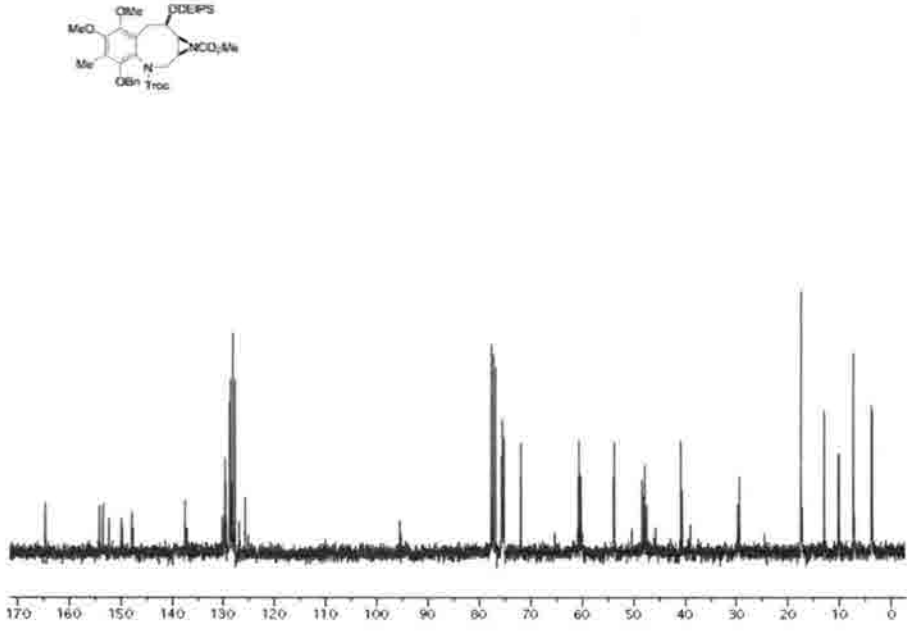
(1a*S*,9*R*,9a*S*)-1-methyl-3-(2,2,2-trichloroethyl)-4-(benzyloxy)-9-(diethyl(isopropyl) silyloxy)-6,7-dimethoxy-5-methyl-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]-azocine-1,3(8*H*)-dicarboxylate (276)



To a solution of **275** (285 mg, 0.38 mmol) and tributylphosphine (170 μL, 0.68 mmol) in dry toluene (20 mL) was added TMAD (98 mg, 0.57 mmol). The solution turned immediately deep yellow and turned to a light brown color as the reaction progressed. The mixture was stirred under argon at room temperature and monitored by TLC until complete consumption of the starting material (2h). The reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (9:1 hexanes/ethyl acetate) to afford the benzazocine **276** (134 mg, 48%) as a white foam. Note: Proton and carbon NMR spectra exists as a mixture of rotamers that did not coalesce under VTNMR experiments. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.57-0.92 (m), 0.96-1.12 (m), 2.16 (s), 2.17 (s), 2.19 (s), 2.44-2.47 (m), 2.54-2.60 (m), 2.70-2.75 (m), 2.88-2.98 (m), 3.39-3.47 (m), 3.71 (s), 3.72 (s), 3.73 (s), 3.74 (s), 3.78 (s), 3.80 (s), 3.81 (s), 3.83 (s), 3.85 (s), 3.87 (s), 4.09-4.14 (m), 4.53 (dd, J = 11.7, 8.7 Hz), 4.66-4.74 (m), 4.77-4.79 (m), 4.82-4.89 (m), 4.97 (d, J = 11.7 Hz), 7.28-7.43 (m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 3.7, 3.8, 3.9, 7.4, 10.1, 10.2, 12.9, 13.0, 17.5, 29.4, 29.7, 40.6, 40.8, 47.4, 47.8, 48.0, 48.4, 53.8, 53.9, 60.3, 60.4, 60.6, 60.8, 61.1, 72.0, 75.2, 75.5, 75.6, 75.7, 95.6, 125.8, 127.0, 127.8, 128.2, 128.5, 128.7, 128.8, 128.9, 129.6, 129.7, 129.8, 130.2, 137.2, 137.5, 147.7, 147.9, 149.7, 149.9, 152.3, 153.4, 154.1, 164.6.

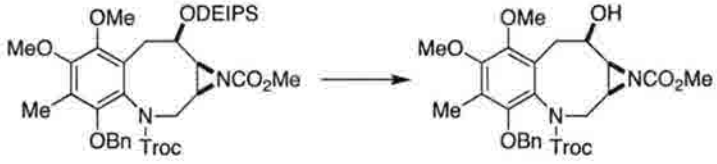


^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-297

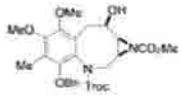


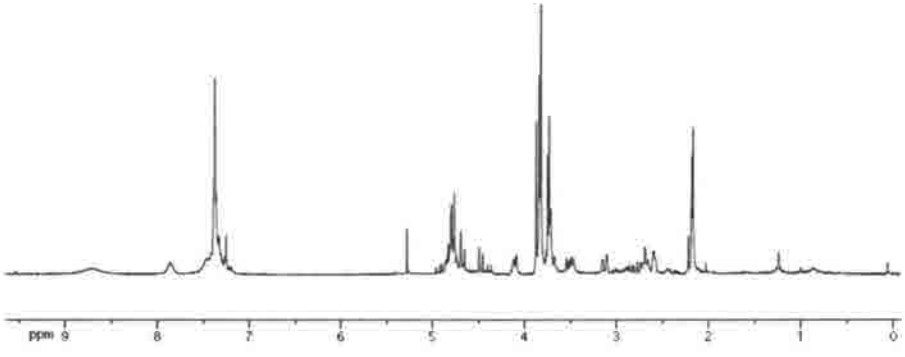
^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-296-13C

(1a*S*,9*R*,9a*S*)-1-methyl-3-(2,2,2-trichloroethyl)-4-(benzyloxy)-9-hydroxy-6,7-dimethoxy-5-methyl-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (277)



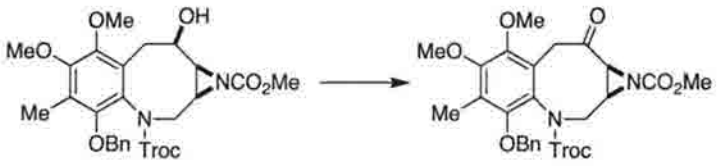
To benzazocine **276** (130 mg, 0.18 mmol), was added 5 mL of a 1.5 M of pyridinium hydrofluoride solution (prepared by addition of 1.0 g HF·Pyr to 20 mL of dry THF containing 5 mL pyridine) and stirred at room temperature for 4 hrs. The solution was then diluted with ethyl acetate, washed (1 x sat. aq. $NaHCO_3$, 1 x brine), dried (Na_2SO_4), filtered and concentrated. The crude alcohol **277** (119 mg, 93%) obtained as a white foam, was taken on without further purification.



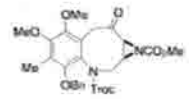


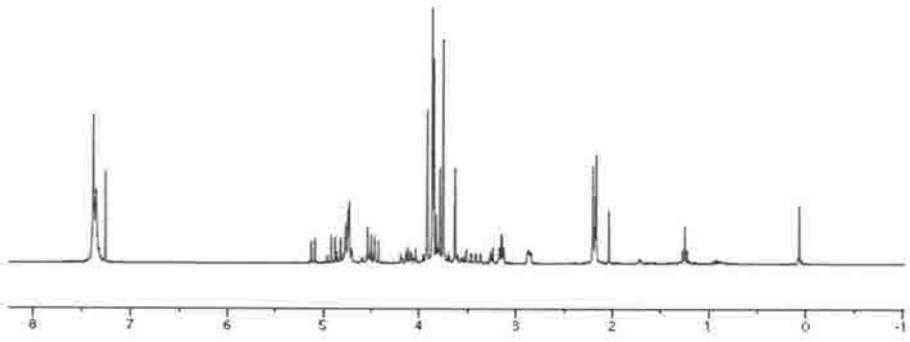
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-298-crude

(1a*S*,9a*S*)-1-methyl 3-(2,2,2-trichloroethyl) 4-(benzyloxy)-6,7-dimethoxy-5-methyl-9-oxo-1a,2,9,9a-tetrahydro-1*H*-azirino[2,3-*f*]benzo[*b*]azocine-1,3(8*H*)-dicarboxylate (278)

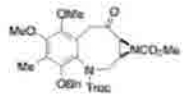


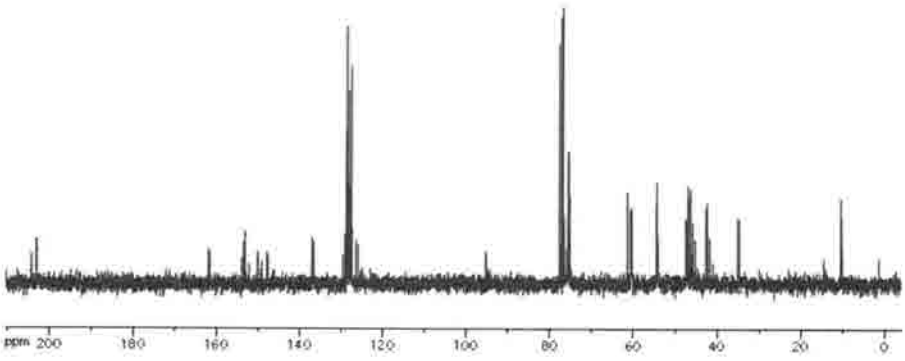
To alcohol **277** (100 mg, 0.17 mmol) in dichloromethane (5 mL) and molecular sieves (4Å, 350 mg) was added pyridinium dichromate (187 mg, 0.50 mmol) and stirred at room temperature for 16 hrs. The reaction mixture was run through a pad of silica using ethyl acetate as eluant and then concentrated. Further purification of the crude material by column chromatography (70:30 hexanes/ethyl acetate) gave benzazocane **278** (88 mg, 88%) as a white foam. Note: Proton and carbon NMR spectra exists as a mixture of rotamers that did not coalesce under VTNMR experiments. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.17 (s), 2.18 (s), 2.19 (s), 2.20 (s), 2.84-2.88 (m), 3.12-3.17 (m), 3.25 (dd, J = 6.9, 3.3 Hz), 3.36 (d, J = 0.9 Hz), 3.41 (d, J = 0.9 Hz), 3.46 (d, J = 0.9 Hz), 3.51 (d, J = 0.9 Hz), 3.63 (s), 3.75 (s), 3.85 (s), 3.86 (s), 3.87 (s), 3.96 (s), 4.04-4.19 (m), 4.48 (dd, J = 19.5, 12.0 Hz), 4.71-4.79 (m), 4.89 (d, J = 12.0 Hz), 5.10 (d, J = 12.0 Hz), 7.32-7.40 (m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 10.0, 10.1, 10.2, 10.3, 34.6, 35.0, 41.7, 42.3, 42.6, 45.2, 45.8, 46.1, 46.8, 46.9, 47.2, 47.5, 54.1, 54.3, 54.4, 60.3, 60.4, 60.5, 60.6, 61.3, 61.4, 75.3, 75.5, 75.6, 75.7, 127.6, 128.0, 128.2, 128.4, 128.6, 128.9, 129.0, 136.7, 137.0, 137.2, 147.8, 148.0, 150.1, 150.3, 153.3, 153.5, 153.7, 154.0, 161.8, 162.0, 203.1, 203.2, 204.4.





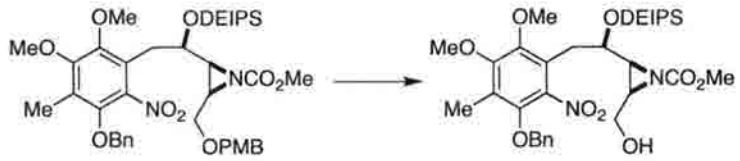
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-299



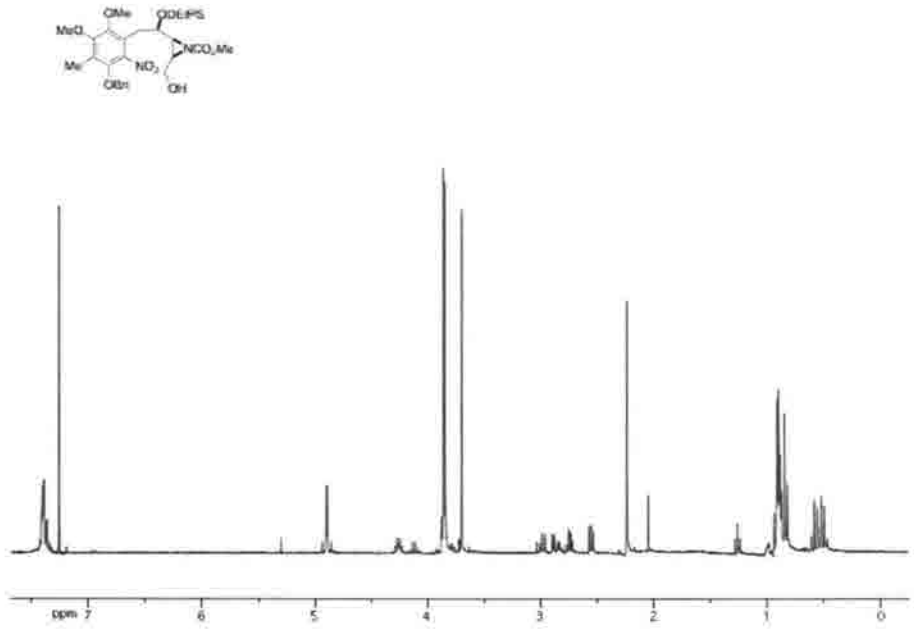


^{13}CNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-299-13C

(2*S*,3*R*)-methyl-2-((*R*)-2-(3-(benzyloxy)-5,6-dimethoxy-4-methyl-2-nitrophenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-(hydroxymethyl)aziridine-1-carboxylate (280)

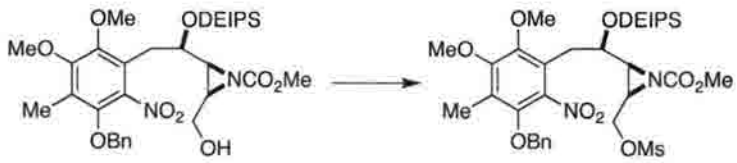


To a stirred solution of the DEIPS ether **276** (200 mg, 0.27 mmol) in 15:1 solution of dichloromethane/water (3 mL) was added DDQ (81 mg, 0.36 mmol) in one portion. The reaction mixture immediately turned green and over the course of 1.5 h the mixture turned orange. After 2 h, the reaction mixture was passed through a short pad of neutral alumina using 10:1 dichloromethane/methanol as eluant. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography (7:3 hexanes/ethyl acetate) to afford the alcohol **280** (126 mg, 77%) as a yellow oil. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.46-0.61 (4H, m), 0.78-0.94 (13H, m), 2.24 (3H, s), 2.55 (1H, dd, J = 6.3, 5.1 Hz), 2.71-2.77 (1H, m), 2.86 (1H, dd, J = 13.8, 5.7 Hz), 2.99 (1H, dd, J = 13.8, 8.4 Hz), 3.70 (3H, s), 3.84 (3H, s), 3.86 (3H, s), 4.23-4.29 (1H, m), 4.89 (2H, dd, J = 13.5, 7.8 Hz), 7.35-7.43 (5H, m).



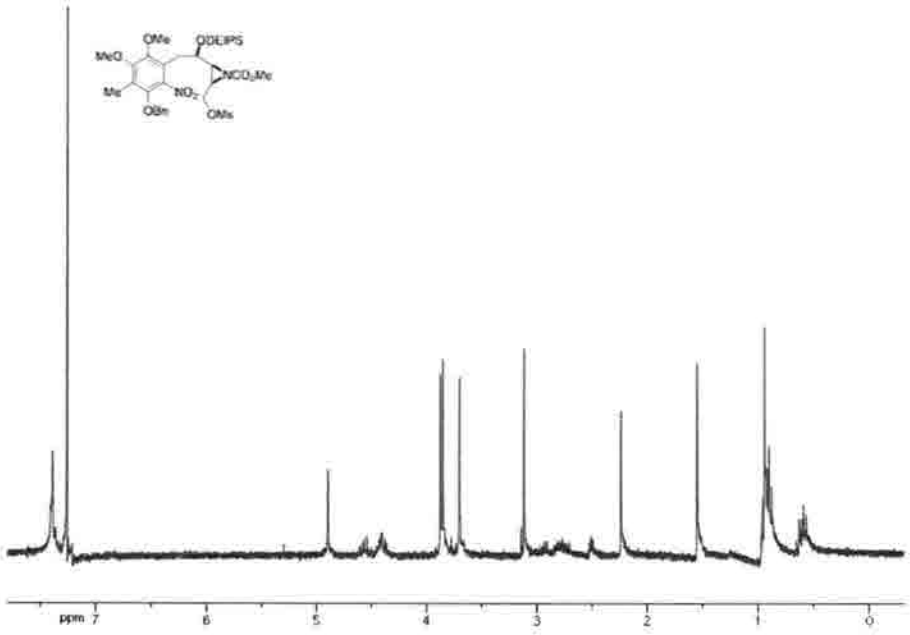
^{1}HNMR, $CDCl_3$, 300 MHz; Filename: Dag-IV-288

(2*S*,3*R*)-methyl 2-((*R*)-2-(3-(benzyloxy)-5,6-dimethoxy-4-methyl-2-nitrophenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-((methylsulfonyloxy)methyl)aziridine-1-carboxylate (281)



To alcohol **280** (125 mg, 0.21 mmol) and triethylamine (87 μL, 0.62 mmol) in dichloromethane (4 mL) at 0 °C was added methanesulfonyl chloride (24 μL, 0.32 mmol,) slowly dropwise and stirred at this temperature for 30 minutes. The reaction was then quenched (brine), extracted (4x dichloromethane), washed (brine), dried (Na_2SO_4) and concentrated. Purification by column chromatography gave mesylate **281** (124 mg,

87%) as a yellow foam. ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 0.49-0.66 (4H, m), 0.88-0.98 (13H, m), 2.24 (3H, s), 2.51 (1H, dd, J = 6.3, 4.2 Hz), 2.70-2.84 (2H, m), 2.94 (1H, dd, J = 13.8, 6.3 Hz), 3,12 (3H, s), 3.71 (3H, s), 3.86 (3H, s), 3.88 (3H, s), 3.36-4.36 (1H, m), 4.54-4.61 (1H, m), 4.90 (2H, s), 7.36-7.43 (5H, m).



^{1}HNMR, $CDCl_3$, 75 MHz; Filename: Dag-IV-289

Appendix 1: Publications

Graphical Abstract

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Fonts or abstract dimensions should not be changed or altered.

Synthetic studies towards the mitomycins: Construction of the tetracyclic core *via* a reductive aminocyclization reaction

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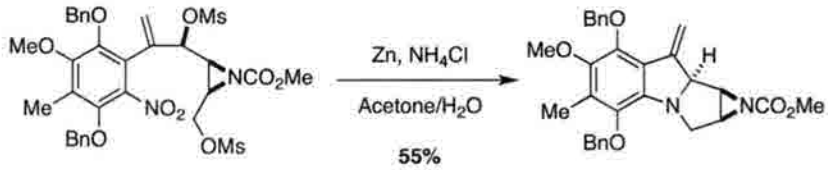
Daniel A. Gubler[a], and Robert M. Williams[a,b*]
Department of Chemistry, Colorado State University, Fort Collins, CO 80523, USA

The tetracyclic skeleton of the mitomycins has been constructed in one step from an acyclic precursor *via* a reductive amino-cyclization reaction. Details and the utility of this reaction are discussed herein.



Pergamon

TETRAHEDRON
LETTERS

Synthetic studies towards the mitomycins: Construction of the tetracyclic core *via* a reductive aminocyclization reaction

Daniel A. Gubler[a], and Robert M. Williams[a,b*]

[a]*Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523, USA*

[b]*University of Colorado Cancer Center, Aurora, Colorado 80045, USA*

Abstract— The tetracyclic core of the mitomycin family of natural products has been formed in one step from an acyclic precursor *via* a reductive aminocyclization reaction. Additionally, the 8-membered benzazocine can be prepared without the need for prior activation of the aniline. Construction of a mitomycin K analogue lacking the C9a methoxy moiety is also reported herein.

The mitomycin family of natural products (Figure 1) have been of interest to the scientific community since their isolation over 50 years ago.[1] Members of this family exhibit potent activity against a variety of cancer cell lines, and were found to be particularly active against solid tumors.[2] The mode of action of these compounds arises from their ability to form interstrand DNA-DNA[3] as well as DNA-protein[4] cross-links. Mitomycin C (Figure 1) has been widely used clinically for over 40 years, and is still routinely employed today.[5]

These molecules present a synthetic challenge due to their densely functionalized nature, as well as the difficulty in maintaining the vulnerable structural elements (i.e. aziridine, quinone, exo-methylene) as the synthesis unfolds. Despite the numerous reported synthetic efforts towards the mitomycins, only Kishi[6], Fukuyama[7], Danishefsky[8], and Jimenez[9] have been successful. *It is important to note that all the syntheses mentioned above are racemic in.*

Our research is centered on the development of an asymmetric total synthesis of the mitomycins in addition to our recently completed asymmetric total synthesis of (+)-FR900482[10]. These efforts are fueled in part by our interest in both the biosynthesis and mode of action of these compounds.[11] Herein we report efficient formation of the tetracyclic core of the mitomycins *via* a reductive aminocyclization reaction.

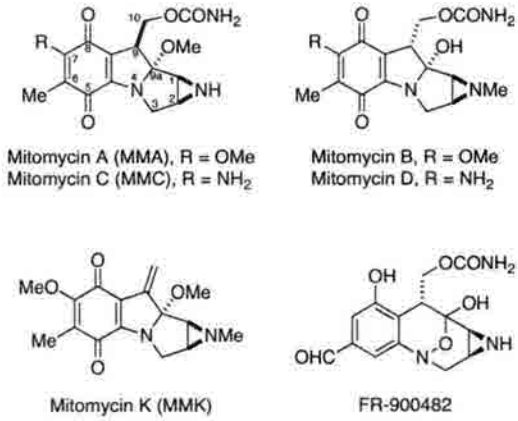


Figure 1. The mitomycin family of natural products

Previously, we reported the synthesis of benzazocines with the mitomycin substitution on the aromatic ring *via* an intramolecular Mitsunobu cyclization reaction.[12] We reported that prior activation of the aniline as the corresponding sulfonamide or carbamate was requisite for Mitsunobu cyclization to occur (Scheme 1). Through this method, benzazocines and benzazocanes containing all the key elements of the mitomycins were efficiently obtained. However, conversion of these benzazocanes to the natural products proved difficult.

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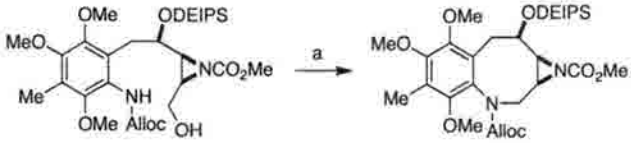
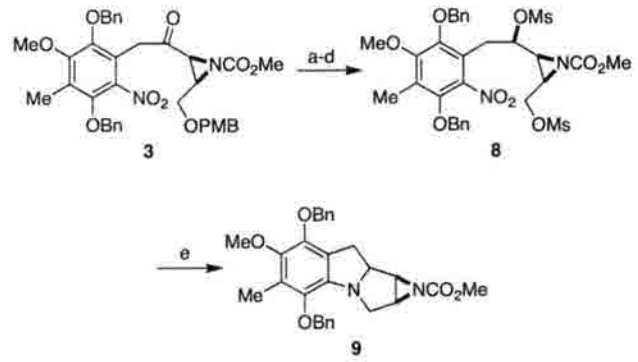


Scheme 1. (a) $(CH_3)_2NCON{=}NCON(CH_3)_2$ (1.5 equiv), PBu_3 (1.8 equiv), toluene, rt, 6 h; 85%.

As part of our continuing efforts to access this family of natural products, a new strategy was developed. This strategy employed the use of benzyl ethers as protecting groups on the arene ring, as well as installation of the exocyclic methylene prior to cyclization (Scheme 2). The synthesis commenced by coupling of nitro arene **1**[13] and aziridine aldehyde **2**[14], followed by oxidation of the resultant alcohol (obtained as a 2:1 mixture of diastereomers R vs. S) to ketone **3**. Methylenation and removal of the PMB ether with DDQ proceeded in excellent yield over two steps to provide primary alcohol **4**. Conversion of alcohol **4** to the corresponding mesylate and reduction of the ketone under Luche conditions gave secondary alcohol **5** in high yield as a single diastereomer. Reduction of the nitro group using zinc dust did not provide the expected aniline **6**, but rather gave benzazocine **7** in near quantitative yield as the product of a reductive aminocyclization reaction. To the best of our knowledge, this is the first example of a cyclization reaction in this family of compounds that proceeds without the need for prior activation of the aniline.

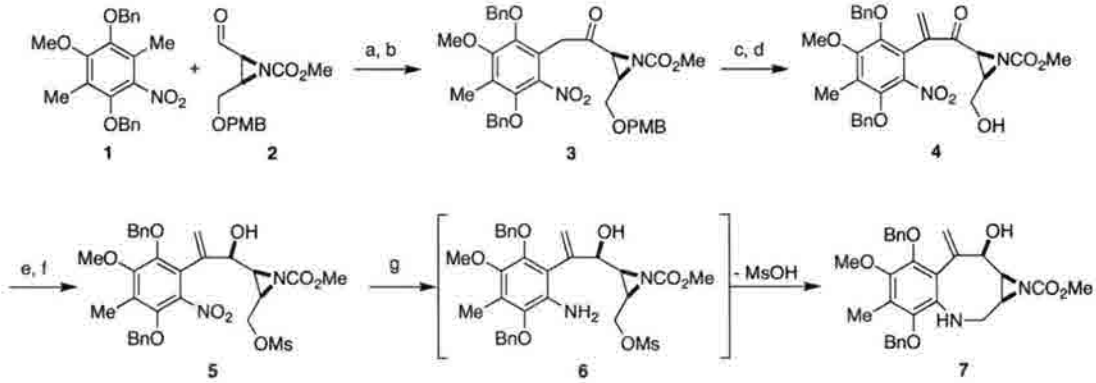
Having found an efficient method for formation of benzazocine **7**, we were interested in examining the utility and scope of this transformation. Specifically, we investigated the possibility of forming the tetracyclic core of the mitomycins in one step from an acyclic precursor *via* this newly discovered reductive aminocyclization reaction. Accordingly, ketone **3** was transformed to bis-mesylate **8** in four straightforward steps consisting of PMB ether removal, mesylation, reduction of the ketone, and a second mesylation (Scheme 3). Treatment of bis-mesylate **8** under identical nitro reduction conditions furnished tetracyclic indoline **9** in 53% yield. Attempted conversion of indoline **9** to the mitomycins is now in progress.



Scheme 3. (a) DDQ (1.3 equiv), CH_2Cl_2/water (95:5), rt, 2 h, 85%; (b) MsCl (1.5 equiv), Et_3N (3.0 equiv), 0 °C, 30 min, 53%; (c) $CeCl_3{\cdot}7H_2O$ (5.0 equiv), $NaBH_4$ (3.0 equiv), 0 °C, 30 min, 93%; (d) MsCl (1.5 equiv), Et_3N (3.0 equiv), 0 °C, 30 min, 75%; (e) Zn dust (5.0 equiv), NH_4Cl (10.0 equiv), acetone/water (4:1), rt, 3 h, 53%.

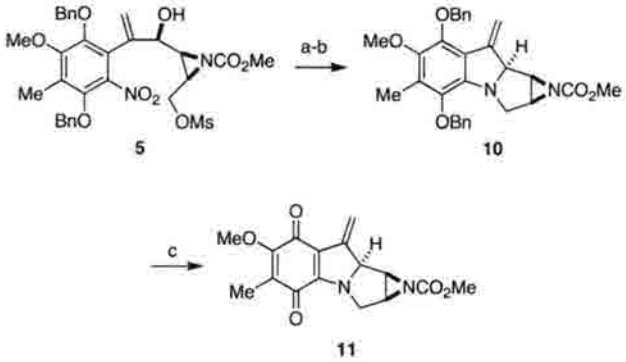
With indoline **9** in hand, we decided to pursue the synthesis of mitomycin K using the same reaction, with prior installation of the C10 exocyclic olefin. Accordingly, mesylation of alcohol **5** under standard conditions provided the aminocyclization precursor bearing the exocyclic olefin (Scheme 4). Reductive aminocyclization conditions used previously did provide tetracycle **10** without any isomerization of the exocyclic olefin to the corresponding indole (mitosene) adduct.[15] Treatment of tetracycle **10** under hydrogenation conditions gave quinone **11** in moderate yield. Quinone **11** comprises the core skeleton of mitomycin K, lacking only the C9a methoxy group. Installation of the requisite methoxy moiety may be accomplished by an allylic C-H activation strategy, and efforts in this vein are in progress.

In summary, benzazocines were synthesized in high yield by use of a reductive aminocyclization reaction without the need for prior activation of the aniline. The tetracyclic core of the mitomycins was also accomplished in a single



Scheme 2. (a) **1** (2.0 equiv), **2** (1.0 equiv), $ZnCl_2$ (1.5 equiv), NaHMDS (2.0 equiv), DMF, -45 °C, 2 h, 85%; (b) Dess-Martin (1.6 equiv), CH_2Cl_2, rt, 2h, 98%; (c) formalin (112 equiv), LiOH (0.4 equiv), THF/water (20:3), rt, 20 h, 99%; (d) DDQ (1.3 equiv), CH_2Cl_2/water (95:5), rt, 2 h, 92%; (e) MsCl (1.3 equiv), Et_3N (3.0 equiv), 0 °C, 30 min, 85%; (f) $CeCl_3{\cdot}7H_2O$ (5.0 equiv), $NaBH_4$ (3.0 equiv), 0 °C, 30 min, 92%; (g) Zn dust (5.0 equiv), NH_4Cl (10.0 equiv), acetone/water (4:1), rt, 3 h, 99%.

step from an acyclic precursor using this methodology. This strategy was used in formation of the tetracyclic indoline compound **9**, as well as the core structure of mitomycin K bearing an exocyclic olefin (i.e. **10**). Attempted conversion of compounds **7**, **9**, and **11** into the natural products are in progress will be reported in due course.



Scheme 4. (a) MsCl (1.5 equiv), Et_3N (3.0 equiv), 0 °C, 30 min, 53%; (b) Zn dust (5.0 equiv), NH_4Cl (10.0 equiv), acetone/water (4:1), rt, 3 h, 55%; (c) 10 % Pd/C (120 wt. %), Et_3N (6.0 equiv), EtOAc, H_2, rt, 30 min, then O_2, 50%.

Acknowledgments

This work was supported by National Institutes of Health (CA51875). D.A.G is grateful to Eli Lilly for a graduate fellowship. Mass spectra were obtained on instruments supported by the NIH Shared Instrumentation Grant GM49631. Mr. Donald Dick is acknowledged for mass spectrometric data.

References

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15. The general procedure for the reductive aminocyclization reaction is illustrated in the conversion of bis-mesylate (from alcohol **5**) to tetracycle **10**: To bis-mesylate (230 mg, 0.32 mmol, 1.0 equiv.) in 8.5 mL acetone/water (4:1) was added zinc dust (104 mg, 1.6 mmol, 5.0 equiv.), ammonium chloride (171 mg, 3.2 mmol, 10.0 equiv.), and then stirred at room temperature for 2 days. The reaction mixture was then concentrated, diluted (ethyl acetate), washed, (water and then brine), dried (sodium sulfate), filtered, and concentrated. Purification by column chromatography (3:1 hexanes/ethyl acetate) gave tetracycle **10** (87 mg, 55%) as a white foam. $[\alpha]^{20}_D$ +14.6 (c = 2.00, $CHCl_3$); ^{1}HNMR (300 MHz, $CDCl_3$) δ TMS: 2.14 (3H, s), 3.28 (1H, dd, J = 4.5, 2.1 Hz), 3.36 (1H, dd, J = 12.9, 2.1 Hz), 3.42 (1H, dd, J = 4.5, 2.7 Hz), 3.65 (3H, s), 3.82 (3H, s), 4.21 (1H, d, J = 12.9 Hz), 4.58 (1H, m), 4.70 (1H, d, J = 11.4 Hz), 5.09 (1H, m), 5.28 (1H, d, J = 2.1 Hz), 5.92 (1H, d, J = 2.4 Hz), 7.57-7.35 (10H, m); ^{13}CNMR (75 MHz, $CDCl_3$) δ 10.0, 36.8, 44.4, 46.1, 51.1, 53.7, 60.9, 69.9, 72.2, 74.2, 120.1, 127.7, 127.9, 128.0, 128.1, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 129.0, 137.7, 137.9, 138.6, 142.9, 145.2, 145.3, 145.5, 162.6; IR (neat) 3030, 2928, 1726, 1634, 1498, 1279 cm^{-1}; HRMS (FAB) m/z calcd for $C_{30}H_{30}N_2O_5$ $(M+H)^+$ 498.21, found 498.21.

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Letter

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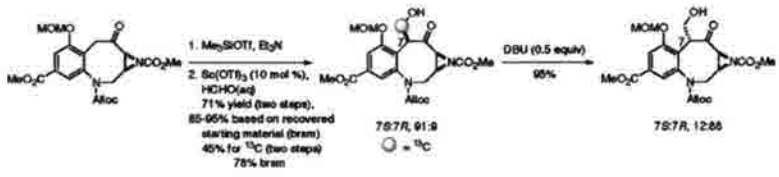

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Synthetic and Biosynthetic Studies on FR900482 and Mitomycin C: An Efficient and Stereoselective Hydroxymethylation of an Advanced Benzazocane Intermediate

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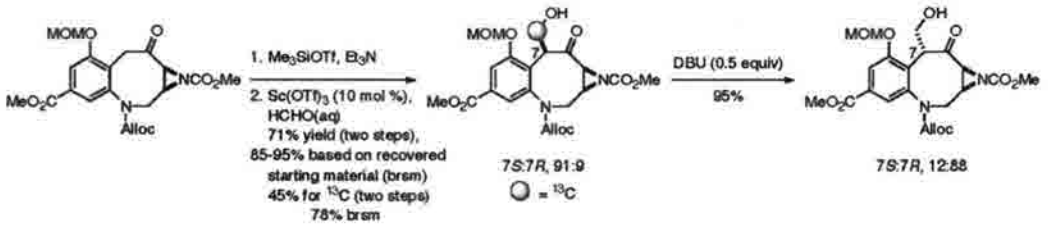
Received August 22, 2007

ABSTRACT



We report a simple, efficient, and stereoselective Mukaiyama aldol approach to install the key hydroxymethyl moiety into the benzazocane framework of FR900482. Synthetic investigations revealed that the reaction is highly dependent upon the electronics of the aromatic ring. This approach enabled the economical introduction of a [^{13}C] label to study the biosynthesis of these structurally and biogenetically related natural products. Epimerization of the initially formed β-hydroxy ketone may enable access to mitomycin C or FR900482 biosynthetic congeners.

FR900482 (**2**) and congeners FR66979 (**3**), FK973 (**4**), and FK317 (**5**) have enormous potential as anticancer agents (Figure 1).[1] These new prospective drugs are cytotoxic to tumors but do not appear to exhibit the deleterious side effects characteristic of the structurally related drug mitomycin C (MMC, **1**).[2] For example, myelosuppression and hemolytic uremic syndrome have plagued the broad clinical utility of MMC since 1974.[3] The allure of less toxic and more active chemotherapeutic agents has spawned numerous total and formal syntheses of FR900482 and its congeners.[4]

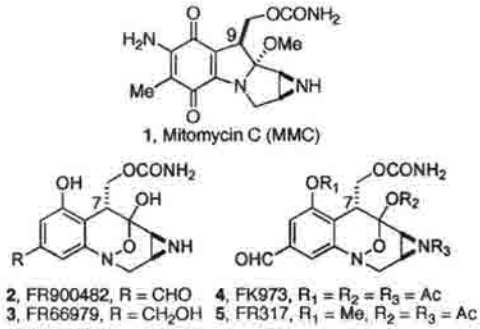


Figure 1. Structures of mitomycin C, FR900482, and congeners.

In particular, we recently described a stereoselective, convergent total synthesis of FR900482 (**2**)[5] as well as an improved synthesis of the benzazocane core of both FR900482

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(1) (a) Boger, D. L.; Wolkenberg, S. E. *Chem. Rev.* **2002**, *102*, 2477. (b) Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723.

(2) (a) Nelson, S. M.; Ferguson, L. R.; Denny, W. A. *Cell & Chromosome* **2004**, *3:2*. (b) Beckerbauer, L.; Tepe, J.; Eastman, R. A.; Mixter, P. F.; Williams, R. M.; Reeves, R. *Chem. Biol.* **2002**, *9*, 427. (c) Beckerbauer, L.; Tepe, J.; Cullison, J.; Reeves, R.; Williams, R. M. *Chem. Biol.* **2000**, *7*, 805.

(3) Bradner, W. T. *Cancer Treat. Rev.* **2001**, *27*, 35.

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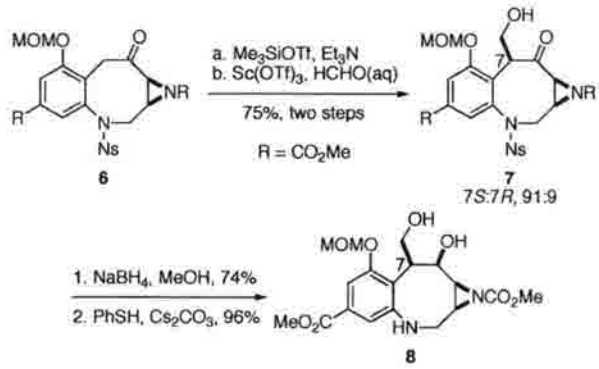
and mitomycin C.[4] Although our 33-step asymmetric route is among the most concise syntheses of FR900482 (**2**) extant, our interest in exploring the biogenesis of **1** and **2** has mandated that we improve the unselective hydroxymethylation aldol condensation reaction (dr ≈ 1:1).[5]

Throughout the course of our studies toward the synthesis of putative biosynthetic intermediates of both FR900482 and the mitomycins, it became necessary to investigate the hydroxymethylation of substrates such as **6** (Scheme 1).[6]

Scheme 1. Hydroxymethylation of Benzazocane **6**

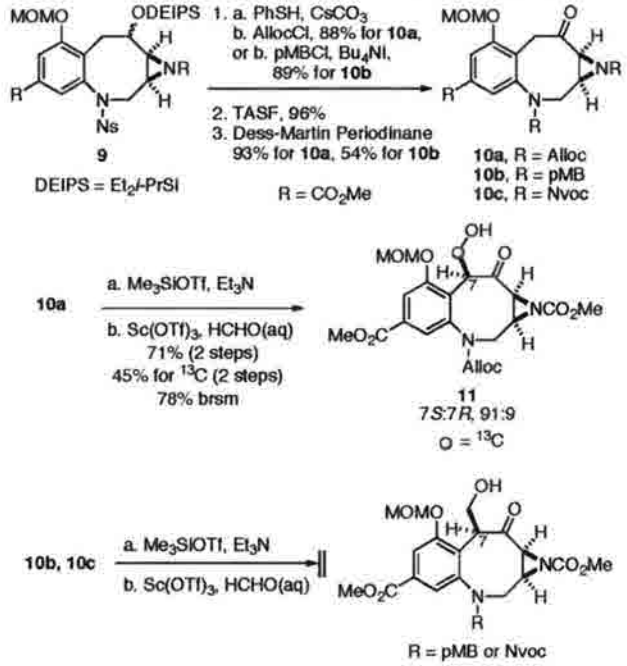


Hydroxymethylation of benzazocane intermediates en route to FR900482 under basic conditions often resulted in undesired elimination of the initially formed hydroxymethylation product. In conjunction with their synthetic studies, Rapoport[7] and Danishefsky[8] demonstrated that epoxidation and reductive ring opening of the *exo*-methylene in their respective eight- or six-membered ring substrates afforded the requisite hydroxymethyl compound indirectly. In a recent full paper describing his total synthesis of FR900482 (**2**), Fukuyama reported a one-step, stereoselective, base-catalyzed hydroxymethylation requiring 115 equiv of aqueous formaldehyde.[9] Such a large molar excess is impractical in the context of the preparation of putative biosynthetic intermediates where an expensive isotopically labeled source of formaldehyde would be employed.[10]

With benzazocane **6** in hand, we explored alternate hydroxymethylation conditions. We were especially intrigued by the lanthanide triflate-catalyzed Mukaiyama aldol reaction of enoxysilanes developed by Kobayashi.[11] Accordingly, treatment of benzazocane **6** with trimethylsilyl triflate provided the silyl enol ether which was used without purification in the hydroxymethylation step. Exposing the enoxysilane to a catalytic amount of ytterbium(III) triflate and 5 equiv of formaldehyde over 48 h provided alcohol **7** in 70% yield as a 84:16 ratio of diastereomers. Scandium(III) triflate was a superior catalyst and furnished the alcohol **7** in 82% yield (75% over two steps) as a 91:9 ratio of diastereomers in only 3 h (Scheme 1). The major diastereomer in the aldol reaction was determined to be the 7*S*-stereoisomer by X-ray crystallographic analysis of a derivative, diol **8**. This derivative was prepared by stereoselective carbonyl reduction and removal of the *N*-nosyl residue of the initial hydroxymethylation product, alcohol **7**.[12]

Having developed an efficient method for the hydroxymethylation of benzazocane **6**, we were interested in exploring the scope and generality of this protocol with more substrates. Specifically, we were interested to see the effect, if any, different protecting groups on the benzazocane nitrogen might have on the selectivity and general outcome of the aldol reaction. Consequently, *N*-alloc benzazocane **10a** was prepared from compound **9**[4] (Scheme 2). Treatment of the

Scheme 2. Hydroxymethylation of Benzazocanes **10a**−**c**



N-alloc benzazocane **10a** to identical hydroxymethylation conditions described earlier to prepare compound **7** provided the 7*S*-stereoisomer of alcohol **11** in 71% yield over two steps with 94:6 dr. We also attempted the same aldol reaction of **10a** with 5 equiv of aqueous ^{13}C-labeled formaldehyde to furnish labeled alcohol ^{13}C-**11** in slightly lower yield (45% for two steps, 78% brsm, unoptimized). Somewhat surprisingly, treatment of *N*-pMB benzazocane **10b**[5] to the same

(4) Ducept, P.; Gubler, D. A.; Williams, R. M. *Heterocycles* **2006**, *67*, 597 and references cited therein.

(5) (a) Judd, T. C.; Williams, R. M. *J. Org. Chem.* **2004**, *69*, 2825. (b) Judd, T. C.; Williams, R. M. *Angew. Chem., Int. Ed.* **2002**, *41*, 4683.

(6) Prepared in two steps (TASF-mediated desilylation, Dess−Martin oxidation) from the corresponding silyl ether **12a** reported in ref 4.

(7) Paleo, M. R.; Aurrecoechea, N.; Jung, K.-Y.; Rapoport, H. *J. Org. Chem.* **2003**, *68*, 130.

(8) The benzylic, exocyclic olefin in Danishefsky's synthesis of FR900482 was the product of a Heck cyclization: Schkeryantz, J. M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 4722.

(9) Suzuki, M.; Kambe, M.; Tokuyama, H.; Fukuyama, T. *J. Org. Chem.* **2004**, *69*, 2831.

(10) For example, $^{13}CH_2O$ solution, 20 wt % in H_2O, 99% ^{13}C, CAS No. 3228-27-1, Aldrich Catalog No. 489417, costs US$266.00/gram.

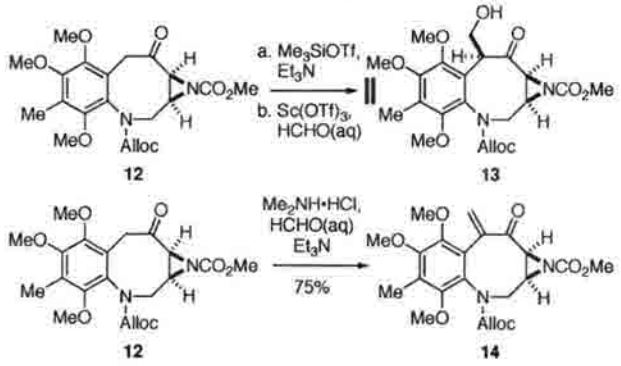
(11) (a) Kobayashi, S.; Hachiya, I. *J. Org Chem.* **1994**, *59*, 3590. (b) Ishikawa, S.; Hamada, T.; Manabe, K.; Kobayashi, S. *J. Am. Chem. Soc.* **2004**, *126*, 12236.

(12) X-ray data for compound **8** are included as Supporting Information.

conditions did not provide any of the desired alcohol. Even more startling was that *N*-Nvoc (Nvoc = 6-nitroveratryloxycarbonyl or 3,4-dimethoxy-6-nitrobenzyloxycarbonyl) benzazocane **10c**[13] did not undergo hydroxymethylation under these conditions. Attempted hydroxymethylation of **10b** and **10c** was apparently unsuccessful since, in each case, the requisite enoxysilane intermediate could not be formed under a variety of conditions.

With these interesting results in hand for benzazocanes constituted with the FR900482 framework, we were intrigued whether this methodology could be useful in our studies toward the asymmetric total synthesis of the mitomycins and their corresponding biosynthetic precursors. As shown in Scheme 3, employing identical hydroxymethylation condi-

Scheme 3. Failed Hydroxymethylations of Benzazocane **12**

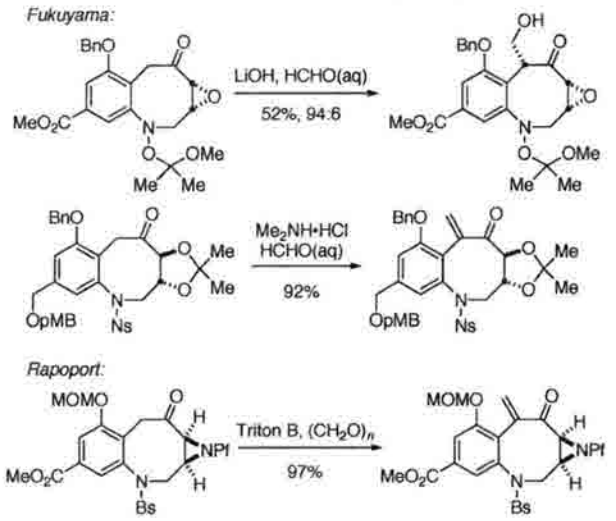


tions as before failed to provide the desired alcohol from benzazocane **12**.[14] In tandem with unsuccessful results observed for FR900482-based benzazocanes **10b** and **10c**, our lack of success in forming the enoxysilane intermediate likely hindered hydroxymethylation of these substrates. Alternatively, attempts to engender enoxysilane formation under basic conditions (LHMDS, Me_3SiCl) also failed. Carbamates other than *N*-alloc (*N*-Boc and methyl) were prepared but also failed to undergo hydroxymethylation. Curiously, attempted hydroxymethylation of **12** using conditions developed by Fukuyama,[9] as well as other conditions, only afforded enone **14** (Scheme 3).

From the hydroxymethylation results for both FR900482- and mitomycin-based benzazocanes, we surmise that the electronics of the substrate play a crucial role in the success of this reaction. This observation is not without precedent for this family of molecules.

Illustrated in Scheme 4 are hydroxymethylation conditions used by Fukuyama[9] and Rapoport[7] in their total syntheses of FR900482, respectively. The major differences among these substrates that may affect the hydroxymethylation reaction are the substituents on the arene ring and the protecting group on the benzazocane nitrogen. From the

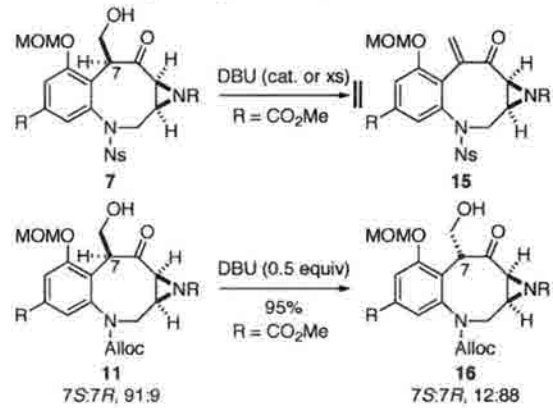
Scheme 4. Electronic Effects on Hydroxymethylation



examples shown in Scheme 4, as well as our own, we may conclude that the more electron-rich the substrate, the more prone it is toward elimination. Curiously, formation of an enoxysilane intermediate from an electron-rich arene appears to be considerably more difficult for application in the scandium(III) triflate-mediated hydroxymethylation reaction which we describe.

One powerful and unanticipated consequence of the Lewis acid catalyzed Mukaiyama aldol approach is that it may be used for the construction of either the FR900482 or the MMC scaffold. Because the hydroxymethylation reaction is stereoselective and kinetically controlled, the initially formed 7*S*-stereoisomer of compound **11** matches the C-9 stereochemistry of MMC (**1**). On the other hand, equilibration of compound **11** to the thermodynamically favored 7*R*-isomer **16** under carefully controlled, base-catalyzed conditions should allow access to FR900482, potential late-stage biosynthetic intermediates and their congeners (Scheme 5).

Scheme 5. Attempts to Epimerize Hydroxymethylation Products



Unfortunately, all attempts to epimerize *N*-nosylsulfonamide **7*S*-7** gave the undesired enone **15** resulting from elimination.

(13) Williams, R. M.; Rollins, S. B.; Judd, T. C. *Tetrahedron* **2000**, *56*, 521.

(14) Prepared in two steps (TASF-mediated desilylation, Dess–Martin oxidation) from the corresponding silyl ether **13b** reported in ref 4.

Successful epimerization of compound **11** (7*S*:7*R*, 91:9) to 7*R*-isomer **16** (7*S*:7*R*, 12:88), but not *N*-nosylsulfonamide **7**, supports our assertion (vide infra) that electronics play a significant role in the chemistry of the benzylic position in these benzazocane architectures.

We attempted to develop a rationale to accommodate why the 7*S*-configuration predominates in successful hydroxymethylation reactions of FR900482-derived templates. Because reactions of medium-ring olefins are known to proceed from the periphery, the major, kinetic product of the hydroxymethylation reaction results from addition to the lowest energy conformation of the ring.[15,16] The possible low-energy conformations of the intermediates in these reactions were determined and analyzed using computational methods (Figure 2).[17] Conformational analysis of the likely intermediate in these reactions revealed that electrophilic addition of formaldehyde to the only available face of the electron-rich olefin in the lowest energy conformer should afford the 7*S*-isomer we observed. Furthermore, analysis of successful hydroxymethylation reactions from our group[5,13] and results reported by Fukuyama[9] suggests that electrophilic approach may proceed via a late transition state.[18] In particular, the development of $A_{1,3}$-strain[19] between the incoming hydroxymethyl group and the aryl alkoxy group as the reaction proceeds may alter the energy barrier for each conformer in the reaction. This unfavorable steric interaction in the transition state may explain differences between experimental selectivities observed and predictions we can make on the basis of computational analysis.

In summary, we report an efficient method for the hydroxymethylation of benzazocanes en route to putative synthetic and late-stage biosynthetic intermediates of FR900482 and MMC. Electronics play a major role in this reaction, as *N*-Nvoc and *N*-PMB benzazocanes of FR900482 fail to undergo hydroxymethylation. Efforts to prepare

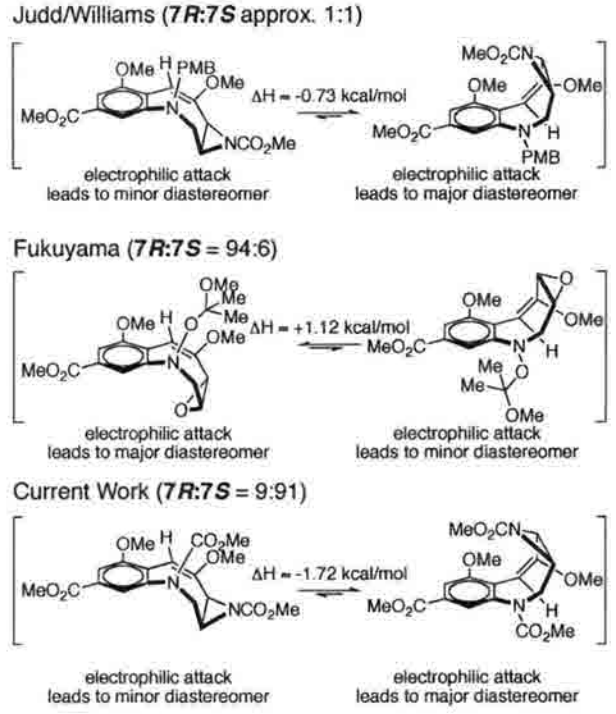


Figure 2. Comparative experimental and computational analysis of intermediates in the hydroxymethylation reaction.

putative biosynthetic intermediates of FR900482 and the mitomycins, as well as the asymmetric total synthesis of the mitomycins, are currently under investigation in these laboratories.

Acknowledgment. Dedicated to the memory of Professor Albert I. Meyers. This work was supported by the National Institutes of Health (CA51875). We also acknowledge a Graduate Student Fellowship from Eli Lilly (to D.A.G.). Mass spectra were obtained on instruments supported by the NIH Shared Instrumentation Grant GM49631.

Supporting Information Available: Complete experimental procedures, characterization data, and spectral data for new compounds, details of theoretical experiments, and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

OL701960V

(15) Still, W. C.; Galynker, I. *Tetrahedron* **1981**, *37*, 3981.

(16) This statement is valid if we assume that the energy barrier of reaction between both conformations of the olefin and the electrophile is nearly identical, according to Curtin−Hammett/Winstein−Holness kinetics: Seeman, J. I. *J. Chem. Educ.* **1986**, *63*, 42.

(17) The lowest energy conformations of the eight-membered ring possessing a *cis*-olefin were determined by a Monte Carlo conformational search. These structures were refined using AM1, then HF/6-31G*. Last, the energies shown represent single-point HF/6-31G** calculations. Certain structural simplifications were made to simplify our qualitative computational experiments. These simplifications reduce computational time but do not, in our opinion, significantly alter the results. Further details and a rationale for the computational methods used are included in the Supporting Information.

(18) Hammond, G. S. *J. Am. Chem. Soc.* **1955**, *77*, 334.

(19) Hoffmann, R. W. *Chem. Rev.* **1989**, *89*, 1841.

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IMPROVED SYNTHESES OF THE FR900482 AND MITOMYCIN BENZAZOCINE RING CORE *VIA* MITSUNOBU CYCLIZATION

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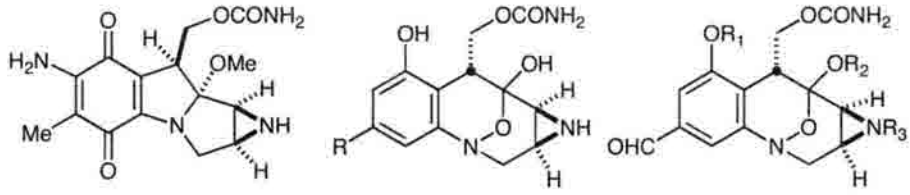
Abstract – An asymmetric synthesis of a highly functionalized benzazocine core of FR900482 and the mitomycins has been achieved in 20 and 19 steps, respectively. Key features of the synthesis include a highly chemoselective reduction of a nitro group and a Mitsunobu cyclization using either sulfonamides or carbamates. Removal of the protecting groups afforded the free benzazocine.

INTRODUCTION

Antitumor drugs restricting uncontrolled cellular growth by acting at the level of DNA replication and transcription are an important class of clinical agents.[1] Among these agents, mitomycin C (**1**) has been widely used in cancer chemotherapy for solid tumors for over 30 years despite its high hematotoxicity.[2] More recently, in the search for superior anticancer agents, Fujisawa Pharmaceutical Co isolated from the fermentation broth of *Streptomyces sandaensis* two new highly potent natural antitumor antibiotic alkaloids FR900482 (**2**) and FR66979 (**3**), which are structurally related to the mitomycins (Figure 1).[3] Similar to the mitomycins, studies on the mode of action of **2** and **3** have established that the cytotoxicity of these new agents derives from the formation of covalent DNA interstrand as well as DNA-protein cross-links both *in vitro* and *in vivo* as a result of the reactive mitosene intermediate generated upon reductive activation.[4] Semi-synthetic derivatives such as FK973 (**4**) and FK317[5] (**5**) were subsequently developed in the search for potentially superior clinical candidates.

Due to their potent antitumor activity, intriguing mode of action and densely functionalized structure, mitomycin C, FR900482 and congeners have attracted considerable interest from synthetic chemists. As a result, many original approaches to these compounds have been described in the literature resulting in a number of total syntheses of both the mitomycins[6] and FR900482 alkaloids.[7]



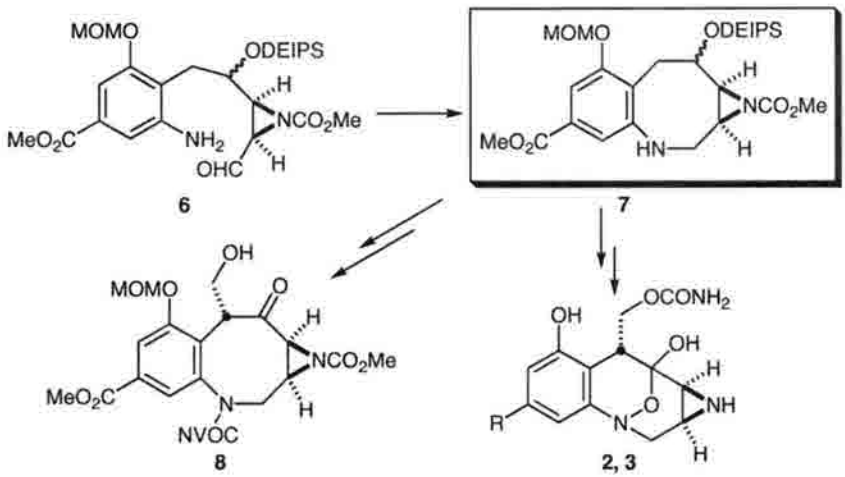
1, mitomycin C

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

4, FK973, R_1=R_2=R_3=Ac
5, FK317, R_1=Me, R_2=R_3=Ac

Figure 1 Structure of mitomycin C, FR900482 and congeners

Most of the strategies that have been deployed rely upon the formation of the benzazocine ring system as a key intermediate, since, in principle, both the mitomycin and FR900482 frameworks can be accessed by varying the oxidation state of the benzazocine nitrogen.[8] New procedures for the synthesis of highly functionalized benzazocines are therefore of great interest. Synthetic efforts in our group were engaged and ultimately culminated in the concise enantioselective total synthesis of FR900482 (**2**) and FR66979 (**3**).[7d,g] A key intermediate in our synthesis was the aziridine containing benzazocine (**7**) that was subsequently used as a late stage intermediate in synthetic routes to **2** and **3** in addition to the photo-triggered mitosene progenitor (8)[9] (Scheme 1).

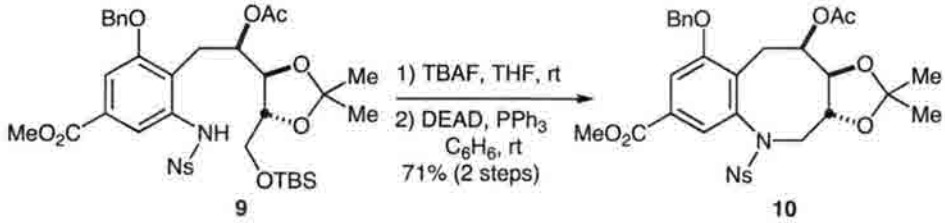


Scheme 1 Benzazocine (**7**) as FR900482/pro-mitosenes intermediate

RESULTS AND DISCUSSION

Compound (**7**) was obtained in moderate yield (50-70%) upon reductive amination of the anilinoaldehyde (**6**). Unfortunately, despite extensive optimization efforts, this reaction suffered from extensive dimerization upon scale up and therefore could only be carried out under dilute conditions (typically ~1 mM) and on a relatively small scale (200-300 mg). In order to address these shortcomings we envisioned a new synthetic route to the benzazocine involving an intramolecular Mitsunobu[10] cyclization of a highly functionalized aziridine alcohol.

In 1995, Fukuyama and co-workers described the preparation of 2- and 4-nitrobenzenesulfonamides (Ns amide) and their use in Mitsunobu reactions for the synthesis of secondary amines, and later for the formation of macrocycles.[11] The value of this strategy was ultimately demonstrated in synthetic efforts towards the enantioselective total synthesis of FR900482 in which the ω-hydroxy-2-Ns amide (**9**) gave the benzazocine (**10**) in good yield *via* an intramolecular Mitsunobu cyclization (Scheme 2).[7h]



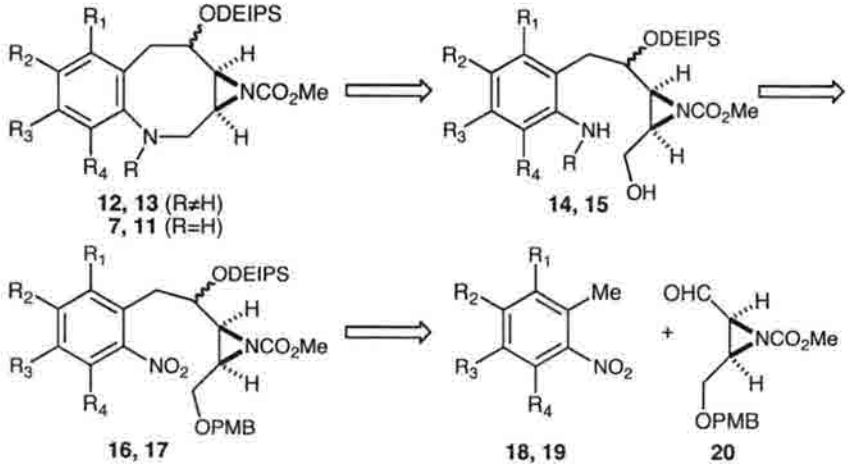
Scheme 2 Fukuyama's Mitsunobu cyclization

It should be mentioned that a similar strategy involving a Mitsunobu cyclization had been attempted in early synthetic efforts in our group utilizing a free aniline similar to **6**, but failed as a result of the lack of aniline activation.[12] With these observations in mind and in light of Fukuyama's successful cyclization, it became clear that the aniline nitrogen atom had to be substituted in a manner that would serve two purposes in our synthesis: (i) activation of the aniline through lowering the pK_a and, (ii) protection of the

aniline during the oxidative removal of the *O*-pMB ether to furnish the corresponding alcohol. Herein, we report a synthesis of the core benzazocine ring system for both the FR900482 and mitomycin families of antitumor agents utilizing an intramolecular Mitsunobu cyclization of a highly functionalized aziridine alcohol with either a *N*-sulfonamide or *N*-carbamoyl moiety. Deprotection strategies to obtain the free anilines (**7**) and (**11**) will also be discussed.

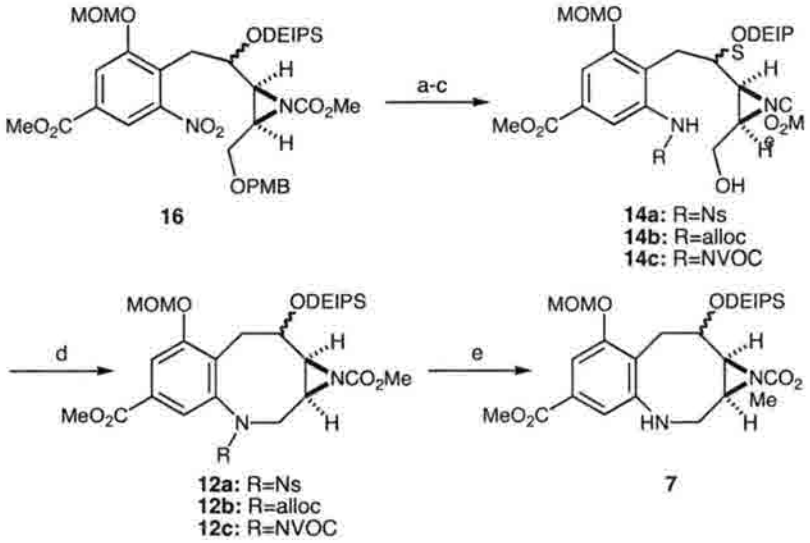
Our initial strategy for both the FR900482 and mitomycin series is outlined in Scheme 3. The nitro compounds (**16**) and (**17**), which would be prepared from coupling of the optically active aziridine (**20**)[13] and the appropriate nitrotoluene derivatives **18**[13] or **19**,[14] would then be elaborated to the Mitsunobu precursors (**14**) and (**15**) in a three-step procedure. With **14** and **15** in hand, the Mitsunobu cyclization if successful, would afford the protected benzazocines (**12**) and (**13**) for both the FR900482 and mitomycin series.

Compound (**16**) was synthesized as previously described,[7g] as a mixture of diastereomers, which were separated by chromatography and subsequently processed independently. The first challenge was to effect the efficient and chemo-selective reduction of the nitro group of **16** in the presence of the pMB ether and the sensitive aziridine. After extensive investigation, catalytic transfer hydrogenation[15] proved to be the most efficient method and cleanly afforded the desired aniline derivative in high yield. This is a notable improvement over the reduction conditions used by Fukuyama *et al.* on a similar substrate[7h] (20 min, 93% vs 52 h, 65%). Protection of the aniline as the corresponding nosyl (Ns) amide by treatment with 2-NsCl in pyridine followed by removal of the pMB group with DDQ afforded the desired alcohol (**14a**). Reaction of **14a** under standard Mitsunobu conditions (DEAD, Ph_3P, benzene) gave the desired benzazocine (**12a**) in 80% yield for both diastereomers (Scheme 4).



FR series: 7, 12, 14, 16, 18: R_1=OMOM, R_2=R_4=H, R_3=CO_2Me
MMC series: 11, 13, 15, 17, 19: R_1=R_2=R=$_4$=OMe, R_3=Me

Scheme 3 Retrosynthesis of mitomycin/FR900482 benzazocines



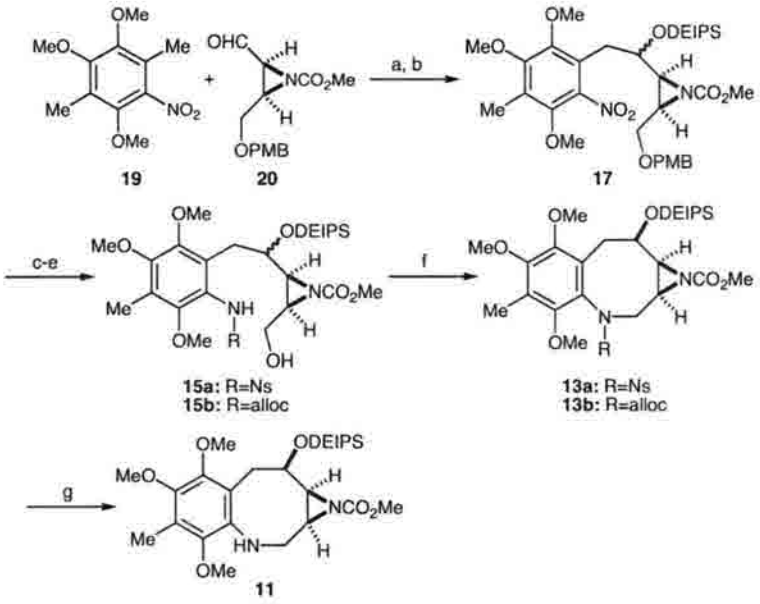
Scheme 4 Mitsunobu cyclization. Conditions: (a) 5% Pd-C 0.1 equiv, HCO_2NH_4 5 equiv, MeOH, rt, 20 min, 93%. (b) NsCl, pyridine, rt, 73% or RCOCl, $NaHCO_3$ aq, CH_2Cl_2, rt (R=alloc 80%, R=NVOC 78%). (c) DDQ, CH_2Cl_2:H_2O 15:1, rt, (R=Ns 73%, R=alloc 89%, R=NVOC 75%). (d) DEAD, Ph_3P, toluene, rt, (R=Ns 95%, R=NVOC 83%) or TMAD, PBu_3, toluene, rt (R=alloc 82%). (e) Cs_2CO_3, PhSH, MeCN, rt, 90%.

Interestingly, a more detailed investigation revealed that toluene was a slightly more effective solvent than benzene, resulting in a reduced reaction time (15 min) and slightly higher yield (95%). Using these optimized conditions, the cyclization was successfully performed on multigram scale. This result represents a considerable improvement over our previous cyclization route[7g] (15 min, 95% vs 72 h, 50-70%). Removal of the nosyl group using cesium carbonate and thiophenol[7h,16] cleanly afforded the benzazocine (**7**), which was spectroscopically identical to an authentic sample.[7g] Although the number of steps from **16** to **7** (5 steps) is identical to that of our reductive amination route, this procedure possesses several advantages including scale-up, short reaction times and ease of purification.

Having demonstrated the feasibility of the intramolecular Mitsunobu cyclization for the construction of the eight-membered ring, we then became interested in determining if this procedure could be applied to (i) groups other than a sulfonamide, such as carbamates and, (ii) synthesis of the core benzazocine (**13**) precursor to the mitomycins.

Reaction of the aniline derived from **16** with allyl or 6-nitroveratryl chloroformates and sodium hydrogen carbonate afforded the desired carbamates in high yield. Removal of the pMB group gave the alcohols (**14b,c**) (Scheme 4). Mitsunobu coupling using the DEAD/Ph_3P system gave the desired cyclized products (**12b,c**, **12c**) that proved identical to a sample previously prepared.[9c] Interestingly, the cyclization of **14b** using the above conditions was much slower than for **14c** under similar condition and did not go to completion even after extended reaction time (40 h). Harsher conditions (90°C, 36 h, 79%) were required

for efficient conversion to proceed. This observation likely reflects the higher pK_a of the carbamate group of **14b** as compared to **14c**.



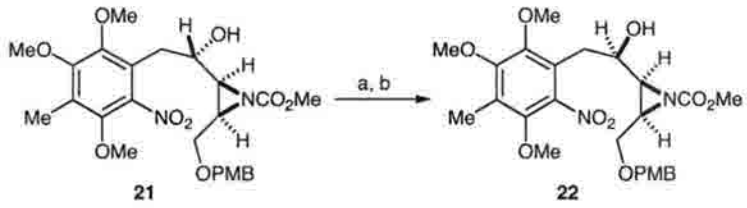
Scheme 5 Mitomycin benzazocine synthesis. Conditions: (a) NaHMDS, $ZnCl_2$, DMF, -45°C, 87%. (b) DEIPSCl, Im, CH_2Cl_2, rt, 89%. (c) 5% Pd-C 0.1 equiv, HCO_2NH_4 5 equiv, MeOH, rt, 80%. (d) NsCl, pyridine, rt, 79% or allocCl, $NaHCO_3$, CH_2Cl_2-H_2O, rt, 75%. (e) DDQ, CH_2Cl_2:H_2O 15;1, rt (R=Ns 87%, R=alloc 87%). (f) DEAD, Ph_3P, toluene, 70% (R=Ns) or TMAD, PBu_3, toluene, rt, 83% (R=alloc). (g) $Pd(Ph_3P)_4$ 5%, HCO_2H 4 equiv, THF, rt, 72%.

To overcome this reactivity problem we searched for alternative reagents for the Mitsunobu cyclization. A variety of Mitsunobu systems have been designed to allow for the use of nucleophiles possessing higher pKa values (pK_a=11-13).[17] Consequently, several reagents were screened and it was determined that the TMAD/PBu_3 system[18] allowed the cyclization of **14b** to proceed at room temperature in a reduced reaction time (6 h). This new approach to carbamate substituted benzazocines is two steps shorter than our previous synthesis[9c] and should allow the efficient preparation of various novel mitosene progenitors with alternative triggering mechanisms.

Finally, we turned our attention to utilizing this newly developed chemistry to construct the core benzazocine ring precursor to the mitomycins. Coupling of the nitrotoluene derivative **19** and the aziridine aldehyde (**20**) gave the desired alcohol as a 2:1 mixture of diastereomers, which were separated by column chromatography. Protection of the alcohols furnished the required precursor (**17**). Following the procedure used for the FR900482 series, we were pleased to find that the same sequence of reactions furnished the desired Mitsunobu products (**13a,b**) in all cases (Scheme 5).

As first observed for **14b**, the cyclization of the Alloc carbamate (**15b**) was noticeably slower than for the Ns amide (**15a**) and did not go to completion even after prolonged reaction time (24 h, 50% conversion). The use of TMAD/PBu_3, as before, proved to be a superior system for the Mitsunobu cyclization and

provided **13b** much more efficiently (6 h, 83%). Although many similarities were observed with respect to the cyclization of intermediates in both the FR900482 and mitomycin series, we noticed several interesting differences. A surprising result was the stereochemical requirement for the Mitsunobu cyclization in the mitomycin series. It was found that the minor diastereomer of **15b** did not undergo cyclization even after prolonged heating in refluxing toluene. To rationalize this result, both diastereomers of **15b** were analyzed by molecular modeling. MM2 energy minimization showed that the C-N bond distance for the minor diastereomer (4.1 Å) was considerably greater than for the major diastereomer (3.0 Å) and likely accounts for the lack of reactivity. Fortunately, the minor diastereomer (**21**), obtained after coupling of **19** and **20**, could be easily converted in excellent yield to the major diastereomer (**22**) by Dess-Martin oxidation[19] to the corresponding ketone followed by reduction with $NaBH_3CN$ (Scheme 6).



Scheme 6 Epimerization. Conditions: (a) DMP, CH_2Cl_2, rt (b) $NaBH_3CN$, AcOH, CH_2Cl_2:MeOH 2:1, 4 °C, 95% (2 steps)

With an efficient method in hand for the construction of the requisite benzazocine ring, we then focused our efforts on the deprotection of the nitrogen position. Unfortunately, attempts to remove the Ns group of **13a** using a variety of conditions such as Cs_2CO_3/PhSH, $HSCH_2CO_2H/Et_3N$ or *n*-$PrNH_2$[20] failed to afford the desired benzazocine (**11**) even under forcing conditions. To the best of our knowledge this is the first example in which these conditions have failed to unmask a nosyl protected nitrogen. Alternatively, efforts to introduce a 2,4-dinitrosulfonamide[20b,21] moiety in an effort to facilitate the deprotection were also unsuccessful. In light of these results, we focused on the removal of the Alloc carbamate group in **13b**. Upon treatment of **13b** with tetrakis(triphenylphosphine)palladium in the presence of formic acid in THF[22] we were pleased to obtain the desired aniline (**11**) in 72% yield, whose data matched a sample prepared by an alternative procedure (unpublished result).

In conclusion, we have shown that the aziridine containing benzazocine ring precursor to the FR900482 and mitomycin alkaloids can be efficiently prepared by an intramolecular Mitsunobu reaction with either sulfonamides or carbamates. The free benzazocine could be obtained in each series. The use of benzazocine (**7**) to prepare stable and radioisotopically labeled FR900482 analogues as well as conversion of **13b** to achieve the first asymetric total synthesis of mitomycin K,[6c,d] A and C[6a,b] is currently being investigated and will be reported on in due course. In addition, this procedure, which is readily conducted

on a preparative scale, provides access to gram quantities of various mitomycin and FR900482 analogues for biological testing.

EXPERIMENTAL

General Considerations. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ (^{1}H, 7.27; ^{13}C, 77.0 ppm) on Varian Inova-300 and Inova-400 spectrometers unless otherwise noted. When appropriate, the multiplicity of a signal is denoted as "br" to indicate the signal was broad. IR spectra were recorded on a Nicolet Avatar 320 spectrometer. MS spectra were obtained on a Fisons VG Autospec. Optical rotation values were measured using a Rudolph Research Autopol III automatic polarimeter referenced to the sodium D-line.

1,2,4-Trimethoxy-3,6-dimethyl-5-nitrobenzene (19)

To a solution of 1,3,4-trimethoxy-2,5-dimethylbenzene (20.0 g, 102 mmol) in acetic anhydride (400 mL) at 0°C was added cupric nitrate trihydrate (47.4 g, 204 mmol). After stirring for 45 min at rt, the reaction mixture was passed through a short plug of silica gel, eluted with EtOAc and the filtrate concentrated. The residual oil was dissolved in CH_2Cl_2 (200 mL), washed with sat. aq $NaHCO_3$ (150 mL) and brine (150 mL). The combined organics were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (90:10 hexanes:EtOAc) to afford **19** (24.0 g, 50%) as a yellow crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.14 (3H, s), 2.17 (3H, s), 3.76 (3H, s), 3.77 (3H, s), 3.82 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.5, 10.6, 60.3, 60.5, 62.5, 122.3, 124.7, 142.9, 146.1, 147.6, 153.5. IR (NaCl neat) (cm^{-1}): 2997, 2942, 2854, 1571, 1532, 1471, 1370, 1253, 1094, 955. MS: m/z = 241 (100), 154 (90), 137 (72). HRMS (FAB+): m/z Calcd 241.0950 $(MH)^{+}$, Found 241.0954. mp: 26-27°C

Methyl (2*R*,3*S*)-2-((4-methoxybenzyloxy)methyl)-3-(1-hydroxy-2-(2,3,5-trimethoxy-4-methyl-6-nitro-phenyl)ethyl)aziridine-1-carboxylate (21, 22)

Prior to the reaction, compounds (**19**) (8.6 g, 36 mmol) and **20** (5.0 g, 18 mmol) were dehydrated three times by azeotropic distillation using anhydrous toluene and lastly dried under vacuum for 1 h before use. A solution of **20** in dry DMF (15 mL) was then added to freshly fused zinc chloride (2.9 g, 21.5 mmol). The resulting solution was stirred under argon for 1 h at rt. In a separate flask, NaHMDS (36 mL of a 1 M solution in THF, 36 mmol) was added dropwise to a solution of **19** in dry DMF (50 mL) cooled to -45°C. The mixture immediately turned deep red. To this solution was added dropwise the aziridine solution

prepared above and the resulting mixture was stirred at -45°C until completion of the reaction by TLC analysis. The reaction mixture was quenched with sat. aq NH_4Cl (50 mL), allowed to warm to rt and H_2O (50 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (5x150 mL), the combined organics dried over Na_2SO_4 and concentrated under reduced pressure. The crude oil was purified by flash column chromatography (60:40 hexanes:EtOAc) to afford the alcohol (8.3 g, 89%) as a pale yellow oil (2:1 mixture of separable diastereomers (**21**) and (**22**)). Major diastereomer: $[\alpha]_D^{20}$=-21.1°, (*c*=1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 2.22 (3H, s), 2.60 (1H, dd, *J* = 8.0, 6.0 Hz), 2.83 (1H, q, *J* = 6.0 Hz), 2.92-3.03 (3H, m), 3.46 (1H, dd, *J* = 10.5, 7.5 Hz), 3.61-3.68 (1H, m), 3.70 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 3.83 (3H, s), 3.85 (3H, s), 4.50 (2H, d, *J* = 1.5 Hz), 6.85 (2H, dt, *J* = 8.5, 2.5 Hz), 7.23 (2H, dt, *J* = 8.5, 2.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.7, 31.5, 39.8, 45.1, 53.7, 55.2, 60.2, 60.6, 62.5, 67.6, 69.6, 72.9, 113.8, 122.4, 125.9, 129.2, 129.5, 143.0, 146.2, 147.8, 153.3, 159.2, 162.9. IR (NaCl neat) (cm^{-1}): 3479, 3002, 2944, 2856, 1730, 1612, 1533, 1466, 1370, 1299, 1115, 755. MS: m/z = 521 (100), 493 (46), 491 (26), 474 (49). HRMS (FAB+): m/z Calcd 521.2135 $(MH)^+$, Found 521.2134

Minor diastereomer: $[\alpha]_D^{20}$=+6.1° (*c*=1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 2.20 (3H, s), 2.45 (1H, d, *J* = 5.0 Hz), 2.64 (1H, t, *J* = 7.0 Hz), 2.78-2.95 (3H, m), 3.28 (1H, dd, *J* = 11.0, 5.0 Hz), 3.50 (1H, dd, *J* = 11.0, 7.0 Hz), 3.71-3.75 (1H, m), 3.76 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 3.82 (3H, s), 3.83 (3H, s), 4.47 (1H, d, *J* = 11.5 Hz), 4.56 (1H, d, *J* = 11.5 Hz), 6.87 (2H, dt, *J* = 8.5, 2.5 Hz), 7.27 (2H, dt, *J* = 8.5, 2.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.7, 31.3, 41.4, 45.9, 54.0, 55.3, 60.2, 60.6, 62.6, 67.0, 69.5, 72.6, 113.8, 121.5, 126.5, 129.6, 130.0, 143.1, 146.3, 147.9, 153.5, 159.3, 163.4. IR (NaCl neat) (cm^{-1}): 3364, 3009, 2937, 2893, 1729, 1611, 1532, 1249, 1090, 1035. MS: m/z = 521(100), 493 (44), 491 (27), 474 (49), 154 (52), 137 (36). HRMS (FAB+): m/z Calcd 521.2135 $(MH)^+$, Found 521.2135

Methyl (2*R*,3*S*)-2-((4-methoxybenzyloxy)methyl)-3-(1-(diethyl(isopropyl)silyloxy)-2-(2,3,5-trimethoxy-4-methyl-6-nitrophenyl)ethyl)aziridine-1-carboxylate (17)

To a solution of the alcohols (**21**) and (**22**) obtained above (5.2 g, 10.0 mmol) and imidazole (2.4 g, 35 mmol) in dry DCM (250 mL) was added dropwise chlorodiethylisopropylsilane (3.3 g, 20 mmol). The mixture was stirred at rt for 24 h. The reaction was quenched with sat. aq $NaHCO_3$, diluted with DCM and the layers separated. The aqueous layer was extracted with EtOAc (2x50 mL). The combined organics were washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (80:20 hexanes:EtOAc) to afford **17** as a pale yellow oil (6.15 g, 89%). Major diastereomer: $[\alpha]_D^{20}$=-19.9° (*c*=1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 0.42-0.65 (4H, m), 0.75-1.02 (13H, m), 2.21 (3H, s), 2.49 (1H, t, *J* = 6.0 Hz), 2.76 (1H, q, *J* =

6.0 Hz), 2.83-2.95 (2H, m), 3.61-3.77 (2H, m), 3.72 (3H, s), 3.78 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 3.83 (3H, s), 4.11 (1H, dt, J = 10.0, 5.0 Hz), 4.53 (1H, d, J = 11.5 Hz), 4.66 (1H, d, J = 11.5 Hz), 6.88 (2H, d, J = 8.5 Hz), 7.32 (2H, J = 8.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 3.4, 3.6, 6.8, 6.9, 9.6, 12.7, 17.1, 33.1, 41.4, 45.0, 53.6, 55.2, 60.0, 60.5, 62.5, 67.5, 68.5, 72.5, 113.7, 122.8, 125.7, 129.5, 130.3, 143.4, 146.2, 148.2, 153.3, 159.2, 163.7. IR (NaCl neat) (cm^{-1}): 2943, 2875, 1731, 1612, 1533, 1464, 1290, 1114, 1093, 727. MS: m/z = 649 (100), 605 (20), 469 (22), 122 (90) . HRMS (FAB+): m/z Calcd 649.3156 $(MH)^+$, Found 649.3147

General procedure for the nitro reduction

Methyl (2*R*,3*S*) 2-((4-methoxybenzyloxy)methyl)-3-(2-(2-amino-4-(methoxycarbonyl)-6-(methoxymethoxy) phenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)aziridine-1-carboxylate

To a stirred suspension of **16** (3.36 g, 5.07 mmol) and 5% Pd-C (0.6 g) in dry MeOH (40 mL) was added anhydrous ammonium formate (1.6 g, 25.3 mmol) in one portion. The mixture was stirred at rt and monitored by TLC (50:50 hexanes:EtOAc). After complete consump-tion of the starting material (20-30 min), the catalyst was removed by filtration through a pad of celite and the filtrate was concentrated under reduced pressure. To the residue was added H_2O (40 mL) and the aqueous phase was extracted with DCM (2x40 mL). The combined organics were dried over $MgSO_4$ and concentrated under reduced pressure to afford clean aniline as a pale yellow oil (2.89 g, 90%) which was used without further purification. For analytical purposes, the aniline was purified by flash column chromatography (60:40 hexanes:EtOAc). Major diastereomer: $[\alpha]_D^{20}$=-6.7° (c=1.53, $CHCl_3$)

^{1}H (300 MHz, $CDCl_3$): δ = 0.50 (4H, app sext, J = 7.5 Hz), 0.77-0.92 (13H, m), 2.53 (1H, dd, J = 6.0, 5.0 Hz), 2.76 (1H, q, J = 6.0 Hz), 2.97 (2H, d, J = 6.0 Hz), 3.45 (3H, s), 3.72 (3H, s), 3.74 (2H, d, J = 6.5 Hz), 3.81 (3H, s), 3.87 (3H, s), 4.15 (2H, br s), 4.26 (1H, q, J = 6.0 Hz), 4.51 (1H, d, J = 11.5 Hz), 4.61 (1H, d, J = 11.5 Hz), 5.16 (1H, d, J = 6.5 Hz), 5.19 (1H, d, J = 6.5 Hz), 6.88 (2H, dt, J = 8.5, 2.0 Hz), 7.03 (1H, d, J = 1.5 Hz), 7.12 (1H, d, J = 1.5 Hz), 7.29 (2H, dt, J = 8.5, 2.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.4, 3.5, 6.7, 6.8, 12.7, 17.1, 32.0, 40.8, 44.9, 52.0, 53.6, 55.2, 56.1, 67.7, 68.7, 72.8, 94.2, 104.3, 110.9, 113.8, 117.3, 129.4, 129.6, 129.8, 147.3, 156.0, 159.3, 163.8, 167.1. IR (NaCl neat) (cm^{-1}): 3449, 3376, 2952, 2875, 1720, 1584, 1513, 1437, 1343, 1298, 1240, 1077, 730. MS: m/z = 633 (88), 394 (34), 345 (20), 224 (66), 154 (26), 136 (35), 121 (100). HRMS (FAB+): m/z Calcd 633.3207 $(MH)^+$, Found 633.3221

Minor diastereomer: $[\alpha]_D^{20}$=+23.8° (c=0.55, $CHCl_3$). ^{1}H (300 MHz, $CDCl_3$): δ = 0.46 (2H, q, J = 7.5 Hz), 0.61 (2H, q, J = 7.5 Hz), 0.78-0.98 (13H, m), 2.66 (1H, q, J = 8.0 Hz), 2.71-2.75 (1H, m), 2.81-2.85 (2H, m), 3.34 (3H, s), 3.35 (1H, dd, J = 11.5, 6.5 Hz), 3.45 (1H, dd, J = 11.5, 4.0 Hz), 3.74 (3H, s), 3.75-3.82

(1H, m), 3.80 (3H, s), 3.87 (3H, s), 4.12 (2H, br s), 4.53 (1H, d, J = 11.5 Hz), 4.64 (1H, d, J = 11.5 Hz), 5.06 (1H, d, J = 7.0 Hz), 5.09 (1H, d, J = 7.0 Hz), 6.87 (2H, dt, J = 9.0, 2.5 Hz), 7.07 (1H, s,), 7.14 (1H, s), 7.29 (2H, dt, J = 9.0, 2.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.5, 3.6, 6.8, 6.9, 12.8, 17.1, 17.2, 31.5, 41.3, 46.0, 51.9, 53.5, 55.2, 56.0, 67.2, 71.4, 72.2, 94.1, 104.6, 111.1, 113.7, 116.9, 129.2, 129.4, 129.9, 147.2, 156.0, 159.2, 163.5, 166.9. IR (NaCl neat) (cm^{-1}): 3435, 3368, 2952, 2875, 1720, 1513, 1240, 1065, 730. MS: m/z = 633 (10), 394 (10), 121 (100). HRMS (FAB+): m/z Calcd 633.3207 $(MH)^+$, Found 633.3221

Methyl (2*R*,3*S*)-2-((4-methoxybenzyloxy)methyl)-3-(2-(2-amino-3,5,6-trimethoxy-4-methylphenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)aziridine-1-carboxylate

The experiment was carried out by the general procedure for the nitro reduction with **17** (0.72 g, 1.08 mmol), 5% Pd-C (0.13 g) and anhydrous ammonium formate (0.35 g, 5.57 mmol) in dry MeOH (6 mL). The crude product was purified by flash column chromatography (70:30 hexanes:EtOAc) to yield the aniline (0.53 g, 80%) as a pale yellow oil. Major diastereomer: $[\alpha]_D^{20}$=-7.3° (c=5.6, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.48 (4H, app sext, J = 8.0 Hz), 0.77-0.92 (13H, m), 2.17 (3H, s), 2.60 (1H, dd, J = 6.0, 5.0 Hz), 2.75 (1H, q, J = 6.0 Hz), 2.88 (2H, d, J = 7.0 Hz), 3.59-3.68 (2H, m), 3.67 (3H, s), 3.70 (3H, s), 3.73 (3H, s), 3.77 (3H, s), 3.80 (3H, s), 4.04 (2H, br s), 4.15 (1H, q, J = 6.0 Hz), 4.51 (1H, d, J = 11.5 Hz), 4.61 (1H, d, J = 11.5 Hz), 6.87 (2H, d, J = 8.5 Hz), 7.29 (2H, d, J = 8.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 3.4, 3.5, 6.7, 6.8, 9.2, 12.6, 17.1, 32.1, 41.0, 45.0, 53.5, 55.1, 59.3, 40.2, 60.2, 67.5, 69.6, 72.6, 113.6, 115.4, 123.0, 129.5, 129.9, 135.9, 141.6, 143.1, 148.2, 159.1, 163.8. IR (NaCl neat) (cm^{-1}): 3436, 3361, 2952, 1731, 1613, 1513, 1464, 1248, 1090, 1014, 821, 730. MS: m/z = 618 (100), 380 (24), 210 (46), 180 (17). HRMS (FAB+): m/z Calcd 618.3336 $(MH)^+$, Found 618.3361

General procedure for the nosylation

Methyl (2*R*,3*S*)-2-((4-methoxybenzyloxy)methyl)-3-(1-diethyl(isopropyl)silyloxy)-2-(4-(methoxycarbonyl) -2-(methoxymethoxy)-6-(2-nitrophenylsulfonamido) phenyl)ethyl)aziridine-1-carboxylate

To a stirred solution of aniline derived from **16** (0.06 g, 0.095 mmol) in dry pyridine (0.4 mL) was added 2-nitrobenzenesulfonyl chloride (0.084 g, 0.38 mmol) portion wise over 20 min. After the end of the addition, the reaction was stirred at rt and monitored by TLC until complete consumption of the starting material. The reaction mixture was diluted with EtOAc and washed with brine (5 mL), 1N HCl (5 mL) and brine (5 mL). The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure.

The residue was purified by flash column chromatography (70:30 hexanes:EtOAc) to afford the sulfonamide as a pale yellow oil (0.056 g, 73%). Major diastereomer: $[\alpha]_D^{20}$=-6.4° (*c*=0.56, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 0.39 (2H, m), 0.48 (2H, q, *J* = 7.5 Hz), 0.69-0.86 (13H, m), 2.54 (1H, dd, *J* = 13.5, 11.0 Hz), 2.73 (1H, t, *J* = 6.5 Hz), 2.80 (1H, q, *J* = 6.0 Hz), 2.86 (1H, dd, *J* = 13.5, *J* = 3.5 Hz), 3.33 (3H, s), 3.50 (2H, m), 3.65-3.70 (1H, m), 3.67 (3H, s), 3.80 (3H, s), 3.93 (3H, s), 4.48 (1H, d, *J* = 11.5 Hz), 4.57 (1H, d, *J* = 11.5 Hz), 5.07 (2H, s), 6.86 (2H, dt, *J* = 8.5, 2.0 Hz), 7.24 (2H, dt, *J* = 8.5, 2.0 Hz), 7.55 (1H, dt, *J* = 7.5, 1.0 Hz), 7.60 (1H, d, *J* = 1.5 Hz), 7.70 (1H, dt, *J* = 7.5, 1.5 Hz), 7.74 (1H, dd, *J* = 7.5, 1.5 Hz), 7.95 (1H, dd, *J* = 8.0, 1.5 Hz), 7.97 (1H, d, *J* = 1.5 Hz), 8.83 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.3, 3.4, 6.4, 6.6, 12.6, 16.9, 17.0, 31.3, 41.0, 45.6, 52.3, 53.7, 55.2, 56.1, 67.5, 69.0, 72.7, 94.4, 112.4, 113.7, 122.1, 125.9, 128.0, 129.5, 129.6, 129.8, 130.6, 132.5, 133.5, 133.7, 137.1, 147.8, 155.1, 159.3, 163.0, 166.3. IR (NaCl neat) (cm^{-1}): 3269, 2954, 1726, 1543, 1306, 1244, 1174, 1087, 1061, 732. MS: m/z = 818 (8), 307 (19), 154 (68) 137 (48), 121 (100). HRMS (FAB+): m/z Calcd 818.2990 $(MH)^+$, Found 818.2980

Methyl (2*R*,3*S*)-2-((4-methoxybenzyloxy)methyl)-3-(1-(diethyl(isopropyl)silyloxy)-2-(2,3,5-trimethoxy-4-methyl-6-(2-nitrophenylsulfonamido)phenyl) ethyl)aziridine-1-carboxylate

The experiment was carried out by the general procedure for nosylation with the aniline derived from **17** (0.120 g, 0.19 mmol), 2-nitrobenzenesulfonyl chloride (0.172 g, 0.77 mmol) in dry pyridine (1 mL). The crude product was purified by flash column chromatography (70:30 hexanes:EtOAc) to afford the sulfonamide as a pale yellow oil (0.118 g, 79%). Major diastereomer: $[\alpha]_D^{20}$=+17.7° (*c*=1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 0.40-0.54 (4H, m), 0.75-0.91 (13H, m), 2.09 (3H, s), 2.61 (1H, t, *J* = 6.5 Hz), 2.69 (1H, dt, *J* = 7.0, 4.5 Hz), 2.78 (1H, dd, *J* = 13.5, 9.0 Hz), 2.96 (1H, dd, *J* = 13.5, 5.0 Hz), 3.36 (3H, s), 3.48-3.55 (2H, m), 3.66 (3H, s), 3.71 (3H, s), 3.73 (3H, s) 3.77 (3H, s), 3.80-3.83 (1H, m), 4.47 (1H, d, *J* = 11.5 Hz), 4.59 (1H, d, *J* = 11.5 Hz), 6.83 (2H, d, *J* = 8.5 Hz), 7.24 (2H, d, *J* = 8.5 Hz), 7.62 (1H, dt, *J* = 7.5, 1.5 Hz), 7.67 (1H, dt, *J* = 7.5, 1.5 Hz), 7.77 (1H, s), 7.89 (1H, dd, *J* = 9.0, 1.5 Hz), 7.97 (1H, dd, *J* = 9.0, 1.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.3, 6.7, 9.5, 12.8, 17.0, 17.3, 29.6, 33.0, 40.9, 45.2, 53.6, 55.1, 59.4, 59.7, 60.1, 67.5, 69.8, 72.5, 113.6, 124.3, 124.8, 125.0, 125.2, 128.5, 129.4, 129.9, 130.3, 131.0, 132.3, 132.8, 135.4, 136.4, 147.6, 151.3, 151.9, 159.1, 163.5. IR (NaCl neat) (cm^{-1}): 3319, 2930, 1728, 1542, 1464, 1409, 1251, 1172, 1082, 732. MS: m/z = 804 (15), 618 (89), 574 (15), 472 (13), 380 (100), 376 (55), 330 (22), 234 (30). HRMS (FAB+): m/z Calcd 804.3197, Found 804.3191

General procedure for carbamate formation

Methyl (2*R*,3*S*)-2-((4-methoxybenzyloxy)methyl)-3-(2-(2-(allyloxycarbonyl)-4-(methoxycarbonyl)-6- (methoxymethoxy)phenyl)-1-(diethyl(isopropyl)-silyloxy)ethyl)aziridine-1-carboxylate)

To a stirred solution of aniline derived from **16** (0.180 g, 0.284 mmol) and allyl chloroformate (90 μL, 0.852 mmol) in DCM (5 mL) was added sat. aq $NaHCO_3$ (0.8 mL). The reaction was vigorously stirred at rt and monitored by TLC until complete consumption of the starting material. The reaction mixture was diluted with EtOAc and brine (10 mL) was added. The phases were separated and the aqueous phase was extracted with EtOAc (3x10 mL). The combined organics were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (70:30 hexanes:EtOAc) to afford the carbamate as a colorless oil (0.163 g, 80%). Major diastereomer: $[\alpha]_D^{25}$=+7.9° (*c*=1.07, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.37-0.45 (2H, m), 0.51 (2H, q, *J* = 8.0 Hz), 0.71-0.90 (13H, m), 2.64 (1H, t, *J* = 6.0 Hz), 2.80 (1H, q, *J* = 6.0 Hz), 2.91 (1H, dd, *J* = 14.0, 10.0 Hz), 3.21 (1H, dd, *J* = 14.0, 3.0 Hz), 3.41 (3H, s), 3.58 (1H, dd, *J* = 10.5, 5.5 Hz), 3.66 (1H, dd, *J* = 10.5, 6.5 Hz), 3.76 (3H, s), 3.80 (3H, s), 3.89 (3H, s), 4.05 (1H, ddd, *J* = 9.0, 5.0, 3.0 Hz), 4.51 (1H, d, *J* = 11.5 Hz), 4.60 (1H, d, *J* = 11.5 Hz), 4.62-4.74 (2H, m), 5.17 (2H, s), 5.25 (1H, dd, *J* = 10.5, 1.5 Hz), 5.36 (1H, app dq, *J* = 17.0, 1.5 Hz), 5.98 (1H, m), 6.87 (2H, dt, *J* = 8.5, 2.0 Hz), 7.26 (2H, dt, *J* = 8.5, 2.0 Hz), 7.49 (1H, d, *J* = 1.5 Hz), 7.93 (1H, br s), 8.15 (1H, br s). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 3.8, 7.0, 7.2, 13.1, 17.5, 32.6, 41.4, 45.7, 52.6, 54.2, 55.7, 56.6, 66.3, 67.9, 70.0, 73.2, 94.9, 110.4, 114.2, 117.6, 118.4, 124.2, 129.9, 130.1, 130.2, 133.0, 139.0, 154.1, 155.7, 159.8, 164.0, 167.1. IR (NaCl neat) (cm^{-1}): 3358, 2952, 1732, 1589, 1436, 1249, 1214, 1083, 1018, 728. MS: m/z = 717 (100), 404 (28), 154 (100). HRMS (FAB+): m/z Calcd 717.3418 $(MH)^+$, Found 717.3436

Methyl (2*S*,3*R*)-2-(2-(2-((4,5-dimethoxy-2-nitrobenzyloxy)carbonyl)-4-(methoxycarbonyl)-6-(methoxymethoxy)phenyl)-1-(diethyl(isopropyl) silyloxy)ethyl)-3-((4-methoxybenzyloxy)-methyl)aziridine-1-carboxylate

The experiment was carried out by the general procedure for carbamate formation with the aniline derived from **16** (0.062 g, 0.098 mmol) and 6-nitroveratryl chloroformate (0.081 g, 0.294 mmol) in CH_2Cl_2 (0.7 mL) and sat. aq $NaHCO_3$ (0.3 mL). The crude product was purified by flash column chromatography (60:40 hexanes:EtOAc) to afford the carbamate (0.066 g, 78%) as a pale yellow oil. Major diastereomer: $[\alpha]_D^{20}$=-5.9° (*c*=1.65, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.41 (2H, m), 0.49 (2H, q, *J* = 7.5 Hz), 0.70-0.83 (13H, m), 2.63 (1H, t, *J* = 6.0 Hz), 2.80 (1H, q, *J* = 6.0 Hz), 2.92 (1H, dd, *J* = 13.5, 10.0 Hz), 3.21 (1H, dd, *J* = 14.0, 3.0 Hz), 3.40 (3H, s), 3.57 (1H, dd, *J* = 10.5, 5.5 Hz), 3.64 (1H, dd, *J* = 10.5, 6.5

Hz), 3.74 (3H, s), 3.78 (3H, s), 3.90 (3H, s), 3.95 (6H, br s), 4.01-4.10 (1H, m), 4.47 (1H, d, J = 11.5 Hz), 4.56 (1H, d, J = 11.5 Hz), 5.17 (2H, s), 5.60 (2H, s), 6.83 (2H, d, J = 8.5 Hz), 7.04 (1H, br s), 7.22 (2H, d, J = 8.5 Hz), 7.51 (1H, d, J = 1.0 Hz), 7.71 (1H, s), 8.03 (1H, br s), 8.07 (1H, br s). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 3.5, 6.7, 6.8, 12.8, 17.1, 17.2, 32.4, 41.0, 45.4, 52.3, 53.9, 55.3, 56.2, 56.4, 56.5, 62.8, 64.0, 67.5, 69.5, 72.8, 94.4, 108.1, 108.2, 110.3, 110.6, 110.9, 113.7, 127.5, 129.4, 129.6, 132.2, 138.1, 139.9, 148.1, 153.4, 155.2, 159.2, 163.4, 166.4. IR (NaCl neat) (cm^{-1}): 3362, 2953, 1727, 1520, 1277, 1068. MS: m/z = 872 (5), 196 (20), 121 (100). HRMS (FAB+): m/z Calcd 872.3637 (MH)+, Found 872.3650

Methyl (2*R*,3*S*)-2-((4-methoxybenzyloxy)methyl)-3-(2-(2-(allyloxycarbonyl)-3,5,6-trimethoxy-4-methylphenyl)-1-(diethyl(isopropyl)silyloxy) ethyl)aziridine-1-carboxylate

The experiment was carried out by the general procedure for carbamate formation with the aniline derived from **16** (0.25 g, 0.40 mmol) and allyl chloroformate (68 μL, 0.80 mmol) in CH_2Cl_2 (2 mL) and sat. aq $NaHCO_3$ (0.8 mL). The crude product was purified by flash column chromatography (70:30 hexanes:EtOAc) to afford the carbamate (0.21 g, 75%) as a colorless oil. Major diastereomer: $[\alpha]_D^{25}$=-3.1° (c=3.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.49 (4H, app sext, J = 8.0 Hz), 0.75-0.94 (13H, m), 2.15 (3H, s), 2.56 (1H, t, J = 6.0 Hz), 2.68 (1H, q, J = 6.0 Hz), 2.85 (1H, dd, J = 13.0, 9.0 Hz), 2.98 (1H, dd, J = 13.5, 4.5 Hz), 3.52-3.57 (2H, m), 3.65 (3H, s), 3.69 (3H, s), 3.72 (3H, s), 3.73 (3H, s), 3.77 (3H, s), 3.98 (1H, br s), 4.45 (1H, d, J = 11.5 Hz), 4.59 (1H, d, J = 11.5 Hz), 4.62-4.68 (2H, m), 5.18 (1H, d, J = 10.0 Hz), 5.31 (1H, d, J = 16.5 Hz), 5.93 (1H, m), 6.83 (2H, d, J = 7.0 Hz), 6.99 (1H, br s), 7.24 (2H, d, J = 6.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.4, 3.5, 6.7, 6.8, 12.7, 17.1, 32.9, 41.0, 53.7, 55.2, 59.9, 60.2, 65.8, 67.4, 70.0, 72.5, 113.7, 117.2, 124.3, 126.5, 129.4,129.9, 133.0, 147.6, 150.4, 150.9, 154.6, 159.2, 163.8. IR (NaCl neat) (cm^{-1}): 3330, 2939, 1724, 1613, 1513, 1465, 1228, 1090, 757, 729. MS: m/z = 703 (100), 464 (22), 452 (33), 415 (27), 318 (32), 294 (53), 253 (58), 221 (36). HRMS (FAB+): m/z Calcd 703.3626 $(MH)^+$, Found 703.3613

General procedure for the PMB deprotection

Methyl (2*S*,3*R*)-2-(diethyl(isopropyl)silyloxy)-2-(4-(methoxycarbonyl)-2-methoxymethoxy-6-(2-nitro phenylsulfonamido)phenyl)ethyl)-3-(hydroxymethyl)-aziridine-1-carboxylate (14a)

To a stirred solution of sulfonamide derived from **16** (4.29 g, 5.25 mmol) in 15:1 solution of DCM-H_2O (50 mL) was added DDQ (1.55 g, 6.83 mmol) in one portion. The reaction mixture immediately turned green and over the course of 1.5 h the mixture turned brown-orange. After 2 h, the reaction mixture was passed through a short pad of activated alumina using 10:1 DCM:MeOH as eluant. The filtrate was

concentrated under reduced pressure and the crude product was purified by flash column chromatography (40:60 hexanes:EtOAc) to afford the alcohol (**14a**) (3.55 g, 97%). Major diastereomer: $[\alpha]_D^{20}$=-15.1° (c=0.9, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 0.41 (2H, m), 0.50 (2H, q, J = 8.0 Hz), 0.66-0.91, (13H, m), 1.75 (1H, br s), 2.60 (1H, dd, J = 13.5, 10.5 Hz), 2.78 (1H, dd, J = 6.0, 5.5 Hz), 2.79 (1H, dd, J = 6.0, J = 5.5 Hz), 2.92 (1H, dd, J = 13.5, 3.5 Hz), 3.40 (3H, s), 3.60-3.68 (1H, m), 3.69 (3H, s), 3.84-3.94 (2H, m), 3.93 (3H, s), 5.13 (1H, d, J = 7.0 Hz), 5.18 (1H, d, J = 7.0 Hz), 7.57 (1H, dt, J = 7.5, 1.5 Hz), 7.62 (1H, d, J = 1.5 Hz), 7.71 (1H, dt, J = 8.0, 1.5 Hz), 7.77 (1H, dd, J = 8.0, 1.5 Hz), 7.94 (1H, d, J = 1.5 Hz), 7.95 (1H, dd, J = 8.0, 1.5 Hz), 8.80 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.4, 3.5, 6.5, 6.6, 12.7, 16.9, 31.3, 43.1, 46.3, 52.4, 53.8, 56.3, 60.2, 69.3, 94.5, 112.5, 122.1, 125.8, 127.9, 129.9, 130.7, 132.5, 133.5, 133.8, 137.0, 147.8, 155.2, 163.1, 166.3. IR (NaCl neat) (cm^{-1}): 3515, 3265, 2954, 1725, 1543, 1308, 1240, 1061, 1010, 732. MS: m/z = 698 (100), 512 (17), 154 (85) 136 (60), 129 (35). HRMS (FAB+): m/z Calcd 698.2415 $(MH)^+$, Found 698.2389

Methyl (2*S*,3*R*) 2-(2-(2-allyloxycarbonyl-4-methoxycarbonyl-6-(methoxymethoxy)phenyl)-1-(diethyl(isopropyl)silyloxy)ethyl)-3-(hydroxymethyl)-aziridine-1-carboxylate (14b)

The experiment was carried out by the general procedure for the PMB deprotection with the alloc carbamate (0.160 g, 0.223 mmol), DDQ (0.061 g, 0.268 mmol) in 15:1 CH_2Cl_2-H_2O (3 mL). The crude product was purified by flash chromatography (60:40 petroleum ether:EtOAc) to yield **14b** as a colorless oil (0.118 g, 89 %). Major diastereomer: $[\alpha]_D^{20}$=+3.1° (c=0.65, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 0.42 (2H, m), 0.52 (2H, q, J = 8.0 Hz), 0.73-0.89 (13H, m), 1.97 (1H, br s), 2.73 (1H, t, J = 6.0 Hz), 2.78 (1H, q, J = 6.0 Hz), 2.93 (1H, dd, J = 13.5, 10.0 Hz), 3.24 (1H, dd, J = 14.0, 3.0 Hz), 3.45 (3H, s), 3.77 (3H, s), 3.78-3.87 (2H, m), 3.89 (3H, s), 4.15 (1H, ddd, J = 9.0, 5.5, 3.0 Hz), 4.69 (2H, m), 5.20-5.29 (3H, m), 5.38 (1H, dt, J = 17.5, 1.5 Hz), 5.99 (1H, m), 7.50 (1H, s), 7.93 (1H, br s), 8.15 (1H, br s). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 3.4, 6.6, 6.7, 12.7, 17.0, 22.6, 29.7, 32.1, 43.0, 46.1, 52.2, 53.9, 56.3, 60.2, 65.9, 69.8, 94.4, 94.5, 110.0, 117.1, 118.0, 123.6, 129.7, 132.5, 138.5, 153.6, 155.2, 163.6, 166.7. IR (NaCl neat) (cm^{-1}): 3500, 3366, 2953, 1728, 1590, 1436, 1215, 1038, 1017. MS: m/z = 597 (100), 565 (30), 307 (49), 288 (32). HRMS (FAB+): m/z Calcd 597.2843 $(MH)^+$, Found 597.2832

Methyl (2*S*,3*R*)-2-(2-(2-((4,5-dimethoxy-2-nitrobenzyloxy)carbonyl)-4-(methoxycarbonyl)-6-(methoxymethoxy)phenyl)-1-(diethyl(isopropyl)-silyloxy)ethyl)-3-(hydroxymethyl)-aziridine-1-carboxylate (14c)

The experiment was carried out by the general procedure for the PMB deprotection with the NVOC carbamate (0.064 g, 0.073 mmol), DDQ (0.020 g, 0.088 mmol) in 15:1 CH_2Cl_2-H_2O (0.7 mL). The crude product was purified by flash chromatography (40:60 hexanes: EtOAc) to yield **14c** as a yellow oil (0.041 g, 75 %). Major diastereomer: $[\alpha]_D^{20}$=-14° (*c*=0.1, $CHCl_3$)

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.39 (2H, m), 0.50 (2H, q, *J* = 8.0 Hz), 0.72-0.85 (13H, m), 2.17 (1H, br s), 2.68 (1H, t, *J* = 6.0 Hz), 2.77 (1H, q, *J* = 6.0 Hz), 2.96 (1H, br t, *J* = 11.5 Hz), 3.22 (1H, dd, *J* = 14.0, 3.0 Hz), 3.45 (3H, s), 3.72 (3H, s), 3.82-3.90 (3H, m), 3.88 (3H, s), 3.93-4.00 (3H, m), 3.96 (3H, s), 4.21 (1H, dt, *J* = 10.5, 5.0 Hz), 5,22 (1H, d, *J* = 6.5 Hz), 5.24 (1H, d, *J* = 6.5 Hz), 5.61 (2H, s), 7.07 (1H, br s), 7.52 (1H, d, *J* = 1.0 Hz), 7.72 (1H, s), 8.09 (1H, br s). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 3.3, 3.4, 6.5, 6.7, 12.7, 16.9, 32.1, 42.6, 45.8, 52.2, 53.9, 56.3, 56.4, 60.2, 64.0, 69.5, 94.5, 108.2, 110.2, 111.1, 117.2, 123.7, 127.3, 129.7, 138.2, 140.0, 148.3, 153.5, 155.3, 163.5, 166.5 IR (NaCl neat) (cm^{-1}): 3512, 3361, 2953, 1727, 1587, 1522, 1326, 1278, 1214, 1067, 756. MS: m/z = 752 (12), 196 (100), 154 (29), 136 (24). HRMS (FAB+): m/z Calcd 752.3062 $(MH)^+$, Found 752.3053

Methyl (2*S*,3*R*)-2-(1-(diethyl(isopropyl)silyloxy)-2-(2,3,5-trimethoxy-4-methyl-6-(2-nitrophenyl sulfonamido)phenyl)ethyl)-3-(hydroxymethyl)-aziridine-1-carboxylate (15a)

The experiment was carried out by the general procedure for the PMB deprotection with the Ns sulfonamide (0.052 g, 0.065 mmol), DDQ (0.02 g, 0.084 mmol) in 15:1 CH_2Cl_2-H_2O (0.8 mL). The crude product was purified by flash column chromatography (50:50 hexanes:EtOAc) to afford the alcohol **15a** (0.039 g, 87%) as a colorless oil. Major diastereomer: $[\alpha]_D^{25}$=+24.2° (*c*=1.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.45-0.63 (4H, m), 0.81-0.95 (13H, m), 2.07 (3H, s), 2.69 (2H, m), 2.91 (1H, dd, *J* = 13.0, 8.5 Hz), 3.08 (1H, dd, *J* = 13.5, 5.5 Hz), 3.32 (3H, s), 3.65 (3H, s), 3.74 (3H, s), 3.78 (3H, s), 3.83-3.89 (1H, m), 4.00 (1H, m), 7.64 (1H, td, *J* = 7.5, 1.5 Hz), 7.68 (1H, td, *J* = 7.5, 1.5 Hz), 7.79, (1H, s), 7.90 (1H, dd, *J* = 9.0, 1.5 Hz), 7.98 (1H, dd, *J* = 9.0, 1.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.6, 3.7, 6.8, 9.5, 13.0, 17.1, 33.0, 42.4, 45.8, 53.7, 59.4, 59.8, 60.3, 70.3, 124.4, 124.6, 124.9, 128.5, 131.1, 132.4, 132.8, 135.4, 147.5, 147.7, 151.3, 151.8, 163.5. IR (NaCl neat) (cm^{-1}): 3319, 3099, 2940, 2877, 1727, 1694, 1514, 1464, 1382, 1296, 1079, 1016, 733, 653. MS: m/z = 684 (20), 498 (100), 454 (25), 380 (39), 352 (46), 210 (26). HRMS (FAB+): m/z Calcd 684.2622 $(MH)^+$, Found 684.2615

(2*S*,3*R*)-Methyl 2-(2-(2-allyloxycarbonyl-3,5,6-trimethoxy-4-methylphenyl)-1-(diethyl(isopropyl)silyl oxy)ethyl)-3-(hydroxymethyl)-aziridine-1-carboxylate (15b)

The experiment was carried out by the general procedure for PMB deprotection with PMB ether (0.19 g, 0.26 mmol) and DDQ (0.80 g, 0.34 mmol) in 15:1 CH_2Cl_2-H_2O (3 mL). The crude product was purified by flash column chromatography (50:50 hexanes:EtOAc) to afford the alcohol (**15b**) (0.13 g, 87%) as a colorless oil. Major diastereomer: $[\alpha]_D^{25}$=-13.6° (*c*=1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.50 (2H, q, *J* = 7.5 Hz), 0.56 (2H, q, *J* = 7.5 Hz), 0.81-0.98 (13H, m) 1.97 (1H, br s), 2.15 (3H, s), 2.61-2.67 (2H, m), 2.91 (2H, dd, *J* = 13.5, 8.0 Hz), 3.00 (1H, dd, *J* = 13.5, 5.5 Hz), 3.65 (3H, s), 3.67 (3H, s), 3.74 (3H, s), 3.78 (3H, s), 3.80 (1H, d, *J* = 2.0 Hz), 4.08-4.13 (1H, m), 4.63 (2H, d, *J* = 2.0 Hz), 5.20 (1H, d, *J* = 10.0 Hz), 5.34 (1H, d, *J* = 17.0 Hz), 5.95 (1H, m), 6.91 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.8, 3.9, 4.1, 6.9, 7.0, 9.7, 13.1, 13.3, 17.4, 33.2, 43.1, 46.0, 54.0, 60.1, 60.5, 63.4, 66.1, 70.5, 94.6, 108.8, 117.7, 124.7, 126.6, 133.1, 147.9 150.8, 151.2, 163.9. IR (NaCl neat) (cm^{-1}): 3346, 2942, 2877, 1728, 1602, 1504, 1464, 1230, 1090, 1045, 728. MS: m/z = 583 (43), 464 (41), 379 (36), 362 (70), 253 (75), 236 (100), 194 (48), 147 (75), 129 (78). HRMS (FAB+): m/z Calcd 583.3050, Found 583.3034

General procedure for the Mitsunobu cyclization

Method A (DEAD/Ph_3P):

Methyl (3*S*,4*R*)-5-(diethyl(isopropyl)silyloxy)-7-methoxymethoxy-1-(2-nitrophenylsulfonyl)-(3,4)-[*N*-(methoxycarbonyl)aziridino]-1,2,3,4,5,6-hexahydro benzo[*b*]azocine-9-carboxylate (12a)

To a stirred solution of **14a** (0.10 g, 0.143 mmol) and triphenylphosphine (0.068 g, 0.258 mmol) in dry toluene (7 mL) was added DEAD (94 μL of a 40% solution in toluene, 0.215 mmol). The mixture was stirred at rt and monitored by TLC until complete consumption of the starting material (30 min). The reaction mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (70:30 hexanes:EtOAc) to afford the benzazocine (**12a**) (0.092 g, 95%) as a white foam. Major diastereomer: $[\alpha]_D^{20}$=-9.1° (*c*=1.44, $CHCl_3$) Note: ^{1}H NMR and ^{13}C NMR are reported as a mixture of rotamers at both room temperature and 323K. ^{1}H NMR (300 MHz, DMSO, 393K): δ = 0.52 (2H, q, *J* = 8.0 Hz), 0.68 (2H, q *J* = 8.0 Hz), 0.92-1.04 (13H, m), 2.58 (1H, dd, *J* = 6.5, 3.5 Hz), 2.65 (1H, dd, *J* = 6.5, 3.5 Hz), 2.62-2.69 (1H, m), 2.90-3.03 (1H, m), 3.465 ((2/3)3H, s), 3.47 ((1/3)3H, s), 3.64 ((2/3)3H, s), 3.67 ((1/3)3H, s), 3.79 ((1/4)3H, s), 3.80 ((3/4)3H, s), 4.00-4.27 (3H, m), 4.46 (1H, app p, *J* = 4.5 Hz), 5.28 (1H, d, *J* = 6.5 Hz), 5.31 (1H, d, *J* = 6.5 Hz), 7.10 ((1/3)H, d, *J* = 1.5 Hz), 7.14 ((2/3)H, d, *J* = 1.5 Hz), 7.63 ((1/3)H, d, *J* = 1.5 Hz), 7.66 ((2/3)H, d, *J* = 1.5 Hz), 7.74-7.94 (4H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.5, 6.9, 12.7, 17.1, 30.6, 40.3, 46.3, 51.8, 52.2, 53.6, 56.4, 94.6, 114.7, 124.0, 124.4, 131.2, 132.4, 132.5, 133.8, 139.2, 147.9, 155.8, 163.7, 165.6. IR (NaCl neat) (cm^{-1}): 2954, 2876, 1727, 1545,

1437, 1372, 1297, 1243, 1077, 730. MS: m/z = 680 (100), 636 (41), 494 (23), 348 (42), 154 (59), 136 (57). HRMS (FAB+): m/z Calcd 680.2309 $(MH)^+$, Found 680.2310

Methyl (3*S*,4*R*)-1-((4,5-dimethoxy-2-nitrobenzyloxy) carbonyl)-5-(diethyl(isopropyl)silyloxy)-7-methoxy methoxy-(3,4)-[*N*-(methoxycarbonyl)aziridino]-1,2,3,4,5,6-hexahydrobenzo[1,2-b] azocine-9-carboxylate (12c)

The experiment was carried out by the general procedure for the Mitsunobu cyclization (method A) with **14c** (0.021 g, 0.028 mmol), DEAD (7 μL, 0.042 mmol) and Ph_3P (0.013 g, 0.05 mmol) in dry toluene (0.5 mL). The crude product was purified by PTLC (30:70 petroleum ether:EtOAc) to afford the benzazocine (**12c**) (0.017 g, 83%) as a pale yellow oil. Major diastereomer: $[\alpha]_D^{20}$=+45.2° (*c*=1.3, $CHCl_3$). ^1H NMR (300 MHz, DMSO, 393 K): δ = 0.65-0.75 (4H, m), 0.96-1.02 (13H, m), 2.67 (1H, br s), 2.86 (4H, br s), 2.98 (1H, br s), 3.47 (3H, s), 3.62 (3H, s), 3.80 (3H, s), 3.86 (3H, s), 3.87 (3H, s), 3.92 (1H, t, *J* = 4.0 Hz), 4.35 (1H, br s), 5.28 (1H, d, *J* = 7.0 Hz), 5.32 (1H, d, *J* = 6.5 Hz), 5.41 (2H, s), 6.93 (1H, br s), 7.46 (1H, d, *J* = 2.0 Hz), 7.63 (1H, d, *J* = 1.5 Hz), 7.65 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) (note: at room temperature, this compound exists as an equilibrating mixture of conformational isomers of the alloc urethane on the NMR time scale): δ = 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) (cm^{-1}): 2951, 1725, 1581, 1522, 1438. MS: m/z = 734 (15), 196 (100), 154 (22). HRMS (FAB+): m/z Calcd 734.2956 $(MH)^+$, Found 734.2941

Methyl (3*R*,4*S*)-5-(diethyl(isopropyl)silyloxy)-7,8,10-trimethoxy-9-methyl-1-(2-nitrophenylsulfonyl)-[*N*-(methoxycarbonyl)aziridino]-1,2,3,4,5,6-hexahydrobenzo[*b*]azocine (13a)

The experiment was carried out by the general procedure for the Mitsunobu cyclization (method A) with alcohol (**15a**) (0.04 g, 0.057 mmol), DEAD (14 μL, 0.086 mmol) and triphenylphosphine (0.027 g, 0.10 mmol) in dry toluene (2.5 mL). The crude product was purified by flash column chromatography (80:20 hexanes:EtOAc) to afford the benzazocine **13a** (0.026 g, 70%) as a colorless oil. Major diastereomer: $[\alpha]_D^{25}$= +4.8° (*c*=1.0, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 0.65 (4H, m), 0.79-0.85 (2H, m), 0.92-0.97 (17H, m), 2.09 (3H, s), 2.35-2.40 (2H, m), 2.70 (1H, dd, *J* = 13.5, 9.5 Hz), 2.97 (1H, dd, *J* = 13.5, 4.5 Hz), 3.29 (1H, s), 3.64 (3H, s), 3.68 (3H, s), 3.81 (6H, s), 3.88 (1H, d, *J* = 2.5 Hz), 3.91 (1H, s), 4.06-4.16 (2H, m), 4.49 (2H, dd, *J* = 14.5, 5.0 Hz), 7.50-7.55 (2H, m), 7.58-7.64 (2H, m), 7.89 (1H, dd, *J* = 9.0, 1.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.4, 3.5, 7.0, 10.2, 12.7, 14.4, 17.2, 31.4, 40.0, 45.9,

50.4, 53.5, 60.1, 60.5, 60.8, 69.4, 123.5, 124.4, 127.0, 128.3, 130.9, 131.0, 132.1, 133.2, 133.3, 147.0, 148.1, 152.7, 153.2, 153.9. IR (NaCl neat) (cm^{-1}): 3450, 3314, 2926, 1731, 1545, 1463, 1372, 1260, 942, 731. MS: m/z = 666 (100), 622 (50), 480 (84), 436 (45), 334 (70) 234 (37), 221 (42). HRMS (FAB+): m/z Calcd 666.2516 $(MH)^+$, Found 666.2498

Allyl (3*R*,4*S*)-5-(diethyl(isopropyl)silyloxy)-7,8,10-trimethoxy-9-methyl-[*N*-(methoxycarbonyl) aziridino] -3,4,5,6-tetrahydrobenzo[*b*]azocine-1(2*H*)-carboxylate (13b)

The experiment was carried out by the general procedure for the Mitsunobu cyclization (method A) with alcohol (**15b**) (0.180 g, 0.31 mmol), TMAD (0.081 g, 0.56 mmol) and tributylphosphine (140 μL, 0.56 mmol) in dry toluene (12 mL). The solution immediately turned deep purple and slowly changed to a light brown color as the reaction progressed. The crude product was purified by flash column chromatography (80:20 hexanes:EtOAc) to afford the benzazocine (**13b**) (0.142 g, 81%) as a colorless oil. Major diastereomer: $[\alpha]_D^{25}$=+28.1° (*c*=1.2, $CHCl_3$) **Note:** ^1H NMR and ^{13}C NMR spectra are reported as a 1.4:1 mixture of rotamers at room temperature. At 323K onset of coalescence was observed. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.70-0.91 (m), 1.04-1.10 (m), 1.26 (d, *J* = 3.0 Hz), 2.18 (s), 2.17 (s), 2.42 (m), 2.52, (dd, *J* = 8.5, 2.0 Hz), 2.57, (dd, *J* = 9.0, 2.0 Hz), 2.73 (dd, *J* = 9.5, 3.0 Hz), 2.89 (d, *J* = 1.5 Hz), 2.91 (d, *J* = 3.0 Hz), 3.28, (dd, *J* = 7.5, 1.5 Hz), 3.32 (dd, *J* = 7.0, 1.5 Hz), 3.60 (d, *J* = 1.5 Hz), 3.66 (s), 3.69 (s), 3.73 (d, *J* = 1.5 Hz), 3.83 (s), 3.84 (s), 3.85 (s), 4.10 (m), 4.54 (dq, *J* = 9.5, 1.5 Hz), 4.64 (dt, *J* = 8.0, 1.5 Hz), 4.68 (dt, *J* = 9.0, 1.5 Hz), 4.76 (dd, *J* = 19.0, 3.5 Hz), 4.86 (dd, *J* = 19.0, 3.5 Hz), 5.04 (t, *J* = 1.5 Hz), 5.08 (dq, *J* = 11.0, 1.5 Hz), 5.13 (q, *J* = 5.0, 1.5 Hz), 5.23 (dq, *J* = 14.5, 1.5 Hz), 5.39 (m), 5.81 (m), 6.01 (m). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.7, 3.9, 7.3, 9.7, 9.8, 13.0, 17.5, 29.5, 29.7, 40.7, 40.9, 47.5, 47.6, 47.9, 53.7, 53.8, 60.3, 60.4, 60.6, 60.8, 61.0, 66.3, 66.7, 71.8, 71.9, 117.2, 117.5, 122.9, 124.8 125.1, 129.7, 130.6, 132.2, 133.0, 133.3, 147.4, 151.8, 155.2, 164.6, 164.9, 168.3, 168.5, 168.7. IR (NaCl neat) (cm^{-1}): 2951, 2876, 1730, 1710, 1467, 1245, 1094, 721. MS: m/z = 565 (100), 521 (48), 421 (26), 334 (17), 221 (20), 136 (10). HRMS (FAB+): m/z Calcd 565.2945 $(MH)^+$, Found 565.2935

Method B ($TMAD/PBu_3$):

Methyl (3*S*,4*R*)-1-(allylloxycarbonyl)-5-(diethyl (isopropyl)silyloxy)-7-methoxymethoxy-(3,4)-[*N*-(methoxycarbonyl)aziridino]-1,2,3,4,5,6-hexahydro benzo[1,2-*b*]azocine-9-carboxylate (12b)

To a solution of **14b** (0.036 g, 0.06 mmol) and tributylphosphine (26 μL, 0.102 mmol) in dry toluene (1.5 mL) was added TMAD (0.016 g, 0.09 mmol). The solution immediately turned yellow and slowly changed to a white cloudy color as the reaction progressed. The mixture was stirred under argon at rt and monitored by TLC until complete consumption of the starting material (6 h). The reaction mixture was

concentrated under reduced pressure and the residue was purified by flash column chromatography (70:30 hexanes:EtOAc) to afford the benzazocine (**12b**) (0.028 g, 82%) as a colorless oil. Major diastereomer: $[\alpha]_D^{25}$=+26.1° (*c*= 0.67, $CHCl_3$). ^{1}H NMR (300 MHz, DMSO, 393 K): δ = 0.66-0.75 (4H, m), 0.95-1.04 (13H, m), 2.63-2.71 (2H, m), 2.82-3.07 (4H, m), 3.48 (3H, s), 3.66 (3H, s), 3.75-3.86 (1H, m), 3.87 (3H, s), 4.06-4.20 (1H, br s), 4.33-4.42 (1H, m), 4.56 ((3/4)H, d, *J* = 5.0 Hz), 4.62 ((1/4)H, d, *J* = 5.0 Hz), 5.13-5.23 (1H, m), 5.27 (1H, d, *J* = 6.5 Hz), 5.31 (1H, d, *J* = 6.5 Hz), 5.89 (1H, m), 7.41 (1H, d, *J* = 1.5 Hz), 7.62 (1H, d, *J* = 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) (note: at room temperature, this compound exists as an equilibrating mixture of conformational isomers of the alloc urethane on the NMR time scale): δ = 3.4, 3.5, 7.0, 12.7, 17.2, 29.2, 29.4, 38.8, 40.4, 45.9, 47.2, 48.4, 49.7, 52.2, 52.3, 53.5, 53.6, 56.3, 66.2, 66.7, 70.2, 94.4, 94.8, 113.0, 113.5, 113.8, 116.9, 117.2, 117.7, 122.6, 123.0, 130.3, 130.8, 131.5, 131.8, 132.4, 132.6, 142.1, 142.5, 154.5, 154.9, 155.0, 155.5, 155.8, 156.0, 164.3, 166.0. IR (NaCl neat) (cm^{-1}): 2953, 1727, 1583, 1437, 1240, 1105, 730. MS: m/z = 579 (84), 307 (29), 154 (100), 137 (69). HRMS (FAB+): m/z Calcd 579.2738 $(MH)^+$, Found 579.2737

General procedure for the nosyl deprotection:

Methyl (3*S*,4*R*)-5-(diethyl(isopropyl)silyloxy)-7-methoxymethoxy-(3,4)-[*N*-(methoxycarbonyl) aziridino]-1,2,3,4,5,6-hexahydrobenzo[*b*]azocine-9-carboxylate (7)

To a solution of **12a** (0.62 g, 0.91 mmol) in dry MeCN (15 mL) were successively added thiophenol (106 μL, 1.03 mmol) and cesium carbonate (0.875 g, 2.68 mmol). The reaction mixture immediately turned yellow and after 1 h the solution was partitioned between EtOAc and 10% citric acid. The organic phase was washed with brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (70:30 hexanes:EtOAc) to afford the benzazocine (**7**) as a pale yellow oil (0.397 g, 88%). Major diastereomer: $[\alpha]_D^{20}$=+60.6° (*c*=1.2, $CHCl_3$). ^{1}H NMR (400 MHz, C_6D_6): δ = 0.74-0.86 (4H, m), 1.08-1.19 (13H, m), 2.10 (1H, m), 2.20 (1H, t, *J* = 6.0 Hz), 2.94 (1H, dt, *J* = 13.5, 10.5 Hz), 3.15 (3H, s), 3.18-3.25 (1H, m), 3.35 (1H, dd, *J* = 13.5, 6.0 Hz), 3.36 (3H, s), 3.40-3.48 (1H, m), 3.52 (3H, s), 4.66 (1H, dt, *J* = 12.5, 6.0 Hz), 4.87 (1H, d, *J* = 6.5 Hz), 4.90 (1H, d, *J* = 6.5 Hz), 7.05 (1H, d, *J* = 1.5 Hz), 7.61 (1H, d, *J* = 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 3.4, 3.5, 7.0, 12.9, 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) cm^{-1}: 3380, 2953, 1724, 1587, 1437. MS: m/z = 495 (100), 451 (40), 393 (46), 349 (59), 248 (42), 204 (75), 136 (76). HRMS (FAB+): m/z Calcd 495.2527 $(MH)^+$, Found 495.2524

General procedure for the alloc deprotection

To a solution of **12a** (0.159 g, 0.159 mmol) in dry THF (10 mL) were successively added acetic acid (46 μL, 0.795 mmol), triphenylphosphine (0.042 g, 0.159 mmol) and tetrakis (triphenylphosphine)palladium (0.046 g, 40 μmol). The reaction mixture was stirred at rt for 20 min and was diluted with Et_2O (15 mL). The organic phase was washed with $NaHCO_3$ (10 mL) then brine (10 mL). The combined organics were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (70:30 hexanes:EtOAc) to afford the benzazocine (**7**) (0.063 g, 81%) as a pale yellow oil.

(3*R*,4*S*)-5-(Diethyl(isopropyl)silyloxy)-7,8,10-trimethoxy-9-methyl-(3,4)-[*N*-(methoxycarbonyl) aziridino]-1,2,3,4,5,6-hexahydrobenzo[*b*]azocine (11)

The experiment was carried out by the general procedure for the alloc deprotection with benzazocine (**13b**) (0.025 g, 0.035 mmol), acetic acid (6 μL, 0.14 mmol) and tetrakis(triphenylphosphine)palladium (0.02 g, 1.8 μmol) in dry THF (0.3 mL). The crude product was purified by column chromatography (70:30 hexanes:EtOAc) to afford the benzazocine (**11**) (0.013 g, 72%) as a white solid. Major diastereomer: $[\alpha]_D^{25}$=+38.1° (*c*=0.21, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 0.72-0.79 (4H, m), 1.02-1.09 (13H, m), 2.17 (3H, s), 2.51-2.57 (2H, m), 2.82 (1H, dd, *J* = 13.5, 10.0 Hz), 3.13 (1H, dd, *J* = 14.0, 6.0 Hz), 3.44-3.64 (1H, m), 3.67 (3H, s), 3.68 (3H, s), 3.76 (3H, s), 3.83 (3H, s), 3.79-3.86 (1H, m), 4.51 (1H, dt, *J* = 10.5, 5.5 Hz), 4.60 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 3.5, 3.6, 7.1, 9.5, 13.0, 17.3, 31.8, 41.5, 42.9, 46.5, 53.3, 59.8, 60.2, 60.5, 69.0, 115.5, 122.9, 136.7, 142.8, 143.5, 148.0, 163.8. IR (NaCl neat) (cm^{-1}): 3390, 2952, 2875, 1730, 1490, 1464, 1293, 1229, 1090, 730. MS: m/z = 480 (100), 394 (13), 307 (30), 289 (14), 154 (95), 136 (72). HRMS (FAB+): m/z Calcd 480.2656 $(MH)^+$, Found 480.2649. Mp = 131-133°C

Procedure for the alcohol epimerization

To a stirred solution of the alcohol (**21**) (3.0 g, 5.8 mmol) in dry DCM (200 mL) was added DMP (2.97 g, 7.0 mmol) in one portion. The reaction was stirred at rt and monitored by TLC until complete consumption of the starting material (2 h). The reaction was diluted with Et_2O (200 mL) and a solution of aqueous $Na_2S_2O_3$ was added. The reaction mixture was vigorously stirred until the solution becomes clear. The phases were separated and the aqueous phase was extracted with EtOAc (3x10 mL). The combined organics were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (70:30 hexanes:EtOAc) to afford the ketone as a pale yellow oil (2.9 g, 97%). $[\alpha]_D^{25}$=-44.6° (*c*=1.3, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ = 2.24 (3H, s), 3.05 (1H, dd, *J* = 12.5, 5.5 Hz), 3.40 (1H, d, *J* = 7.0 Hz), 3.53 (1H, dd, *J* = 11.0, 5.5 Hz), 3.63 (1H, dd, *J* =

11.0, 5.5 Hz), 3.77 (3H, s), 3.78 (3H, s), 3.81 (3H, s), 3.83 (3H, s), 3.84 (3H, s), 3.92 (2H, d, J = 8.5 Hz), 4.50 (1H, d, J = 11.5 Hz), 4.53 (1H, d, J = 8.5 Hz), 6.87 (2H, d, J = 8.5 Hz), 7.28 (2H, d, J = 8.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.8, 39.0, 43.1, 44.1, 54.1, 55.3, 60.2, 60.6, 62.6, 66.3, 72.9, 113.9, 119.3, 127.2, 129.7, 129.8, 142.3, 146.8, 153.8, 159.4, 162.0, 198.9. MS: m/z = 519 (100), 503 (90), 460 (15), 307 (35), 154 (85), 137 (70). HRMS (FAB+): m/z Calcd 519.1979 $(MH)^+$, Found 519.1964

A stirred solution of the ketone (2.0 g, 3.9 mmol) in DCM:MeOH 2:1 (300 mL) was cooled to 0°C with an ice bath for 15 min. $NaBH_3CN$ (0.73 g, 11.6 mmol) and acetic acid (0.66 mL, 11.6 mmol) were then added sequentially. The reaction mixture was stirred at 5°C and monitored by TLC (50:50 hexanes:EtOAc) until complete consumption of the starting material. The reaction was quenched with sat. aq $NaHCO_3$ (100 mL). The phases were separated and the aqueous phase was extracted with DCM (3x100 mL). The combined organics were washed with brine (100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography (70:30 hexanes:EtOAc) to afford the alcohol (**22**) as a pale yellow oil (1.97 g, 98%).

ACKNOWLEDGEMENTS

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Appendix 2: Research Proposal

Specific Aims: *The aim of this proposal is the development of polyamide antagonists of the heat shock transcription factor-1 (HSF-1) that down-regulate production of the alpha isoform of heat shock protein 90 kDa (Hsp90α).* Under cellular stress, HSF-1 traffics to the nucleus, binds to DNA heat shock elements (HSE), and activates transcription of the Hsp90α gene. Hsp90 is a member of the chaperonin family of proteins responsible for folding and stabilization of associated client proteins.[1-2] In addition to the traditional role as a chaperone protein, Hsp90 has also been shown to act in various signal transduction cascades.[3] Many of the client proteins shown to interact with Hsp90 are known to be oncogenic, such as Polo-1[4], Bcr-Abl[5], Akt[6], B-Raf[7], Cdk4[8], Met[9], hTERT[10] and others.[11] Several transcription factors vital to the growth and progression of cancer are also associated with Hsp90 such as mutant p53[12], HIF-1α[13], androgen receptor[14], and the estrogen receptor[15]. **Due to the myriad number of proteins and processes in which it is associated, Hsp90 has been termed the "master regulator" of cancer.**[16] Over the past 10 years inhibitors of Hsp90 have been developed that have shown therapeutic promise.[17-18] Targeting Hsp90 represents a paradigm shift in drug development where an upstream regulator of *multiple processes* inherent to cancer is targeted as opposed to the traditional *one drug one target approach.* In addition, HSF-1 has been effectively targeted using siRNA technology and also holds therapeutic promise.[19] In light of these encouraging findings, we aim to down-regulate gene expression of Hsp90 by disruption of the HSF-1/DNA interface with a hairpin polyamide. Hairpin pyrrole/imidazole polyamides are a class of small molecules that are both cell permeable and programmable to bind a broad repertoire of DNA sequences with affinities and specificities comparable to transcription factors. Polyamides modulate gene expression through interruption of the transcription factor/DNA interface. *In contrast with the use of siRNA[20] or zinc fingers[21] to modulate gene expression, polyamides are cell permeable and enter living cells without the need for the lipophilic transfection agents required for these methods.*

The Specific Aims for this Proposal are the Following:

1. Synthesize, by solid phase methodology, polyamides targeting the HSF-1 binding site 5'-TCCGGA-3' sequence in the HSE promoter region of Hsp90α.
2. Evaluate the binding affinities of the polyamides for the HSE promoter region sequence mentioned above using quantitative DNase I footprinting titrations.
3. Using commercially available HSF-1 protein, evaluate the ability of the designed polyamides to antagonize HSF-1 binding to a ^{32}P-labelled DNA-HSE sequence by electrophoretic mobility shift assay (EMSA).
4. Investigate the cellular uptake and nuclear localization capability of the polyamides mentioned above in a variety of cell lines using confocal microscopy.
5. Evaluate the ability of designed polyamides to inhibit the production of Hsp90 mRNA as well as the mRNA of other heat shock proteins by use of quantitative

6. real-time PCR (qRT-PCR). Compare the results of the polyamide with siRNA results targeting HSF-1.
7. Demonstrate occupancy of HSF-1 in the HSE promoter region of the Hsp90 gene using chromatin immunoprecipitation (ChIP) assay.
8. Assay for necrosis and apoptosis on a variety of both normal and cancerous cell lines under varying concentration of polyamides.
9. Analyze global effects on Hsp90 gene expression using Affymetrix high-density Human Genome U133 Plus 2.0 microarrays. Compare this with the gene expression profile by inhibition of Hsp90 using geldanamycin.

Background and Significance: Heat shock proteins (HSPs) were first discovered in connection with the discovery of the heat shock response, and are members of the chaperonin family of proteins.[22] Members of the HSP family (named according to their size in kDa) include: Hsp27, Hsp40, Hsp60, Hsp70, Hsp90 and others. HSPs, induced by heat or other stressful situations where protein denaturation might occur, have many roles in the cell regarding the fate and function of proteins.[23] Regulatory roles of HSPs include: 1.) Protein folding 2.) Protein transport 3.) Protein repair/degradation 4.) Re-folding of misfolded proteins and 5.) Involvement in signal transduction pathways.[24] HSP induction occurs in response to cellular stress by activation of heat shock transcription factor-1 (HSF-1).[1] In its latency period, HSF-1 exists as a monomer bound to Hsp90 in the cytosol. Upon activation HSF-1 is released from Hsp90, forms a trimer with itself, travels to the nucleus, binds to heat shock elements (HSE) in the promoter and activates transcription of the Hsp90α gene. The three HSE-DNA sequences that are bound and recognized by the HSF-1 trimeric complex are 5'-TTCTTCCGGAA-3', 5'-AGTTCGGGAGG-3' and 5'-CTTCTGGAAAA-3'.[25] X-ray crystallography revealed HSF-1 to have a 'winged' helix-turn-helix motif, the crystal structure of which is shown in `Figure 1.[26] Downstream from the HSE are also a

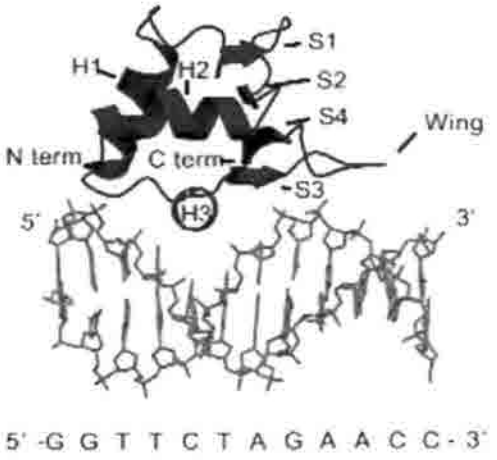

Figure 1. Crystal Structure (1.75 Å) of the *Kluyveromyces lactis* HSF-1 bound to the HSE. (adapted from reference 25)

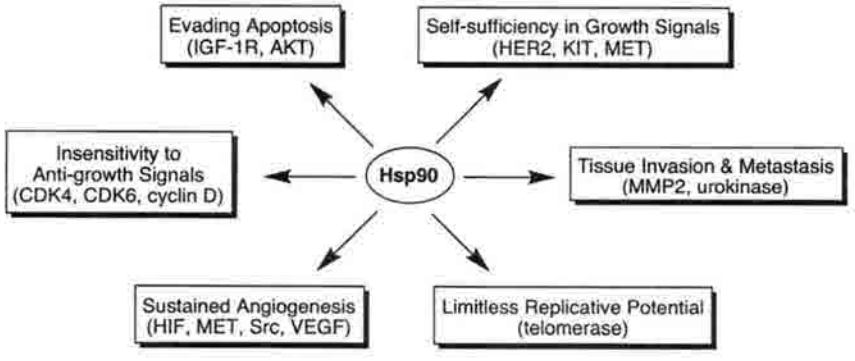

Figure 2. The Six Hallmarks of Cancer, with client proteins of Hsp90 related to each hallmark shown in parantheses.[29]

GAGA box and TATA box promoter sequence that must be activated in order for formation of the mediator complex and transcription to occur.[27]

Hsp90, a member of the HSP family of proteins, has been shown to be of particular interest in the development and progression of cancer. There are two major cytoplasmic isoforms of Hsp90 named Hsp90α and Hsp90β. Hsp90α is the inducible/major form whereas Hsp90β is the constitutive form/minor form.[28] Hsp90α and Hsp90β are ubiquitously expressed in normal cells (1-2% of total cellular protein content) with significantly higher concentrations in cancer cells (4-6%). Hsp90 has been shown to help stabilize cancer cells in several ways including: 1.) Acting as a biochemical buffer to allow the cell to still function under the high stress environment inherent to cancer. 2.) Stabilizing mutant oncoproteins, thus allowing them to retain or regain function. 3.) Effecting gene expression and adding to tumor heterogeneity.[28] In 2000 Hanahan and Weinberg published a landmark paper in which they reviewed and defined six characteristics of cancer termed "The Hallmarks of Cancer".[30] The numerous client proteins stabilized by Hsp90 (shown in parentheses in Figure 2) are involved in every hallmark of cancer mentioned by the authors. Due to its broad spectrum of activity, it is no wonder why Hsp90 has been termed the "master regulator" of cancer and has been a hot target in the search for new cancer therapies.

Hsp90α in particular, has been shown to be an important target in cancer therapy. It has been shown that overexpression of Hsp90α is associated with a poor prognosis of many types of cancer.[31] Most recently Jay and coworkers showed that extracellular Hsp90α is required for tumor invasiveness in two different cancer cell lines. It was found that Hsp90α interacts with metalloproteinase 2 (MMP2) and was proposed to activate MMP2, thus increasing digestion of the extracellular matrix enhancing cancer metastasis.[32]

In light of the benefits of inhibiting Hsp90, it is not surprising that much work has been done in this field since the discovery of Hsp90 inhibitors roughly 10 years ago.[333] Two natural products, geldanamycin (GDA) and radicicol (RDC), have been found to be inhibitors of Hsp90.[34] These molecules, as well as synthetic inhibitors that have been developed, act as non-reversible competitive inhibitors by binding in the N-terminal ATP binding pocket of Hsp90 with high affinity.[35-36] GDA has also been useful in the study and elucidation of the various client proteins associated with Hsp90. Hsp90 inhibitors are important in cancer research and might also be applicable to other diseases linked to over-expression of Hsp90.[37-38] **One drawback of all current small molecule inhibitors of Hsp90 in development (due to the highly conserved nature of the nucleotide binding pocket in both Hsp90α and Hsp90β) is the inability of these small molecules to be isoform selective inhibitors for either Hsp90α or Hsp90β.**

Polyamides are a class of programmable small molecules developed in the Dervan lab that can be designed to bind sequence-specific regions of DNA with affinities rivaling that of native DNA binding-proteins. This class of molecules comprised of *N*-methylimidazole (Im), *N*-methylpyrrole (Py), and 3-hydroxy-*N*-methylpyrrole (Hp) bind to the minor groove of DNA (Figure 3b) and localize unaided to the nuclei of living cells. Inspired by the natural products distamycin A and netropsin, sequence specificity of polyamides is determined by the side-by-side pairing of Im, Py, and Hp as follows: Im/Py recognizes G·C over C·G, Py/Im recognizes C·G over G·C, Hp/Py recognizes T·A over A·T, and Py/Hp recognizes A·T over T·A (Figure 3a).[39] Due to these pairing rules, many

sequence-specific polyamides have been synthesized and have been used in a variety of diverse applications including modulators of gene expression.[40]

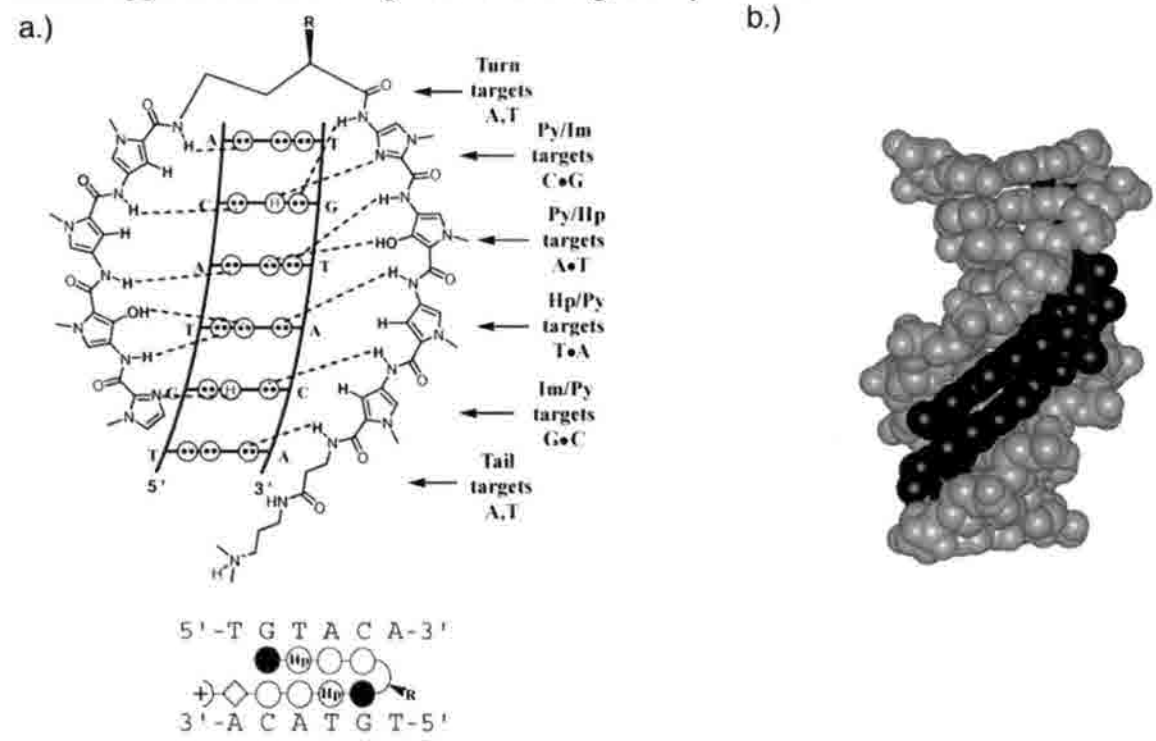

Figure 3: a.) General structure of a hairpin polyamide and pairing rules for sequence-specific recognition of polyamides with the minor groove of DNA. b.) Crystal structure of a polyamide bound to the minor groove of DNA (PBD 407D).

As mentioned above, research has shown that polyamides enter and localize to the nucleus of living cells. A pivotal study in this area was conducted by the Dervan group in which over 120 polyamide-fluorophore conjugates were synthesized and their cellular uptake profiles were assayed across 13 mammalian cell lines using confocal microscopy.[41] This study showed that elements conducive for polyamide uptake in cells include the following: an eight-ring hairpin polyamide binding domain, one or more positive charges on the turn residue of the linker, and a conjugated fluorescein fluorophore (FITC) on the tail of the polyamide. A recent study has shown the ability of polyamides with a non-fluorescent tail, isophthalic acid (IPA), to be taken up by cells and displayed comparable binding affinity and activity as the polyamide-fluorophore conjugates.[42] Negative elements for cellular uptake included the β-alanine residue in the tail portion and lack of a cationic alkyl amine moiety. The end result of this study was a dataset of 1500 entries that established guidelines for nuclear uptake of polyamides over a range of different cell lines.[42]

The long-term goal of the Research in the Dervan group is towards the development of polyamides as clinically used therapeutic agents. The goal of the proposed research (an incremental step towards realization of the long-term goal) is to develop polyamides that are capable of disrupting the interaction of HSF-1 with the HSE promoter region (by targeting the 5'-TCCGGA-3' sequence), and thus down-regulate Hsp90α gene expression (Figure 5). Realization of this goal and the ability to modulate gene

expression could have many applications relevant to both the biological and the medical community. Targeting Hsp90 with a polyamide could be a useful method in helping to shed more light on the function of Hsp90 as well as its associated client proteins. **Polyamide-mediated modulation of gene expression by selectively targeting the transcription factor/DNA interface of Hsp90α could be a novel therapeutic strategy in order to access an isoform specific inhibitor of Hsp90α**. This approach might also be applicable to a variety of different pathological conditions thus adding to the growing field of transcription therapy.[43] Additionally, inhibition of Hs90α by targeting a specific DNA sequence could be advantageous over a small molecule in that the DNA sequence should be less prone to mutation as opposed to the protein targeted by a small molecule. Modulating Hsp90 gene expression by antagonizing the HSF-1/DNA interface could be an interesting parallel approach to the siRNA gene knock-down strategies currently employed. **Polyamides have the advantage over traditional gene therapy approaches of being cell permeable without the need for lipophilic transfection agents.**

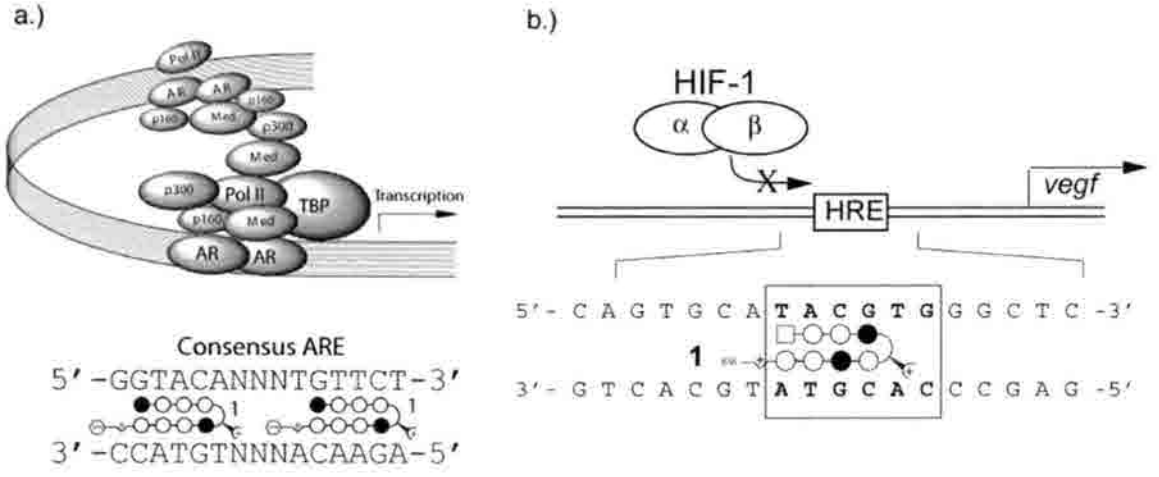

Figure 4: Previous systems on which polyamides have been successfully used to modulate gene expression. a.) Targeting the androgen receptor by a polyamide and down-regulation of PSA gene expression. b.) Polyamide binding to the HRE causing inhibition of HIF-1 binding and VEGF transcription. Abbreviations and symbols used: PSA = prostate-specific antigen, ARE = androgen response element, HRE = hypoxia response element, HIF-1 = hypoxia inducible factor 1, vegf = vascular endothelial growth factor, N = any nucleotide, open circles = Py, filled circles = Im, square = chlorothiophene, curved line = γ-aminobutyric acid, half diamond with plus sign = 3,3'-diamino-*N*-methyldipropylamine (adapted from ref. 44).

Preliminary Studies: The foundation for the proposed research is based on two landmark studies recently published from the Dervan group in which modulation of endogenous gene expression in cell culture was shown. In the first study (shown in Figure 4a), sequence specific hairpin polyamides were developed that bound to androgen response elements (ARE), the target of the androgen receptor (AR), and down-regulated androgen-induced gene expression of the prostate-specific antigen (PSA).[44] Polyamides that bound to the ARE sequence 5'-TGTTCT-3' were synthesized and exhibited excellent binding affinities and specificities as shown by quantitative DNase I footprinting. The

AR plays an essential role in the normal development of the prostate, and has been implicated in the progression of prostate cancer.[45] The designed polyamides were shown to reduce occupancy of AR at the PSA promoter and to inhibit mRNA production of PSA. Down-regulation of PSA was shown to be comparable with the known anti-androgen, bicalutamide. Global effects on androgen-induced gene expression was determined and then compared between the designed polyamides and bicalutamide.

The second study showed the utility of using polyamides to down-regulation gene expression of the pro-angiogenic factor vascular endothelial growth factor (VEGF) by disruption of the hypoxia inducible factor (HIF-1α)/DNA interface (Figure 4b).[46] HIF-1α is involved in many processes in the cell including tumor angiogenesis and metathesis as well as cell-death pathways. Polyamides targeting the sequence 5'-ATACGT-3', found in the VEGF hypoxia response element (HRE), were developed and found to possess both high affinity and specificity for the desired sequence. The ability of the designed polyamides to reduce promoter occupancy of HIF-1α and VEGF mRNA expression was shown as in the AR study above. Additionally, global effects on gene expression were compared between the designed polyamides, HIF-1α siRNA, and a DNA-binding natural product (echinomycin). It was found that the number of genes affected by the designed polyamide and HIF-1α siRNA was about the same.

Past experiences and protocols from the studies described above as well as current projects in the group will be drawn upon in order to help increase the probability for success of the proposed research. The proposed research is part of an expanded effort to see how generally applicable this principle of polyamide-mediated modulation of endogenous gene expression in cell culture is to a variety of different systems. Recent and ongoing research in the Dervan lab has shown the uniqueness of each system and has given great insight into the subtleties and nuances associated with modulation of gene expression in different systems.

Research previously conducted in the Dervan lab has shown the feasibility of targeting the DNA sequence 5'-WCWWGW-3' (where W = A/T), with the polyamides synthesized and their binding affinities shown in Figure 5. Historically, targeting DNA sequences with a terminal C (pyrrole) cap has proved difficult due to the low specificity between match and mismatched pairs. In 2001 it was found that substitution of β-alanine in place of Py on the hairpin polyamide to give **1** resulted in K_a values favoring the match over the mismatch sequence by roughly 3-5 fold (Figure 5).[47] More recent work in the group has targeted a similar sequence (5'-WCGWGW-3') and, using standard Py, Im, and β-alanine components, have obtained polyamides with both excellent binding affinity and specificity for the match over mismatch at the C-terminal end.[48] This important precedence provides a solid platform for development of polyamides that target 5'-TCCGGA-3' (5'-WCWGGW-3').

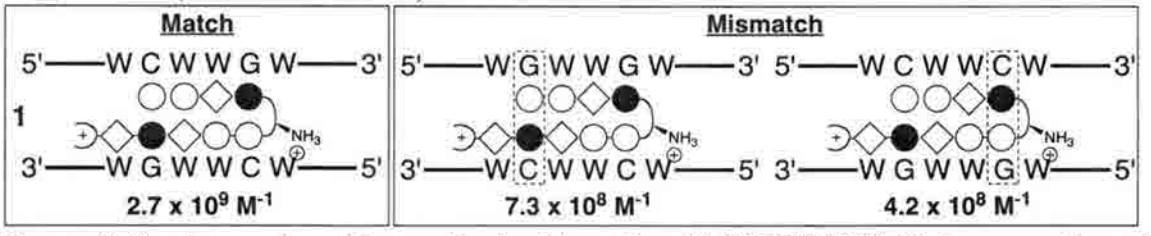

Figure 5: Previous polyamides synthesized targeting 5'-WCWWGW-3'. Incorporation of two β-alanine residues induces better selectivity (3-5 fold) between match and

mismatched polyamides. Ka values are shown in bold below the sequence and reflect numbers determined by quantitative DNase I footprinting. Abbreviations and symbols used: W = A/T, open circles = Py, filled circles = Im, curved line = γ-aminobutyric acid, diamond = β-alanine, half-circle with plus sign = ((dimethyl)amino)propylamine

Research Design and Methods:

Chronological Timetable of the Proposed Research: (Proposed length of research is two years)

*Specific Aims to be completed within the **first year**:*

1. The solid phase synthesis of polyamides.
2. Evaluation of binding affinities using quantitative DNase I footprinting.
3. Use of EMSA to probe ability of polyamides to antagonize HSF-1 binding to a DNA-HSE sequence.
4. Use of confocal microscopy to determine cell permeability and nuclear localization of polyamides.

*Specific Aims to be completed within the **second year**:*

5. Use of qRT-PCR to show polyamides decrease production of Hsp90 mRNA.
6. Chromatin immunoprecipitation (ChIP) assay to show HSF-1 occupancy of HSE promoter region.
7. Evaluate ability of polyamides to induce apoptosis in both cancerous and normal cell lines.
8. Determination of global gene expression using microarray technology.

Detailed Research Plans, Designs, and Methods:

1. Synthesize, by solid phase methodology, polyamides targeting the HSF-1 binding site 5'-TCCGGA-3' sequence in the HSE promoter region.

We intend to modulate Hsp90 gene expression through design of a polyamide capable of binding two of the three HSE's on the Hsp90β gene with high affinity. A selection of genes containing HSE's in the promoter region along with the specific HSE sequence is shown in Table 1. A number of genes other than those encoding HSP's have HSE's in their promoter regions. **However, it is essential to note that the 6 bp sequence to be targeted by our designed polyamide (i.e. 5'-TCCGGA-3') is found only in the HSE of Hsp90α.**

Gene	HSE Sequence
Human Hsp90α[49]	5'-TTCT**TCCGGA**A3', 5'-AGTCGGGAGG-3', and 5'-CTTCTGGAAAA
Human Hsp90β[50]	5'-GTTCTGGAAGATTCA-3', 5'-GTTCTGGAAGCTTCT-3', and 5'-CTTCCAGATCTTTCT-3'
Human Hsp70[51]	5'-CCCTGGAATATTCCC-3'
Human Hsp83[52]	5'-CTCTAGAAGTTTCTA-3'
Human Hsp68[52]	5'-ATCTCGAATTTTCCC-3'

Table 1: Genes containing the heat shock response element (HSE). The 6bp sequence to target is in bold.

We aim to target Hsp90α expression through design of a polyamide **2** that can bind the HSE sequence 5'-TCCGGA-3' with high affinity. As mentioned in Section C, polyamides targeting sequences similar to the 5'-WCWGGW-3' (where W = A/T) sequence inherent to the Hsp90 HSE promoter region have already been synthesized.[47-48] Synthesis of polyamide **2**, shown in Figure 6, should be relatively straightforward using the solid phase methodology already developed in the Dervan group.[53-54] The isophthalic acid (IPA) polyamide tail will be used in the synthesis of **2**, as it has proved beneficial in the previous gene regulation studies. Polyamides of type **2** bearing the FITC (fluorescein isothiocyanate) will also be made, as polyamides with the FITC tail have shown an increased ability to traffic into the nucleus. The polyamide tail might need to be re-designed depending on nuclear localization studies that will be performed in specific aim four. Other developments that surface during the time of the proposed research for the synthesis of polyamides with better binding affinities, specificities, and/or nuclear localization properties will be used and incorporated into the synthesis. Purification of polyamides will be performed using preparative reverse-phase high performance liquid chromatography (RP-HPLC). Polyamides will be characterized by analytical HPLC for purity and by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) for correct chemical composition.

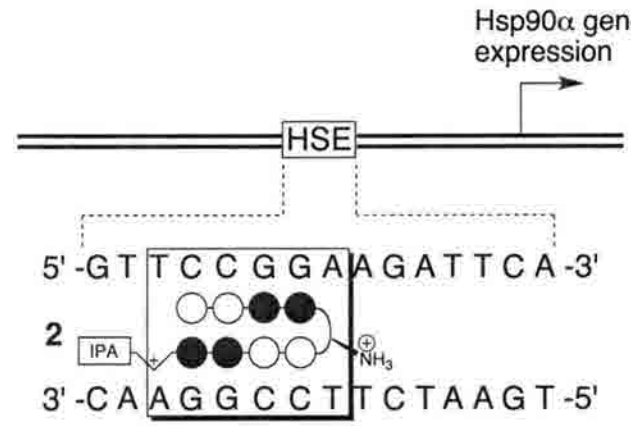

Figure 6: Polyamide **2** bound to the 5'-TCCGGA-3' sequence of the HSE. Abbreviations and symbols used: HSE = heat shock element, and IPA = isophthalic acid.

2. Evaluate the binding affinities of the polyamides for the HSE promoter region sequence mentioned above using quantitative DNase I footprinting titrations.

After completion of the synthesis of the desired polyamides detailed in specific aim 1, the binding affinities of the polyamides to the HSE sequence will be determined by quantitative DNase I footprinting. These affinities and specificities will be compared with the similar polyamides previously made in the group and are expected to be quite high (see Figure 7). The specificities on polyamides for the matched over the mismatched sequences will be compared. Footprinting will be carried out according to published procedures already developed in the Dervan lab.[55]

3. Using commercially available HSF-1 protein, evaluate the ability of the designed polyamides to antagonize HSF-1 binding to a ^{32}P-labelled DNA-HSE sequence by electrophoretic mobility shift assay (EMSA).

Once polyamides that bind to the HSE with high specificity have been made and identified, these polyamides will then be used to see if they can antagonize HSF-1 binding to the promoter sequence of the HSE using EMSA. Similar EMSA experiments have been done previously in the Dervan lab on HIF-1α and the androgen receptor and

the general procedures already outlined will be followed.[44, 46] Commercially available HSF-1 will be obtained and used for the experiment as well as a ^{32}P-labelled DNA fragment from the Hsp90α gene containing the HSE promoter sequence.[56]

4. Investigate the cellular uptake and nuclear localization capability of members of the polyamide library mentioned above in a variety of cell lines using confocal microscopy.

After completion of aims 1-3, the next task will be determination of the ability of these designed polyamides to traffic inside cells and localize in the nucleus.[57] Polyamides of type **3** or **4** bearing a 5'-C-terminal pyrrole linkage have not been investigated for their cellular uptake properties. Confocal microscopy will be used for this experiment following the established guidelines in the Dervan group.[39] Several cell lines, both cancerous and non-cancerous will be used in attempting to establish general trends for the cellular uptake of the selected polyamides. Cell lines to be used for this study include the following: HeLa, LnCAP, PC-3, U251, AU565, DU145, and NHDF. Since Hsp90 is ubiquitously expressed in the cell at high levels[58], most cell lines would be an appropriate choice for this experiment. Polyamides bearing the FITC tail will be examined first and, as needed, will be modified with the IPA residue so as to improve cellular uptake in a wide variety of cell lines.

5. Evaluate the ability of designed polyamides to inhibit the production of Hsp90α mRNA as well as the mRNA of other heat shock proteins by use of quantitative real-time PCR (qRT-PCR). Compare the results of the polyamide with siRNA results targeting HSF-1.

Quantitative real-time PCR (qRT-PCR) will be used following the procedures previously outlined in the androgen receptor[44] and HIF-1α projects[46], in order to determine if polyamides can suppress production of Hsp90α mRNA. This result will be compared with siRNA of HSF-1. Cell lines used for this experiment will be the same ones used in the nuclear localization studies. The following primers (s = sense, as = antisense), which have been previously employed and are known to work[59], will be used for this experiment:

Hsp70; (s) 5'-CAACACCCTTCCCACCGCCACTC-3', (as) 5'-TGCAGCCGCACAGGTTCGCTCT

Hsp90; (s) 5'-GGCGATTGAGGGAAGGTTG, (as) 5'-GGCTAAGTGACCGCACAGGA-3'

6. Demonstrate occupancy of HSF-1 to the HSE promoter region of the Hsp90α gene using chromatin immunoprecipitation (ChIP) assay.

As in previous studies in the Dervan group, a ChIP assay will be used to show occupancy of HSF-1 in the HSE promoter region according to already established protocols in the group[44, 46] as well as other groups[59]. The primers used for this experiment will be the same as for the qRT-PCR experiment (shown above). The experiment will be run both with and without polyamide so as to confirm that the polyamide is actually binding the HSE sequence and disrupting the HSE/HSF-1 binding interface.

7. Assay for necrosis and apoptosis on a variety of both normal and cancerous cell lines under varying concentration of polyamides.

Hsp90 inhibitors, like geldanamycin, have a unique mode of action in that they

selectively induce apoptosis in cancerous cells but not normal cells. Many speculations exist as to why this might be the case but has not been definitively proven. It is thought that enhanced tumor-cell recognition is due to specific modification of Hsp90.[60] *Polyamide-mediated inhibition of Hsp90 could help to answer this question of selective apoptosis in cancer cells over normal cells in the presence of geldanamycin.* Due to the different mechanism of inhibiting production and function of Hsp90, results from this experiment might be a big step forward in elucidating this origin of selectivity. Several cell lines, both normal and cancerous, will be incubated with polyamide and these results will be compared with incubation of these same cell lines with geldanamycin. Normal cell lines to be used for this test include: NHDF, Ke, CEM, and Sf9. Cancer cell lines to be used are: LnCap, PC-3, SKBR-3, DU145, and AU565. Depending on the outcome of these results, other cell lines will also be used. Various stains (trypan blue, Wright-Giesma, etc.) will be used to detect general cell death and the cells will be counted by flow cytometry. Propidium iodide stain will be used to determine cell viability. A commercially available ELISA assay will be used to screen and discern between apoptosis and necrosis.

8. Analyze global effects on Hsp90 gene expression using Affymetrix high-density Human Genome U133 Plus 2.0 microarrays. Compare this with the gene expression profile by inhibition of Hsp90 using geldanamycin.

Global effects on Hsp90 gene expression will be profiled by use of Affymetrix high-density Human Genome U133 Plus 2.0 microarray technology using manufacturer procedures and those already used in the Dervan group.[44, 46] In this experiment over 20,000 genes will be scanned for variability in Hsp90 gene expression after treatment with polyamide or geldanamycin. The results of gene expression will be compared between the two methods and the similarities/differences will be analyzed. This result should provide information on the toxicity effect of polyamides that bind to 5'-TCCGGA-3' sequences outside of the HSE promoter region. Additionally, this experiment will give us important information regarding specificity, and will tells us how many genes are being altered by the polyamide. *In lieu of the androgen and HIF-1α results we expect that binding of polyamides to sequences outside of the promoter region will not be toxic to cells.* This experiment might also help to reveal further information on the genes affected by this "master regulator" of cancer. New client proteins or other proteins associated with Hsp90 might be identified through this method and add to the growing list of Hsp90 interactors.[11]

Potential Difficulties and Limitations with this Proposal:

Invariably, as with any scientific undertaking, difficulties will be encountered. Listed below are some potential difficulties along with proposed solutions:

1. Hairpin polyamides lack sufficient specificity to successfully modulate Hsp90 gene expression.

This problem seems unlikely due to the previous studies in the Dervan group of successful modulation of gene expression using hairpin polyamides. In the case of both the androgen receptor[44] and HIF-1α[46] (mentioned in Preliminary Studies section above), global gene analysis confirmed that only a limited number of genes are affected. In the

HIF-1α work, it was shown that the number of genes affected by the hairpin polyamide was comparable to siRNA targeting HIF-1α (1626 genes for the polyamide versus 1523 genes for siRNA). **Despite targeting 6 base pairs, hairpin polyamides have shown excellent functional specificity in two previous cases.** It should also be mentioned that Nature's transcription factors target specific DNA sequences usually 4-8 base pairs in length.

2. The designed polyamides are not able to localize in the nucleus of the specific cell lines to be tested.

Many of the cell lines to be used in the proposed research have already shown good cellular uptake profiles with polyamides.[42] If this problem is encountered, new polyamides with different tails will be synthesized. The binding affinities, specificities, and nuclear localization capabilities will then be assessed. This process will be repeated as long as needed until polyamides are found that are taken up by a majority of the cell lines tested. If necessary, additional cell lines will be tested to accurately determine the cellular uptake profile for the designed polyamides.

3. Inhibition of Hsp90 gene expression is not selective for cancer cells.

This would still provide useful information as to the origin of selectivity of Hsp90 inhibitors to induce apoptosis in tumor cells.

4. Use of Polyamides to target gene expression is too toxic towards cells for any clinical utility.

Recent research using siRNA targeting HSF-1 and small molecule inhibitors have shown good success without being overly toxic to the cell.[19] These findings suggest that inhibition using polyamides to modulate gene expression should not be overly toxic to the cell. **It should be noted that in previous studies polyamides have not shown any cellular toxicity at micromolar concentrations.**

Closing Remarks:

In conclusion this proposal describes a novel method for modulation of the Hsp90 gene, known as the "master regulator" of cancer. *Related to* this, *it has been shown that almost every protein involved in oncogenic processes related to cell growth and development are regulated by Hsp90.*[61] Consequently inhibitors of Hsp90 have attracted considerable attention over the past ten years with several lead compounds now in clinical trials. **The approach shown above is novel from traditional small molecule inhibitors in that this would be the first example of a isoform selective inhibitor of Hsp90.** Alternate ways to modify Hsp90α gene expression (such as this proposed research) could have great utility as medicinal agents for cancer and various other diseases as well as an added tool to gain more information about HSPs and the pathways related to them. The scientific basis for this proposal is solidly grounded on previous work done in the Dervan group that should greatly increase the probability for a successful outcome to the proposed research. Polyamides will be synthesized that to bind the 5'-TCCGGA-3' DNA sequence on the HSE using solid-phase methodology and will be assessed for sequence specific binding affinity by DNase I footprinitng. Confocal microscopy will be used to determine cellular uptake of polyamides. The ability of the

designed polyamides to antagonize HSF-1 binding will be determined using EMSA. Determination of polyamides to displace HSF-1 from the HSE promoter region and HSF-1 promoter occupancy will be analyzed using ChIP. Levels of mRNA expression of genes affected by HSF-1 will be shown using qRT-PCR. Effects of polyamides on induction of apoptosis in cancer cells and microarray analysis to profile gene expression will then be performed. Upon successful completion of the above objectives, future directions include taking this same system into animal models, and further investigation into the therapeutic utility of using polyamides to target Hsp90α as a novel approach to the treatment of cancer.

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