DISSERTATION

SYNTHESIS OF A PHOTO-ACTIVATED ANALOG OF THE ANTITUMOR ANTIBIOTIC FR 900482

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SAMUEL BURKE ROLLINS ENTITLED SYNTHESIS OF A PHOTO-ACTIVATED ANALOG OF THE ANTITUMOR ANTIBIOTIC FR 900482 BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Adviser

Department Head

ABSTRACT OF DISSERTATION

SYNTHESIS OF FR 900482 ANALOGS

A novel synthetic route to the benzazocine framework of the antitumor antibiotic FR 900482 is presented. The synthesis of the benzazocine ring is characterized by a convergent asymmetric strategy. Although the benzazocine ring was not elaborated into the bicyclic skeleton of the natural product, synthesis of a hitherto unknown class of latent mitosenes was accomplished. Acylation of the benzazocine nitrogen with a photo cleavable protecting group allowed for the formation of the highly reactive mitosene under non-reductive conditions.

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Schemes

List of Abbreviations

Ac	acetyl
Ac_2O	acetic anhydride
ACCN	1,1'-azobis(cyclohexylcarbonitrile)
AIBN	2,2'-azobis(isobutyronitrile)
Alloc	allyloxycarbonyl
9-BBN	9-borabicyclo[3.3.1]nonane
Boc	*tert*-butoxycarbonyl
Bn	benzyl
Bu	butyl
Bz	benzoyl
CAN	ceric ammonium nitrate
Cbz	benzyloxycarbonyl
CSA	camphor sulfonic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene

DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL	diisobutylaluminum hydride
DIAD	diisopropyl azodicarboxylate
DMAD	dimethyl acetylenedicarboxylate
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
DMPI	Dess-Martin periodinane
DNBSA	dinitrobenzene sulphonic acid
DPPA	diphenylphosphoryl azide
Et	ethyl
Im	imidazole
KHMDS	potassium hexamethyldisilamide
Me	methyl
MOM	methoxymethyl
Ms	methanesulfonyl
MTPA	α-methoxy-α-trifluoromethylphenylacetic acid
NaHMDS	sodium hexamethyldisilamide
NBS	*N*-bromosuccinimide
NMO	*N*-methyl morphiline oxide
NVOC	6-nitroveratryloxycarbonyl
Ph	phenyl
PhFl	9-phenyl-9-fluorenyl
Piv	pivaloyl
PMB	*p*-methoxybenzyl
Pr	propyl
Py	pyridine

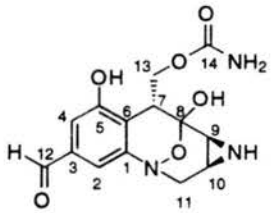
TBAF	tetrabutylammonium fluoride
TBS	*tert*-butyldimethyl silyl
TFA	trifluoroacetic acid
Tf	trifluoromethanesulfonyl
THF	tetrahydrofuran
TIPS	triisopropylsilyl
Troc	2,2,2-trichloroethoxycarbonyl
Ts	*p*-toluenesulfonyl
Xy	xylenes

Chapter 1

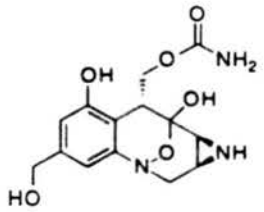
FR 900482 and Related Compounds

1.1 Introduction

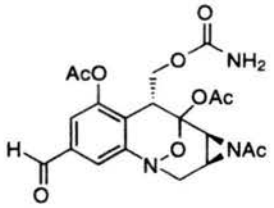
In 1987 the Fujisawa Pharmaceutical Co. in Japan isolated[1] and characterized[2,3] a new antitumor antibiotic, FR 900482 (**1**),[4,5] from the fermentation broth of *Streptomyces Sandaensis* No. 6897. Two years later, the dihydroderivative, FR 66979 (**2**), was isolated from the same streptomyces strain.[6] The semi-synthetic triacetyl derivative of FR 900482, FK 973 (**3**), possessed promising activity against various transplanted murine and human tumors.[7,8] These substances are structurally related to mitomycin C (MMC) (**4**) but lack the quinone moiety and contain a novel hydroxylamine hemiketal.



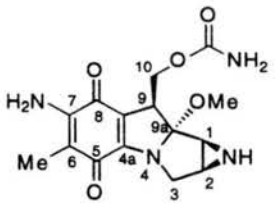
FR 900482 (**1**)



FR 66979 (**2**)



FK 973 (**3**)

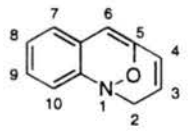


Mitomycin C (**4**)

Figure 1. FR-series of compounds and mitomycin C

All of these substances (**1-3**) behave similarly to MMC (**4**) in that they cross-link DNA.[9,10] Studies of the *in vitro* DNA-DNA interstrand cross-linking reaction of FR 66979 and FR 900482 have determined the *in vitro* site of cross-linking (5'-CpG) and sequence selectivity.[11-13] In addition, several studies have provided strong evidence[12,14-16] for the proposal of Fukuyama and Goto[17] that the FR 900482 series of compounds undergoes a two electron reduction cleaving the N-O bond and subsequently dehydrates to yield a mitosene like intermediate. The mitosene formed is responsible for the drug's DNA damaging activity by cross-linking double stranded DNA. As a result, the FR 900482 series of compounds are latent reductively activated mitosenes.

Endeavors aimed at reaching FR 900482, or substructures thereof, by *de novo* chemical synthesis could be justified solely on the basis of its activity. Additional impetus for synthesizing FR 900482 is provided by its unique structure: the 1,5-epoxybenzazocine ring system (Figure 2) was at the time of isolation unknown in the chemical literature. Several different approaches to the core nucleus of **1** have been



2*H*-1,5-epoxy-1-benzazocine

Figure 2. Chemical Abstracts parent structure.

published[17-28] and three groups have successfully completed the total synthesis.[29-33] Concurrent with the work of others, our laboratory was engaged in attempts to design and synthesize molecules that mimic or combine the cross-linking activity of the FR 900482. The synthetic efforts in our labs have been focused on constructing a natural product analog that is not reductively activated but photochemically, oxidatively, or hydrolytically activated to form a reactive mitosene. The following is a profile of the FR-

series of compounds, as well as a full account of the trial and errors associated with the implementation of our synthetic program.

1.2 Isolation

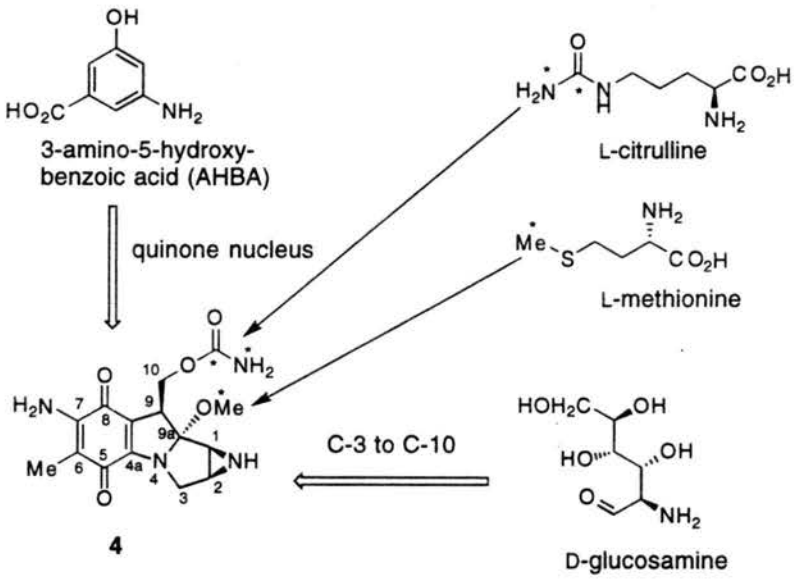
FR 900482 was discovered by the Exploratory Research Laboratories of the Fujisawa Pharmaceutical Co., Ltd., (Japan) as part of a routine screening for new antitumor substances. The strain of actinomycete that produces FR 900482 was isolated from a soil sample obtained from the Hyogo Prefecture in Sanda-shi, Japan.[1] The strain was enumerated No. 6897, and based on morphological characteristics and cell wall type it was included in the genus *Streptomyces*. Furthermore, from the results of comparative studies of strain No. 6897 with cultures of *Streptomyces aburaviensis* IFO 12830 and *Streptomyces nitrosporeus* IFO 12803, strain No. 6897 was considered a new species within the genus *Streptomyces*. In reference to the soil obtained at Sanda-shi from which the organism was isolated, the new strain was finally denoted as *Streptomyces sandaensis* No. 6897.

Production of FR 900482 by fermentation began by the inoculation of a seed medium containing soluble starch, glucose, cotton seed meal, dried yeast, corn steep liquor, and $CaCO_3$ with a loop full of mature slant culture of *Streptomyces sandaensis* No. 6897.[2] The seed culture was kept at 30 °C for 48 h and then inoculated into a production medium containing soluble starch, dried yeast, peanut powder, and soybean meal. The broth was fermented at 31 °C for 96 h. Filtration of the fermentation broth through Dianion and ion-exchange columns, solvent extraction of the filtrate, and column chromatography provided a crude powder containing the active material. Finally, HPLC gave pure FR 900482 as a colorless solid. In a typical large scale production, 1600 L of fermentation broth yielded 1 gr of FR 900482.

1.3 Biosynthesis

Only one study has been published to date concerning the precursors in the biosynthesis of FR 900482[34] while many studies of the biosynthetic precursors of the mitomycins have been published.[35-39] Outlined in Scheme 1, biosynthetic studies of the mitomycins have shown that the carbon skeleton arises biogenetically from two key intermediates: 3-amino-5-hydroxybenzoic acid (AHBA)[37,39] and D-glucosamine.[35,36] The AHBA provides the 4a-amino-6-methylbenzoquinone nucleus, and the C-1 and C-6 of D-glucosamine becomes the aziridine-substituted six-carbon chain C-3 to C-10. The various *O*- and *N*-methyl groups are introduced by transmethylation from methionine,[38] and the carbamate function is derived from L-citrulline.[36]

Scheme 1. Biosynthesis of mitomycins



Due to the structural similarities between the mitomycins and FR 900482, initial experiments in the biosynthesis of FR 900482 probed the role of AHBA and D-glucosamine.[34] When D-[uniformly labeled-^{14}C] or D-[1-^{14}C]-glucosamine and [7-^{14}C]-AHBA were added to culture broth of *Streptomyces Sandaensis* No. 6897, both

components were effectively incorporated into FR 900482 (Scheme 2). Further, fermentation medium supplementation with D-glucosamine or AHBA increased FR 900482 formation although the extent was not reported in the study. As discovered in earlier mitomycin studies, the feeding experiments with labeled D-glucosamines indicated that the entire hexose was incorporated as an intact unit. For example, when D-[1-^{13}C]-glucosamine was added to fermentation broths, the ^{13}C NMR spectrum of isolated FR 900482 exhibited large enhancements of signals corresponding to the C-11. This revealed that the C-1 of glucosamine was incorporated into the 11-position of FR 900482. The remaining carbon at C-14 and the terminal nitrogen of the urethane were unaccounted for by labeling studies.

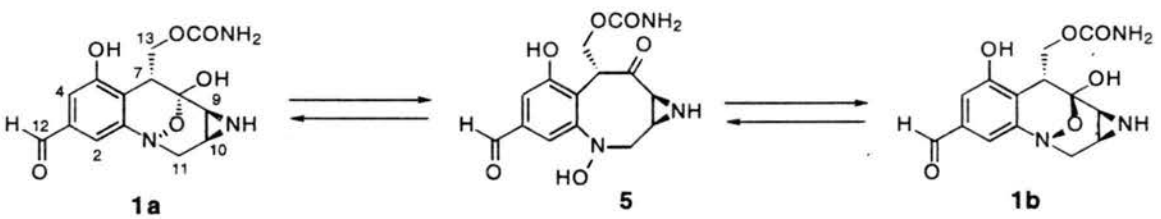
Scheme 2. Biosynthesis of FR 900482

1.4 Physical, Chemical, and Structural Characteristics

Researchers at the Fujisawa Pharmaceutical Co., Ltd. reported the following characteristics of FR 900482:[2,3] amphoteric white powder, soluble in water and methanol, insoluble in acetone, ethyl acetate, and chloroform, mp ~175 °C (decomposition), $[\alpha]_D^{23}$ +8 (*c*=1.0, H_2O), -26.5 (*c*=1.0, 0.1 N HCl). Elemental analysis calcd for $C_{14}H_{15}N_3O_6 \cdot H_2O$: C 49.56, H 5.05, N 12.38, found: C 49.73, H 4.83, N 12.52; UV λ max (MeOH) nm (ε): 236 (19,200), 281 (6,100), 330 (2,200). IR ν (KBr) 3600~3000, 1690, 1580, 1400, 1340, 1080 cm^{-1}.

The ^1H NMR spectra and TLC behavior revealed that FR 900482 exists as a mixture of two diastereomers, **1a** and **1b** (*ca.* 2:1 at neutral pH, See Scheme 3) which likely interconvert via the ring-open tautomer **5**. The TLC behavior is as follows: $CHCl_3$-MeOH (4:1) R_f 0.20 (major) and 0.45 (minor); *i*-PrOH-H_2O (9:1) R_f 0.55 (major) and 0.65 (minor). Color reactions: positive to sulfuric acid, potassium permanganate, 2,4-dinitrophenylhydrazine, iodine vapor, and negative to Sakaguchi reactions. ^1H NMR (D_2O) (400MHz) (δ TMS): **1a** 2.69 (dd, J = 6.5, 3.5 Hz, 10-H), 2.72 (d, J = 6.5 Hz, 9-H), 3.52 (dd, J = 6.0, 1.0 Hz, 7-H), 3.79 (d, J = 3.5 Hz, 11-H_2), 4.68 (dd, J = 11.0, 1.0 Hz, 13-H), 5.13 (dd, J = 11.0, 6.0 Hz, 13-H), 7.05 (d, J = 1.3 Hz, 2-H), 7.08 (d, J = 1.3 Hz, 4-H), 9.74 (s, 12-H). **1b** 2.51 (dd, J = 7.0, 2.0 Hz, 10-H), 2.89 (d, J = 7.0 Hz, 9-H), 3.42 (dd, J = 5.5, 2.0 Hz, 7-H), 3.63 (d, J = 14.5 Hz, 11-H), 3.86 (dd, J = 14.5, 2.0 Hz, 11-H), 4.45 (dd, J = 11.5, 2.0 Hz, 13-H), 4.66 (dd, J = 11.5, 5.5 Hz, 13-H), 6.96 (d, J = 1.3 Hz, 2-H), 7.12 (d, J = 1.3 Hz, 4-H), 9.75 (s, 12-H).

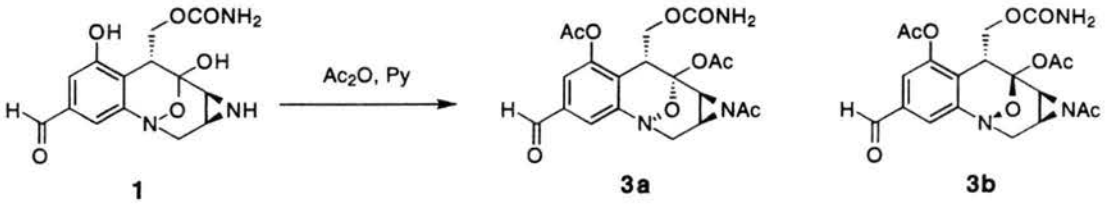
Scheme 3. Interconversion of FR 900482 diastereomers



The mixture of diastereomers and the relatively small number of protons, some of which are separated by a quaternary center, precluded full structural determination by NMR experiments. Acetylation of FR 900482 (Ac_2O, Py) gave a mixture of (*ca.* 10:1) of triacetates **3a** and **3b** (FK 973), which could be separated (Scheme 4). Standard NMR experiments conducted on the major isomer gave a partial structure with the position of one oxygen remaining uncertain as shown in Figure 3. Examination of the existing spectroscopic and chemical data did not conclusively distinguish between the possible

structures. Finally, X-ray crystallographic analysis of the major acetate provided the complete structure.

Scheme 4. Synthesis of FK 973



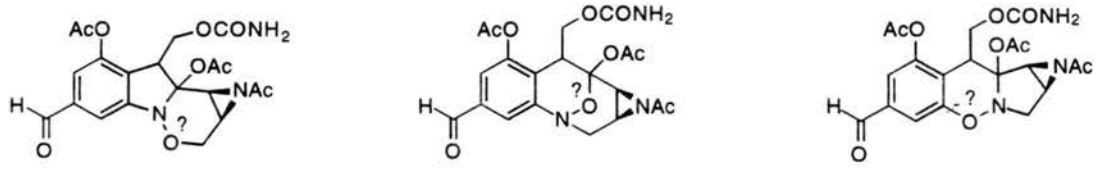


Figure 3. Possible positions of oxygen in FK 973 based on NMR data

The absolute configuration of FR 900482 has been demonstrated by Terashima and coworkers[31] in their total synthesis of FR 900482 (See Section 1.7.9). Using L-diethyl tartrate as their starting material, they demonstrated that the absolute configuration is as drawn in Scheme 3. In the mitomycin series it was eventually concluded that the absolute stereochemistry as determined by X-ray analysis of the 1-*N*-*p*-bromobenzoyl derivative was consistent with the biosynthetic incorporation of D-glucosamine unit with complete retention of the C-2 amino configuration.[40,41] The biosynthesis of FR 900482 exhibits the same fidelity.

1.5 Biological Activity

The unique pharmacological activity of FR 900482 was the reason for its isolation from the culture broths and structural determination. Most of the work related to FR 900482 was the result of its favorable biological activity. The natural product first demonstrated its activity in assays sensitive to antitumor agents, prompting further testing to determine its possible viability as a candidate for the treatment of neoplastic diseases. Drugs such as mitomycin C (MMC) (**4**) and adriamycin (ADR) are widely used in the treatment of various neoplastic diseases,[42] and they provide good sources for comparison with prototype drugs like FR 900482. While both MMC and ADR possess potent antitumor activity, one of the major side effects is myelosuppresion, the functional inhibition of the normal blood forming ability of the bone marrow, expressed as leukopenia (an abnormally low concentration of white blood cells) or thrombopenia (an abnormally low concentration of platelets in the blood). This side effect limits their clinical usefulness.

As a result, the criteria for a successful new drug candidate are more than potent activity, but minimal adverse side effects as well. Initial *in vitro* testing of FR 900482 against a variety of transplantable experimental tumors in mice showed antitumor activity greater than or equal to MMC (**4**).[4] More importantly, the hematotoxic and myelosuppressive effects of FR 900482 were weaker than those associated with MMC (**4**) in mice.[5] In search for compounds with still superior activity and toxicity profiles, derivatives of the parent FR 900482 were prepared by semi-synthesis or degradation. Of all the candidates tested, the semi-synthetic triacetate, FR 66973, was given the highest consideration because it retained low toxicity and showed improved activity. FR 66973 was then submitted for Phase I clinical trials bearing the new designation FK 973 (**3**).[7] Because the results of these trials are proprietary information belonging to the Fujisawa Pharmaceutical Co., information regarding the progression of the clinical trials is not available. There are, however, published reports indicating the spectrum of carcinoma to

which FK 973 might be applied. Described below are extracts from the general study initially conducted to evaluate FK 973.

As part of the selection process for clinical trial candidates, an expansive study of the antitumor activity of FK 973 was performed by the researchers at the Fujisawa Pharmaceutical Co.[7] The following murine and human carcinomas were tested: murine P388 leukemia, murine L1210 leukemia, murine B16 melanoma, murine Lewis lung carcinoma, murine colon 38 carcinoma, murine M5076 reticulum cell carcinoma, murine colon 26 adenocarcinoma, murine MH134 hepatoma, murine Ehrlich carcinoma, MMC-resistant P388 leukemia, cyclophosphamide (CPM)-resistant P388 leukemia, vincristine (VCR)-resistant P388 leukemia, ADR-resistant leukemia, human LX-1 lung carcinoma, human MX-1 mammary carcinoma, human SC-6-JCK stomach carcinoma, human CCRF-CEM leukemia, human PC10 lung carcinoma, and human MKN45 stomach carcinoma. FK 973 showed strong antitumor activity against the large majority of these carcinomas including the drug resistant P388 leukemia, P388/VCR, P388/MMC, P388/ADR, and P388/CPM. In addition, over a wide dosage range FK 973 had greater antitumor activity than MMC against murine ascitic tumors, P388 and L1210 leukemia, B16 melanoma, M5076 reticulum cell carcinoma, colon 26 carcinoma, Ehrlich carcinoma, and MH134 hepatoma (tumor of the liver).

Along with this promising range of activity, FK 973 exhibited relatively low virulence in mice. Since most antitumor chemotherapeutic drugs induce hematotoxicity, it is important with potential drug candidates to determine to what extent the blood will be intoxicated. In tests conducted with mice, although both MMC and FK 973 decreased the number of peripheral white blood cells uniformly, a disparity existed in the observed number of platelets: animals treated with FK 973 showed no decrease in their platelet count. The implication was that FK 973 might not cause the usual hematoxic side effects of thrombocytopenia (an abnormally low concentration of platelets in the blood).

Also monitored were the hematopoietic cells, the cells responsible for the production and development of blood cells. To this end, a number of colony forming units in the spleen and in culture of the bone marrow cells of mice were measured. The results would give an indication of the degree of myelosuppression to expect. In comparative tests with MMC, FK 973 was categorically less myelosuppressive.

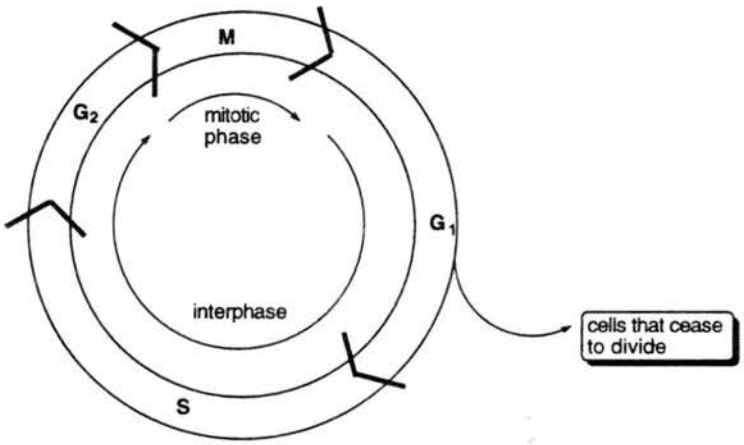
1.6 Mode of Action

In the preceding section the biological activity of FR 900482 (**1**) and FK 973 (**3**) were discussed in terms of their cytotoxicity and ability to inhibit cell growth. In this section the mode of action is defined as the manner or mechanism by which the drug exhibits its cytotoxicity and retards cellular proliferation. In the chemotherapeutic treatment of cancer where the objective is to discriminate between normal healthy cells and cancerous cells, an understanding of the mode of action of a drug can prove to be extremely important. Since cell death can be achieved by obstructing different phases of the cell life cycle, determination of when cell death occurs as a result of a drug treatment is essential in combined drug therapy wherein the objective is sometimes to interrupt the cell cycle at different phases and times. In clinical application and experimental tumor models, it is well known that an enhanced antitumor effect can be obtained by the combined use of various types of antitumor drugs which act on different phases of the cell cycle.[42] Thus, before discussing current understanding of FR 900482's mode of action, it is appropriate to briefly review the cell life cycle of a typical eukaryotic cell.

Cells reproduce by duplicating their contents and then dividing in two. The cell-division cycle is the fundamental means by which all living things are propagated. The duration of the cell cycle varies greatly from one cell to another, and the eukaryotic cell cycle is traditionally divided into four distinct and successive phases (Scheme 5).[43] The processes of nuclear division (mitosis) and cell fission (cytokinesis) which are together called M phase (M = mitotic) typically occupy only a small fraction of a cell cycle. In

most cells M phase takes only about an hour. The much longer period that elapses between one M phase and the next is known as interphase. During interphase the cell grows continuously. Replication of nuclear DNA usually occupies only a portion of interphase called the S phase of the cell cycle (S = synthesis). The interval between the completion of M phase and the beginning of S phase is called the G_1 phase (G = gap), and the interval between the S phase and the beginning of the M phase is called the G_2 phase. The G_1 and G_2 phases provide additional time for growth and mass doubling before a cell divides. Cells that do not divide, such as fully mature muscle or red blood cells, have no need to replicate their genetic material. These cells usually spend the remainder of their lives arrested in the G_1 phase.

Scheme 5. Phases of standard eukaryotic cell cycle



Because of its structural resemblance to the mitomycins, the mode of action of FK 973 was preconceived to be one characteristic of a bifunctional alkylating agent. As such, the effect of FK 973 on the cell life cycle of murine leukemia cell line L1210 was compared to several chemotherapeutic drugs known to produce DNA lesions that ultimately lead to cell death.[10] Parallel experiments were run comparing FK 973 to nitrogen mustards, MMC (**4**), *cis*-diaminedichloride platinum, adriamycin (ADR), and

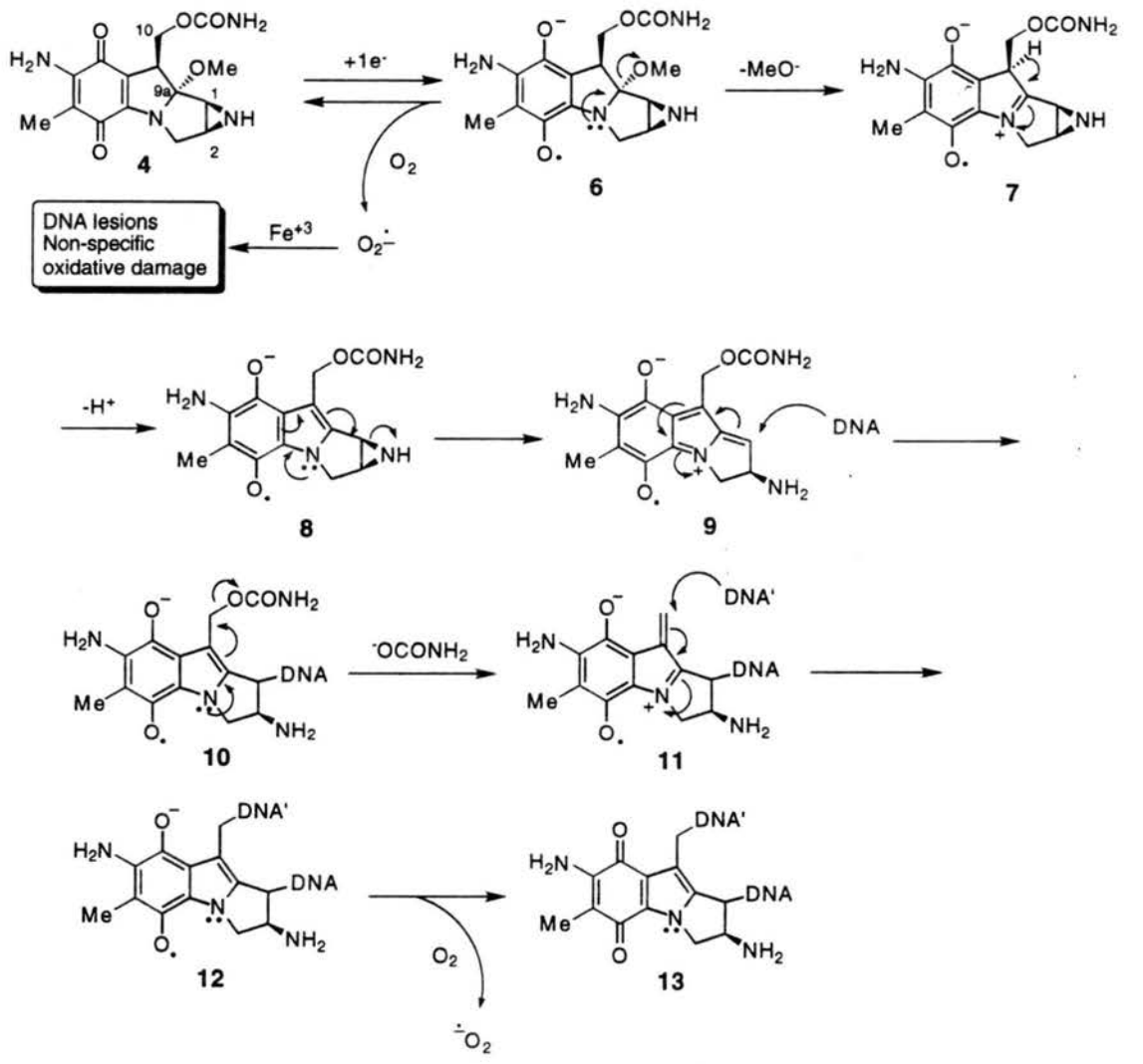
bleomycin. The nitrogen mustards and MMC were known interstrand DNA-DNA and DNA-protein cross-linking agents. *Cis*-diaminedichloride platinum was an intrastrand DNA cross-linking agent, and ADR and bleomycin were DNA cleaving agents. The effects of FK 973 most resembled those of MMC by forming DNA-DNA cross-links. Experimental data also showed FK 973 is gradually activated after it is incorporated into cells. Single strand cleavage of DNA by FK 973 was not detected. Further studies comparing FK 973 and MMC demonstrated FK 973 was threefold more potent than MMC in inhibiting L1210 cell growth. FK 973, like MMC, arrested cells in the G_2 phase[8] which is the expected point of interruption by a bifunctional alkylating agent.[44-48]

With these behavioral similarities between MMC and FK 973, it is appropriate to discuss the vast quantity of existing knowledge on the mode of action of MMC. It is well established that MMC is reductively activated to afford a species capable of facile DNA alkylation[44-48] as well as superoxide production[49] and subsequent oxidative DNA damage. When the molecule is in the quinone oxidation state, it is extraordinarily stable in the absence of exogenous reducing agents since the lone pair of the indole nitrogen is delocalized into the conjugated π-system of the quinone. Upon enzymatic or chemical reduction of the quinone either by a one or a two electron process, a cascade of spontaneous transformations ensues (Scheme 6). The lone pair of the indole nitrogen, no longer delocalized in the quinone system, extrudes the C-9a methoxide (**6**). After rearrangement to form the mitosene **8** and aziridine ring opening, the unstable vinylogous quinone methide **9** is produced revealing the first electrophilic site at C-1. When quinone methide **9** is alkylated by DNA at C-1, a second site of alkylation develops at C-10 by reverse Michael elimination of the carbamate. Alkylation of the C-10 by another DNA nucleophile and oxidation gives the cross-linked adduct **13**.

The fact that MMC is reductively activated is important in the selectivity of its antitumor activity. Many solid tumors are hypoxic (oxygen starved) compared to normal tissues. The oxidative inhibition is a manifestation of superoxide production by MMC[49]

resulting from the reduction of molecular oxygen by the semi-quinone radical anion intermediates (**6**, **8**, or **10**). Subsequent Haber-Weiss/Fenton cycling of superoxide produces hydroxyl radical and related reactive oxidants capable of causing indiscriminate tissue damage. Since activation of MMC is inhibited by an oxidizing environment, MMC has selective toxicity for hypoxic solid tumors and potentially suppresses their growth. The essential features of this scheme are supported by observations in many laboratories, but particularly concisely by the isolation and characterization of **13** from enzymatic digests of MMC treated DNA.[47]

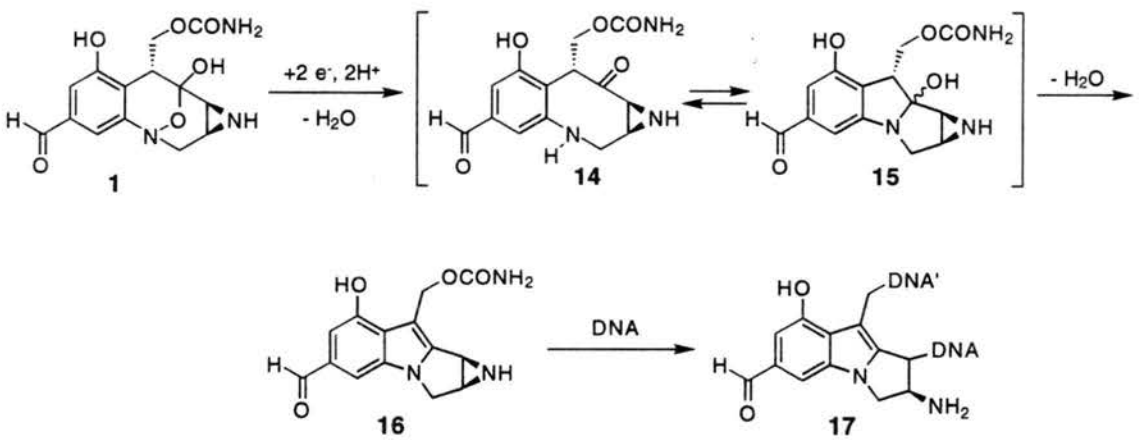
Scheme 6. Mitomycin C reductive activation cascade



Due to the structural and behavior similarities between FK 973 and MMC, initial mechanistic proposals pertaining to the mode of action of FK 973 relied on a mitomycin-like pathway. Particularly intriguing was the means by which activation to the DNA reactive species might occur. The aromatic portion of FK 973 is already at the proper oxidation state to undergo this cascade of reactions, but cascade initiation is precluded by the bridging oxygen. Therefore, to participate in a mechanism resembling the mitomycin model, some type of transformation of FK 973 would be required. As discovered in preliminary mechanistic work on FK 973, the drug did not cross-link DNA in isolated nuclei, but did cross-link DNA in whole cells (L1210).[10] These results were interpreted to indicate that the drug must first be chemically activated in the cytoplasm prior to forming a reactive species.

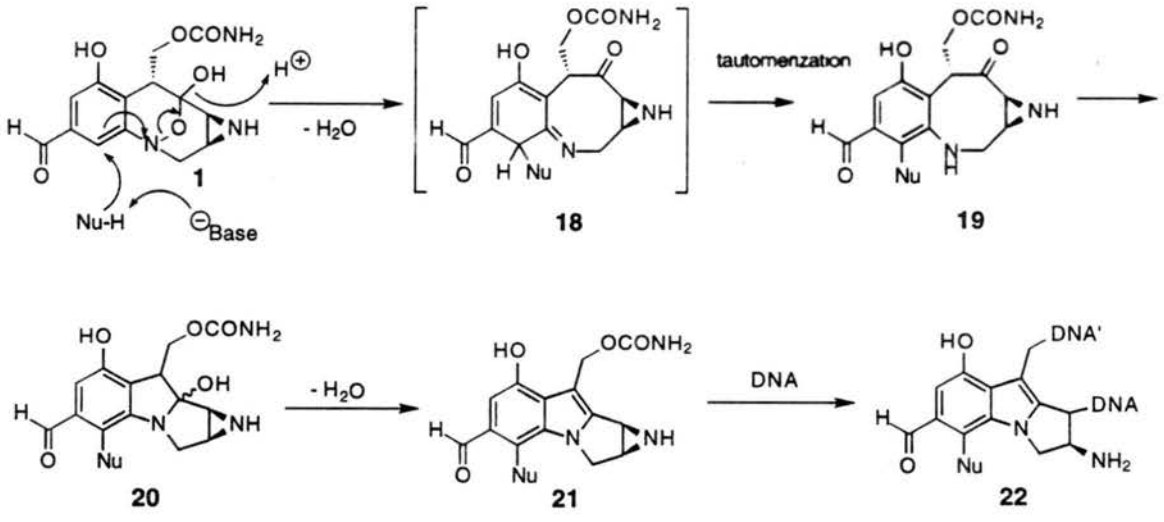
In 1989 Fukuyama and Goto were the first to propose that FR 900482 (and by analogy FK 973) experiences reductive activation *in vivo* (Scheme 7).[17] This proposal holds that reduction of the N-O bond generates aniline species **14** and initiates a reaction cascade which ultimately yields the mitosene-like nucleus **16** presumed to cross-link DNA (**17**).

Scheme 7. Fukuyama's proposed reductive activation of FR 900482

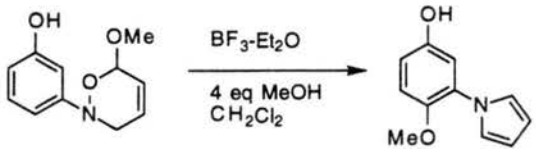


Alternatively, Danishefsky and McClure proposed that the activation of the FR 900482 class of compounds might follow a nucleophilically triggered motif.[27] Addition of some external nucleophile to the aromatic nucleus (presumably a proteinaceous thiol, amine, or hydroxyl group) at C-2 (FR 900482 numbering) is envisioned to induce heterolytic cleavage of the hydroxylamine hemiketal with concominant loss of water (**18**) (Scheme 8). Following re-aromatization by tautomerization (**19**), the expected ring closure would yield carbinolamine **20**. Dehydration of **20** would then afford the highly reactive aziridinomitosene **21**, capable of bis-alkylation at the activated C-1 and C-10 positions (mitomycin numbering). DNA alkylation by this species would yield a lesion similar to that invoked by the Fukuyama proposal with the notable exception of a substitution at the aryl C-2 position. To date, there is no evidence to support this solvolytic mechanism, only some preliminary experiments performed during initial model studies on the synthesis of the FR 900482 core skeleton which suggest the possibility of solvolytic activation (Scheme 9).[27]

Scheme 8. Danishefky's proposed nucleophilic activation of FR 900482



Scheme 9. Solvolytic activation of N-O bond

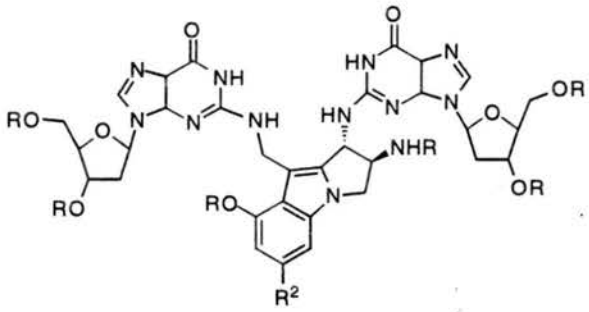


In 1992 two laboratories began to independently study *in vitro* DNA-DNA interstrand cross-linking reactions of FR 900482 (**1**) and FR 66979 (**2**). While many observations on the mode of action of FR 900482 were confirmed by both laboratories, several pieces of conflicting data on the mode of action of FR 66979 arose but were eventually reconciled. Each lab showed that FR 900482 and FR 66979 each shared the following features with MMC: (i) the drugs specifically cross-linked at 5'-d(CG) sites on duplex DNA; (ii) the cross-links involved a bridge between two proximate dG residues on complimentary strands, connected by a bond to the N2 amino group of the guanines; (iii) the efficiency of the cross-linking reaction was shown to be a function of the flanking sequences of the 5'-d(CG) sites with the relative efficiency in the order 5'-d(ACGT)>>5'-d(TCGA)≈5'-d(CCGG).[13,14]

Although both laboratories observed DNA-DNA interstrand cross-linking by FR 900482 only upon the addition of exogenous reducing agents such as 2-mercaptoethanol[12] or sodium dithionite,[14] divergent conclusions concerning the activation of FR 66979 were initially reached by Williams and Hopkins. Williams' laboratory found FR 66979 efficiently cross-linked double stranded DNA in the absence of exogenous reducing agents,[12] while Hopkins' laboratories found reductive activation to be essential for cross-linking activity.[11,14] Through collaborative efforts, the source of this discrepancy was traced to the different methods of synthesis of FR 66979 by the two groups.[15] Due to the low abundance of FR 66979 from fermentation, the drug was prepared by the reduction of FR 900482. Each laboratory selected different reductants (Williams used H_2/Pd-C, Hopkins used $NaBH_4$) and relied solely upon the one reaction

to produce FR 66979. The activity of FR 66979 in the absence of exogenous reducing agents seen by Williams was traced to the reactivity of some minor by-product of the hydrogenation of FR 900482 that was not removed upon purification. The structure of this highly reactive agent has not yet been established, but is most likely a reduced relative of FR 66979. It should be noted that it has recently been discovered that metal ions such as iron are a previously unrecognized, critical component for the *in vitro* activation of FR 900482 and FR 66979.[16]

The covalent connectivity of the DNA interstrand cross-link formed by FR 900482 and FR 66979 was unequivocally established as **23** and **24**, the nuclei of the DNA-DNA interstrand cross-link formed by reductively activated FR 900482 and FR 66979, respectively.[11,14] The connectivity was determined by the isolation and structural characterization of the peracetylated derivative **25** of **24**. Mass spectral analysis of **24** and **25** gave molecular ion peaks and fragmentation patterns consistent with the proposed structures. The UV data offered support for the putative changes in the



23 R = H, R^2 = CHO
24 R = H, R^2 = CH_2OH
25 R = $COCH_3$, R^2 = CH_2OCOCH_3

Figure 4. Isolated lesions from cross-linking reactions

aromatic chromophores from a substituted phenol to a substituted hydroxyindole. Particularly diagnostic in the UV spectrum of **23** was a long wavelength absorbance of

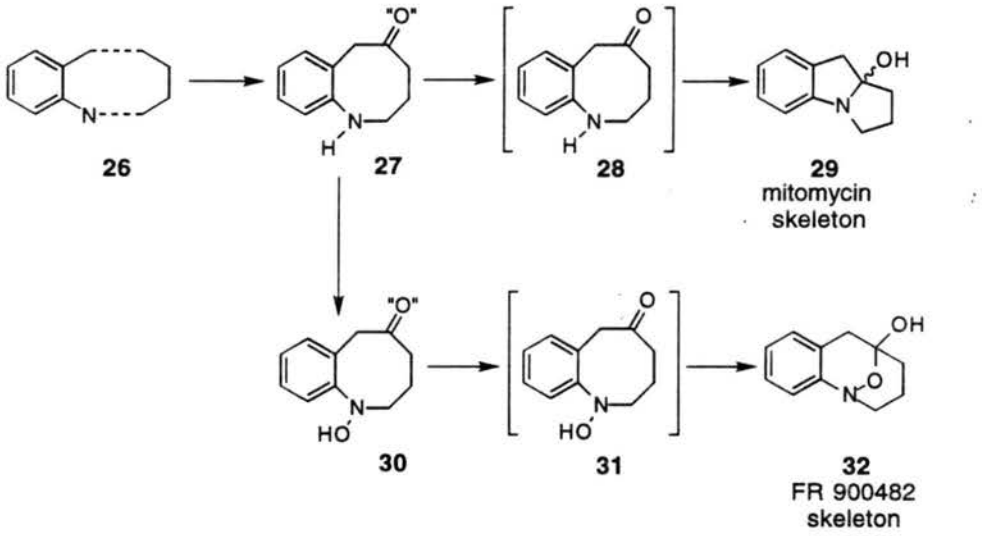
λ_{max} = 372 nm which is attributed to the hydroxyindolecarboxaldehyde n $\rightarrow$ π^* transition. This band appears at λ_{max} = 330 nm in FR 900482 and is the expected consequence of the conversion of a hydroxy*benzene*carboxaldehyde to a hydroxy*indole*carboxaldehyde and thus supports the formation of the pyrrole ring in **23**. Analysis of the UV spectrum of **24** was complicated by overlap but was likewise qualitative in support of the indole structure. The ^{1}H NMR spectrum of the **25** at 500 MHz was readily assigned on the basis of the results of phase-sensitive COSY experiments. Several 1-dimensional nOe experiments aided in resolving residual ambiguities. The relative stereochemistry of the substituents at C-1 and C-2 (mitomycin numbering) was resolved to be *trans* using several nOe experiments. This structural determination provided further support for the *in vitro* model proposed by Fukuyama and Goto[17] for the interstrand cross-linking reaction of this family of compounds. It also established directly the close parallel of the reactions of the reductively activated MMC (**4**) and FR 66979 (**2**) and by analogy those of FR 900482 (**1**) and FK 973 (**4**).

Clearly, both the mechanism of bioactivation and mode of action for this group of drugs remains to be fully understood and several questions remain unanswered: what are the reasons for the varying degrees of activity *in vivo* and *in vitro* for FK 900482, FR 66979, and FK 973? What are the reasons that the induction period (8 h) before the onset of significant biological activity is much longer for FK 973 than MMC? Nevertheless, most experimental data support the notion that these drugs manifest their cytotoxicity in a manner similar to MMC by being reductively activated to form a bis-electrophilic mitosene species that cross-links duplex DNA.

1.7 Synthetic Studies Toward the Total Synthesis of FR 900482

Several communications were reported before and during the course of our investigations revealing the approaches others were employing for the construction of FR 900482. While the details of the respective disclosures were different, the overall strategies were reminiscent of the first total synthesis of the mitomycins. The work by Kishi[50] on the mitomycins provided many clues to the proper sequence of synthetic steps needed to construct FR 900482. The two central ideas reaped from Kishi's synthesis were that a properly functionalized benzazocine ring system should be built first (**26** to **27**) and, that having done so, a carbonyl group or equivalent should be unveiled (**28**) and trapped by the transannular amine (**29**) (Scheme 10). Applied to FR 900482, this protocol would require oxidation of the amine to the corresponding hydroxylamine (**30**) before unmasking the ketone (**31**) to eventually generate the FR 900482 skeleton **32**.

Scheme 10. Synthetic Analysis of Mitomycins and FR 900482



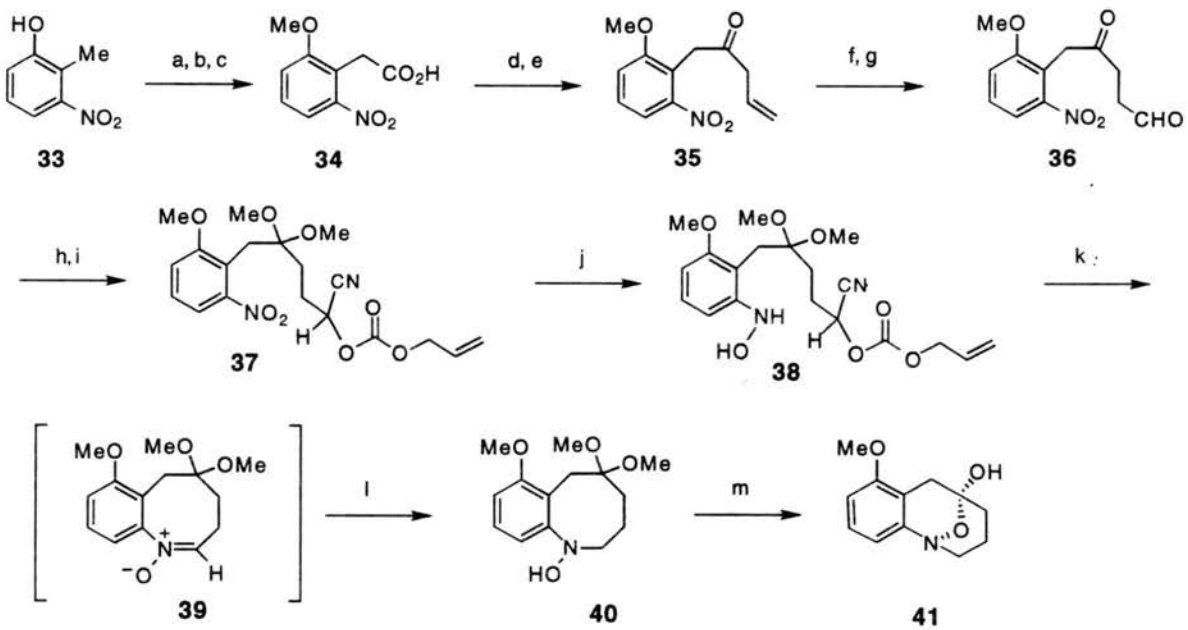
The synthetic work by Williams,[18] Rapoport,[19] Martin,[20] Grubbs,[21] Fukuyama,[17,29] and Terashima[31-33] can all be characterized as adaptations of this philosophy. In a clever variation, Dmitrienko,[24,28] Sulikowski,[22,23] and Ziegler[51] took

the mitomycin strategy a step further and postponed the amine oxidation until the pyrroloindole ring system was formed. Then, an oxidative ring expansion was used to generate the 1,5-epoxybenzazocine. Finally, in a departure from this conventional thinking, Danishefsky's[25,27,30,51] approach to FR 900482 exploited the major structural differences with the mitomycins.

1.7.1 Williams' Model Study

Williams and Yasuda published the first synthetic model study of FR 900482 in 1989 (Scheme 11).[18] 2-Methyl-3-nitroanisole (**33**) was converted into acid **34** (74% yield for three steps) which was subsequently allylated to form the β, γ-unsaturated ketone **35** (68% yield for two steps). Hydroboration and oxidation furnished the

Scheme 11. Williams' model study



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

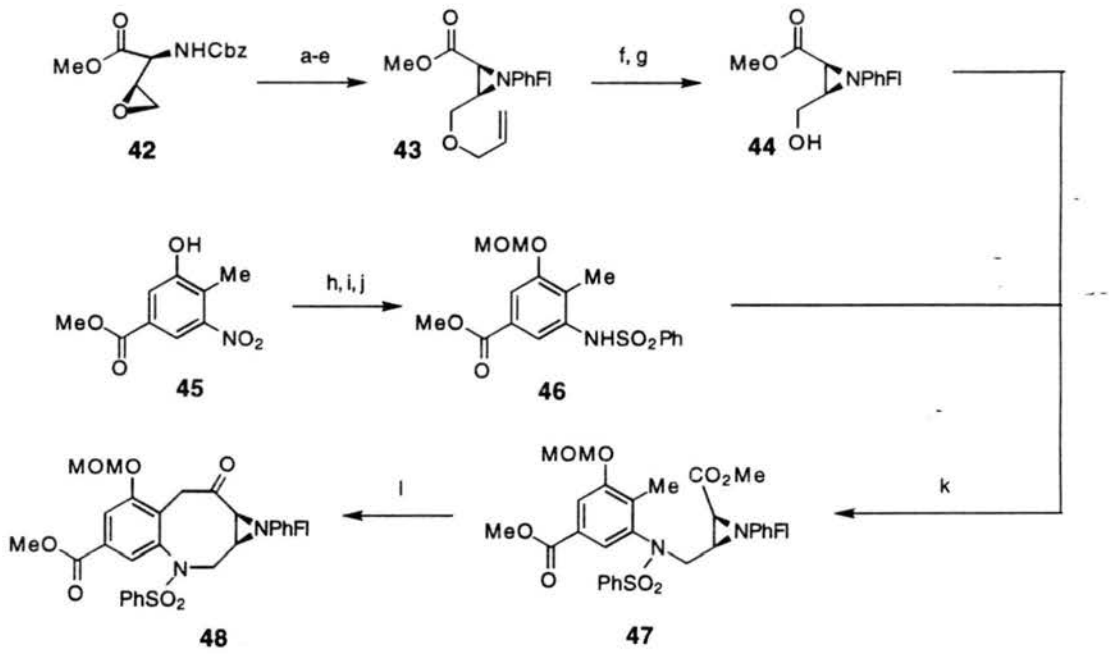
γ-alcohol which was oxidized under Swern conditions to produce the key keto-aldehyde **36** (74% yield for three steps). Protection of the aldehyde with the novel cyanohydrin allyl carbonate protecting group and protection of the ketone as the dimethyl acetal gave **37** (83% yield for two steps). The allyl carbonate was used to allow the selective unmasking of the aldehyde for the intramolecular reductive amination. Zinc reduction of the nitro group afforded labile hydroxylamine **38** (75% yield). Deprotection of the cyanohydrin allyl carbonate resulted in the spontaneous formation of the cyclic nitrone **39** which was immediately reduced to the eight-membered hydroxylamine **40** in 51% yield for two steps. Treatment of **40** with HCl in THF unmasked the ketone and afforded the bicyclic hydroxylamine hemiketal **41** in 64% yield. These investigations demonstrated a method for constructing the novel ring system of FR 900482 and avoided the issue of oxidation of the benzazocine nitrogen by performing a careful reduction of the arylnitro compound **37** to the hydroxylamine **38**. Compound **38,** however, lacked many of the functionalities necessary for the synthesis of the natural product.

1.7.2 Rapoport's model study

A second approach to FR 900482 was reported by Jones and Rapoport using a convergent strategy (Scheme 12).[19] Rather than constructing the eight-membered ring and then installing the aziridine functionality, these researchers synthesized a four carbon piece with a protected aziridine and coupled it to a fully functionalized aromatic piece. In doing so, they hoped to quickly and efficiently access the core skeleton of FR 900482. The starting material for the aliphatic portion was the optically pure Cbz epoxide **42**, derived from L-methionine in four steps in 33% overall yield. Exchanging the Cbz group for a Boc group, opening the epoxide with allyl alcohol, and forming the protected aziridine produced **43** (23 to 30% yield for five steps). Removal of the allyl ether gave **44** in 55% yield for two steps. The aromatic portion **46** was prepared in three steps in 84% yield overall. In a departure from typical intramolecular reductive amination procedures

for the construction of the carbon nitrogen bond of the eight membered ring, Mitsunobu coupling of the benzenesulfonimide **46** and aziridino alcohol **44** afforded the coupled (phenylsulfonyl)anilide **47** as a mixture of conformational isomers in 87% yield.

Scheme 12. Rapoport's model study



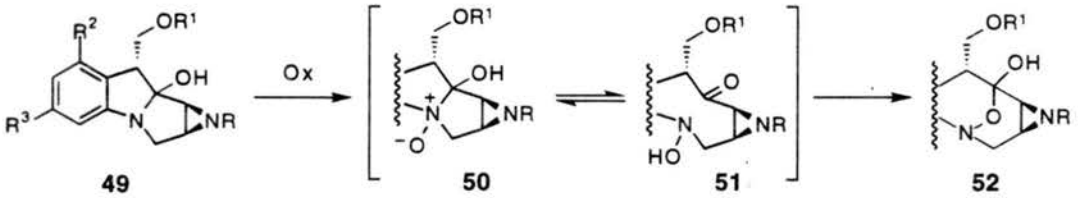
Key: (a) 10% Pd/C, H_2 (1 atm), $(Boc)_2O$, MeOH, 70-90%; (b) $HClO_4$, allyl alcohol, 40%; (c) MsCl, Et_3N; (d) TFA; (e) PhFlBr, K_3PO_4, $Pd(NO_3)_2$, 84%; (f) $RhCl(PPh_3)_3$, DBU, EtOH; (g) TsOH, 55% for two steps; (h) MOMCl, K_2CO_3, acetone, 97%; (i) Pd/C, H_2 (1 atm), 94%; (j) $PhSO_2Cl$, Py, DMAP, 92%; (k) Ph_3P, DMAD, THF, 87%; (l) KHMDS, THF, -10 to 5 °C, 52%.

Treatment of the Mitsunobu product **47** with KHMDS provided the ketone **48** in 52% yield. Rapoport's synthesis demonstrated the convergent construction of the benzazocine ring with a protected aziridine remaining intact. Challenging aspects to the synthesis of FR 900482 remained, such as the installation of the (carbamoyloxy)methyl group alpha to the ketone of **48** with the correct stereochemistry and the oxidation of the amine to an hydroxylamine in the presence of an aziridine. Clearly, the intention was to eventually carry out such operations, but no mention of progress in this area has been reported in the literature to date.

1.7.3 Dmitrienko's ring expansion model study

Dmitrienko's unique approach to the core skeleton of FR 900482 is based on the assumption that the novel hydroxylamine hemiketal ring system might be generated by an oxidative ring expansion of appropriately substituted pyrrolo[1,2a]indoles (**49**) as illustrated in Scheme 13.[28] This idea stems from the likely existence of tautomer **51** (or **5**, see Scheme 3) used to explain the interconversion of FR 900482 to its natural thermodynamic mixture. The *N*-oxide aminal **50** would be a kinetic intermediate derived from the alternative attack of the lone pair of the aryl nitrogen on the carbonyl, rather than the hydroxylamine oxygen. Central to this idea was the notion that if a pyrrolo[1,2a]indole **49** could be constructed and oxidized to generate the *N*-oxide **50**, it should then revert to the more favorable hydroxylamine hemiketal **52**. Such a process should provide a bridge between the extensive chemical methodology for the construction of pyrrolo[1,2a]indoles, developed for the synthesis of the mitomycins, and the FR 900482 system.

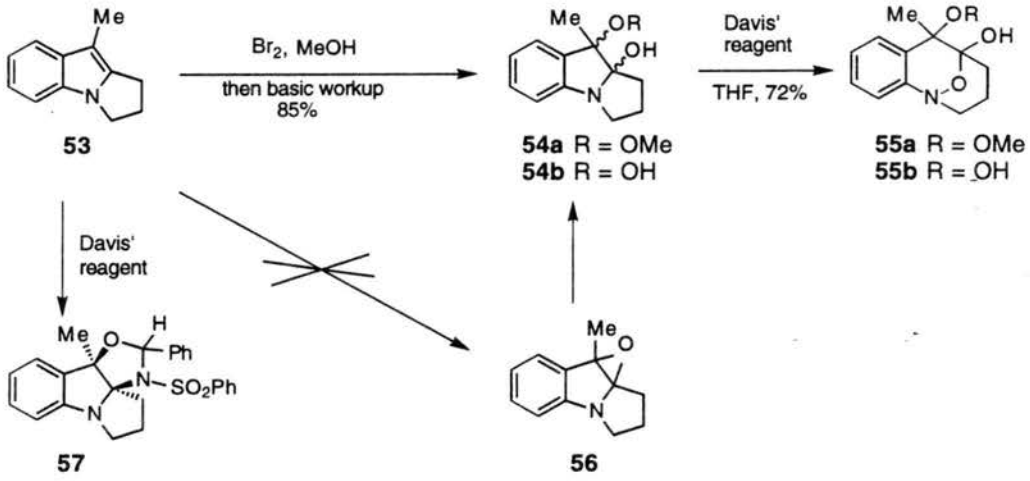
Scheme 13. Dmitrienko's proposed oxidative expansion



Starting from indole **53**, the bicyclic ring system was constructed through bromohydrin formation of **54a** (R = OMe) followed by oxidation with Davis' reagent to generate **55a** (R = OMe) (Scheme 14).[28] Further attempts to simplify the transformation of **53** into the FR 900482 ring system failed. Hoping **53** would react with Davis' reagent to yield diol **54b** (R = OH), via epoxide **56**, and undergo oxidative ring expansion to **55b** (R = OH), researchers found instead, that **53** reacts with Davis' reagent to yield the

unusual 1,3-oxazolidinoindole ring system **57**.[24] Dmitrienko's investigations demonstrated a unique method for constructing the novel ring system of FR 900482 and provided interesting overtones relevant to a possible unified theory of biogenesis for FR 900482 and the mitomycins.

Scheme 14. Dmitrienko's ring expansion model study



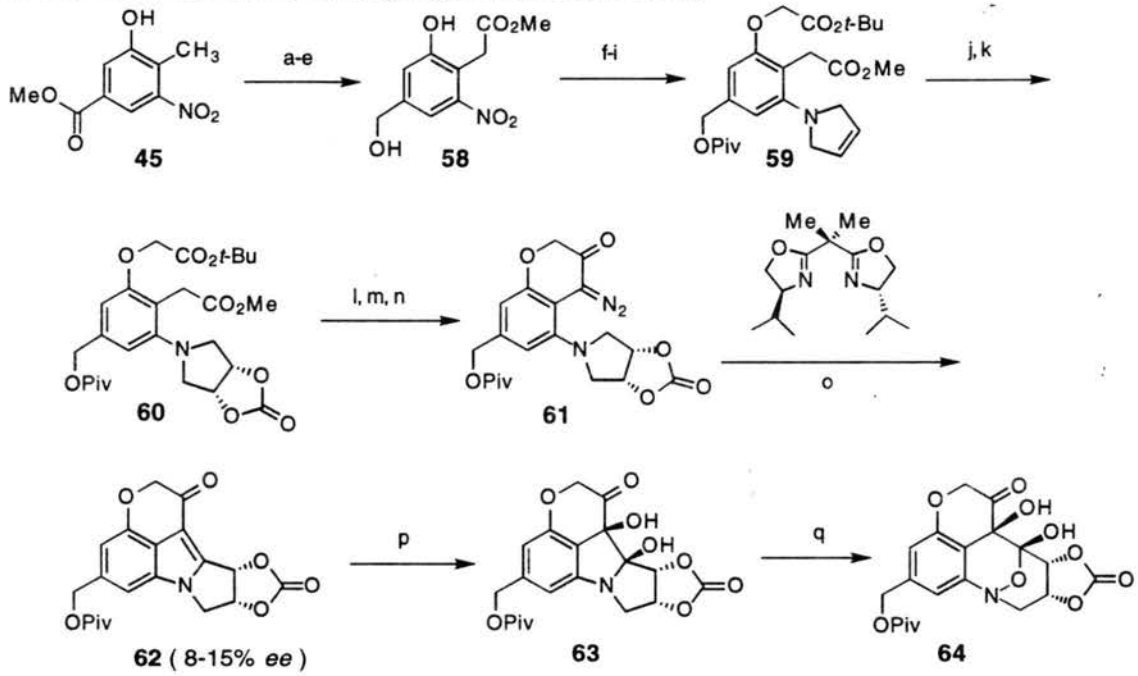
1.7.4 Sulikowski's model study

Using the same ring expansion approach as Dmitrienko, Lim and Sulikowski reported the oxidative expansion of a fully functionalized core structure of FR 66979 (Scheme 15).[22] The model study started with phenol **45**, the same starting material used in Rapoport's model study (See Scheme 12). Phenol **45** was converted into benzylic alcohol **58** in five steps (26% yield overall). Conversion of **58** to **59** was accomplished in another four steps in 68% yield overall. Dihydroxylation of **59** followed by treatment with phosgene led to formation of the meso carbonate **60** (64% yield). Intramolecular Dieckmann cyclization of **60** followed by decarboxylation and diazotonation produced the key diazoketone **61** (42% yield for three steps).

In previous model studies, Lim and Sulikowski had that demonstrated the copper(I) catalyzed cyclization of a diazoester derivative related to **61** occurred smoothly

at room temperature to provide the corresponding dihydroindole.[23] In contrast, cyclization of diazoketone **61** required forcing conditions ($CHCl_3$, reflux) and unexpectedly formed indole (or mitosene) **62**. The oxidation was assumed to be copper(I) dependent since a large amount of copper(I) triflate (50 mol%) was consumed. The cyclization of **61** also proceeded with a low level of asymmetric induction (8-15% ee). Dihydroxylation of **62** gave pyrrolo[1,2a]indole **63** (33% yield) and set the stage for the oxidative ring expansion. Treatment of **63** with dimethyldioxirane effected oxidative ring expansion to the core structure **64** in 62% yield. In contrast to Dmitrienko's model study, this study demonstrated the construction of the fully functionalized core structure of FR 66979.

Scheme 15. Sulikowski's ring expansion model study

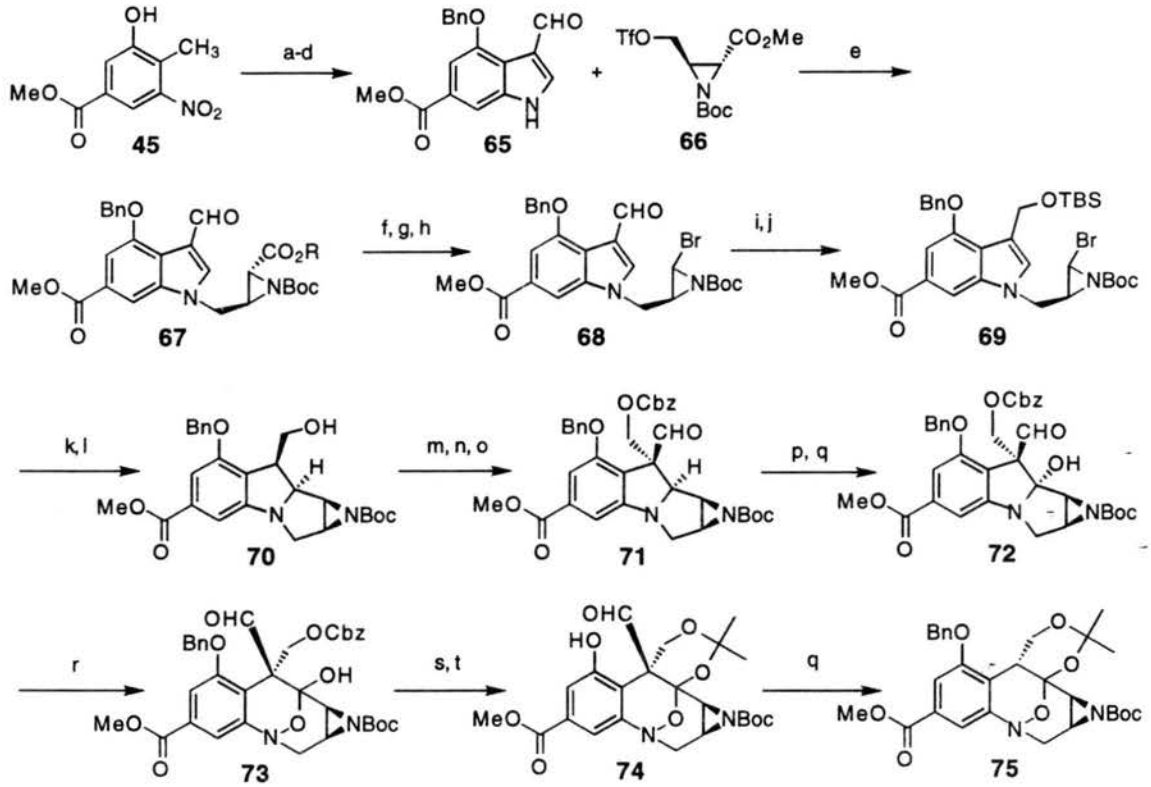


Key: (a) TBSCl, Im, DMF; (b) NBS, AIBN, PhH; (c) DIBAL, CH_2Cl_2, -78 °C, 77% for three steps; (d) KCN, DMSO, 57%; (e) 1% H_2SO_4, MeOH, reflux, 59%; (f) $BrCH_2CO_2t$-Bu, K_2CO_3, acetone; (g) PivCl, Py, CH_2Cl_2, 91% for two steps; (h) Pd/C, H_2, MeOH; (i) $NaHCO_3$, $(MsOCH_2CH)_2$, DMF, 75% for two steps; (j) OsO_4, t-BuOOH, Et_4NOAc, acetone; (k) phosgene, Py, CH_2Cl_2; 64% for two steps; (l) NaHMDS, THF, -78 °C, 92%; (m) p-TsOH, PhH, reflux, 75%; (n) NaHMDS, THF, -78 °C, then DNBSA, -78 to 20 °C, 62%; (o) Cu(I)OTf, $CHCl_3$, 4Å mol sieves, reflux, 15 h, 51%; (p) OsO_4, Py, then H_2S, 33%; (q) dimethyldioxirane, acetone, 62%.

1.7.5 Ziegler's model study

Ziegler *et al.* also used an oxidative ring expansion reaction based on Dmitrienko's initial work to access a fully functionalized core structure of FR 900482.[51] In contrast to Sulikowski, Ziegler utilized the cyclization of an aziridinyl radical with a functionalized indole nucleus to construct an appropriately functionalized dihydroindole. The model study, illustrated in Scheme 16, started with phenol **45**, the same starting material used in Rapoport's and Sulikowski's model studies (see Scheme 12 and 15). Modification of phenol **45** into target indole **65** proceeded smoothly using precedented literature protocols (46% yield for four steps). Indole **65** was alkylated with triflate **66**, which was derived from D-isoascorbic acid. The resultant ester **67**, prepared in 66% yield, was converted into a mixture of *trans* and *cis* bromoaziridines **68** by visible light photolysis (W-lamp) of the intermediate thiohydroxamic acid anhydride. The formation of the mixture of bromoaziridines was of little consequence since both isomers were amenable to subsequent reductive cyclization. Reduction of the aldehyde of **68** followed by silylation of the resulting alcohol formed **69** and set the stage for the reductive cyclization. Accordingly, when **69** was treated with a solution of n-Bu_3SnH and ACCN in toluene, the dihydroindole **70** was formed in 56% yield for four steps from **68**. Note that oxidation of **70** to the indole was avoided, thus circumventing many of the synthetic problems encountered by Dmitrienko and Sulikowski (Scheme 14 and 15). Oxidation of alcohol **70** followed by aldol condensation with formaldehyde and Cbz protection of the resultant alcohol gave **71** (61% yield for three steps) with the correct relative stereochemistry for elaboration to the (carbamoyloxy)methyl side chain of the natural product.

Scheme 16. Ziegler's radical cyclization model study

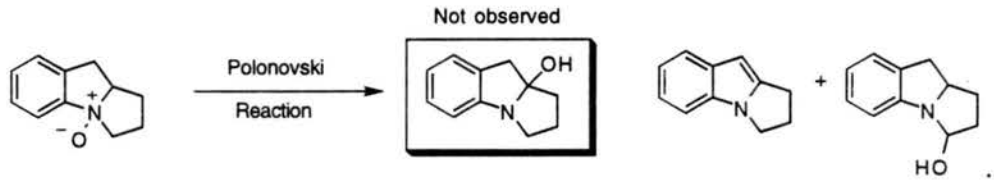


Key: (a) BnCl, K_2CO_3, DMF, 100 °C, 90%; (b) pyrrolidine, DMFDMA, 110 °C; (c) NH_2NH_2, Raney Ni, THF/MeOH, 56% for two steps; (d) $POCl_3$, DMF, 0 °C, then H_2O, 92%; (e) NaHMDS, THF, -30 °C, 1 h, 66%; (f) LiOH, 96%; (g) 2,2'-dithiobis(pyridine *N*-oxide), n-Bu_3P; (h) hν (visible), $BrCCl_3$, 53% for two steps; (i) $NaBH_4$, MeOH, 0 °C; (j) TBSCl, Im, CH_2Cl_2; (k) PhMe, ACCN, n-Bu_3SnH, 116 °C, 1 h; (l) TBAF, THF, 56% for four steps; (m) DMPI, CH_2Cl_2; (n) 37% CH_2O/H_2O, $NaHCO_3$, CH_2Cl_2/MeOH, 2 h, 86% two steps; (o) 1,1'-carbonyldiimidazole, CH_3CN, rt, 3.5 hr, then BnOH, DMAP, 63 °C, 3 h, 71%; (p) *m*-CPBA, CH_2Cl_2, 0 °C, 2 h, 98%; (q) Ac_2O, 24 h, 0 °C, then H_2O, 73%; (r) *m*-CPBA, 0 °C, 1.5 h, 81%; (s) 10% Pd/C, H_2, EtOH, 30 min, 92%; (t) dimethoxypropane, *p*-TsOH•H_2O, CH_2Cl_2, 20 min, 70%; (q) $(Ph_3P)_3RhCl$, Xy, 130 °C, 3.75 h, 77%.

Introduction of the two oxygen atoms to form the hydroxylamine hemiketal relied on several previous studies by other research groups. The first oxygen was installed by *m*-CPBA oxidation of **71** to form the *N*-oxide. Subsequent Polonovski rearrangement (Ac_2O, THF, 24 h, then H_2O) produced carbinolamine **72** (73% yield). In comparison, during a synthetic study on the mitomycin series[52] researchers hoped a Polonovski rearrangement of an *N*-oxide might install the angular C-9a oxygen to produce a carbinolamine (Scheme 17). Instead, Danishefsky and Fiegelson observed that a

Polonovski rearrangement led to aromatization via the C-9a iminium salt and C-3 oxidation (mitomycin numbering) in near equal amounts. Dmitrienko's oxidative ring expansion of the carbinolamine **72** installed the second oxygen atom and

Scheme 17. Polonovski rearrangement



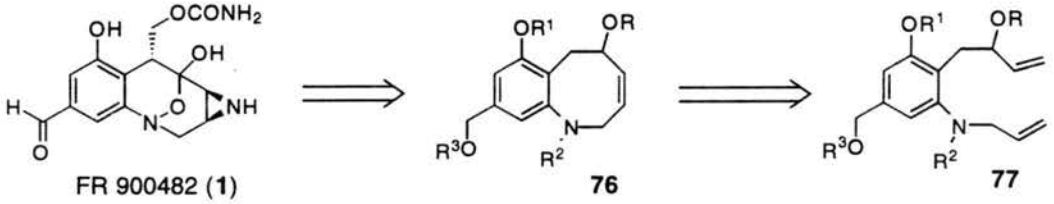
produced the hydroxylamine hemiketal **73** (57% yield for three steps). Hydrogenolysis of both benzyl groups of **73** and conversion of the intermediate triol to its acetonide provided **74**. Finally, attention was focused towards decarbonylation of **74** with retention of configuration. Stoichiometric decarbonylation with Wilkinson's catalyst produced the desired product **75**. Unfortunately, the reaction proved to be capricious and failed to give consistent results. With this noted, Ziegler's study constructs the most advanced structure to date using an oxidative ring expansion reaction. It also demonstrates the high potential for such a process to provide a bridge between the methodology for the construction of pyrrolo[1,2a]indoles, developed for the synthesis of the mitomycins, and the FR 900482 system.

1.7.6 Ring Metathesis Reactions

In 1995, two different model studies were published simultaneously by Martin *et al.*[20] and Grubbs and co-workers.[21] As shown in Scheme 18, each study used a ring closing metathesis (RCM) reaction to access intermediates related to compounds used in Fukuyama's total synthesis[29] (See Scheme 21, compound **93**) of FR 900482. In addition,

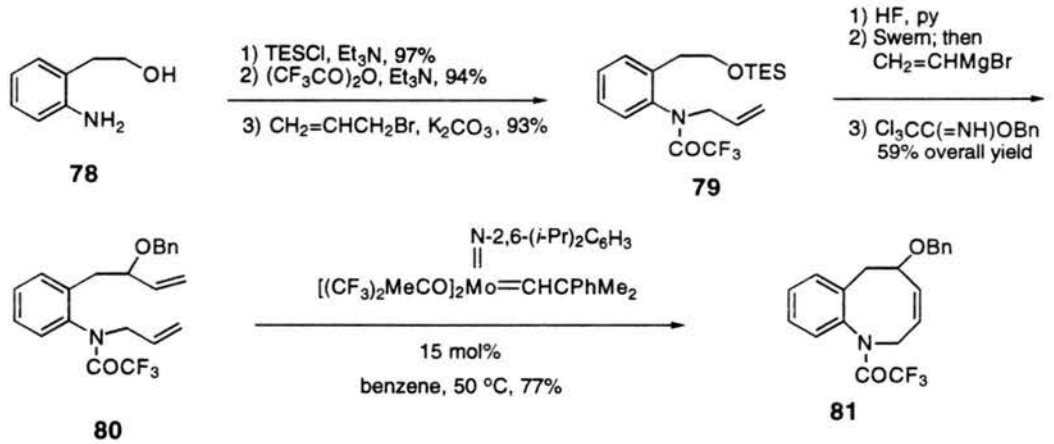
suitably functionalized structures related to benzazocine **76** can be converted into mitomycin analogs as demonstrated in Kishi's synthesis of the mitomycins.[50]

Scheme 18. Retro-synthesis using a ring closing metathesis reaction



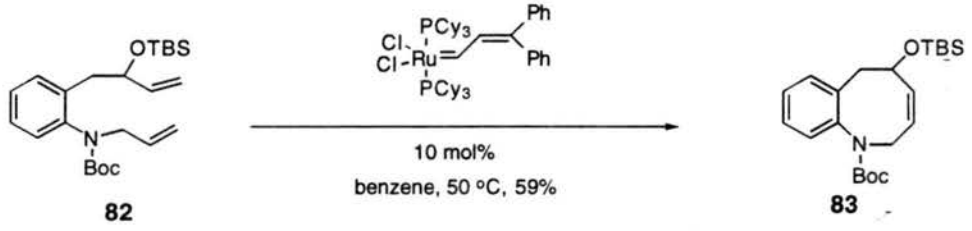
To test the key step in Martin's approach to FR 900482, the α,ω-diene **80** was prepared in good overall yield from amino alcohol **78** by a straightforward sequence of reactions illustrated in Scheme 19.[20] Following protection of the primary alcohol in **78**, the requisite allyl group was introduced by *N*-allylation of the trifluoroacetamide (85% yield for three steps). Deprotection of the alcohol in **79** followed by oxidation, Grignard addition, and *O*-protection gave **80** (59% yield for three steps). Upon treatment with the molybdenum carbene complex, **80** underwent facile RCM in 77% yield to produce benzazocine **81**.

Scheme 19. Martin's ring closing metathesis model study



Grubbs tested the application of the RCM reaction to this class of structures by synthesizing diene **82** and subjecting it to RCM (Scheme 20).[21] When **82** was treated with the ruthenium catalyst, compound **83** was isolated in 59% yield. Since RCM reactions tolerate a variety of functional groups, these investigations by Martin and Grubbs demonstrated a possible method for swiftly constructing extremely advanced benzazocine intermediates for the synthesis of FR 900482.

Scheme 20. Grubbs' ring closing metathesis model study

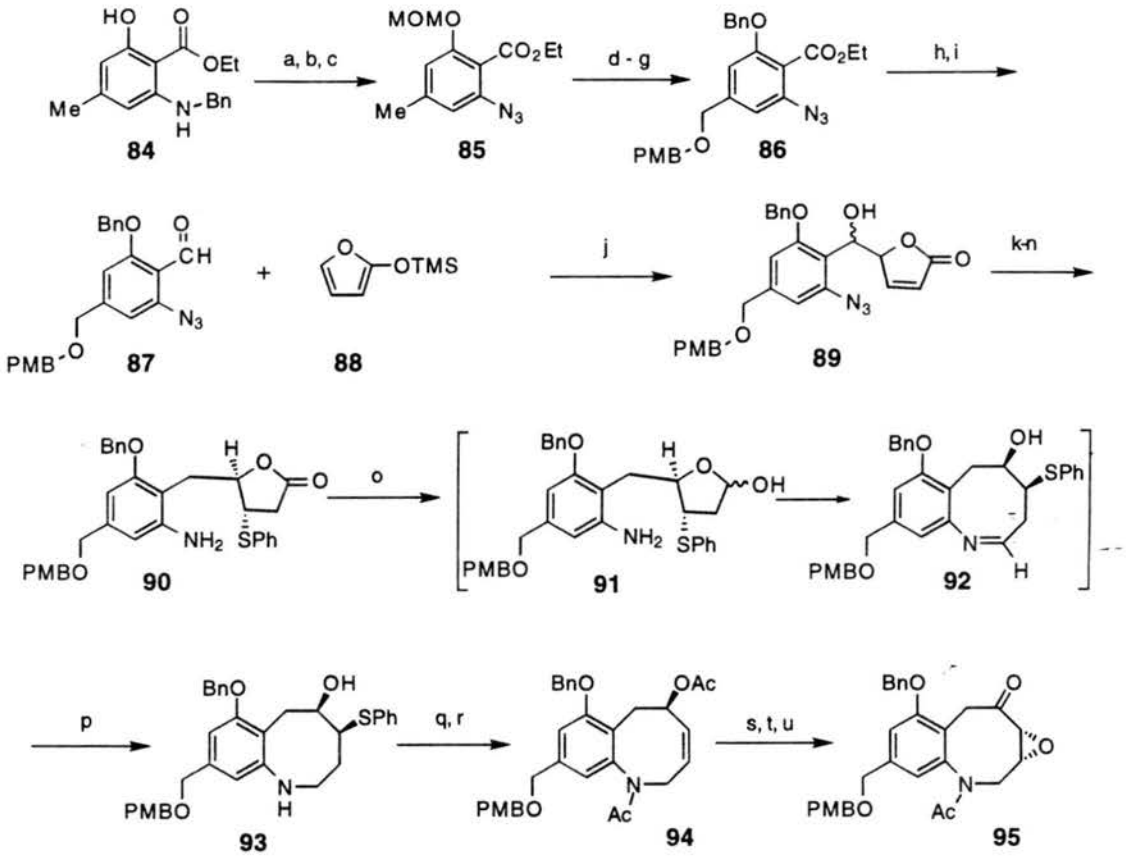


1.7.7 Fukuyama'a total synthesis

In 1992, Fukuyama *et al.* were the first to publish a total synthesis of racemic FR 900482.[29] Although quite lengthy (43 steps) and linear, the approach to the natural product successfully capitalized on the abundance of information available from the synthetic studies of the mitomycins by Fukuyama[53,54] and Kishi.[50] It also closely followed the synthetic outline in Scheme 10. The first part of the total synthesis is depicted in Scheme 21.

Transformation of **84** using standard synthetic methods produced aldehyde **87** in 44% yield for nine steps.[29] Addition of 2-(trimethylsilyloxy)furan to aldehyde **87** occurred smoothly to give a diastereomeric mixture of butenolides **89** in 96% yield. It was that found protection of the butenolide was necessary, and this was effected by Michael addition of thiophenol. Acetylation and reductive removal of the benzylic acetate provided a single isomer of the azido lactone whose azide group was further reduced with zinc to give amine **90** (47% yield for four steps). As in Fukuyama's model

Scheme 21. Fukuyama's synthesis of a functionized benzazocine ring



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

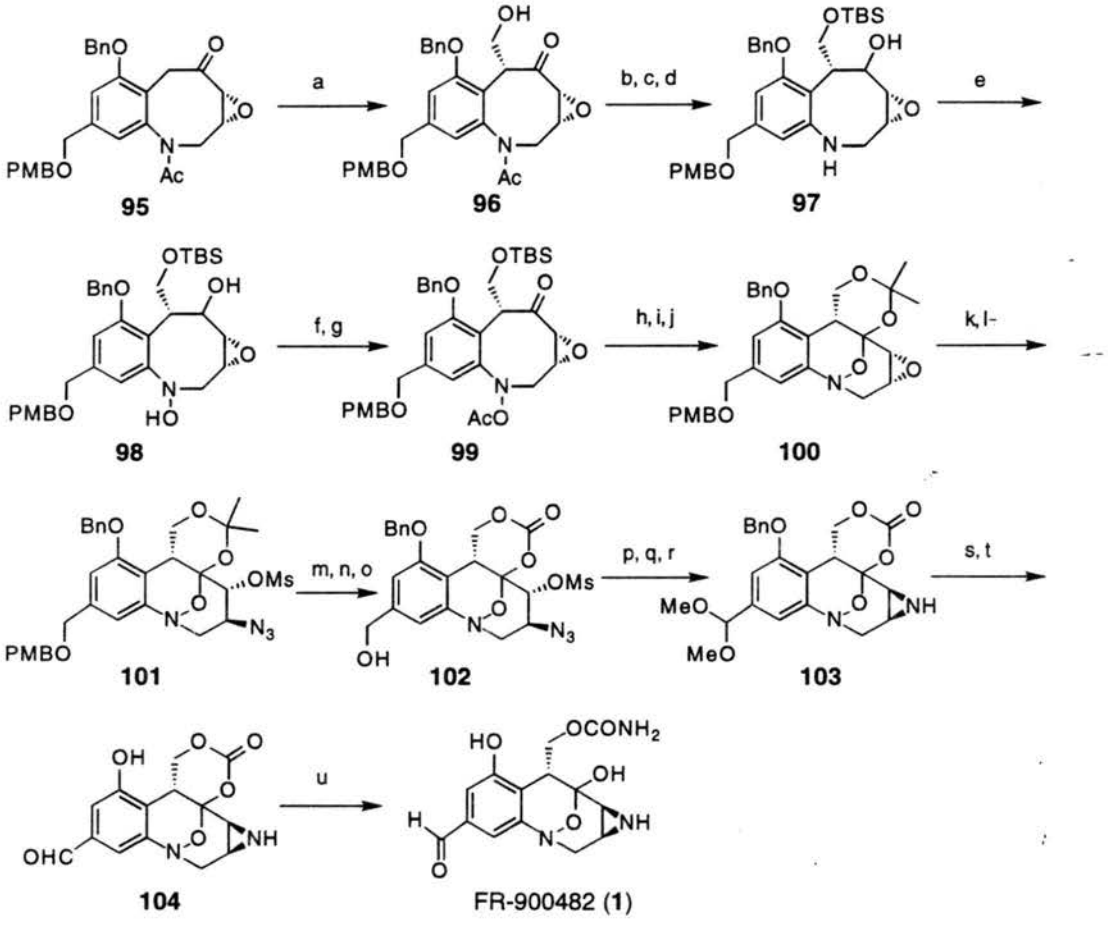
study of FR 900482,[17] the critical transformation of **90** into the desired benzazocine **93** was achieved by sequential reduction of the lactone, with DIBAL(**90** → **91**) and with sodium cyanoborohydride of the resulting imine, in 83% yield for two steps. Protection of amine **93**, oxidation of the sulfide, and subsequent thermolysis of the resultant

sulfoxide restored the masked olefin **94** (71% yield for two steps). Conversion of **94** into epoxy ketone **95** through hydrolysis of the acetate, epoxidation, and Swern oxidation (92% for three steps) was necessary to successfully install the hydroxymethyl side chain.

Hydroxymethylation of ketone **95** proceeded stereospecifically to give a single stereoisomer **96** with the correct stereochemistry relative to the epoxide (Scheme 22). No explanation was given for the direction and degree of selectivity for this aldol reaction. The unstable ketone **96** was immediately reduced with $NaBH_4$, the primary alcohol was selectively protected, and the acetamide was deprotected to give amine **97** in 45% yield for four steps. While Davis' reagent was the only oxidizing agent that successfully converted secondary amines into hydroxylamines in model studies,[17] Davis' reagent failed to oxidize amine **97** to the desired hydroxylamine. Facile and clean oxidation of **97** to hydroxylamine **98** was achieved by treatment with *m*-CPBA. The labile hydroxylamine was selectively protected as an acetate, and subsequent Swern oxidation of the secondary alcohol yielded ketone **99** (68% yield for three steps). Exposure of ketone **99** to excess hydrazine cleaved the acetate group and effected the key transannular cyclization. Deprotection of the TBS ether and protection of the diol as an acetonide gave **100** as a single isomer in 96% yield for three steps. To form the desired aziridine, epoxide **100** was cleaved with NaN_3, and the resultant alcohol was converted to mesylate **101** (89% yield for two steps). After recognizing the extreme lability of aziridines under acidic conditions, all acid requiring steps were carried out prior to the aziridine formation. Acetonide **101** was converted into the carbonate and treatment with CAN deprotected the benzylic alcohol to give **102** in 74% yield for three steps. Alcohol **102** was oxidized to the aldehyde which was protected as the dimethyl acetal **103**. Reduction of the azide with triphenylphosphine in the presence of *i*-Pr_2NEt furnished aziridine **103** in 71% yield. Hydrogenolysis of the benzyl ether followed by mild acidic cleavage of the dimethylacetal afforded **104** without appreciable decomposition of the aziridine. Finally,

regioselective ammonolysis of the cyclic carbonate gave exclusively (±)-FR 900482, which was identical with an authentic sample of the natural product.

Scheme 22. Fukuyama's total synthesis of (±)-FR 900482

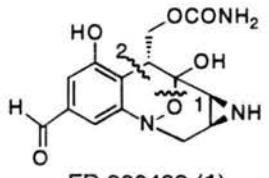


Key: (a) CH_2O, LiOH, THF/H_2O, 0 °C, 2 h; (b) $NaBH_4$, EtOH, -78 to 23 °C, 71% for two steps; (c) TBSCl, Im, DMAP, CH_2Cl_2, 23 °C, 92%; (d) DIBAL, PhMe, -78 °C, 64%; (e) *m*-CPBA, CH_2Cl_2, 23 °C; (f) Ac_2O, 23 °C, 10 h, 83% for two steps; (g) DMSO, $(ClCO)_2$, CH_2Cl_2, then Et_3N, 83%; (h) NH_2NH_2,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

1.7.8 Danishefsky's total synthesis

In 1995, Danishefsky *et al.* published the second total synthesis of racemic FR 900482.[30] The Fukuyama total synthesis[29] was founded on the logic implied by the disconnection of the hydroxylamine hemiketal at line 1 in Figure 5 and by the

Figure 5. Disconnections of FR 900482



FR 900482 (1)

logic depicted in Scheme 10. In constrast, the Danishefsky synthesis relied on a radically different construction, implied by line 2 in Figure 5, wherein the bicyclic ring system was established by the intramolecular arylation of a suitably substituted system bearing an aziridine. An interesting feature of the synthesis was the formation of the protected hydroxylamine hemiketal through a novel hetero Diels-Alder cycloaddition reaction.

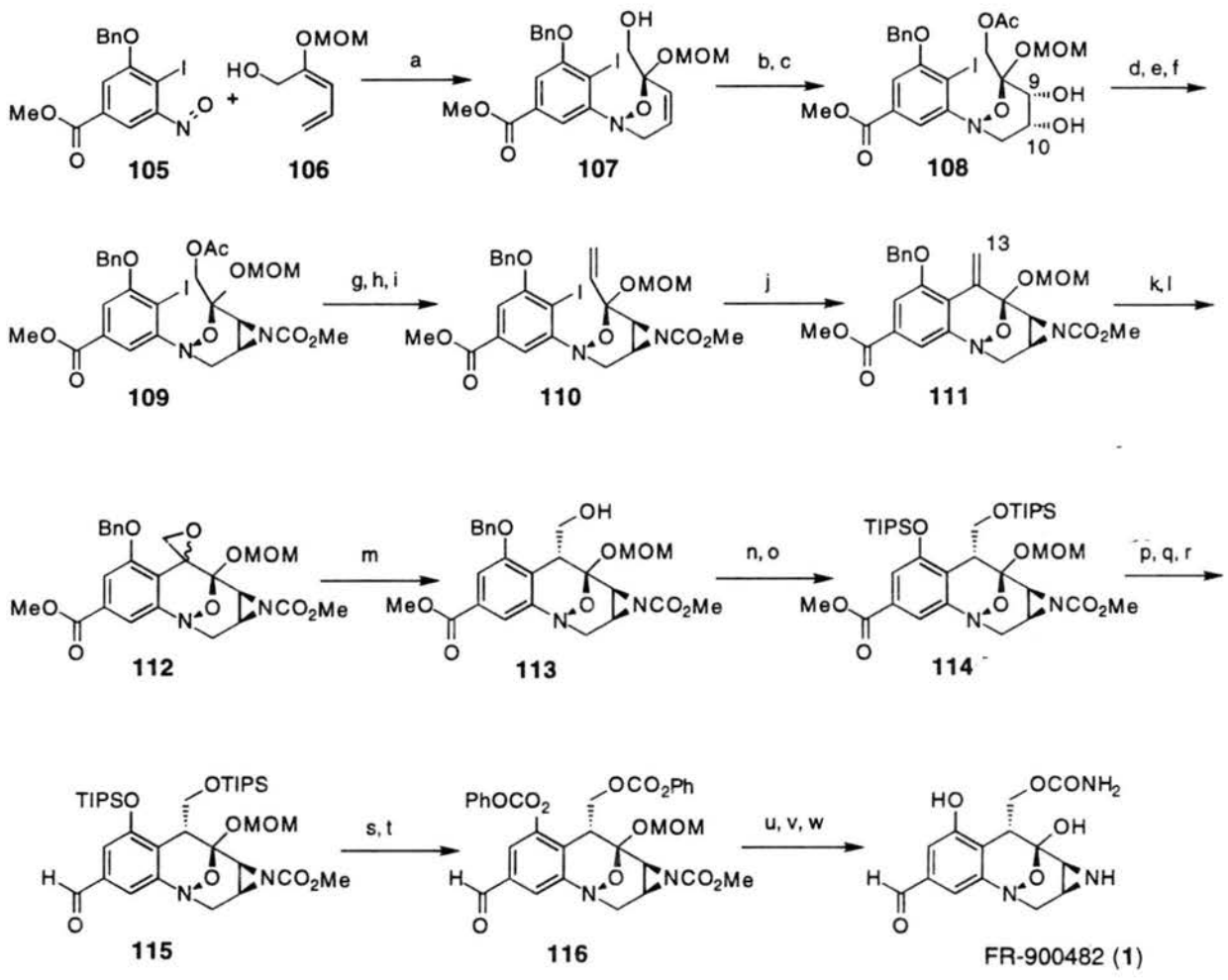
The requisite heterodienophile **105** was prepared following eight straight forward steps (32% overall yield) from methyl vanillate.[30] Smooth cycloaddition of **105** with **106** produced **107** in an 80% yield (Scheme 23). Acetylation and stereospecific dihydroxylation of the olefin gave diol **108** (65% yield for two steps) and set the stage for the installation of the aziridine functionality based on precepts founded by Kishi[50] in the synthesis of the mitomycins. Selective triflation at C-10 (FR 900482 numbering) followed by azidolysis of the triflate allowed for the triflation of the C-9 alcohol. Reduction of the azide with triphenylphosphine followed by the hydrolysis of the iminophosphorane and protection of the resultant aziridine afforded compound **109** in 54% yield for four steps. Conversion of **109** to the cyclization precursor **110** was accomplished by acetate hydrolysis, Swern oxidation of the free primary alcohol, and

Wittig olefination of the resultant aldehyde (75% yield for three steps). Intramolecular Heck arylation ($Pd(Ph_3P)_4$, Et_3N, CH_3CN, 90 °C) of **110** gave **111** in 93% yield.

Introduction of the hydroxy function at C-13 of **111** proved to be more difficult than expected but was accomplished by dihydroxylation and epoxide formation. Reduction of epoxide **112** with samarium diiodide afforded **113** in excellent yield (71%) with the correct relative stereochemistry. Hydrogenolysis of the benzyl group was followed by protection of the two hydroxyl groups as TIPS ethers (**114**) in 91% yield for two steps. Reduction of the ester and removal of the carbomethoxy group with excess DIBAL, followed by selective reprotection of the aziridine and oxidation (MnO_2, CH_2Cl_2), led to benzaldehyde **115** (73% for three steps). Remarkably, the aldehyde was maintainable unprotected for the duration of the synthesis. The two TIPS groups were cleaved, and two phenyl carbonate groups were introduced to give **116** in 100% yield for two steps. Finally, removal of the MOM function (Ph_3CBF_4, di-*tert*-butylpyridine), ammonolysis of the phenyl carbonates, and hydrolysis of the aziridine protecting group afforded fully synthetic racemic FR 900482.

Considering the complexities of the natural product, this synthesis provided a reasonably direct route to the target compound in 34 steps (1.2% overall yield) from methyl vanillate. Additionally, the aziridine was installed extremely early in the synthesis and successfully carried through intact the remainder of the total synthesis (16 steps). Although not addressed by Danishefsky, the synthesis was racemic, but an asymmetric dihydroxylation of **108** could be used to synthesize the natural product in optically active form.

Scheme 23. Danishefsky's total synthesis of (±)-FR 900482



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

1.7.9 Terashima's asymmetric total synthesis

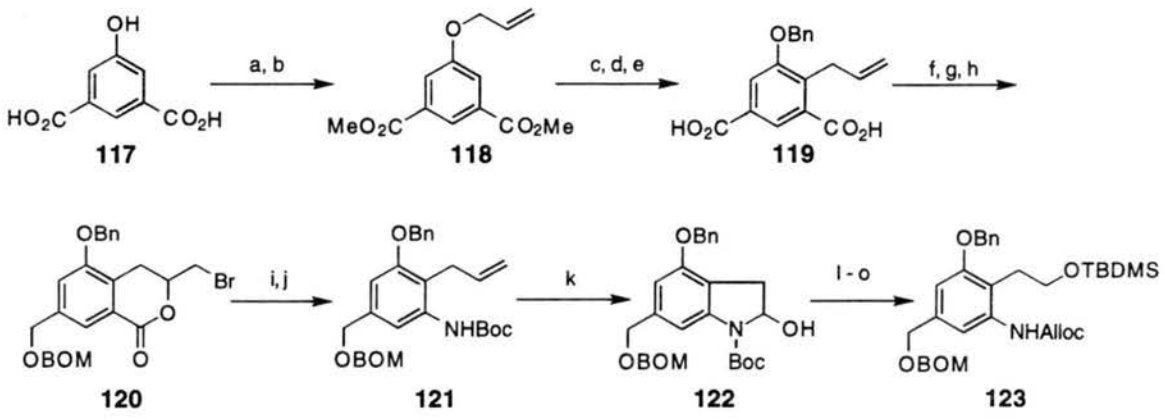
In 1996, Terashima and co-workers published the first asymmetric total synthesis of (+)-FR 900482 in a series of three papers.[31-33] Using a similar retrosynthetic plan as Rapoport *et al.*,[19] researchers constructed an optically active four carbon aliphatic fragment with appropriate functionality to quickly construct the aziridine function. The aliphatic fragment was coupled to an aromatic fragment in hopes of quickly and efficiently accessing the natural product. As in the Rapoport synthesis, the aliphatic fragment was constructed from a chiral pool reagent.

One unique feature in the reports of the total synthesis by Terashima is the relay synthesis.[31] The key intermediate **141** was synthesized from FK 973 (**3**) and successfully reconverted into FR 900482. These studies demonstrated that **141** was a suitable synthetic target for the total synthesis and that the crucial final sequence of reactions (**141** $\rightarrow$ FR 900482) involving delicate deprotection and oxidation steps could be realized.

To initially pursue the synthesis of the aromatic fragment (Scheme 24),[33] **117** was converted to the allyl ether followed by formation of the diester to give **118** in 98% yield for two steps. Claisen rearrangement of **118** produced the tetra-substituted aromatic ring and protection of the resulting phenol followed by ester hydrolysis produced the diacid **119** in 67% yield for three steps. In order to differentiate between the carboxylate groups, **119** was converted to the corresponding bromolactone (72% yield). The remaining carboxyl group was reduced to give the benzyl alcohol and protected to furnish **120** (69% yield for two steps). Reductive cleavage of the bromolactone released the carboxylic acid function which subsequently was converted to the Boc protected aniline **121** using a modified Curtius rearrangement (DPPA, Et_3N, *t*-BuOH, reflux, 76% yield). Oxidative cleavage of the terminal olefin in **121** (OsO_4, $NaIO_4$, dioxane/H_2O, rt, 73% yield) resulted in the formation of aminal **122**. Finally, **122** was converted to **123** (87% yield for four steps) by sequential reduction, silylation of the resulting alcohol, and exchange of the Boc group with an Alloc protecting group.

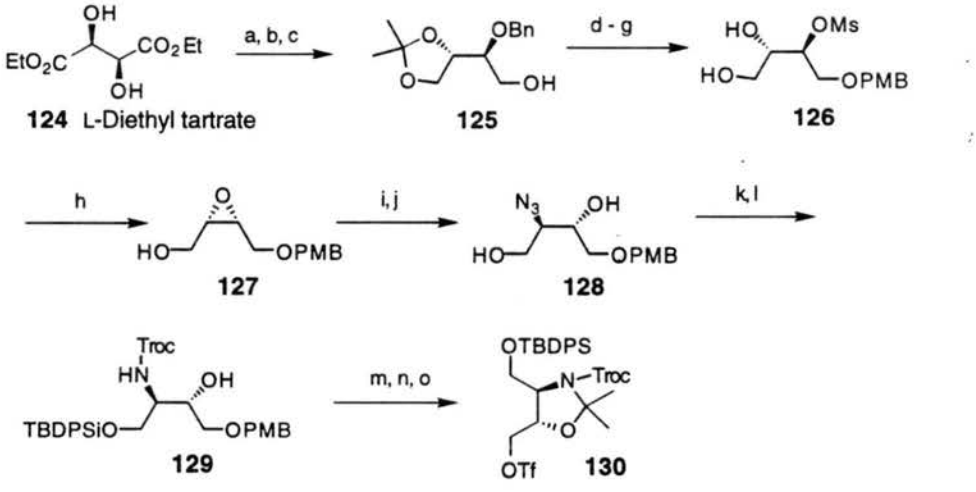
To synthesize the aliphatic fragment (Scheme 25), Terashima and co-workers[33] started with L-diethyl tartrate (**124**) and through previously published synthetic steps, transformed it into alcohol **125** in 72% overall yield. Protection of the primary alcohol followed by chemoselective hydrogenolysis of the benzyl ether (Raney Ni, H_2, EtOH, rt) produced the secondary alcohol (90% yield for two steps). The secondary alcohol was converted to epoxy alcohol **127** by a three step sequence of reactions involving mesylation of the secondary alcohol, acidic hydrolysis of the acetonide (**126**), and basic epoxide ring formation (85% overall yield). The optical purity of epoxide **127** was measured to be greater than 98% ee by ^{1}H NMR analysis of MTPA derivatives. Note that epoxide **127** was constructed more directly by Sharpless asymmetric epoxidation of the corresponding allylic alcohol, but the optically purity was of **127** prepared by the Sharpless epoxidation was measured to be 85% ee. Nucleophilic ring opening of epoxide **127** with sodium azide gave a 2:3 mixture of 1,2- and 1,3-diols (92% yield). The mixture was exposed to sodium periodate to separate the desired 1,3-diol **128** from the undesired 1,2-diol. This sequence of steps discarded 50% of a valuable advanced intermediate. Selective protection of the primary alcohol in diol **128** followed by reduction of the azide and protection of the resulting amine as the trichloroethoxy carbamate produced **129** in 90% yield for three steps. Finally, **129** was successfully converted to **130** (81% for three steps) by sequential acetonide formation, deprotection of the PMB group, and triflation of the resulting primary alcohol. With the aromatic and aliphatic fragments in hand, efforts were focused on the synthesis of the benzazocine and installation of the hydroxymethyl side chain.[32] The critical coupling reaction of **123** and **130** (NaH, THF, -78 °C to rt) proceeded cleanly to give a quantitative yield of the desired adduct **131** (Scheme 26). Simultaneous removal of the Troc and acetonide groups in **131** followed by tosylation and mesylation of the resulting amino alcohol produced mesylate **132** in 72% yield for three steps. Treatment of mesylate **132** with sodium hydride formed the *N*-protected aziridine (92% yield). Deprotection of both silyl groups followed by double oxidation of

Scheme 24. Terashima's synthesis of the aromatic portion of FR 900482



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

Scheme 25. Terashima's synthesis of the aliphatic portion of FR 900482

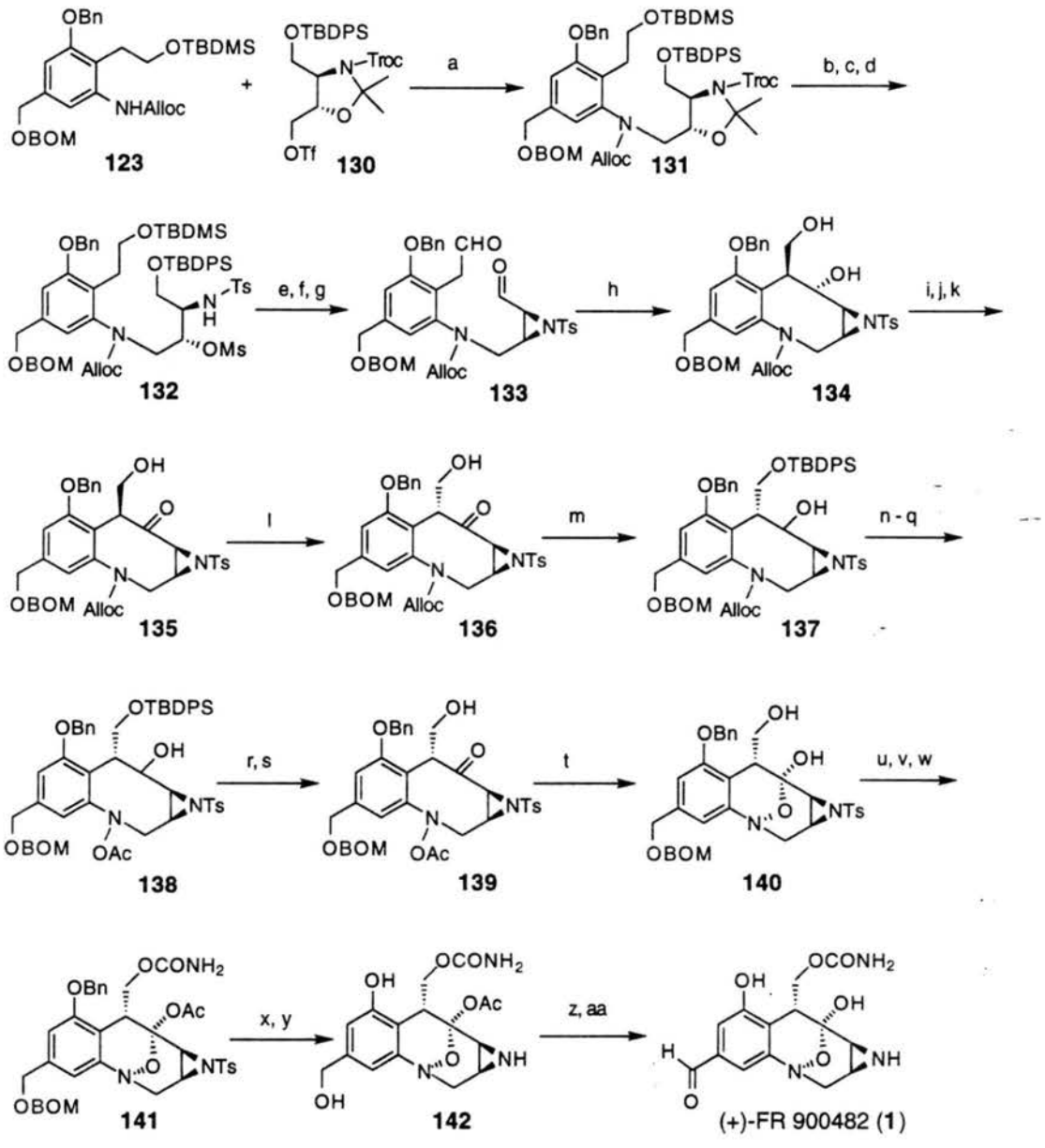


Key: (a) PhCHO, *p*-TsOH, PhH; (b) $LiAlH_4$, $AlCl_3$, THF; (c) dimethoxypropane, acetone, 68% for three steps; (d) NaH, PMBCl, DMF, rt, 97%; (e) Raney Ni, H_2, EtOH, rt, 93%; (f) MsCl, Et_3N, CH_2Cl_2, 0 °C, 100%; (g) conc. HCl, MeOH, rt, 97%; (h) K_2CO_3, MeOH, rt, 88%; (i) NaN_3, NH_4Cl, EtOH, relux,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

the two primary alcohols furnished dialdehyde **133** in 73% yield over two steps and set the stage for the key intramolecular aldol reaction. Aldol cyclization of **133** and immediate reduction provided the 1,3-diol **134** in 42% yield and 33% recovered starting material. The aldol reaction failed to give the desired relative stereochemistry, and the hydroxymethyl group was inverted by base catalyzed epimerization of ketone **135**, derived from **134** by way of a three step sequence (68% yield overall). When ketone **135** was treated with DBU in THF at room temperature for 2 h, an equilibrium mixture of **136** and **135** in a ratio of *ca.* 2:1 was isolated. This mixture was readily separated to give **136** (64% yield) along with starting material **135** (34% yield). Ketone **136** was reduced with $NaBH_4$ to afford diol **137** as a single diastereomer.

With the key intermediate **137** in hand (note similarity to **97** in Scheme 22), efforts were focused on oxidizing the amine and forming the bicyclic hemiketal. Towards this end, **137** was converted to acetate **138** (27% yield for four steps) *via* a sequence involving selective silylation of the primary alcohol, cleavage of the Alloc protecting group, oxidation of the liberated secondary amine with *m*-CPBA, and acetylation of the hydroxylamine. As in Fukuyama's total synthesis (see Scheme 22, **97** $\rightarrow$ **98**), the oxidation of the amine stopped at the hydroxylamine. Oxidation of alcohol **138** followed by desilylation furnished alcohol **139**. As with the Fukuyama and Williams syntheses, the last critical step in this synthetic scheme was the formation of the bicyclic hydroxylamine hemiketal ring system. Removal of the acetyl group of **139** by treatment with potassium carbonate in methanol cleanly produced the free hydroxylamine, which under went spontaneous cyclization to form bicyclic compound **140** in 89% yield. Diol **140** was treated with diphosgene. The resulting cyclic carbonate was subjected to regioselective ammonolysis to form the carbamate and further acetylation of the hemiketal afforded **141** (66% yield for two steps). Compound **141** had previouslybeen shown by a relay synthesis, starting from FK 973 (**3**), to furnish the desired natural

Scheme 26. Terashima's total synthesis of (±)-FR 900482



Key: (a) NaH, THF, -78 °C to rt, 100%; (b) Zn, AcOH, THF/H_2O, rt; (c) TsCl, Et_3N, DMF, 0 °C to rt, 77% for two steps; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 94%; (e) NaH, Im, THF, reflux, 92%; (f) HF•Py, Py, 0 °C, 99%; (g) DMPI, CH_2Cl_2, rt, 98%; (h) LiHMDS, THF, -78 to -5 °C, then $NaBH_4$, H_2O, -5 to 0 °C, 42%; (i) TBSCl, Et_3N, DMAP, CH_2Cl_2, rt, 79%; (j) DMPI, CH_2Cl_2, rt, 93%; (k) HF•Py, Py, 0 °C to rt, 93%; (l) DBU, THF, rt; separation (64% for desired isomer, 43% for undesired); (m) $NaBH_4$, THF/H_2O, 0 °C to rt, 87%; (n) TBSCl, Et_3N, DMAP, CH_2Cl_2, rt, 71%; (o) $(Ph_3P)_4Pd$, Ph_3P, THF, rt, 83%; (p) *m*-CPBA, CH_2Cl_2, -5 °C, 67%; (q) Ac_2O, $NaHCO_3$, rt, 69%; (r) DMPI, CH_2Cl_2, rt, 88%; (s) HF•Py, Py, 0 °C to rt, 88%; (t) K_2CO_3, MeOH, 0 °C to rt, 89%; (u) $ClCO_2CCl_3$, Py, 0 °C to rt, 81%; (v) NH_3, THF, 0 °C to rt, 94%; (w) Ac_2O, Py, DMAP, rt, 87%; (x) sodium naphthalenide, DME, -70 °C, 84%; (y) 10% Pd/C, H_2, EtOAc, rt, 81%; (z) DMSO, $(ClCO)_2$, Et_3N, CH_2Cl_2, -78 °C, 88%; (aa) NH_3, MeOH, rt, 73%.

product.[31] Finally, **141** was converted to the fully synthetic (+)-FR 900482 in the same manner as detailed in the relay synthesis. The oxidation of the benzylic alcohol to an aldehyde using Swern condition is of particular interest since it was accomplished in the presence of the free aziridine.

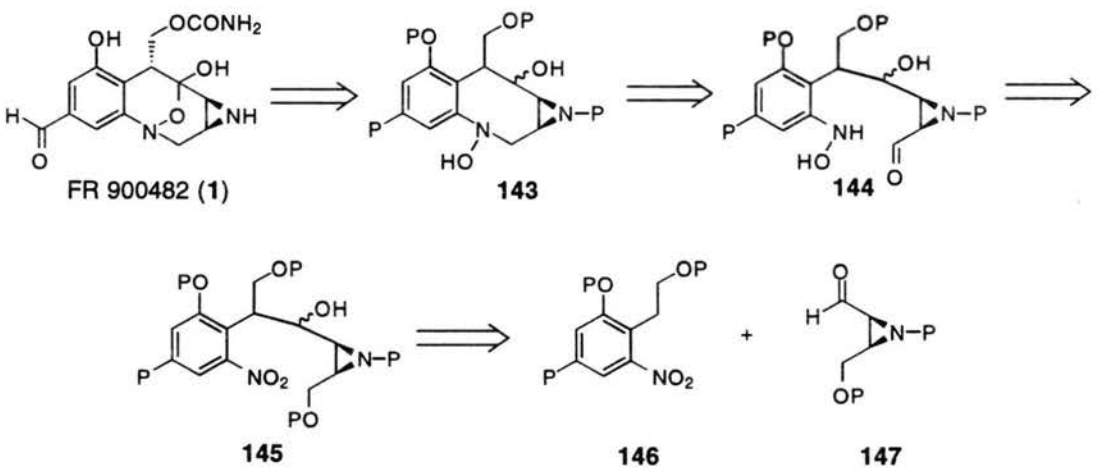
Chapter 2

Synthetic Model Studies

2.1 Synthetic Analysis

In our synthetic analysis of FR 900482 (**1**), the structural similarities between it and MMC (**4**) were noted. As discussed in Section 1.7, the central ideas of Kishi's synthesis of MMC[50] could be applied to the construction of FR 900482. In our own laboratories, the synthetic model study by Yasuda and Williams[18] used an adaptation of Kishi's strategy to synthesize the core skeleton of FR 900482. In a slight departure from conventional methods, Yasuda and Williams avoided the issue of benzazocine nitrogen oxidation by reducing arylnitro compound **37** to the hydroxylamine **38** (See Scheme 11). In an effort to capitalize on this discovery and to efficiently construct the natural product

Scheme 27. Retro synthetic analysis of FR 900482



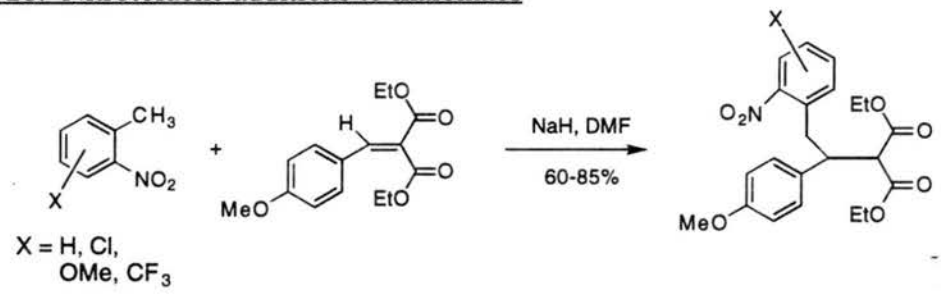
in asymmetric form, a unique approach to the natural product was designed with the retrosynthetic analysis outlined in Scheme 27. Intermediate **143** was viewed as arising from **144** by reductive amination as performed in the Yasuda and Williams model study (See Scheme 11). Through a series of oxidation state modifications and deprotections, **144** would be constructed from **145**. Finally, coupling of **146** and **147** by deprotonation of the benzylic position of **146** and nucleophilic addition to **147** would produce **145**. Addition of the homobenzylic alcohol to the aziridine would install all requisite carbons for future elaboration to the natural product including the (carbamoyloxy)methyl side chain. Aziridine **147** would be constructed from an optically active epoxide whose stereochemistry would be set using Sharpless asymmetric epoxidation[55] methodology. In this synthetic route to the natural product three points were at issue. First, would the aziridine function survive the numerous steps needed to elaborate **145** into FR 900482? Second, would the reductive amination used in the model study work in a fully functionalized system, and finally, what ways could the stereochemistry of the first carbon-carbon bond formation be controlled? Below is a description of the efforts made to implement this synthetic strategy.

2.2 Coupling reactions of nitrotoluene derivatives and functionalized aldehydes

The first key carbon-carbon bond formation involved generating a benzylic anion of a nitrotoluene derivative and condensing it with an aldehyde. While a similar reaction had been used in Williams' model study (See Scheme 11, **33 --> 34**), it was thought the conditions used for this transformation (KOH, DMSO) would hydrolyze an aziridine function if a similar reaction were used to couple derivatives of **146** and **147**. A literature search for reactions using benzylic anions of nitrotoluene derivatives yield several publications.[56-59] One study described the Michael addition of various nitrotoluene derivatives to α,β-unsaturated malonate derivatives using sodium hydride in DMF (Scheme 28).[58] Using similar conditions, commercially available nitrotoluene was

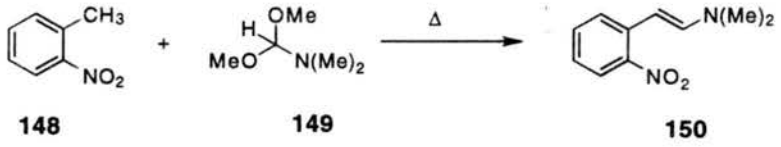
treated with sodium hydride in DMF followed by the addition of benzaldehyde. From the experimental results, it was concluded that the anion of nitrotoluene was generated under these conditions but that the reaction conditions were not suitable for the nucleophilic addition to benzaldehyde.

Scheme 28. Nitrotoluene additions to malonates



In another reference dealing with reactions of nitrotoluenes, the condensation of nitrotoluene **148** with *N,N*-dimethylformamide dimethylacetal (DMFDMA) (**149**) produced enamine **150** (Scheme 29).[57] The transformation is significant since no base is added to the reaction. A catalytic amount of methoxide is generated from the DMFDMA upon heating, and the methoxide deprotonates the methyl group of nitrotoluene.

Scheme 29. Enamine formation



148 **149** **150**

Using this information, several different alkoxide and hydroxide bases were used to generate the benzylic anion of nitrotoluene and couple it with model aldehyde **155** (Table 1). Aldehyde **155** originated from *p*-anisaldehyde (**151**) and 2-butene-1,4-diol (**152**) by the four step sequence shown in Scheme 30. Combination of **151** and **152**

Scheme 30. Formation of epoxy aldehyde

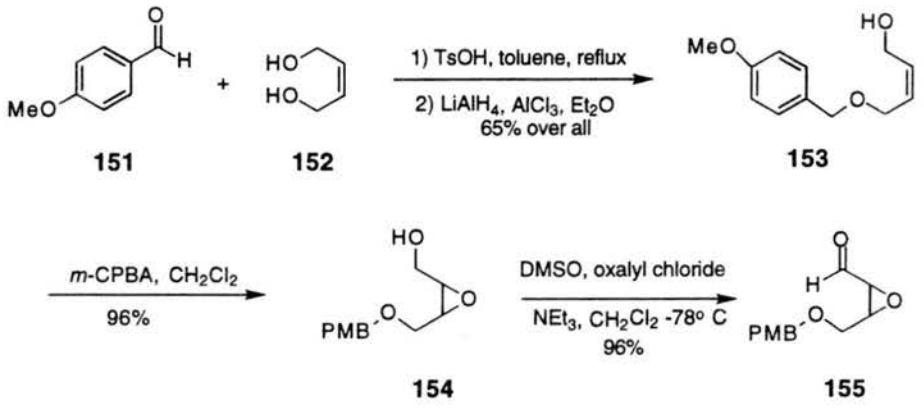
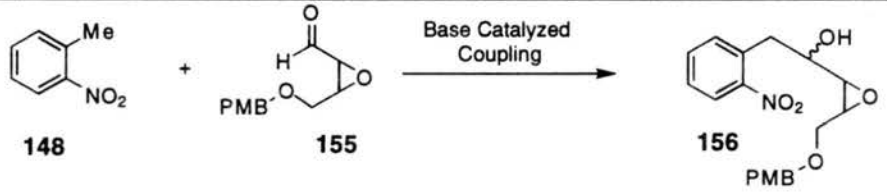


Table 1. Base catalyzed coupling



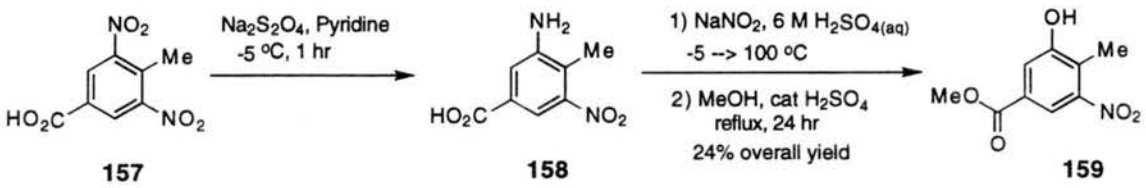
Entry	Conditions	Results
1	NaOH, DMF, 0 °C --> rt	< 5%
2	*t*-BuOK, DMF, -10 °C --> rt	< 5%
3	Et_3N, DMF, rt	No reaction
4	*n*-Bu_4NOH/MeOH, THF, -10 °C	No desired products
5	*n*-Bu_4NOH/H_2O, NaOH, CH_2Cl_2, rt	No desired products
6	*n*-Bu_4NOH/MeOH, DMF, -10 °C,	12%
7	0.1 eq NaOMe, DMF, 4 °C	8%
8	0.5 eq NaOMe, THF, -35 °C --> rt	No desired products
9	NaHMDS, THF, -78 °C	No desired products

formed a cyclic acetal and reduction of the acetal yield the *cis*-monoprotected allylic alcohol **153**. Epoxidation with *m*-CPBA and oxidation under Swern conditions[60] generated the aldehyde **155**. Although this seems to be a convoluted route to aldehyde **155**, the cyclization and reduction steps ensure the facile formation and purification of only the *cis*-monoprotected alcohol **154**. Diol **152** is commercially available only as a 95:5 mixture of *cis/trans* isomers.

A great deal of time was spent investigating methods to perform the coupling reaction shown in Table 1. Although the majority of the reactions listed gave no significant yield of alcohol **156**, one item was gleaned from the poor experimental results which accelerated future efforts to couple similar molecules. Primarily, the types and strengths of bases necessary to form the benzylic anion of nitrotoluene derivatives were determined.

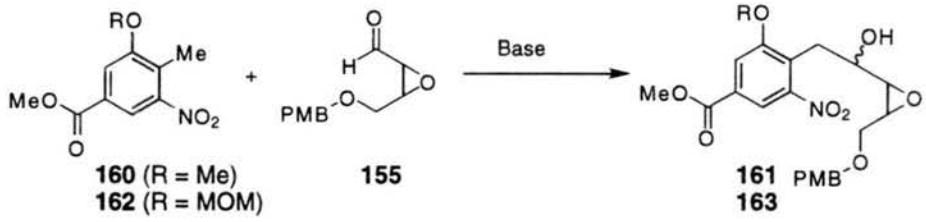
After the failure to successfully couple **148** and **155** in high yields, we decided to attempt the desired condensation with other nitrotoluene derivatives which had the appropriate functionalities in place to eventually form FR 900482. The core structure of the aromatic piece, methyl 3-hydroxy-4-methyl-5-nitrobenzoate (**159**), was prepared from 3,5-dinitro-*p*-toluic acid (**157**) according to the procedure of Neilson *et al.*[61] by successive sodium dithionite reduction, diazotization, and esterification (Scheme 31). In our hands and others,[51] the dithionite reduction proved troublesome. We modified the dithionite reduction by diluting the reaction mixture but were still unable to reproduce the yields reported.[19,61] To circumvent their problems with the dithionite reduction, Ziegler[51] used a modified Zinin reduction (ammonium sulfide) of toluic acid **157** toaccess the intermediate *m*-anthranilic acid (**158**). With phenol **159** in hand, several attempts were made to construct the carbon-carbon bond shown in Scheme 32. The first protecting group used for the phenol function was a methyl group. Although alkoxide bases were known effect the type of transformation shown in Scheme 32, sodium and

Scheme 31. Synthesis of aromatic core structure



potassium bis(trimethylsilyl)amide (NaHMDS and KHDMS) were initially used to form the benzylic anion of **160** (R = Me) due to results of Rapoport (See Scheme 12, **47 --> 48**) with a similar system and transformation. Treatment of **160** under the conditions described by Rapoport followed by the addition of aldehyde **155** failed to produce alcohol **161**.

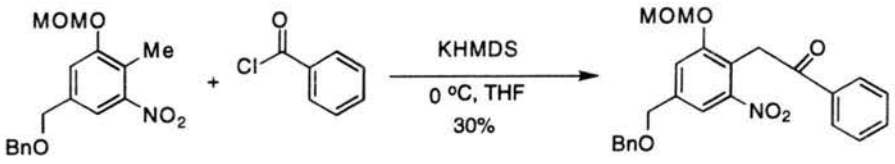
Scheme 32. Coupling with functionalized aromatic piece



After careful examination of other researcher's unpublished results from this laboratory,[62] a note was found describing the coupling reaction shown in Scheme 33. The success of the reaction was attributed to the choice of the methoxymethyl (MOM) protecting group for the phenol function since reactions under identical conditions using other phenol protecting groups (Me or Bn) failed to produce any products. The phenol of **157** was protected with MOMCl (*i*-Pr_2NEt, CH_2Cl_2/THF, 97% yield) to give **162** (R = MOM) and attempts were made to couple it with aldehyde **155** (Scheme 32). The first experiments to effect the connection used KHMDS and NaHMDS in THF to form the benzylic anion of **162**, but these reactions produced no desired products. Finally,

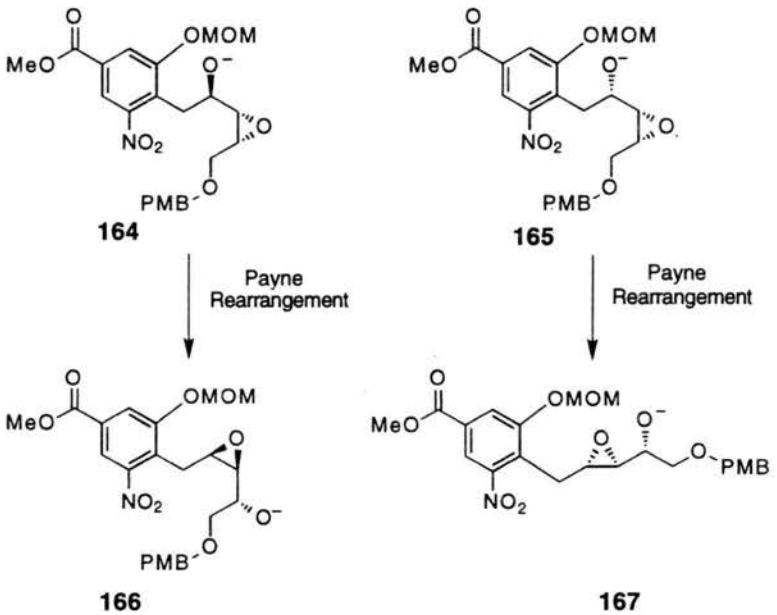
alkoxide bases were used to attempt the coupling reaction between **162** and **155**. The best results were achieved when a solution of **162** in DMF was treated with a solution of sodium methoxide in methanol followed by the dropwise addition of aldehyde **155**. Alcohol **163** was produced in 75% yield as a 1:1 mixture of separable diastereomers.

Scheme 33. Coupling of aromatic piece with ester



One potential problem with this method to form the desired carbon-carbon bond is the possibility of a Payne rearrangement[63-66] of the alkoxide intermediates **164** and **165**. If a Payne rearrangement occurred, the diastereomers **164** and **165** would produce

Scheme 34. Payne rearrangement



epoxides **166** and **167**, respectively (Scheme 34). Due to the S_N2 nature of the reaction, **166** is a *cis*-epoxide while **167** is a *trans*-epoxide. After careful examination of the two isolated products of the condensation of **162** with **155**, no evidence was seen for the formation of the *trans*-epoxide **167**. Typical coupling constants for *trans*-epoxide protons are ~2.5 Hz,[67] and the observed coupling constants for the signals identified as the epoxide protons in **163** and were ~4 Hz, typical coupling constants for *cis*-epoxides.[67] While this observation rules out the formation of **167**, it does not eliminate the possibility of formation of **166**. Future synthetic studies confirmed no rearrangement products were formed.

With the viability of the carbon-carbon bond formation demonstrated with epoxide **155**, efforts were focused on synthesizing an analogous aziridine and attempting similar coupling reactions.

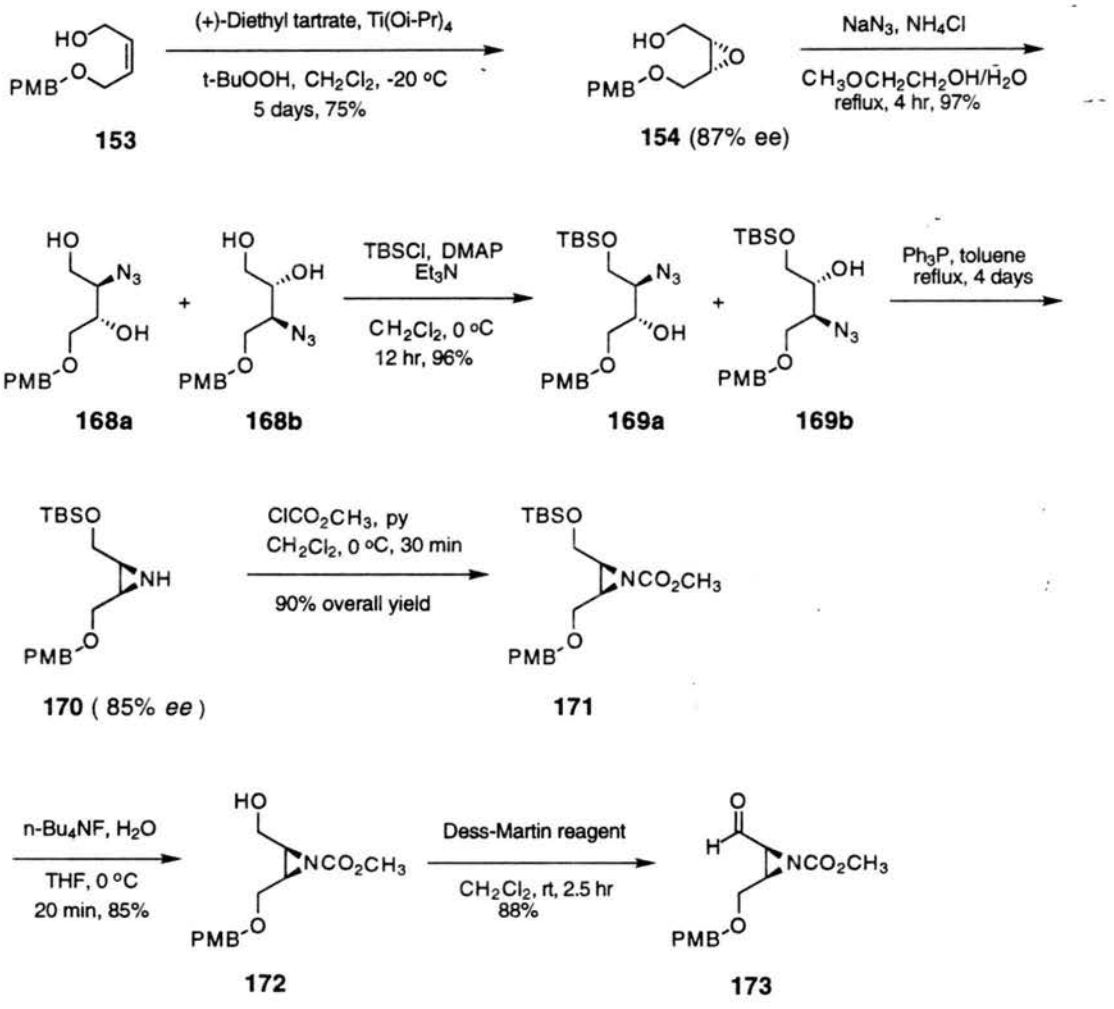
2.3 Synthesis of aziridine piece

The synthesis of the aziridine **173** starts with the Sharpless asymmetric epoxidation[55] of allylic alcohol **153** to produce epoxide **154** in 87% ee (± 2%) as measured by the ^{19}F NMR of the Mosher ester[55,68] derivative of **154** (Scheme 35). The asymmetric induction is consistent with reported data of other researchers[55] who have used the Sharpless reaction on *cis*-disubstituted allylic alcohols similar to **153**. More specifically, epoxide **154** was constructed in Terashima's total synthesis of FR 900482[31] using two different routes. The first route was the same as ours, and the enantiomeric excess was the same as we observed. In a second route (See Scheme 25), researchers started with L-diethyl tartrate, and epoxide **154** was constructed in eight steps (52% overall yield) with an ee of 98%.

Using Sharpless methodology, we found several procedures were necessary to optimize the synthesis of epoxide **154** and to scale up the reaction. First, all reagents had to be freshly distilled before each reaction. Second, the reaction needed to be run at low

temperature (-20 °C) for an unusually long period (4-5 days). Lastly, the reaction was run with stoichiometric amounts of titanium catalyst and thereby increased the time necessary for work up and purification. Typical Sharpless epoxidations of *trans*-disubstituted allylic alcohols are usually performed in a few hours with a catalytic amount of titanium catalyst (5 mol%) with ee's higher than 95%.[55] As a result of these difficulties, racemic **154** was used in initial experimental investigations followed by optically active **154**.

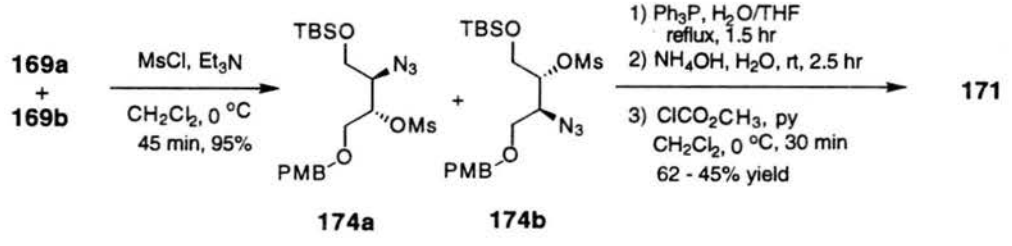
Scheme 35. Synthesis of aziridine piece



Transformation of epoxide **154** into aziridine **173** followed typical literature procedures.[69] Ring opening of epoxide **154** with sodium azide[66,70] in the presence of ammonium chloride resulted in the formation of a mixture of regioisomers **168a** and **168b** in a ratio of *ca.* 3:2. Since both isomers could be used to construct aziridine **173**, they were separated only for characterization. Selective protection of the primary alcohol of **168a** and **168b** afforded silyl ethers **169a** and **169b**. Once again, these isomers were separated only for characterization.

Originally, aziridine **170** was synthesized from **169a** and **169b** by the route shown in Scheme 36. After the formation of mesyl azides **174a** and **174b**, they were treated with triphenylphosphine[71] in water and THF. It was assumed that the putative iminophosphorane intermediate would be readily hydrolyzed to form triphenylphosphine oxide and the free secondary amine. Upon treatment with mild base, the free secondary amine would displace the mesyl group to form aziridine **170** which would be immediately protected with methyl chloroformate. Unfortunately, the iminophosphorane intermediate did not readily hydrolyze. Low and inconsistent yields for the three step transformation from **174a** and **174b** to **171** were seen.

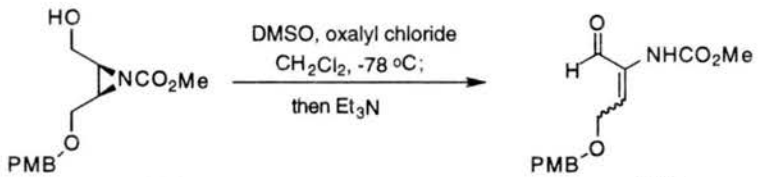
Scheme 36. Initial Staudinger reaction



It was known that compounds similar to **169a** and **169b** had been transformed into aziridines in one step upon treatment with triphenylphosphine under anhydrous conditions.[72,73] These reactions typically were complete in less than 1 h with high

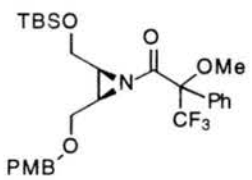
yields. When **169a** and **169b** were treated to similar conditions as those shown in Scheme 35 for 1 h, aziridine **170** was obtained in a low yield. Upon additional investigation, it was seen that a dramatic extension of the reaction time from 1 h to 4 days was necessary to convert **169a** and **169b** into **170** in high yields. Although the reaction time is extremely long and has the extended risk of accidentally destroying the compound if the solvent evaporates, this route is a significant improvement over the initial method used to synthesize **170**. The reaction is easily reproduced and much less labor intensive than the route shown in Scheme 36. Note that aziridine **170** was isolated only for characterization and typically protected with methyl chloroformate to give **171** before purification. The silyl ether **171** was cleanly deprotected with TBAF to produce alcohol **172** which was oxidized with the Dess-Martin periodinane [74-76] to generate the target compound, aziridine **173**. Attempts to oxidize alcohol **173** under Swern conditions[60] resulted in the destruction of the aziridine ring to form the α,β-unsaturated aldehyde **175** (Scheme 37).

Scheme 37. Swern oxidation of aziridine



172 **175**

The optical purity of the unprotected aziridine **170** was measured by formation of the Moser amide[68] derivative **176**. The diastereomeric peaks of an aziridine hydrogen of



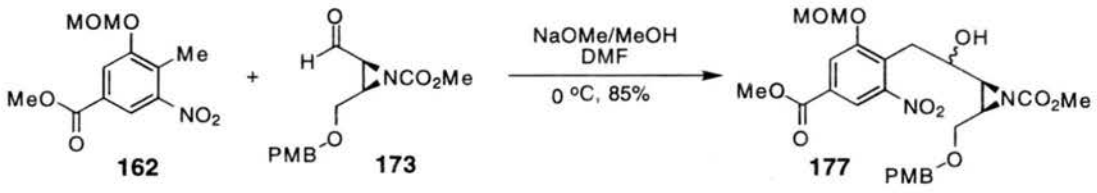
176

176 were resolved by ^{1}H NMR, and the ee was determined to be 85% (± 2%). These results are consistent with the stereospecific formation of the aziridine **170** from epoxide **154** resulting in the net inversion of both stereocenters.

2.4 Synthesis of benzazocine ring

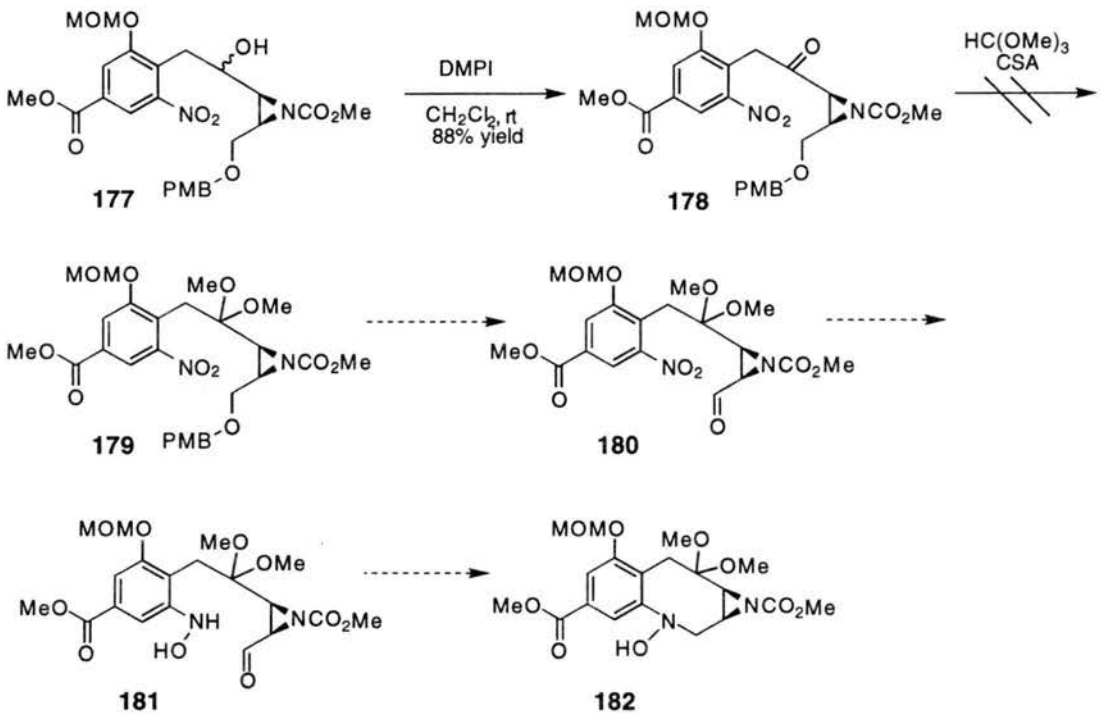
Once aziridine **173** had been synthesized, the coupling reaction between it and nitrotoluene **162** was attempted under the identical conditions used to couple **155** and **162**. As shown in Scheme 38, alcohol **177** was produced as a 4:1 mixture of separable diastereomers. Each diastereomer was fully characterized, and all data was consistent with the desired structure and the previously synthesized analogous alcohol **163**. Although initial yields were moderate (~50%), the reaction was eventually optimized to an 85% yield and performed on a multigram scale.

Scheme 38. Successful coupling reaction

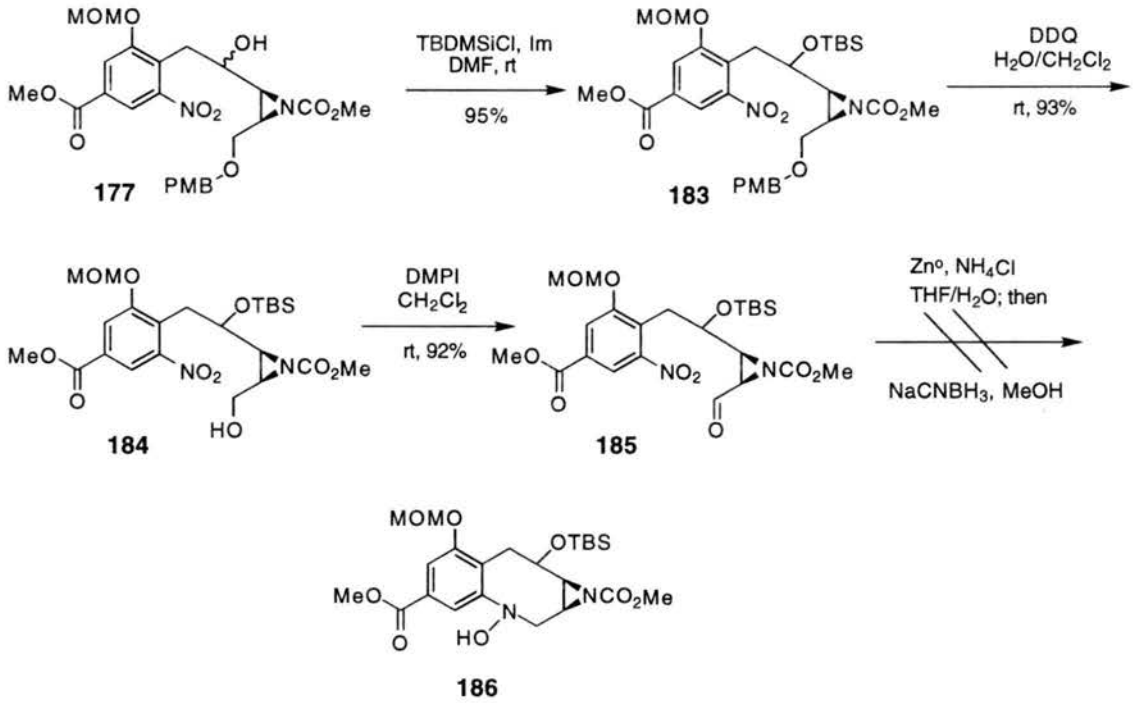


Once a large quantity of alcohol **177** had been synthesized, two routes to form the eight-membered ring following the logic of Yasuda and Williams' model study[18] (See Scheme 11) were investigated. The first route would begin with the oxidation of the secondary alcohol of **177** to yield ketone **178** and protection as the dimethyl ketal would produce ketal **179** (Scheme 39). Removal of the PMB protecting group followed by oxidation would give aldehyde **180**. Reduction of the nitro group of **180** to an hydroxylamine (**181**) and reductive amination of the resulting nitrone would yield the desired benzazocine ring **182**. The first step in this synthetic sequence proceeded

Scheme 39. First proposed route to benzazocine ring

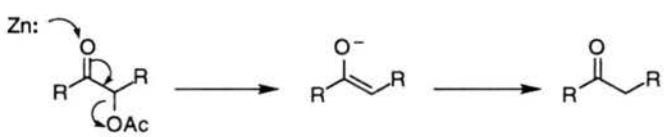


Scheme 40. Second proposed route to benzazocine ring



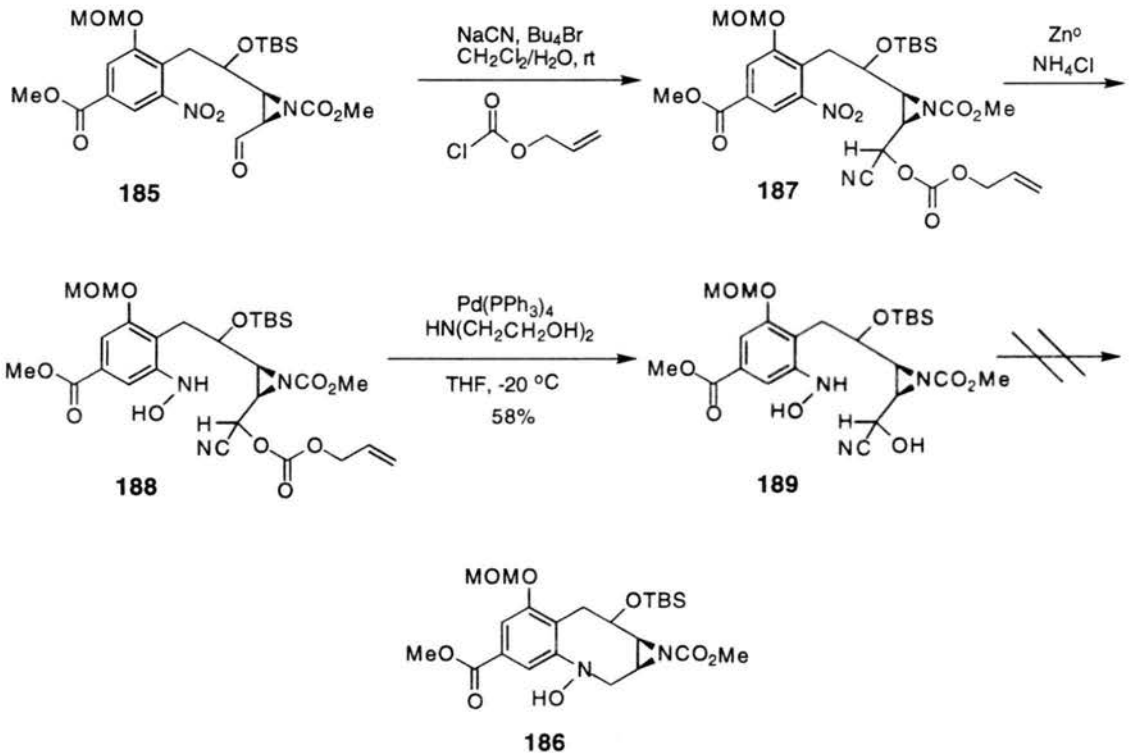
smoothly. The Dess-Martin oxidation[76] of the secondary alcohol **177** produced the corresponding ketone **178** in 88% yield. Unfortunately, attempts to protect ketone **179** using trimethylorthoformate failed. The aziridine function was probably cleaved under the acidic conditions affording several different products. No further attempts were made to protect the ketone. One side note is that oxidation of the diastereomeric mixture of **177** to one ketone proved no Payne rearrangement was taking place during the coupling reaction in Scheme 38. With the problems in protecting ketone **178**, a more direct route to the benzazocine ring was attempted. This route also followed the logic of our published model study,[18] but did not attempt to protect the aldehyde since no ketone functionality would be present. The first three synthetic steps proceeded smoothly. Using a single diastereomer, alcohol **177** was protected as a silyl ether to yield **183** (Scheme 40). The PMB group of **183** was removed[77] producing the free primary alcohol **184** which was oxidized[76] affording aldehyde **185**. The last step involves the selective reduction of a nitro function to an hydroxylamine, formation of a cyclic nitrone, and finally reductive amination to form **186**. All attempts to construct **186** under the conditions described failed. After careful analysis of the many side products from this reaction, it was found that the aziridine was destroyed during the zinc reduction step. Most likely, the zinc cleaves the aziridine ring through a mechanism similar to the reduction of α-aceto-ketones[78] with zinc (Scheme 41). This was our first indication of the extremely reactive and labile nature of the α-aziridine aldehyde function.

Scheme 41. Zinc reduction of α-aceto-ketones



The next effort to synthesize the benzazocine ring followed our published model study[18] more closely and protected the aldehyde before reducing the nitro group. The aldehyde of **185** was protected as the allyloxycarbonyl cyanohydrin to give **187** in high yield as a 1:1 mixture of diastereomers (Scheme 42). The diastereomers were separated and taken on individually. With the aldehyde protected, the nitro group of **187** was reduced using zinc dust yielding hydroxylamine **188**. Characterization of the diastereomers of **188** proved difficult. The heteroatom protons of either diastereomer could not be seen in their ^{1}H NMR spectra in $CDCl_3$ or d_6-benzene, although their IR spectra showed characteristic stretches for an hydroxylamine (broad stretch 3300-3000 cm^{-1}). The carbonyl protecting group was removed from **188** using the same

Scheme 42. Third proposed route to benzazocine ring

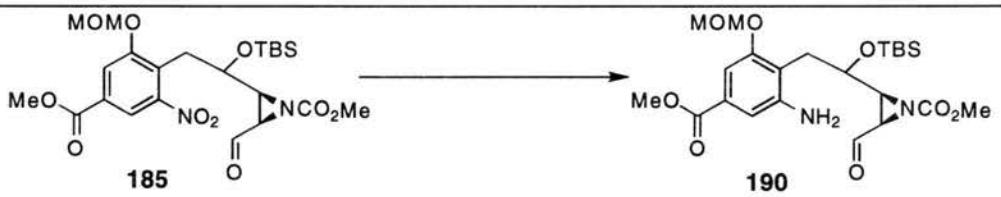


reported by Yasuda and Williams,[18] to produce the stable cyanohydrin **189** in moderate yield (58%) whose ^{1}H NMR spectrum showed the three heteroatom peaks expected. With compound **189** prepared, the ring closure was attempted using $NaCNBH_3$ under neutral, acidic, and basic conditions. The TLC's of the crude reactions were extremely complex, and no major products could be distinguished. The ^{1}H NMR of the crude reactions also showed a complex mixture of products including arylamines and alcohols resulting from the reduction of the hydroxylamine and aldehyde functional groups.

With the failure of the routes to the benzazocine ring based on Yasuda and Williams' model study, a new route to the eight membered ring was planned. Following logic similar to Fukuyama's[29] in his total synthesis of FR 900482 (See Scheme 21), an intramolecular reductive amination between an aniline and an aldehyde would be attempted. Starting with aldehyde **185**, a wide variety of conditions were used in an effort to reduce the nitro group to an amine without disturbing the aldehyde or aziridine functions (Table 2). Reductions under transfer hydrogenation conditions (Entry 1, 2, and 3) led to a complex mixture of products. A simple hydrogenation (Entry 4) reaction gave the desired amino aldehyde **190** but was capricious in nature. The same phenomenon was seen for the reduction of the nitro group with Lindlar's catalyst (Entry 5). Reduction with stannous chloride dihydrate gave only decomposition products (Entry 6 and 7). The failure of these reactions to consistently produce the desired amino aldehyde **190** under mild conditions is presumably due to the activated nature of the aldehyde function.

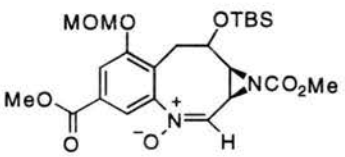
Initially, Lindlar's catalyst reduction of the nitro group was chosen as the preferred method to generate **190**. After many attempts to determine reproducible conditions for the reduction, the reaction proved to be extremely sensitive to the batch of catalyst used, and only one batch out of five (two were purchased commercially and three were prepared[79] in our labs) would produce **190** in high yields. Returning to 5% Pd/C, the most reproducible yields were found when the catalyst was loaded with hydrogen by

Table 2. Reductions of nitro group to an amine



Entry	Conditions	Results
1	5% Pd/C, HCO_2NH_4, MeOH, rt	No desired products
2	5% Pd/C, HCO_2Na, HCO_2H, MeOH, rt	No desired products
3	10% Pd/C, cyclohexene, EtOH, reflux	No desired products
4	5% Pd/C, H_2 (1 atm), MeOH, rt	90 - 50% yield
5	Pd/$CaCO_3$/Pd, H_2 (1 atm), MeOH, rt	90 - 50% yield
6	$SnCl_2$•2 H_2O, EtOAc, reflux	No desired products
7	$SnCl_2$•2 H_2O, EtOH, reflux	No desired products

bubbling the gas through a solution of 5% Pd/C in MeOH for 30 min and then stirring the catalyst under a hydrogen atmosphere for another 30 min. Upon addition of aldehyde **185**, the starting material was converted into **190** in 10 min. Immediate filtration through a Celite pad was necessary to prevent the formation of side products. If the palladium



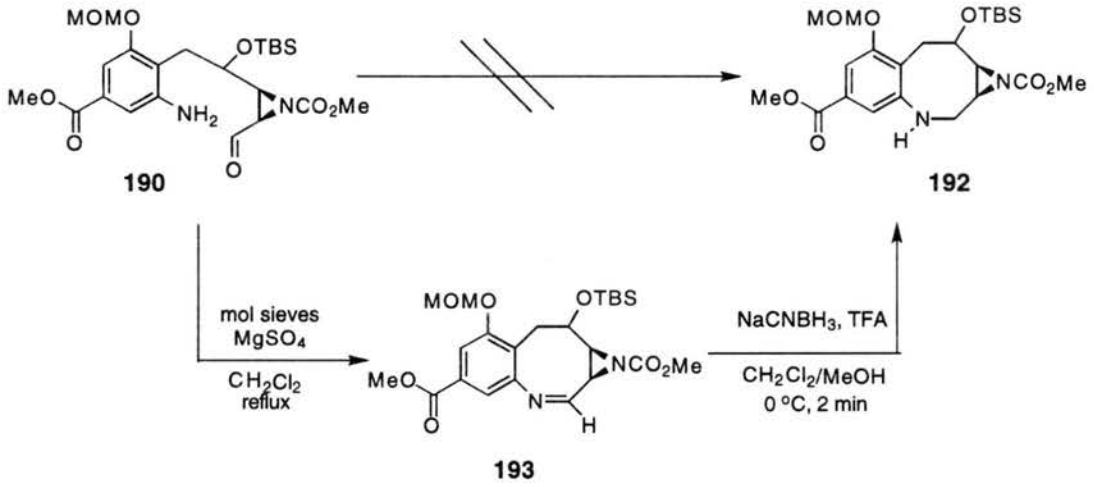
191

catalyst was not loaded with hydrogen before **185** was introduced into the reaction flask, the major product isolated was cyclic nitrone **191**. The structure of this side product was

not identified until it had been synthesized using a different route (vide infra). It was assumed that **191** forms when the nitro function of **185** was reduced to the hydroxylamine and was trapped by the aldehyde before it could be reduced further.

With amino aldehyde **190** constructed, attempts were made to perform an intramolecular reductive amination. We had hoped **190** would cyclize spontaneously to form an imine and be reduced by $NaCNBH_3$. All efforts to proceed directly from **190** to **192** failed (Scheme 43). The major product isolated from these reactions was the reduced aldehyde, again pointing to the activated nature of the aldehyde of **185**. As a result, the sequential formation of the imine, isolation, and reduction was necessary. Formation of cyclic imine **193** from **190** proceeded smoothly under dehydrating conditions (4Å mol sieves, $MgSO_4$) in refluxing CH_2Cl_2. Reaction times of 24 to 36 h gave the best results with longer times producing side products. Reduction of imine **193** was very troublesome (Table 3). When the reduction was performed on a small scale (20 mg) with $NaBH_4$, **192** was isolated, but no yield was calculated. Upon scale-up (Entry 1), large

Scheme 43. Reductive amination



amounts of side products were isolated. The amount of $NaBH_4$ used in the reduction was increased (Entry 2) in hopes of forming **192** before **193** could hydrolyze and form side

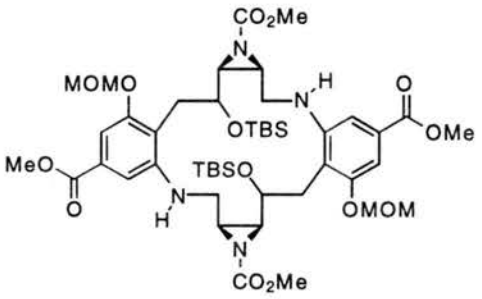
products, but no improvement was seen. Other less traditional reagents were used in an attempt to form **192** (Entry 3 and 4), but no products were isolated. Acidic solutions of sodium cyanoborohydride are typically used to reduce imines to amines.[80] We thought the low pH of these reactions would cleave the aziridine ring, so preliminary reactions using $NaCNBH_3$ were performed under neutral conditions. (Entry 5). The reduction of **193** using $NaCNBH_3$ in MeOH without acid proceeded slowly with a complex mixture of products forming including a small amount of **192**. Finally, the conditions used in Fukuyama's total synthesis[29] of FR 900482 to reduce cyclic imine **92** (See Scheme 21) were used (Entry 6), and **192** was isolated in 60% yield for the three step transformation from **185**.

Table 3. Reductive amination conditions for **193** to **192** in Scheme 43

Entry	Conditions	Results
1	2 eq $NaBH_4$, MeOH, rt, 10 min	35% yield
2	14 eq $NaBH_4$, MeOH, 10 °C --> rt, 2 h	Decomposition
3	$LiAlH(O\text{-}t\text{-}Bu)_3$, THF, 0 °C --> rt, 24 h	Decomposition
4	5% Pd/C, H_2 (1 atm), THF, 24 h	Decomposition
5	2 eq $NaCNBH_3$, MeOH, rt, 4 h	Low yield
6	1 eq $NaCNBH_3$, TFA, MeOH/CH_2Cl_2, 0 °C, 2 min	60% yield from **185**

One of the more interesting side products isolated from the reduction of imine **193** was the 16-membered ring dimer **194**. It was isolated from some reaction mixtures in the same amount as **192**. In an effort to minimize dimer production, the reaction mixture to form imine **193** was diluted to roughly 0.002 M in **185**, and the products were

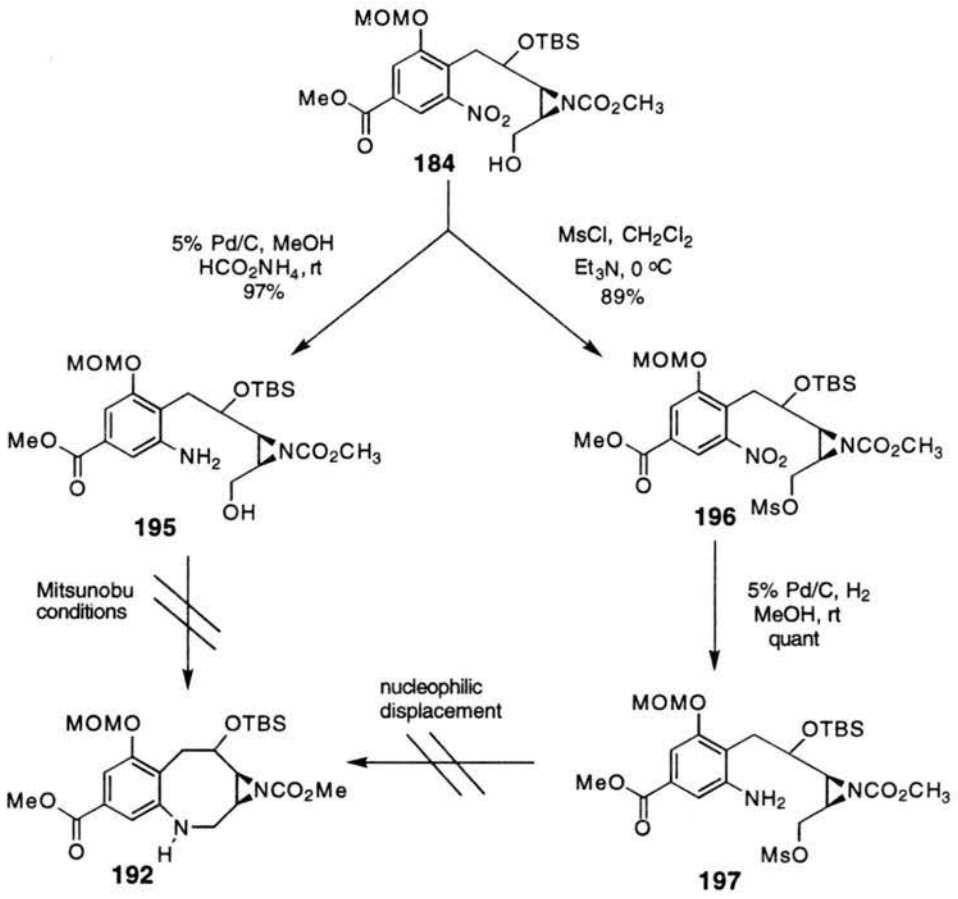
immediately characterized and prepared for the following reaction. Although these steps decreased dimer production, a much larger decrease was seen when dichloromethane, instead of ethyl acetate, was used as the only solvent to facilitate the transfer of imine **193** from flask to flask. The ^{1}H NMR spectrum of **194** is almost identical to **192**, but the R_f of the dimer is much lower than that of the monomer.



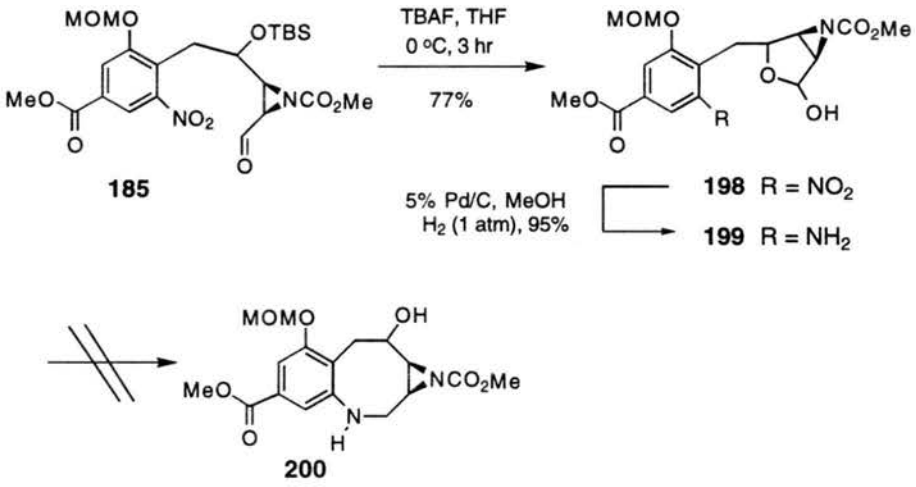
194

While standardizing the cyclization reaction in Scheme 43, other routes to the cyclic amine **192** were investigated but were eventually abandoned in favor of the reductive amination. These reactions are mentioned to illustrate the range of possible approaches to the benzazocine ring that might work if there were slight changes to the molecule, such as the delayed construction of the aziridine ring until the eight membered ring is constructed. The first route investigated involved the formation of amino alcohol **195** from the previously constructed nitro alcohol **184** (Scheme 44). Subjecting amino alcohol **195** to standard Mitsunobu conditions,[81,82] we hoped it would cyclize to form **192**. This type of reaction had been successfully executed during Rapoport's synthesis[19] of the core structure of FR 900482 (See Scheme 12). Unfortunately, when **195** was treated with Mitsunobu conditions, no products were observed and slow decomposition of the starting material resulted after 24 h. It is assumed the amine function of **195** is not acidic or nucleophilic enough to effect the second step of the Mitsunobu reaction. A second unsuccessful route to form **192** also began with nitro alcohol **184** (Scheme 44). Mesylation of **184** followed by smooth reduction of the nitro group produced amine **197**.

Scheme 44. Mitsunobu and nucleophilic cyclizations



Scheme 45. Failed reductive amination



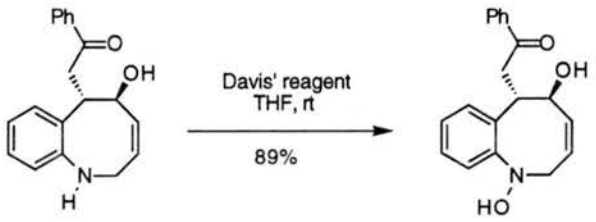
Efforts to cyclize **197** through an intramolecular displacement of the mesyl group failed. Several different bases (Et_3N, NaH, and BuLi) and solvents were used with no success.

The last approach to the benzazocine ring investigated was a variation on the reductive amination route. As shown in Scheme 45, removal of the silyl protecting group of aldehyde **185** formed lactol **198**. No signal for the aldehyde could be seen by ^1H NMR, and only one diastereomer of the lactol was isolated. We thought the reduction of the nitro group of **198** to produce aniline **199** would be difficult due to the problems encountered during investigation of the reduction of **185**. The reduction of **198** went quickly and in high yield with no other products seen by TLC or ^1H NMR. Compound **199** is a stable compound and not prone to dimerization. The contrast in reactivity between **185** and **198** is further evidence of the activated nature of the aldehyde function of **185**. With **199** in hand, efforts to form **200** directly by reductive amination ($NaCNBH_3$, acid) failed. In addition, efforts to form and isolate the cyclic imine under dehydrating conditions in acidic, neutral, and basic conditions saw no change in the starting material.

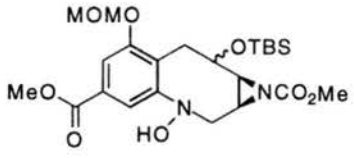
2.5 Oxidation of benzazocine ring

Once benzazocine **192** had been formed, attempts were made to oxidize it to hydroxylamine **201**. Typically Davis' reagent (2-(Phenylsulfonyl)-3-phenyloxaziridine)[83,84] or *m* -CPBA are used to oxidize secondary amines to hydroxylamines. In examples more specific to the synthesis of FR 900482, Fukuyama and Goto[17] showed Davis' reagent successfully oxidized a benzazocine to an hydroxylamine (Scheme 46) in high yields. Oxidation of other benzazocine derivatives with *m*-CPBA to hydroxylamines is seen in Fukuyama's[29] (Scheme 22, **97 --> 98**) and Terashima's[31] (Scheme 24, **137 --> 138**) total syntheses of FR 900482.

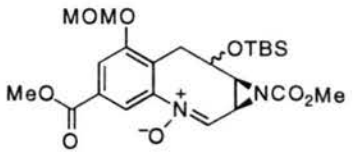
Scheme 46. Oxidation of amine with Davis' reagent



When Davis' reagent was used to oxidize benzazocine **201**, the result was a complex mixture of products not **201**. Next *m*-CPBA (50 - 85%) was used to



201



202

oxidize **192**, and the result was the quantitative conversion of the **192** to nitrone **202** rather than the desired hydroxylamine **201**. Initially, we was thought that over oxidation had occurred because an excess of *m*-CPBA had been used in the reaction. Upon careful addition of *m*-CPBA in less than 0.5 equivalents to a cold solution of **192**, a mixture of starting material and nitrone **202** was produced. This result shows that the rate of amine oxidation is much less than the rate of hydroxylamine oxidation under these conditions. Note that when the TLC of the *m*-CPBA reaction mixture was compared to the TLC of the Davis' reagent reaction mixture, Davis' reagent had also oxidized amine **192** straight to nitrone **202**.

Examination of our results and other researchers' who successfully oxidized benzazocine rings to hydroxylamines revealed that the primary difference was the oxidation state of carbon-12 (FR 900482 numbering). In our system, the carbon was in the acid oxidation state while in other systems it was either not present or in the alcohol

oxidation state. The electron withdrawing nature of the ester must faciliate the oxidation of the hydroxylamine to the nitrone.

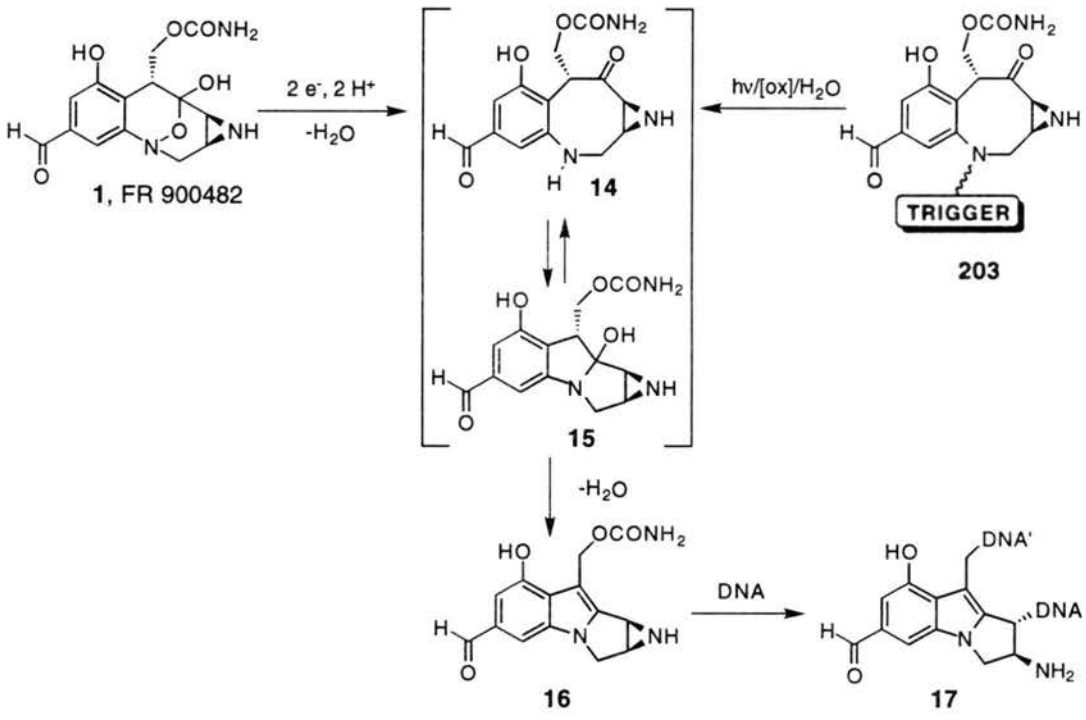
The ^{1}H NMR spectrum of nitrone **202** is quite unusual. The aromatic protons are 1.6 ppm apart with shifts of 8.08 and 6.46 ppm relative to TMS. An explanation for the large difference in chemical shift is that the aromatic proton *ortho* to the nitrone is shielded by the full negative charge on the oxygen. The aromatic proton *para* to the nitrone group is deshielded by resonance. Due to the unusual shifts of the aromatic protons of **202**, several proton decoupling experiments were conducted, and all results were consistent with the proposed structure.

Upon determination of the structure of nitrone **202**, a careful re-examination of the products from the reduction of **185** (Table 2, Entry 4) showed that **202** was formed when the catalyst was not loaded with hydrogen prior to introduction of starting material. If nitrone **202** could be used in the synthesis of FR 900482, taking **185** directly to nitrone **202** would eliminate three steps from the synthesis, so efforts were made to reduce nitrone **202** to hydroxylamine **201** using $NaCNBH_3$ and $NaBH_4$. Attempts to reduce nitrone **202** were performed using the same conditions used to reduce imine **193**: $NaCNBH_3$, TFA, MeOH/CH_2Cl_2, 0 °C. After 35 min, the reaction was quenched and a complex mixture was seen by TLC and ^{1}H NMR analysis with a moderate amount of starting material still present. Next, $NaBH_4$ was used to reduce nitrone **202**. The resulting compound looked promising by ^{1}H NMR with the aromatic peaks shifted to 7.65 and 7.34 ppm. The crude compound was treated with neat Ac_2O in hopes of obtaining the acetate protected hydroxylamine, but the major product isolated was not the desired compound. Other attempts to protect the hydroxylamine using AcCl were not successful.

2.6 Formation of photoactivated alkylating agents

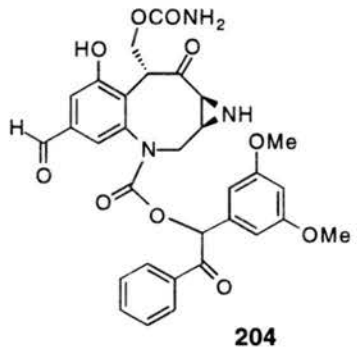
Frustrated by our inability to selectively oxidize the benzazocine ring, but determined to salvage our findings, synthetic chemistry was developed to construct a hitherto unknown class of latent mitosenes. Since both MMC and FR 900482 are reductively triggered mitosenes, we sought to expand the range of conditions used to trigger mitosene formation to potentially include photochemical, oxidative, and hydrolytic triggering. The basic concept is illustrated in Scheme 47 where the nitrogen of benzazocine **203** is protected with various urethane-type groups that can be removed by long wavelength ultraviolet light, by oxidation, or by hydrolysis. When these urethane groups are cleaved or triggered, intermediate **14** is generated which must cyclize and dehydrate to produce the highly reactive mitosene **16**.

Scheme 47. Latent mitosenes

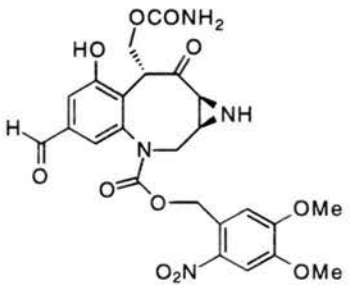


Access to these types of substances has several potentially important uses. These substances can potentially be utilized as new "pro-drug" forms of the mitomycins or FR-series drugs. Since reductive activation will not be required to generate the highly reactive mitosene, undesirable side effects of the reductive activation can be avoided. For example, MMC concomitantly produces oxygen radical species[49] upon reductive activation (See Scheme 6). Secondly, in the case of the photochemically activated species, these substances can serve as useful complements to the psoralen[85] photo-cross-linking agents which cross-link DNA in AT-rich regions at thymine residues. The latent mitosenes will only cross-link DNA in GC-rich regions.

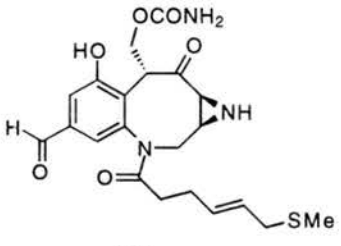
Many possible triggering groups can be envisioned, and a few representative examples are shown in Figure 6. The dimethoxybenzoin carbamate **204** and the *o*-nitroveratryl carbamate **205** can each be cleanly removed with light at >350 nm with little photochemical damage to DNA.[86,87] A potential oxidatively triggered group is allyl



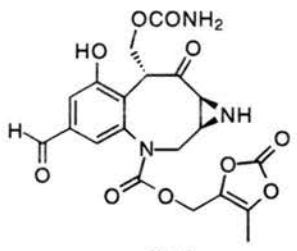
204



205



206



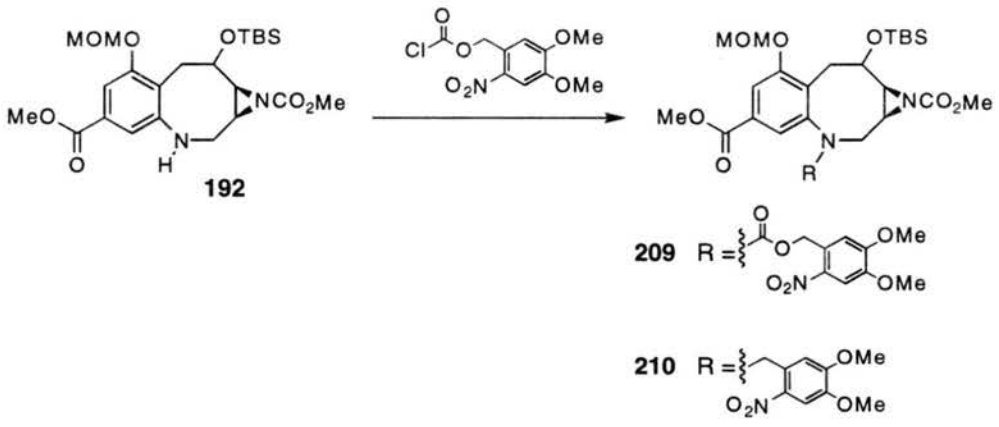
207

Figure 6. Potential latent mitosenes

sulfide **206**. Upon chemical or enzymatic oxidation of the allyl sulfide group to a sulfoxide, a [2,3] sigmatropic rearrangement followed by hydrolysis of the resulting labile sulfinate and loss of butyrolactone will unmask the benzazocine ring.[88] Finally, the (oxodioxolenyl)methyl carbamate **207** is an example of a possible latent mitosene triggered by enzyme catalyzed hydrolysis.[89] The synthesis of the first model trigger compound and its activation with UV light to form a reactive mitosene is described below.

Acylation of the nitrogen of benzazocine **192** with 6-nitroveratryl chloroformate (**208**) was initially troublesome (Scheme 48). The problem was traced to our supply of chloroformate **208** which was obtained by treatment of 6-nitroveratryl alcohol with 20% phosgene in toluene. Chloroformate **208** was isolated as a stable, light orange solid and used without purification. When amine **192** was treated with chloroformate **208**, the isolated product was not the expected *N*-acylated product **209** but the *N*-alkylated product **210**. Although the *N*-alkylated compound **210** could be elaborated into a latent mitosene,

Scheme 48. Acylation of **192**



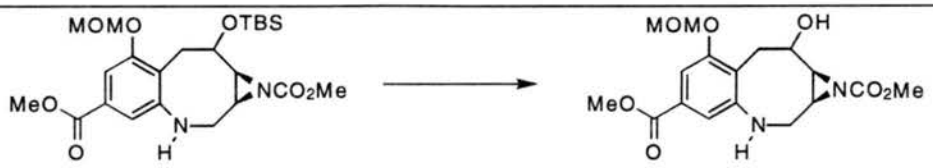
it was produced under these conditions in an unacceptably low yield (37%). As a result of this unexpected product, we decided to use a commercial supplier as the sole source of chloroformate **208** to be used in further experiments. When **192** was treated with

commercial chloroformate **208** (*i*-Pr_2NEt, DMAP, CH_2Cl_2), a smooth conversion to carbamate **209** in high yield (88%) was observed. We assume that chloroformate **208** obtained from the phosgene reaction contained residual HCl that catalyzed its decarboxylation and led to the *N*-alkylated product **210**.

Upon isolation of 6-nitroveratryloxycarbonyl (NVOC) **209**, attempts were made to remove the silyl ether protecting group. Unfortunately, standard conditions (TBAF, THF, rt) gave several products by TLC analysis. Since **209** has a complex ^1H NMR spectrum at room temp due to the rotomers of the aryl carbamate group, interpretation of the ^1H NMR spectra of the isolated products of the desilylation reaction was extremely difficult. As a result, further studies on the removal of the silyl ether protecting group were performed on amine **192**.

As shown in Table 4, a wide variety of reagents were used in our attempts to cleanly remove the silyl protecting group of amine **192**. None of the conditions used afforded the desired alcohol **211**. Conditions typically used to remove silyl groups (Entries 1, 3, and 5) resulted in no reaction or many unidentified products. It was thought that the basic conditions of reactions using TBAF might be responsible for the complex mixture of products. The fluoride ion could be a strong enough base to deprotonate the secondary alcohol of **211** causing a Payne rearrangement[63] and decomposition of the product. Since a Payne rearrangement is a stereospecific reaction, this hypothesis was ruled out when the deprotection was attempted with the minor diastereomer of **211** and similar products were seen. Another hypothesis to explain the complex mixture of products was that the electron withdrawing nature of the aziridine protecting group was the source of the problem. To test this hypothesis, the methyl carbamate of **192** was reductively removed with DIBAL to produce the free aziridine **212** (Scheme 49). Smooth removal of the silyl group of **212** with TBAF in THF to give alcohol **192** showed that the methyl carbamate protecting group was at least partly responsible for the inability of

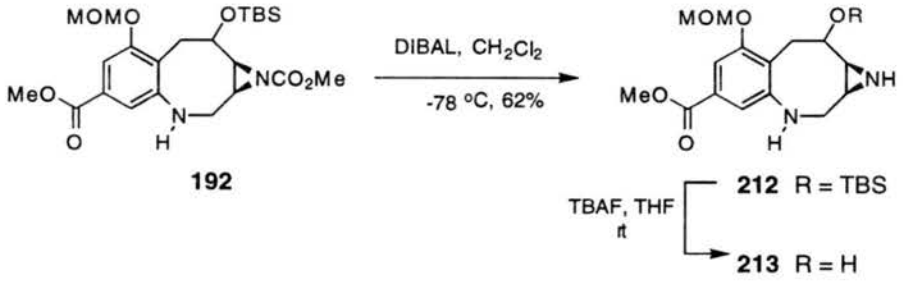
Table 4. Attempts to remove silyl group



192 **211**

Entry	Conditions	Results
1	*p*-TsOH•H_2O, MeOH, rt, 24 hr	No reaction
2	*p*-TsOH, MeOH, rt	Many products and starting material
3	TBAF, THF, 0 °C --> rt	Many products and starting material
4	solid TBAF• x H_2O, CH_2Cl_2, -55 °C --> rt	No reaction
5	aqueous HF, CH_3CN/THF, 0 --> 50 °C	Many products
6	CF_3SO_3H, CH_2Cl_2	Decomposition
7	CF_3SO_3H•Py, CH_2Cl_2	No reaction

Scheme 49. Desilylation of **212**

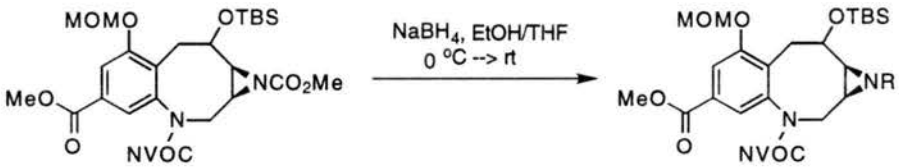


192

TBAF to cleanly remove the silyl group from amine **192**. Other factors must also be involved since the silyl group of **185** was removed without incident (See Scheme 45).

With the successful demonstration of the detrimental nature of the methyl carbamate protecting group, several methods to remove the group from advanced intermediates were investigated. The reduction of amine **192** to **212** Scheme 49 with DIBAL was capricious and produced **212** in erratic yields. Selective removal of the carbamate without side products from the partial or complete reduction of the methyl ester proved difficult. Efforts to remove the carbamate from the aziridine of **209** began with a milder reducing agent, $NaBH_4$ (Scheme 50). Again, rotomers of the starting

Scheme 50. Sodium borohydride reduction of **209**

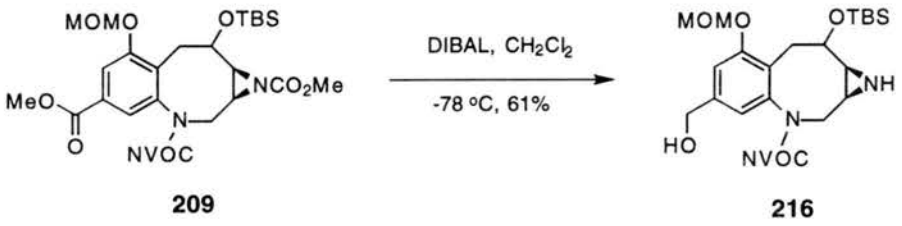


209

214 R = H
215 R = Me

material and the products hampered an exact structural determination. Mass spectral analysis of **214** showed that it was contaminated with a side product of the $NaBH_4$ reduction: the *N*-methylated product **215**. The two compounds could not be separated, so other methods to remove the carbamate group were investigated. Efforts to selectively hydrolyze the carbamate of **209** with potassium carbonate in a solution of $MeOH/CH_2Cl_2$ and a catalytic amount of water failed. The ^1H NMR spectrum of the crude reaction showed loss of the carbamate and ester methyl groups . Finally, the reductive removal of the carbamate and ester groups of **209** with DIBAL consistently produced **216** in an acceptable yield (61%) (Scheme 51). Extremely slow addition of DIBAL was necessary to prevent the reductive removal of the NVOC group.

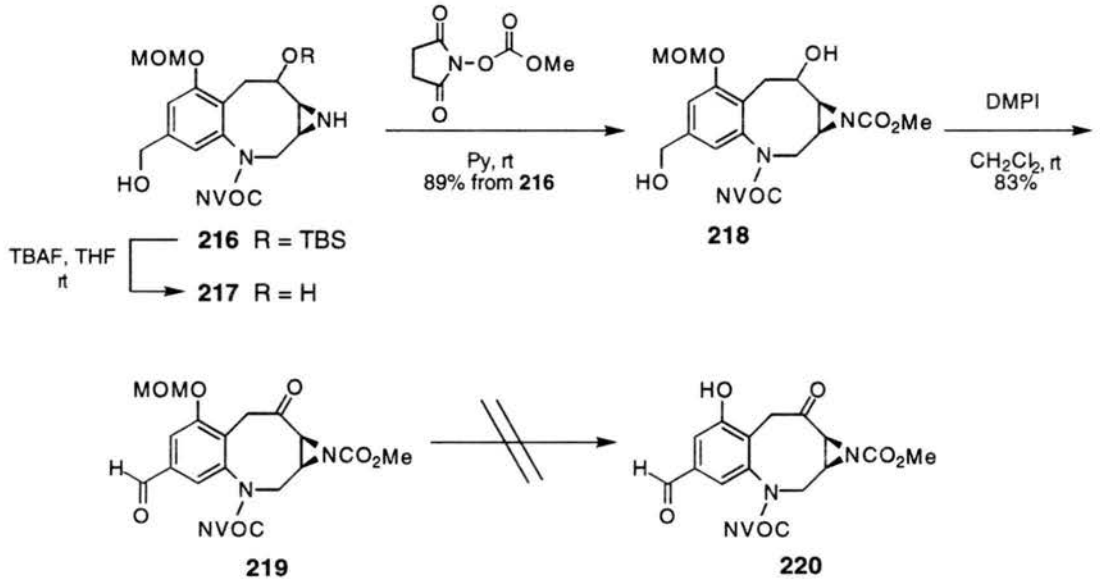
Scheme 51. DIBAL reduction of **209**



209 **216**

With the clean elimination of the carbamate protecting group, efforts were focused on the construction of a benzylic ketone. As a first step, the TBS group of **216** was removed to give diol **217** (Scheme 52). We hoped the primary and secondary alcohols of **217** could be oxidized in the presence of the free aziridine using Swern oxidation conditions[60] (DMSO, oxalyl chloride, CH_2Cl_2, -78 °C, then Et_3N). This

Scheme 52. Synthesis of latent mitosene



219 **220**

proposed reaction had precedent from Terashima's[31] total synthesis of FR 900482 where a benzylic alcohol was oxidized to an aldehyde in the presence of a free aziridine using Swern conditions (See Scheme 26, **142 --> 1**). When we attempted to oxidize **217** using

Swern conditions, a complex mixture of products was seen by TLC. Analysis of the crude reaction by ^1H NMR showed peaks for the expected aldehyde, but no peaks were seen in the range of the expected signals for the aziridine protons (3.0 - 2.5 ppm). Efforts to oxidize **217** using the Dess-martin periodinane[76] resulted in the production of a similar mixture. As a result of our inability to oxidize the alcohols of **217**, the aziridine of **217** was reprotected to form methyl carbamate using the selective acylating reagent *N*-((methoxy)carbonyloxy)succinimide. With the aziridine protected, the two alcohols of **218** were oxidized to give benzylic ketone **219**.

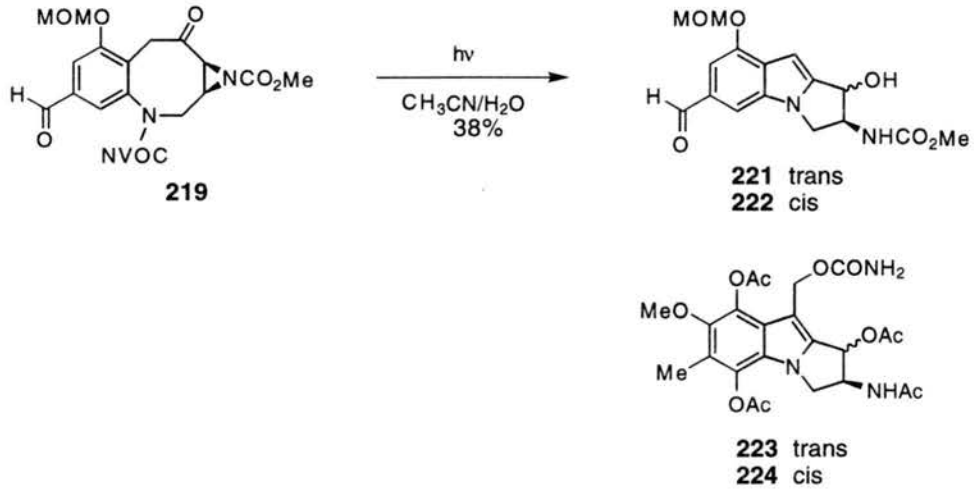
Once the benzylic ketone **219** was constructed, efforts were made to remove the methoxymethyl protecting group from the phenol. We hoped that the MOM group would be easily removed when **219** was treated with trityl tetrafluoroborate since the same protecting group had been removed to unmask an alcohol in Danishefsky's[30] total synthesis of FR 900482 (See Scheme 23, **114 --> 1**). No conditions were found that removed the MOM group from the phenol of **219** using trityl tetrafluoroborate[90,91] (Table 5, Entry 1 and 2). Other attempts to remove the MOM group with 9-BBN-bromide or TFA resulted in the cleavage of the aziridine ring either with HBr (Entry 3 and 4) or water (Entry 5). Efforts to remove the MOM group were abandoned in order to focus on photo reactions.

Table 5. Attempts to remove MOM group

Entry	Conditions	Results
1	PH_3CBF_4, CH_2Cl_2, rt	Many products
2	Ph_3CBF_4, di-*tert*-butylpyridine, CH_2Cl_2, rt	Many products
3	9-BBN-Br, CH_2Cl_2, -78 °C, 1.2 h	HBr addition
4	9-BBN-Br, CH_2Cl_2, -78 °C, 20 min	HBr addition
5	TFA, CH_2Cl_2, rt	H_2O addition

With compound **219** in hand, removal of the NVOC group was effected by treating it with UV radiation (350 nm)[92] for 24 h at room temp in a 3:1 solution of CH_3CN/H_2O (Scheme 53). Upon deprotection, the free amine cyclized with the ketone,

Scheme 53. Photoactivated latent mitosene



and the resultant hemi-aminal dehydrated to give a mitosene. In addition, the aziridine of the mitosene opened and water added to produce an approximately equal mixture of diastereomers **221** (*trans*) and **222** (*cis*) in 38% yield. The stereochemistry of these compounds was assigned by ^1H NMR correlation with diacetates **223** and **224**.[93]

2.7 Conclusion

Our interest in developing a synthesis of FR 900482 and novel analogs stemmed from their interesting structure and promising biological activity. The C-7 to C-8 bond of the benzazocine skeleton was cleanly formed by nucleophilic addition of a fully functionalized aromatic piece with an optically active aziridinyl aldehyde. Several approaches were explored to form the benzazocine ring. The most successful ring forming reaction was an intramolecular reductive amination between aniline and

aldehyde groups. The generality of this approach remains to be seen, and numerous protective group substitutions will be necessary to synthesize the natural product or analogs. For example, reduction of the ester group to an alcohol is required before oxidation of the benzazocine nitrogen. A potential consequence of this transformation is the increased nucleophilicity of the aniline lone pair, and as a result, the potential success of several of our failed routes to the benzazocine ring. The aziridine nitrogen must be protected by a group which is less prone to reduction and does not interfere with the removal of the silyl ether protecting group. Finally, replacement of the MOM group with a more labile protecting group is necessary. This may have an adverse effect on the coupling reaction, so exchange for another group may be required after the coupling reaction.

The ability to prepare a structurally less complex analog of FR 900482 has been demonstrated. The analog forms a reactive mitosene upon exposure to UV light (350 nm). By attaching a photocleavable group or trigger, it has been possible to vary the conditions from reductive to photolysis conditions needed to generate a reactive mitosene. Progress in constructing more complex analogs with an hydroxymethyl side chain is currently underway in our laboratory.

2.8 Future objectives

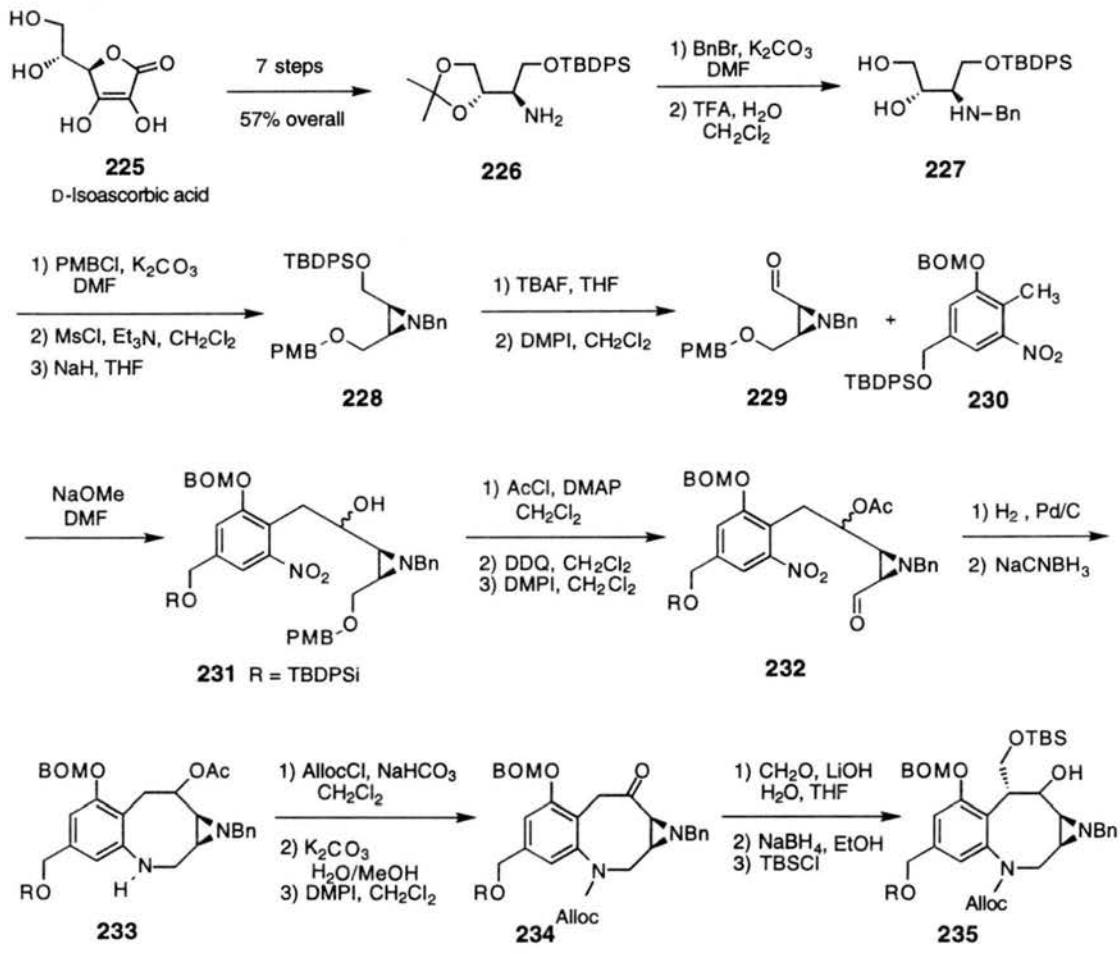
Although we failed to elaborate **192** into the natural product or a fully functionalized photo-activated analog of the natural product, it is believed that incorporation of the suggestions described in Section 2.7 into a route similar to our initial one (See Section 2.1) will allow for construction of these compounds. In Scheme 54, the synthesis of benzazocine **235** is outlined. The proposed synthesis combines the suggestions in Section 2.7 and the strategy used to build **192**. Also, **235** is used to

synthesize FR 900482 (**1**) and a photo-activated analog (**241**) as shown in the proposed routes in Schemes 55 and 56.

Aziridine aldehyde **229** (Scheme 54) is synthesized from D-isoascorbic acid (**225**). Note that **229** is constructed from a chiral pool reagent thus avoiding Sharpless epoxidation of a *cis* olefin, and as a result, the aziridine is formed in high enantiomeric excess. Also, the aziridine function of **229** is protected with a benzyl group rather than an electron withdrawing group making the ring more resistant to reduction and hydrolysis. The synthetic steps to transform **225** into **226** and are taken from a literature procedure.[94] With **226** in hand, the amine is alkylated, and the isopropylidine is removed to give diol **227**. Selective protection of the primary alcohol of **227** followed by mesylation of the secondary alcohol and treatment with sodium hydride forms aziridine **228**. The silyl group of **228** is removed, and the resulting primary alcohol is oxidized to give aziridine aldehyde **229** in 14 steps from isoascorbic acid.

Coupling of **229** with the aromatic piece **230** under the same conditions used to synthesize **192** produces alcohol **231**. Note that the phenol of **230** is protected with a benzyloxymethyl (BOM) group instead of a methoxymethyl. The BOM group will eventually be removed under reducing conditions avoiding potential conflicts with the strong acids needed to remove a MOM group. Also, the BOM group should chelate a sodium ion the same as a MOM group which was necessary to effect the coupling reaction. Protection of the alcohol of **231** and elaboration of primary alcohol to an aldehyde gives **292** and sets the stage for formation of the benzazocine ring. Since the aziridine of **232** is protected with a benzyl group, the aldehyde function should not be activated to reduction. As a result, selective reduction (H_2, Pd/C, MeOH) of the aryl nitro group of **232** followed by reductive amination ($NaCNBH_3$, TFA, CH_2Cl_2/MeOH) should

Scheme 54. Potential route to benzazocine ring

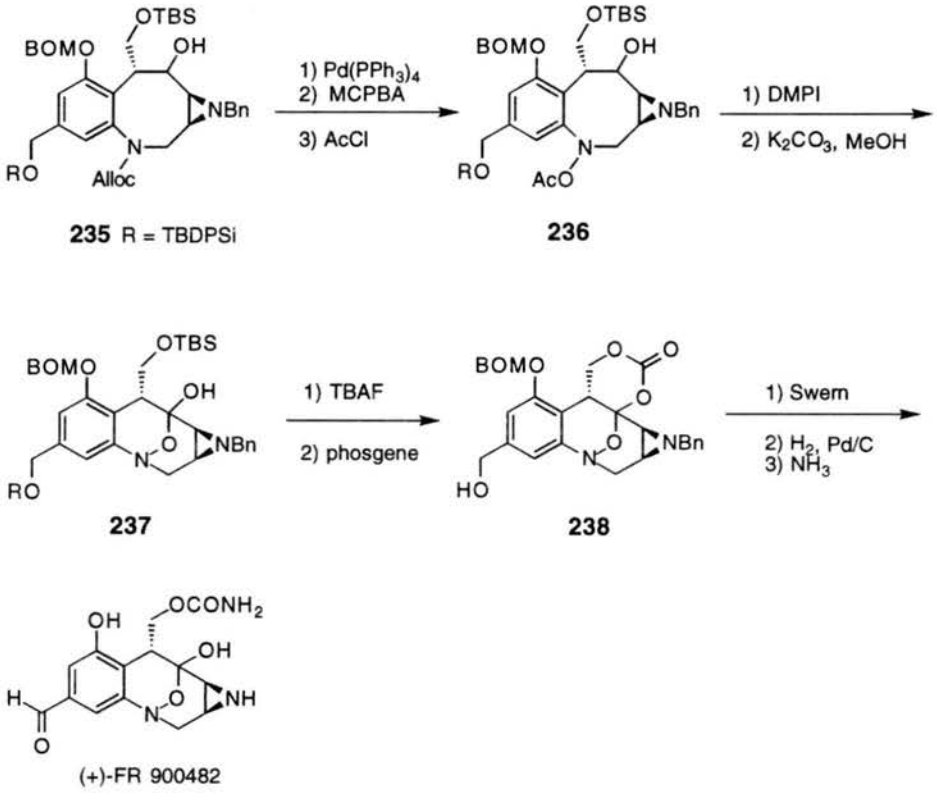


proceed smoothly to give benzazocine **233**. Protection of the secondary amine of **233** and installation of the ketone affords **234**. Finally, installation of the carbomethoxy side chain by an aldol condensation should proceed through an open tub conformation of the eight membered ring to give the correct diastereomer. Reduction of the ketone function of the aldol product is necessary to avoid the elimination of water. Selective protection of the primary alcohol of the resulting 1,3-diol produces the desired key intermediate **235**.

Scheme 55 outlines the proposed synthesis of FR 900482 from benzazocine **235**. Focusing on construction of the bicyclic structure of the natural product, the Alloc protecting group is removed from the benzazocine nitrogen. The resulting free secondary

amine is oxidized to an hydroxylamine and protected to give **236**. Oxidation of **236** to form a ketone sets the stage of transannular cyclization. Hydrolytic removal of the acetate protecting group on the hydroxylamine and spontaneous ring closure produces **237**. Deprotection of both silyl ethers of **237** and treatment of the intermediate triol with phosgene forms the cyclic carbonate **238**. Swern oxidation of the primary alcohol of **X** followed by hydrogenolysis of the BOM and Bn protecting groups and ammonolysis of the carbonate function produces the natural product in asymmetric form.

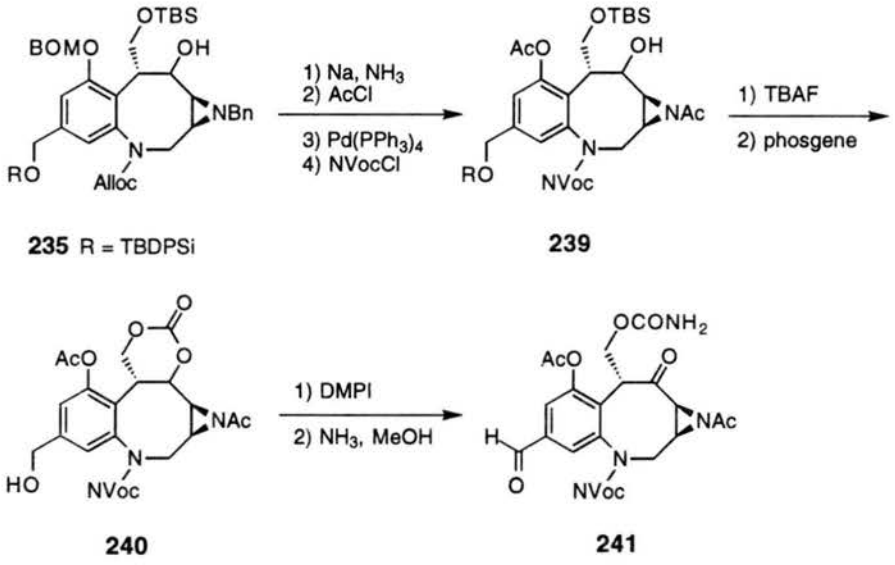
Scheme 55. Potential route to FR 900482



Starting from the same benzazocine intermediate used to synthesize the natural product, Scheme 56 outlines a proposed route to a fully functionalized photo-activated analog of FK 973. Interchange of the BOM and Bn protecting groups on **235** for acetates is necessary to avoid conflict with the NVoc group. Removal of the Alloc group and

acylation of the benzazocine nitrogen with NVocCl produces **239**. Deprotection of both silyl ethers of **239** and treatment of the intermediate triol with phosgene forms the cyclic carbonate **240**. Oxidation of the primary alcohol of **240** followed by ammonolysis of the carbonate function produces the photo-activated FK 973 analog **241** in asymmetric form.

Scheme 56. Proposed synthesis of fully functionalized analog of FR 973



235 R = TBDPSi

239

240

241

Chapter 3
Experimental Section

3.1 General Procedures

Unless otherwise noted materials were obtained from commercially available sources and used without further purification. Diethyl ether (Et_2O) and THF were distilled from sodium benzophenone ketyl under a nitrogen atmosphere. Methylene chloride, triethylamine, pyridine, acetonitrile, and methanol were distilled under a nitrogen atmosphere from calcium hydride. Dimethyl formamide was dried over 4Å molecular sieves. The molecular sieves were activated by heating to 150 °C at 1 mm Hg for 3 h in a vacuum oven.

All reactions involving hydroscopic substances were conducted with flame or oven dried glassware under an inert atmosphere (Ar) dried by passage of atmospheric gases through a column packed with $CaSO_4$. Filtrations of organic extracts were conducted with a cotton plug using gravity, and concentration of the resultant filtrate was performed under reduced pressure (aspirator) using a rotary evaporator.

Chromatographic separations were performed with EM Science TLC plates (silica-gel 60, F_{254}, 20 x 20 cm x 250 μm) or with EM Science 230-400 mesh silica gel using positive air pressure. Reactions and chromatographic fractions were monitored and analyzed with EM Science TLC plates. Visualization on TLC was achieved with ultraviolet light or heating of TLC plates submerged in a 5% solution of phosphomolybdic acid in 95% ethanol. Radial chromatography employed a Chromatotron Model 7954 using 2 or 4 mm silica plates as needed.

Melting points were determined in open-ended capillary tubes with a Mel-Temp apparatus and are uncorrected.

Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR as thin films from dichloromethane and are reported as λ_{max} in wavenumbers (cm^{-1}).

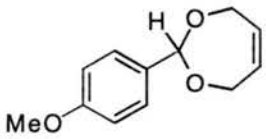
Optical rotations were obtained on a Rudolph Research Autopol III automatic polarimeter at a wave length of 589 nm (sodium "D" line) with a 1.0 dm cell with a volume of 1 mL. Specific rotations, $[\alpha]_D$, are reported at the specified temperature and concentration (c) given in grams per 100 mL in the specified solvent.

Elemental analyses were performed by M-H-W Laboratories, Phenoix, AZ, and are accurate to within ±0.4% of the calculated values. High resolution mass spectra were obtained on a Fisons VG-7070 at University of California Riverside.

Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker AC-300 of JS-300 spectrometer. NMR chemical shifts are given in parts per million (ppm) downfield from an internal tetramethylsilane (TMS) standard or relative to internal $CHCl_3$ or DMSO. Proton NMR (^1H NMR) are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), and coupling constants in hertz. When appropriate, the multiplicity of a signal is denoted as "br" to indicate that the signal was broad. Carbon NMR (^{13}C NMR) are listed with the multiplicity of the ^{13}C-^1H coupled signal.

3.2 Preparation of Compounds

4,7-Dihydro-2-(4-methoxyphenyl)-1,3-dioxepin.



A mixture of *p*-anisaldehyde (**151**) (136 gr, 1.0 mol, 1.0 eq), *cis*-2-butene-1,4-diol (**152**) (105 gr, 1.2 mol, 1.2 eq), and *p*-TsOH (0.20 gr, 1.1 mmol, 0.11 mol%) in 450 mL of benzene was refluxed with azeotropic removal of water. After 1.5 days, the dark brown mixture was cooled to room temperature, diluted with 500 mL of benzene, washed sequentially with 3 x 125 mL H_20 and 1 x 200 mL sat $NaCl_{(aq)}$. The organic solution was concentrated *in vacuo* , and the resulting oil was fractionally distilled under vacuum (1 mm Hg, ~160 °C) to yield 120 gr (58% yield) of 4,7-dihydro-2-(4-methoxyphenyl)-1,3-dioxepin as a clear, colorless, viscous oil (>95% pure).

R_f = 0.50 (5:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 3.79 (3H, s); 4.23 (2H, dd, J=1.7, 15.0 Hz); 4.36 (2H, dd, J=1.7, 15.0 Hz); 5.74 (2H, t, J=1.7 Hz); 5.81 (1H, s); 6.88 (2H, d, J=8.8 Hz); 7.43 (2H, d, J=8.8 Hz).

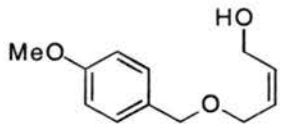
^{13}C NMR (75 MHz) ($CDCl_3$) δ TMS: 55.2 (q), 64.3 (t), 101.9 (d), 113.4 (d), 127.6 (d), 129.9 (d), 131.1 (s), 159.6 (s).

IR (NaCl/neat): 3030, 2938, 2855, 1613, 1586, 1513, 1445, 1248, 1104, 1077, 1035, 821 cm^{-1}.

Anal. Calcd for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84.

Found: C, 69.68; H, 6.69.

***cis*-4-O-(4-Methoxybenzyl)-2-butene-1,4-diol (153).**



To a 1000 mL three-neck flask fitted with a reflux condenser and a thermometer was added 150 mL of THF. The solution was stirred on an ice bath until the solution was 0 °C. Next, anhydrous $AlCl_3$ (14.3 gr, 107 mmol, 4.0 eq) was slowly added to the flask using a solid addition funnel. After stirring for 15 min, 1.0 M $LiAlH_4$ in THF (27 mL, 27 mmol, 1.0 eq) was added to the mixture in a dropwise fashion over 10 min. After stirring the grey solution for an additional 10 min, 4,7-dihydro-2-(4-methoxyphenyl)-1,3-dioxepin (11.1 gr, 53.7 mmol, 2.0 eq) in 20 ml of THF was added to the mixture dropwise over 10 min. While stirring, the mixture was slowly warmed to room temp over 2 h. After TLC analysis showed the reaction to be complete (2:1 Hex/EtOAc), the reaction was quenched by the cautious addition of 50 mL sat $NH_4Cl_{(aq)}$, and the organic solution was decanted away from the grey slurry. The grey slurry was extracted 3 x 100 mL EtOAc, and the combined organic layers were washed with 2 x 50 mL H_2O and 2 x 50 mL sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, and evaporated. The crude oil was purified by distillation using a kugelrohr apparatus (~1 mm Hg, 160 °C) to yield 11.1 gr of **153** (78% yield) as a clear colorless oil (>95% pure).

R_f = 0.40 1:1 Hex/EtOAc.

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.04 (1H, br, D_2O exch.); 3.78 (3H, s); 4.04 (2H, d, J=6.0 Hz); 4.14 (2H, d, J=6.0 Hz); 4.44 (2H, s); 5.76 (2H, m); 6.86 (2H, d, J=8.6Hz); 7.23 (2H, d, J=8.6Hz).

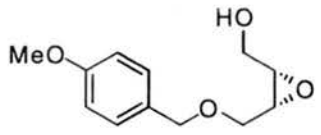
^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 55.2 (q), 58.7 (t), 65.3 (t), 72.1 (t), 113.8 (d), 128.4 (d), 129.5 (d), 129.9 (s), 132.3 (d), 159.3 (s).

IR (NaCl/neat): 3406, 3022, 2934, 2860, 1613, 1586, 1513, 1464, 1302, 1248, 1174, 1074, 820 cm^{-1}.

Anal. Calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74.

Found: C, 68.94; H, 7.56.

(2*S*,3*R*)-2,3-Epoxy-4-O-(4-methoxybenzyl)butane-1,4-diol (154).



Freshly distilled CH_2Cl_2 (200 mL) was added to a 1000 mL 3-neck round bottom flask fitted with a thermomoter and cooled to a temperature range between -20 and -30 °C in an acetonitrile/Dry Ice bath before addition of 5 gr of powdered 4 Å molecular sieves. Next, freshly distilled (+)-diethyl-L-tartrate (8.2 mL, 48.5 mmol, 1.3 eq), freshly distilled titanium isopropoxide (12.2 mL, 41.0 mmol, 1.1 eq), and 3.0 M *tert*-butyl hydroperoxide in toluene (25 mL, 74.6 mmol, 2.0 eq) were added to the flask. The mixture was stirred for 30 min to let the catalyst age. Allylic alcohol **153** (dried over 4 Å molecular sieves) in ~10 mL of CH_2Cl_2 was added dropwise to the mixture over 20 min. The reaction was stirred vigorously for 1 h and placed in a freezer at -20 °C for 5 days. After TLC analysis (1:1 Hex/EtOAc) showed no sign of starting material, the reaction was placed on a -20 °C acetonitrile/Dry Ice bath and quenched with 100 mL of 10% aqueous tartaric acid. The two-phase solution was stirred with a mechanical stirrer for 30 min and then allowed to warm to room temp over 1 h. Approximately 200 mL of water was added to the mixture, and the aqueous solution was extracted. During the extraction, the emulsion due to the mol sieves was removed by filtering the aqueous solution through a cotton plug. The combined organic extracts were dried over $MgSO_4$ and immediately passed through a Celite pad. The concentrated oil was dissolved in 150 mL Et_2O and cooled to 0 °C on an ice bath. Next, 50 mL 1M $NaOH_{(aq)}$, precooled to 0 °C, was added to the organic solution. The biphasic mixture was vigorously stirred for 1.5 h. The organic layer was separated, washed 1 x H_2O, and 1 x sat $NaCl_{(aq)}$, and dried over Na_2SO_4. The concentrated oil was purified by column chromatography (1:1 Hex/EtOAc) to yield 6.05 gr (75% yield) of **154** as a clear colorless oil (>95% pure).

$[\alpha]^{25}_D$ = -25.5 (c = 1.0, $CHCl_3$).

R_f = 0.31 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.04 (1H, br, D_2O exchange); 3.22-3.28 (2H, m); 3.62 (1H, dd, J=5.0, 11.0 Hz); 3.71 (1H, dd, J=5.9, 11.0 Hz); 3.65-3.80 (2H, m); 3.80

(3H, s); 4.46 (1H, d, J=11.4 Hz); 4.55 (1H, d, J=11.4 Hz); 6.87 (2H, d, J=8.6 Hz); 7.26 (2H, d, J=8.6).

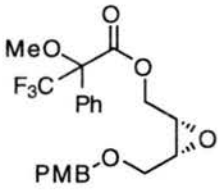
^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 54.7 (d); 55.2 (q); 55.6 (d); 60.7 (t); 67.7 (t); 73.1 (t); 113.9 (d); 129.4 (s); 129.5 (d); 159.4 (s).

IR (NaCl, neat): 3424, 2935, 1612, 1585, 1513, 1463, 1247, 1175, 1086, 1032 cm^{-1}.

Anal. Calcd for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19.

Found: C, 64.49; H, 7.10.

Mosher ester of epoxide 154

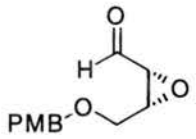


To a 10 mL conical flask was added alcohol **154** (~0.10 mmol, 1.0 eq) and 500 μL of CH_2Cl_2. The solution was stirred until the alcohol had completely dissolved, and DMAP (1.0 eq) and Et_3N (4.0 eq) were added. After stirring for another 2 min, (+)-MTPA-Cl (1.2 eq) was added to the solution. An immediate change to orange was seen, and the reaction was stirred until the reaction was complete by TLC analysis (4:1 Hex/EtOAc). The excess acid chloride was quenched by the addition of dimethylaminopropylamine (5.0 eq), and the mixture was stirred for another 15 min. The mixture was concentrated and passed through a short plug of silica gel (4:1 Hex/EtOAc). The crude oil was analyzed by ^{19}F NMR without further purification.

R_f = 0.60 (4:1 Hex/EtOAc).

^{19}F NMR (282MHz)($CDCl_3$)(ref CF_3CH_2OH -80 ppm) δ TMS : -74.54 (CF_3); -74.60 (CF_3). 87% *ee* (± 2% *ee*)

Epoxy aldehyde 155



To a 50 mL round bottom flask was added CH_2Cl_2 (10 mL) and oxalyl chloride (634 mg, 5.0 mmol, 1.5 eq). The stirred solution was cooled to a temperature range between -50 and -60 °C on an acetone/Dry Ice bath. Dimethyl sulfoxide (781 mg, 10 mmol, 3.0 eq) in ~5 mL CH_2Cl_2 was slowly added to the mixture over 5 min. The solution was stirred for 20 min and epoxide **154** (750 mg, 3.3 mmol, 1.0 eq) in ~5 mL of CH_2Cl_2 was slowly added over 5 min. The whole was stirred for 40 min after which Et_3N (3.33 gr, 33 mmol, 10 eq) was added dropwise. The mixture was stirred for another 30 min, warmed to room temperature, quenched with water, and diluted with CH_2Cl_2. The organic layer was separated and washed 2 x 0.5 M $HCl_{(aq)}$, 1 x sat $NaHCO_{3(aq)}$, and 1 x sat $NaCl_{(aq)}$. The combined aqueous extracts were back extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (3:1 Hex/EtOAc) to give 550 mg (73% yield) of **155** as a clear light yellow oil (95% pure).

$\mathbf{R_f}$ = 0.25 (3:1 Hex/EtOAc).

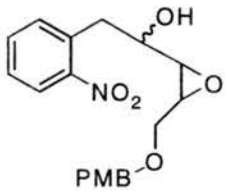
^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 3.41 (1H, dd, J=4.8, 4.8 Hz); 3.49 (1H, ddd, J=3.3, 4.8, 4.8 Hz); 3.74 (1H, dd, J=4.5, 11.7 Hz); 3.81 (1H, dd, J=3.3, 11.7 Hz); 3.81 (3H, s); 4.47 (1H, d, 12.1 Hz); 4.51 (1H, d, 12.1 Hz); 6.88 (2H, d, J=8.6 Hz); 7.23 (2H, d, J=8.6 Hz); 9.42 (1H, d, 4.8 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 55.2 (q); 57.3 (d); 58.0 (d); 65.8 (t); 73.2 (t); 113.9 (d); 129.1 (s); 129.5 (d); 159.5 (s); 197.7 (d).

IR (NaCl, neat): 3000, 2909, 2838, 1722, 1612, 1585, 1513, 1464, 1363, 1302, 1248, 1090, 1032, 847, 820 cm^{-1}.

Mass Spectrum, (EI) m/z (relative intensity)= 222 (M^+, 5.2%); 178(4.4); 135 (11.3); 149 (4.8); 121(C_8H_9O, 100); 109 (6.8); 91 (9.0); 77 (C_6H_5, 21.0).

Alcohol 156



Sodium hydroxide (~10 mg, 0.25 mmol, 0.25 eq) powdered under hexane was added to a 25 mL round bottom flask. The flask was placed on an acetonitrile/Dry Ice bath, and 5 mL of DMF was added. *o*-Nitrotoluene (21 mg, 0.15 mmol, 1.0 eq) was added to the solution, and a faint purple color appeared. After ~2 min, epoxy aldehyde **155** (35 mg, 0.15 mmol, 1.0 eq) in 1 mL of DMF was added, and the purple color disappeared. The reaction mixture was stirred for 2 h at -42 °C, raised to -5 °C for another 2 h, allowed to warm to room temp overnight, and stirred at room temp for 4 days. The reaction was quenched with sat $NaHCO_{3(aq)}$, diluted with water, and extracted with ethyl actetate. The organic extract was washed three times with water, once with sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified using PTLC (10:1 CH_2Cl_2:Et_2O) to give 5 mg (10% yield) of both epimers of **1** as clear oils (85% pure).

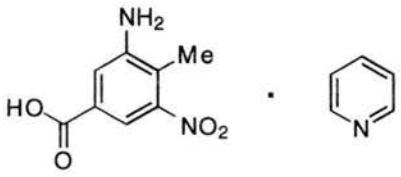
R_f = 0.45 (10:1 CH_2Cl_2:Et_2O).

^{1}H NMR (300 MHz) ($CDCl_3$) δ $CHCl_3$ (7.24): 2.74 (1H, br, D_20 exchange); 3.00 (1H, dd, J=4.3, 7.8 Hz); 3.18 (1H, dd, J=8.5, 13.9 Hz); 3.23 (1H, ddd, J=4.3, 4.3, 6.0 Hz); 3.34 (1H, dd, J=4.2, 13.9 Hz); 3.51 (1H, dd, J=6.2, 10.7 Hz); 3.73 (1H, ddd, J=4.1, 4.1, 8.1 Hz); 3.78 (3H, s); 3.80 (1H, dd, J=5.8, 10.8 Hz); 4.42 (1H, d, J=11.4 Hz); 4.49 (1H, d, J=11.4 Hz); 6.83 (2H, d, J=8.7 Hz); 7.20 (2H, d, J=8.7 Hz); 7.35-7.43 (2H, m); 7.53 (1H, ddd, J=1.3, 7.5, 7.5 Hz); 7.90 (1H, dd, J= 1.3, 8.1 Hz).

IR (NaCl, neat): 3440, 2918, 2850, 1610, 1524, 1461, 1348, 1302, 1246, 1174, 1082, 1030, 820 cm^{-1}.

Mass spectrum, (EI) m/z (relative intensity) = 359 (M^+, 0.02%); 327 (0.03); 307 (0.03); 188 (3.2); 179 (2.2); 137 (20.8); 121(C_8H_9O, 100); 91 (10.6); 77 (C_6H_5, 17.5).

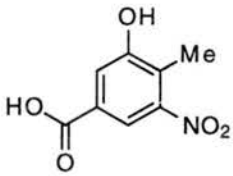
3-Amino-5-nitro-*p*-toluic acid (158)



To a 500 mL 3-neck flask fitted with a thermometer was added 3,5-dinitro *p*-toluic acid (**157**) (11.3 gr, 50 mmol, 1.0 eq) and 55 mL of pyridine. The slurry was heated on a steam bath for 10 min to dissolve the acid. The flask was removed from the steam bath, and the solution allowed to cool to 40 °C when 55 mL of water was added. The slurry was placed on an ice bath and stirred. Once the solution had reached 20 °C, sodium dithionite ($Na_2S_2O_4$) (25.6 gr, 147 mmol, 2.9 eq) was added in portions over 15 min making sure not to let the reaction temperature rise above 24 °C. Once the sodium dithionite was completely added, the golden orange solution was stirred for another 15 min at room temp, and 4 N HCl (200 mL) was slowly added to the flask, again making sure not to let the solution rise above 24 °C. Once added, the reaction stood for 4 days. The precipitate was collected, and the orange solid was air dried to give 4 gr of **158** as a dark yellow pyridinium salt.

^{1}H NMR (300MHz) (d_6-DMSO) δ TMS: 2.13 (3H, s); 7.42 (1H, d, J=1.3 Hz); 7.49 (1H, d, J=1.3 Hz); 8.02 (2H, t, J=6.0 Hz); 8.54 (1H, dt, J=1.1, 7.9 Hz); 8.90 (2H, d, J=1.1, 5.2 Hz).

3-Hydroxy-5-nitro-*p*-toluic acid



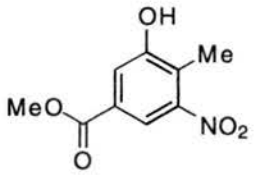
To a 500 mL flask was added 25 mL water and 75 mL of conc. H_2SO_4. The stirred solution was heated to 95 °C on an oil bath and **158** (5.6 gr) was added in portions. Once the addition was complete, the mixture was cooled to 5 °C on an ice bath. Sodium nitrite (3.0 gr, 40 mmol, 2 eq) in 22 mL water was added dropwise to the mixture over 1.75 h. After the addition was complete, the mixture was placed on an oil bath and heated to 100 °C for 4 h, after which it was diluted with 50 mL water and allowed to stand overnight while the product precipitated. The precipitate was collected suction filtration after cooling the crude reaction to 0 °C for 4 h. The light tan solid was air dried to give 2.6 gr (26% yield from 3,5-dinitro-*p*-toluic acid) of 3-hydroxy-5-nitro *p*-toluic acid.

mp (sharp): 210 °C.

^{1}H NMR (300MHz)(d_6-DMSO) δ TMS: 2.28 (3H, s); 7.66 (1H, d, J=1.5 Hz); 7.79 (1H, d, J=1.5 Hz).

Mass Spectrum (ES^-) m/z: 195 (M-1).

Methyl 3-hydroxy-4-methyl-5-nitrobenzoate (159)



To a 25 mL round bottom flask fitted with a reflux condenser was added 15 mL MeOH, 3-hydroxy-5-nitro-*p*-toluic acid (1.55 gr, 7.9 mmol, 1.0 eq), and 12 drops of conc. H_2SO_4. After refluxing for 24 h, the reaction was neutralized with solid Na_2CO_3, and the methanol was removed *in vacuo*. The residue was dissolved in 1 M $HCl_{(aq)}$ and extracted three times with ethyl acetate. The combined organic extracts were washed once with water and sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, and concentrated. The crude solid was purified by column chromatography (5:1 Hex/EtOAc). The white solid was recrystallized from absolute ethanol to yield 1.58 gr (95%) of **159** as orange needles (95% pure).

R_f = 0.25 (5:1 Hex/EtOAc).

mp (range): 162 °C.

^{1}H NMR (300 MHz)(d_6-DMSO) δ TMS: 2.27 (3H, s); 3.85 (3H, s); 7.64 (1H, d, J=1.6 Hz); 7.78 (1H, d, J=1.6 Hz); 10.81 (1H, s, D_2O exchange).

^{13}C NMR (75 MHz)(d_6-DMSO) δ TMS: 11.7 (q), 52.5 (q), 114.7(d), 118.3 (d), 124.3 (s), 128.3 (s), 150.6 (s), 156.9 (s), 164.6 (s).

IR (NaCl/neat): 3399, 3100, 2961, 1704, 1622, 1532, 1436, 1366, 1318, 1269, 1107, 1052, 912, 774 cm^{-1}.

Mass Spectrum, m/z (relative intensity) = 212 (M^+, 4.3%); 211 (37); 194 (100); 180 (41); 166 (34); 135 (12); 134 (46); 106 (44); 77(37).

Methyl 3-methoxy-4-methyl-5-nitro benzoate (160)

To a 100 mL 2-neck flask fitted with a condenser was added **159** (211 mg, 1.0 mmol, 1.0 eq), K_2CO_3 (150 mg, 1.1 mmol, 1.1 eq), 25 mL acetone, and iodomethane (186 µL, 3.0 eq, 3.0 eq). The stirred solution was heated to reflux for 8 h when TLC analysis (4:1 Hex/EtOAc) showed no sign of starting material. The crude reaction was concentrated *in vacuo*, diluted in EtOAc, washed 2 x H_2O, 1 x sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated. The crude residue was crystallized from EtOH/H_2O to give 187 mg of **160** (83% yield) as yellow shards.

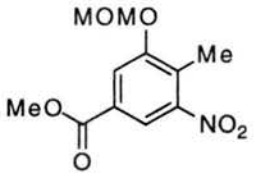
R_f = 0.48 (2:1 Hex/EtOAc).

mp (sharp): 76 °C.

^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 2.38 (3H, s); 3.92 (3H, s); 3.93 (3H, s); 7.66 (1H, d, J=1.5 Hz), 8.04 (1H, d, J=1.5 Hz).

IR (NaCl/neat): 3099, 2955, 1728, 1533, 1463, 1436, 1358, 1291, 1242, 1207, 1112, 1068, 747 cm^{-1}.

Methyl 3-methoxymethyloxy-4-methyl-5-nitrobenzoate (162)



To a 100 mL flask was added **159** (2.0 gr, 9.5 mmol, 1.0 eq), 50 mL CH_2Cl_2, and 10 mL THF. The mixture was cooled to 0 °C on an ice bath, and diisopropylethylamine (3.3 mL, 19.0 mmol, 2.0 eq) was added to the solution. After stirring for 10 min, methoxymethyl chloride (1.07 mL, 14.2 mmol, 1.5 eq) was added to the solution dropwise over 15 min. The stirred mixture was allowed to come to room temp over 6 h. When TLC analysis of the reaction mixture (4:1 Hex/EtOAc) showed complete loss of starting material, the crude reaction was diluted with CH_2Cl_2 and washed with sat $NH_4Cl_{(aq)}$. The organic layer was concentrated, redissolved in EtOAc, washed 2 x sat $NH_4Cl_{(aq)}$, and 1 x sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, and concentrated. The crude product was crystallized from absolute ethanol to yield 2.31 gr (95%) of **162** as white needles (95% pure).

R_f = 0.27 (4:1 Hex/EtOAc).

mp (range): 65 - 66 °C.

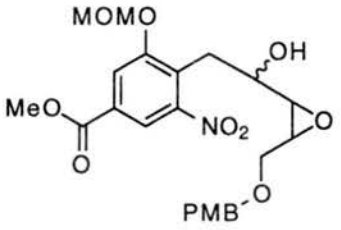
^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.43 (3H, s); 3.49 (3H, s); 3.92 (3H, s); 5.29 (2H, s); 7.90 (1H, d, J=1.7 Hz); 8.10 (1H, d, J=1.7 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 12.2 (q); 52.6 (q); 56.5 (q); 94.9 (t); 117.6 (d); 118.1 (d); 127.7 (s); 129.2 (s); 150.9 (s); 156.1 (s); 165.0 (s).

IR (NaCl/neat): 3099, 2956, 2832, 1729, 1535, 1437, 1357, 1290, 1243, 1207, 1156, 1042, 1002, 986, 748 cm^{-1}.

Mass Spectrum, m/z (relative intensity)= 256 (M^+, 12%); 255 (100); 224 (70); 194 (4); 179 (5); 163 (8); 148 (12); 135 (17); 117 (19); 106 (15); 91 (36); 89 (98); 77 (61).

Alcohol 163



To a 25 mL round bottom flask was added **162** (41 mg, 160 μmol, 1.0 eq) and 15 mL of DMF. The stirred mixture was cooled to -30 °C on a Dry Ice/acetonitrile bath. After cooling, a 0.5 M solution of NaOMe in methanol (32 μL, 15 μmol, 0.1 eq) was added, and the mixture immediately turned bright purple. After 3 min, **155** (36 mg, 160 μmol, 1.0 eq) in 3 mL of DMF was added to the reaction flask, and the mixture was stirred for 7 h as the temperature of the bath was kept below 0 °C. After TLC analysis of the crude reaction mixture showed complete loss of **155**, the reaction was quenched by the addition of 1 mL of sat $NH_4Cl_{(aq)}$. The crude mixture was concentrated *in vacuo*, and the resulting solid residue was dissolved in EtOAc. The organic layer was washed 1 x sat $NH_4Cl_{(aq)}$, 2 x H_2O, 1 x sat $NaCl_{(aq)}$, and dried over Na_2SO_4. The dried organic layer was filtered, concentrated, and purified using Chromatotron (2 mm plate, 10:1 CH_2Cl_2/Et_2O) to yield a total of 54 mg (73% yield) of **163** (1:1 mixture of separable diastereomers, 95% pure) as a clear foamy oil.

Compound **163a**

$\mathbf{R_f}$ = 0.15 (10:1 CH_2Cl_2/ Et_2O).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.5 (1H, br, D_2O exch), 3.10 (1H, dd, J=4.4, 7.4 Hz); 3.16 (1H, dd, J=5.6, 13.3 Hz); 3.22 (1H, ddd, J=5.0, 5.0, 5.0 Hz); 3.29 (1H, dd, J=8.4, 13.3 Hz); 3.38 (2H, d, J=5.1 Hz); 3.42 (3H, s); 3.78 (3H, s); 3.82 (1H, m); 3.92 (3H, s); 4.39 (1H, d, J=11.4 Hz); 4.47 (1H, d, J=11.4 Hz); 5.21 (1H, d, J=6.9 Hz); 5.23 (1H, d, J=6.9 Hz); 6.84 (2H, d, J=8.6 Hz); 7.19 (2H, d, J=8.6 Hz); 7.92 (1H, d, J=1.5 Hz); 8.07 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 30.0 (t); 52.7 (q); 55.2 (q); 56.2 (d); 56.6 (q); 59.2 (d); 67.4 (t); 69.6 (d); 72.9 (t); 95.0 (t); 113.8 (d); 118.0 (d); 118.4 (d); 126.0 (s); 129.4 (d); 129.5 (s); 130.4 (s); 151.4 (s); 156.3 (s); 159.3 (s): 164.7 (s).

IR (NaCl/neat): 3444, 2956, 1727, 1614, 1538, 1514, 1436, 1292, 1247, 1156, 1088, 1032 cm^{-1}.

Compound **163b**

$\mathbf{R_f}$ = 0.25 (10:1 CH_2Cl_2/ Et_2O).

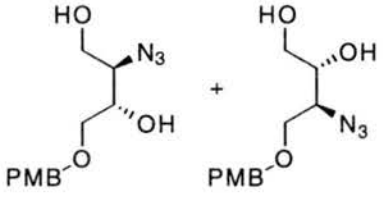
^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.7 (1H, br, D_2O exch.); 3.01 (1H, dd, J=4.3, 8.0 Hz); 3.19 (1H, ddd, J=4.3, 5.8, 5.8 Hz); 3.31 (1H, dd, J=8.2, 13.3 Hz); 3.40 (1H, dd, J=5.0, 13.3 Hz); 3.42 (3H, s); 3.49 (1H, dd, J=5.8, 10.8 Hz); 3.68 (1H, m); 3.72 (1H, dd, J=5.9, 10.8 Hz); 3.77 (3H, s); 3.93 (3H, s); 4.41 (1H, d, J=11.5 Hz); 4.48 (1H, d, J=11.5 Hz); 5.24 (1H, d, J=6.9 Hz); 5.26 (1H, d, J=6.9 Hz); 6.82 (2H, d, J=8.6 Hz); 7.19 (2H, d, J=8.6 Hz); 7.93 (1H, d, J=1.3 Hz); 8.10 (1H, d, J=1.3 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 30.4 (t); 52.7 (q); 54.1 (d); 55.2 (q); 56.5 (q); 58.0 (d); 67.8 (t); 69.1 (d); 73.1 (t); 95.0 (t); 113.9 (d); 117.9 (d); 118.4 (d); 126.5 (s); 129.1 (s); 129.5 (d); 130.2 (s); 151.6 (s); 156.2 (s); 159.4 (s); 164.8 (s).

IR (NaCl/neat): 3496, 2960, 1718, 1613, 1543, 1439, 1296, 1249, 1157, 1099, 1031 cm^{-1}.

(2*R*,3*S*)-2-Azido-4-O-(4-methoxybenzyl)butane-1,3,4-triol (168a)

(2*R*,3*S*)-3-Azido-4-O-(4-methoxybenzyl)butane-1,2,4-triol (168b)



168a **168b**

To a 100 mL flask was added epoxide **154** (1.52 gr, 6.78 mmol, 1.0 eq), NH_4Cl (0.72 gr, 13.5 mmol, 2.0 eq), NaN_3 (2.20 gr, 33.9 mmol, 5.0 eq), 40 mL $CH_3OCH_2CH_2OH$, and 5 mL distilled H_2O. The stirred reaction mixture was heated at reflux for 4 h when TLC analysis (EtOAc) showed complete loss of starting material. The cooled mixture was concentrated *in vacuo*. The resulting orange solid was dissolved in EtOAc, passed through a short plug of silica gel using EtOAc as eluant, concentrated, and dried overnight under vacuum. The cloudy orange oil was used without further purification. For analytical purposes, the mixture of regioisomers was further purified (>95% pure) by column chromatography (EtOAc).

R_f = 0.56; 0.47 (EtOAc).

mixture of isomers:

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.1-2.3 (1H, br, D_2O exch.); 2.4-2.7 (1H, br, D_2O exch.); 3.52 (1H, d, J=1.8 Hz); 3.53 (1H, d, J=2.3 Hz); 3.59 (1/2H, m); 3.64 (1/2H, d, J=5.0 Hz); 3.71 (1/2 H, d, J=5.1 Hz); 3.77 (3/2H, m); 3.79 (3H, s); 3.82 (1/2H, d, J=4.8 Hz); 3.94 (1/2H, q, J=4.3 Hz); 4.47 (1H, s); 4.49 (1H, s); 6.87 (2H, d, J= 8.6 Hz); 7.24 (2H, d, J=8.6 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: **Major Isomer, 168a** 55.2 (q), 62.6 (t), 64.1 (d), 70.6 (d), 70.7 (t), 73.2 (t), 113.9 (d), 129.3 (s), 129.5 (d), 159.4 (s). **Minor Isomer, 168b** 55.2 (q), 62.2 (d), 63.6 (t), 69.8 (t), 71.8 (d), 73.2 (t), 113.9 (d), 129.3 (s), 129.4 (d), 159.4 (s).

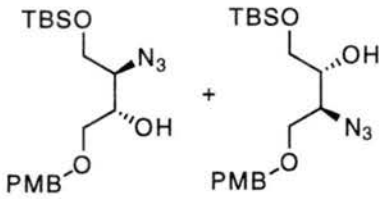
IR (NaCl/neat): 3410, 2935, 2865, 2104, 1612, 1586, 1514, 1464, 1303, 1249, 1175, 1094, 1033 cm^{-1}.

Anal. Calcd for the mixture $C_{12}H_{17}N_3O_4$: C, 53.92; H, 6.41; N, 15.72.

Found: C, 53.71; H, 6.36; N, 15.49.

(2*R*,3*S*)-2-Azido-1-O-(*tert*-butyldimethylsilyl)-4-O-(4-methoxybenzyl) butane-1,3,4-triol (169a).

(2*R*,3*S*)-3-Azido-1-O-(*tert*-butyldimethylsilyl)-4-O-(4-methoxybenzyl) butane-1,2,4-triol (169b).



169a **169b**

To a 50 mL flask was added **169a** and **169b** (1.15 gr, 4.31 mmol, 1.0 eq), and 17 mL CH_2Cl_2. The stirred mixture was cooled on an ice bath for 10 min when Et_3N (1.20 mL, 8.62 mmol, 2.0 eq), TBSCl (943 mg, 6.25 mmol, 1.4 eq), and DMAP (53 mg, 0.43 mmol, 0.1 eq) were added. After stirring for 1 h, the mixture was placed in the refrigerator at 4 °C. After 15.5 h, TLC analysis of the crude reaction mixture (EtOAc) showed complete loss of starting material. The reaction mixture was concentrated *in vacuo* and passed through a short plug of silica gel using 4:1 Hex/EtOAc as eluant to yield 1.60 gr (90% from **154**) of **169a** and **169b** as a cloudy orange oil which was used without further purification. For analytical purposes, the mixture was further purified (>95% pure) by column chromatography (4:1 Hex/EtOAc).

mixture of isomers:

R_f = 0.34; 0.27 (1:1 Hex/ EtOAc).

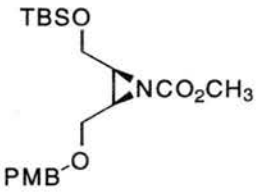
^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 0.05 (1.5H, s); 0.06 (1.5H, s); 0.08 (3H, s); 0.88 (4.5 H, s); 0.89 (4.5H, s); 2.5 (1H, br, D_2O exch.); 3.78 (3H, s); 3.46-3.92 (6H, m); 4.47 (1H, s); 4.50 (1H, s); 6.87 (2H, d, J=8.6 Hz); 7.23 (1H, d, J=8.6 Hz); 7.26 (1H, d, J=8.6 Hz).

IR (NaCl/neat): 3424, 3005, 2935, 2838, 2103, 1613, 1586, 1514, 1464, 1303, 1249, 1175 cm^{-1}.

Anal. Calcd for $C_{18}H_{31}N_3O_4Si$: C, 56.66; H, 8.19; N, 11.01.

Found: C, 56.48; H, 7.89; N, 10.79.

(2*S*, 3*R*)-1-O-(*tert*-Butyldimethylsilyl)-2,3-(methoxycarbonylaziridyl)-4-O-(4-methoxybenzyl)butane-1,4-diol (171).



To a 50 mL 3 neck flask fitted with a reflux condenser was added **169a** and **169b** (1.46 gr, 3.8 mmol, 1.0 eq), 15 mL toluene, and Ph_3P (1.30 gr, 4.9 mmol, 1.3 eq). The stirred reaction was refluxed for 5 days. When TLC analysis (10:1 CH_2Cl_2/MeOH) showed complete loss of starting material, the reaction was cooled, and the solvent was removed *in vacuo*. The resulting oil was placed under vacuum for 12 h. The cloudy white oil was dissolved in 25 mL of CH_2Cl_2 and cooled on an ice bath for ~15 min while stirring. Pyridine (978 μL, 11.4 mmol, 3.0 eq) was added to the mixture, and the mixture was stirred for another 5 min when methyl chloroformate (440 μL, 5.7 mmol, 2.0 eq) was added dropwise over 2 min. The reaction was stirred for 20 min when TLC analysis showed a complete loss of the unprotected aziridine (R_f = 0.73 10:1 CH_2Cl_2/MeOH). To the reaction mixture was added 20 mL sat $NaHCO_{3(aq)}$, and the bilayer was stirred vigorously for 10 min. The mixture was diluted with 100 mL EtOAc, and the two layers were separated. The aqueous layer was extracted 2 x 40 mL EtOAc, and the combined organic layers were washed 1 x 30 mL sat $NaCl_{(aq)}$. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by column chromatography (4:1 Hex/EtOAc) to yield 1.4 gr (92% overall yield from **169a** and **169b**) of **171** as a light yellow oil (>95% pure).

$[\alpha]^{25}_D$ = +9.6 (c = 2.1, $CHCl_3$).

R_f = 0.50 (2:1 hexane/ EtOAc).

^{1}H NMR (500 MHz)($CDCl_3$) δ TMS: 0.03 (3H, s); 0.05 (3H, s); 0.86 (9H, s); 2.69 (1H, ddd, J=6,6,6 Hz); 2.77 (1H, ddd, J=6,6,6 Hz); 3.55 (1H, dd, J=5.5, 11.2 Hz); 3.58 (1H, dd, J=6.3, 11.2 Hz); 3.60 (1H, dd, J=6.1, 11.4 Hz); 3.71 (3H, s); 3.77 (1H, dd, J=5.9, 11.4 Hz); 3.78 (3H, s); 4.46 (1H, d, J=11.5 Hz); 4.59 (1H, d, J=11.5 Hz); 6.85 (2H, d, J=8.6 Hz); 7.26 (2H, d, J=8.6 Hz).

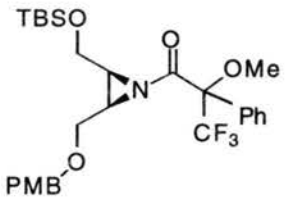
^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.4 (q); -5.3 (q); 18.2 (s); 25.7 (q); 40.4 (d); 41.8 (d); 53.5 (q); 55.1 (q); 61.2 (t); 67.1 (t); 72.4 (t); 113.7 (d); 129.4 (d); 129.9 (s); 159.2 (s); 163.5 (s).

IR (NaCl/neat): 3436, 3001, 2954, 2931, 1732, 1613, 1514, 1464, 1439, 1362, 1298, 1090 cm^{-1}.

Anal. Calcd for $C_{20}H_{33}NO_5Si$: C, 60.73; H, 8.41; N, 3.54.

Found: C, 60.60; H, 8.62; N, 3.45.

Mosher amide of aziridine 170



To a 10 mL conical flask was added aziridine **170** (22 mg, 66 μmol, 1.0 eq) and 500 μl of CH_2Cl_2. The solution was stirred until **170** had completely dissolved, and DMAP (8 mg, 66 μmol, 1.0 eq), and Et_3N (37 μL, 260 μL, 4.0 eq) were added. After stirring for another 2 min, (+)-MTPA-Cl (15 μL, 79 μmol, 1.2 eq) was added to the solution. An immediate change to orange was seen. The reaction was stirred for 20 min when no sign of starting material was seen by TLC (1:1 Hex/EtOAc). The excess acid chloride was quenched by the addition of dimethylaminopropylamine (5.0 eq), and the mixture was stirred for another 15 min. The crude reaction was concentrated *in vacuo* and passed through a short plug of silica gel (2:1 Hex/EtOAc). The crude oil was analyzed by ^{1}H NMR without further purification.

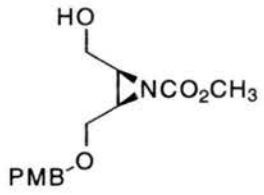
R_f = 0.71 (1:1 Hex/EtOAc).

Peaks used to measure *% ee*

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.29 and 2.12.

85% *ee* (+/- 2% *ee*)

(2*S*,3*R*)-2,3-(methoxycarbonylaziridinyl)-4-O-(4-methoxybenzyl)butane-1,4-diol (172).



To a 200 mL flask was added **171** (2.02 gr, 5.1 mmol, 1.0 eq) and 50 mL THF. The stirred solution was cooled on an ice bath for 15 min, and 1 M TBAF in THF (6.1 mL, 6.1 mmol, 1.2 eq) was added. After 30 min, the reaction was complete by TLC analysis (4:1 Hex/EtOAc). The reaction mixture was removed from the ice bath, quenched by the addition of 25 mL sat $NH_4Cl_{(aq)}$, and stirred vigorously for 5 min. The THF was evaporated, and the aqueous solution was diluted with 25 mL H_2O and extracted with Et_2O (5 x 30 mL). The combined organic layers were dried over Na_2SO_4 overnight. The filtered solution was concentrated, and the resulting oil was purified by column chromatography (2:1 CH_2Cl_2/Et_2O) to yield 1.24 gr (86% yield) of **172** as light yellow oil (>95% pure).

$[\alpha]^{25}_D$ = +36.6 (c=1.3, $CHCl_3$).

R_f = 0.21 (2:1 CH_2Cl_2/Et_2O).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.37 (1H, br, D_2O exch.); 2.81 (2H, m); 3.40 (1H, dd, J=7.2, 10.7 Hz); 3.51 (1H, m); 3.70 (3H, s); 3.78 (3H, s); 3.82 (1H, m); 3.84 (1H, dd, J=5.5, 10.7 Hz); 4.44 (1H, d, J=11.4 Hz); 4.52 (1H, d, J=11.4 Hz); 6.86 (2H, d, J=8.7 Hz); 7.23 (2H, d, J=8.7 Hz).

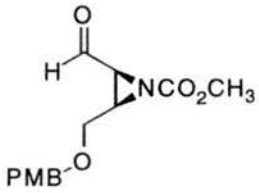
^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 39.5 (d); 41.5 (d); 53.7 (q); 55.2 (q); 60.5 (t); 67.4 (t); 73.0 (t); 113.9 (d); 129.2 (s); 129.6 (d); 159.5 (s); 163.2 (s).

IR (NaCl/neat): 3430, 3003, 2955, 1728, 1613, 1586, 1514, 1440, 1301, 1247, 1175, 1083, 821 cm^{-1}.

Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.78; H, 6.81; N, 4.98.

Found: C, 59.80; H, 7.02; N, 4.82.

Aldehyde 173.



To a 200 mL flask was added **172** (1.29 gr, 4.58 mmol, 1.0 eq), and 45 mL CH_2Cl_2. The mixture was stirred for 5 min when Dess-Martin reagent (3.5 gr, 7.3 mmol, 1.6 eq) was added to the flask in one portion. The mixture was stirred for 2.5 h when TLC analysis (2:1 Hex/EtOAc) showed complete loss of starting material. The reaction mixture was dissolved in 150 mL Et_2O and poured into a solution of 150 mL sat $NaHCO_{3(aq)}$ with seven fold excess of $Na_2S_2O_3 \bullet 5\ H_2O$ (9.0 gr). The biphasic mixture was vigorously stirred for 15 min while the milky color of the organic layer slowly disappeared. The two layers were separated. The organic layer was washed 1 x 25 mL sat $NaHCO_{3(aq)}$ and 1 x 25 mL H_2O. The combined aqueous layers were back extracted 5 x 30 mL Et_2O. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by column chromatography (1.5:1 Hex/EtOAc) to yield 1.20 gr (92% yield) of **173** as a clear colorless oil (>95% pure).

$[\alpha]^{25}_D$ = -80.6 (c=1.3, $CHCl_3$).

$\mathbf{R_f}$ = 0.60 (2:1 CH_2Cl_2/Et_2O).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 3.04 (1H, ddd, J=4.3, 4.4, 6.9 Hz); 3.10 (1H, dd, J=4.5, 6.9 Hz); 3.64 (1H, dd, J=4.3, 11.2 Hz); 3.73 (1H, dd, J=4.4, 11.2 Hz); 3.75 (3H, s); 3.78 (3H, s); 4.45 (1H, d, J=11.5 Hz); 4.47 (1H, d, J=11.5 Hz); 6.85 (2H, d, J=8.7 Hz); 7.19 (2H, d, J=8.7 Hz); 9.31 (1H, d, J=4.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 43.5 (d); 44.7 (d); 54.1 (q); 55.2 (q); 65.8 (q); 73.0 (q); 113.9 (d); 129.2 (s); 129.5 (d); 159.4 (s); 161.8 (s); 196.3 (d).

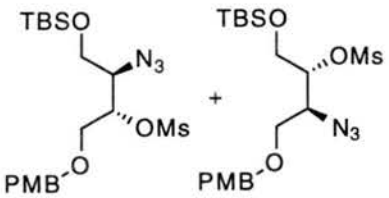
IR (NaCl/neat): 3006, 953, 2834, 1719, 1612, 1586, 1513, 1438, 1327, 1248, 1175, 1089, 819 cm^{-1}.

Anal. Calcd for $C_{14}H_{17}NO_5$: C, 60.21; H, 6.14; N, 5.02.

Found: C, 60.45; H, 6.21; N, 4.96.

(2*R*,3*S*)-2-Azido-1-O-(*tert*-butyldimethylsilyl)-3-O-methanesulfonyl-4-O-(4-methoxybenzyl) butane-1,3,4-triol (174a).

(2*R*,3*S*)-3-Azido-1-O-(*tert*-butyldimethylsilyl)-2-O-methanesulfonyl-4-O-(4-methoxybenzyl) butane-1,2,4-triol (174b).



174a **174b**

To a 25 mL conical flask was added **169a** and **169b** (255 mg, 0.67 mmol, 1.0 eq) and 8 mL CH_2Cl_2. The stirred solution was placed on an ice bath for 15 min when Et_3N (278 µL, 2.0 mmol, 3.0 eq) was added. The mixture was stirred for another 5 min when methanesulfonyl chloride (77 µL, 1.0 mmol, 1.5 eq) was added to the flask dropwise over a minute. After 30 min, TLC analysis (2:1:2 CH_2Cl_2/Et_2O/Hex) of the reaction showed complete loss of starting material. To the reaction mixture was added 10 mL of sat $NaHCO_{3(aq)}$, and the bilayer solution was stirred vigorously for 10 min. Another 10 ml of water and 25 ml of EtOAc was added to the mixture, and the two layers were separated. The aqueous layer was extracted 2 x 15 mL EtOAc, and the combined organic layers were washed 1 x 20 mL sat $NaCl_{(aq)}$. After drying over Na_2SO_4 overnight, the crude mixture was filtered, concentrated, and purified by column chromatography (10:1:10 CH_2Cl_2/Et_2O/Hex) to yield 301 mg (95% yield) of **174a** and **174b** as a light yellow oil (>95% pure).

mixture of isomers:

R_f = 0.44 (2:1:2 CH_2Cl_2/Et_2O/Hex).

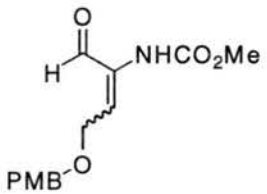
^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 0.03, 0.05, 0.06 (6H, s); 0.86, 0.87 (9H, s); 3.04, 3.05 (3H, s); 3.79 (3H, s); 3.88 - 3.64 (6H, m); 4.47, 4.48 (2H, ABq, J=11.4 Hz); 4.70, 4.80 (1H, q, J=5 Hz); 6.86, 6.87 (2H, d, J=8.6 Hz); 7.22, 7.25 (2H, d, J=8.6 Hz).

IR (NaCl, neat): 2955, 2932, 2108, 1613, 1515, 1465, 1363, 1252, 1177, 838 cm^{-1}.

Anal. Calcd for $C_{19}H_{33}N_3O_6SSi$: C, 49.65; H, 7.24; N, 9.14.

Found: C, 49.86; H, 7.06; N, 8.98.

Aldehyde 175.

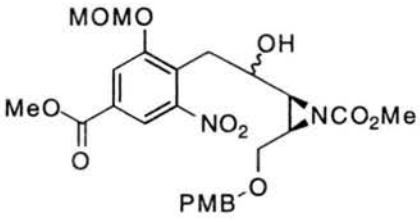


To a conical 2-neck flask was added 3 mL CH_2Cl_2 and oxalyl chloride (17 µl, 0.2 mmol, 1.5 eq). The stirred mixture was cooled to -40 °C on an acetonitrile/Dry Ice bath for 10 min. Next, DMSO (28 µL, 0.4 mmol, 3.0 eq) was added to the mixture. After stirring for another 15 min, **172** (37 mg, 0.13 mmol, 1.0 eq) in 1.5 mL CH_2Cl_2 was slowly added to the reaction over 8 min. The reaction was stirred for 15 min when Et_3N (9.0 µL, 0.65 mmol, 5.0 eq) was added to the reaction. After 10 min, the flask was removed from the bath and allowed to come to room temp. TLC analysis (2:1 CH_2Cl_2/Et_2O) of the warm reaction showed a complete loss of **172**. The mixture was diluted with 15 mL CH_2Cl_2, washed 2 x 10 mL sat $NH_4Cl_{(aq)}$ and 1 x 10 mL sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (3:2 Et_2O/Hex) to yield **175** as a clear colorless oil.

$\mathbf{R_f}$ = 0.31 (2:1 Et_2O/Hex).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 3.70 (3H, s); 3.78 (3H, s); 4.39 (2H, d, J=5.4 Hz); 6.18 (1H, t, J=5.4 Hz); 6.72 (1H, br, D_2O exch.); 6.86 (2H, d, J=8.7 Hz); 7.24 (2H, d, J=8.7 Hz); 9.19 (1H, s).

Alcohol 177.



To a 50 mL flask was added **162** (4.69 gr, 18.4 mmol, 2.0 eq) and 20 mL of DMF. The stirred mixture was cooled on an ice bath for 20 min when 0.5 M NaOMe in MeOH (1.8 mL, 0.9 mmol, 0.1 eq) was added. The clear solution immediately turned dark purple. After the addition of base, **173** (2.58 gr, 9.2 mmol, 1.0 eq) in 10 mL of DMF was added to the reaction mixture in 1 mL aliquots every 5 min. After the additions were complete (50 min), the reaction was stirred for another 3.5 h and quenched with 35 mL of sat $NH_4Cl_{(aq)}$. After 10 min, the reaction was diluted with 20 mL water, and the aqueous mixture was extracted 6 x 50 mL Et_2O, 1 x 25 mL CH_2Cl_2, and 1 x 25 mL EtOAc . The combined organic extracts were washed 1 x 45 mL sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered and concentrated. The crude oil was purified by column chromatography (1:1 Hex/EtOAc) to yield 4.2 gr (85% yield) of **177** as a yellow oil (4:1 mixture of separable diastereomers)(>95% pure).

Major Diastereomer of 177

$[\alpha]^{25}_D$ = -38.4 (c=1.3, $CHCl_3$).

R_f = 0.50 (2:1 CH_2Cl_2/Et_2O).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.65 (1H, dd, J=6.5, 8.4 Hz); 2.80 (1H, ddd, J=5.7, 6.5, 7.9 Hz); 2.98 (1H, br, D_2O exch.); 3.33 (1H, dd, J=7.9, 10.5 Hz); 3.40 (2H, m); 3.42 (3H, s); 3.60 (1H, m); 3.64 (3H, s); 3.76 (3H, s); 3.85 (1H, dd, J=5.7, 10.5 Hz); 3.92 (3H, s); 4.41 (1H, d, J=11.5 Hz); 4.46 (1H, d, J=11.5 Hz); 5.25 (2H, app. sing.); 6.79 (2H, d, J=8.6 Hz); 7.17 (2H, d, J=8.6 Hz); 7.91 (1H, d, J=1.5 Hz); 8.10 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 30.4 (t), 39.2 (d), 44.8 (d), 52.4 (q), 53.5 (q), 54.9 (q), 56.3 (q), 67.3 (t), 69.1 (d), 72.7 (t), 94.7 (t), 113.6 (d), 117.6 (d), 118.1 (d), 126.8 (s), 128.8 (s), 129.4 (d), 129.7 (s), 151.5 (s), 156.0 (s), 159.2 (s), 162.7 (s), 164.7 (s).

IR (NaCl/neat): 3509, 2956, 2923, 2854, 1728, 1613, 1538, 1514, 1438, 1363, 1292 cm^{-1}.

Anal. for the mixture of diastereomers:

Calcd for $C_{25}H_{30}N_2O_{11}$: C, 56.18; H, 5.66; N, 5.24.

Found: C, 55.93; H, 5.83; N, 5.04.

Minor Diastereomer of 177

$[\alpha]^{25}_D = +14.2$ (c=1.3, $CHCl_3$).

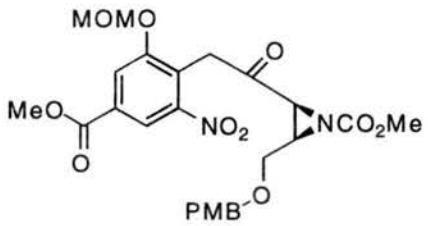
$R_f = 0.45$ (2:1 CH_2Cl_2/Et_2O).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.33 (1H, br, D_2O exch.); 2.66 (1H, dd, J=6.8, 6.8 Hz); 2.81 (1H, ddd, J=5.2, 6.5, 6.8 Hz); 3.20 (1H, dd, J=5.2, 13.4 Hz); 3.36 (2H, m); 3.41 (3H, s); 3.50 (1H, dd, J=6.5, 11.0 Hz); 3.73 (3H, s); 3.78 (3H, s); 3.84 (1H, m); 3.92 (3H, s); 4.43 (1H, d, J=11.4 Hz); 4.50 (1H, d, J=11.4 Hz); 5.21 (2H, app. sing.); 6.84 (2H, d, J=8.6 Hz); 7.21 (2H, d, J=8.6 Hz); 7.92 (1H, d, J=1.5 Hz); 8.08 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 30.9 (t), 41.3 (d), 45.8 (d), 52.7 (q), 53.9 (q), 55.2 (q), 56.6 (q), 66.9 (t), 68.9 (d), 72.6 (t), 95.0 (t), 113.8 (d), 118.0 (d), 118.5 (d), 126.3 (s), 129.4 (d), 129.7 (s), 130.3 (s), 151.5 (s), 156.3 (s), 159.3 (s), 163.3 (s), 164.7 (s).

IR (NaCl/neat): 3509, 2956, 2855, 1728, 1613, 1538, 1514, 1438, 1292, 1246 cm^{-1}.

Ketone 178.



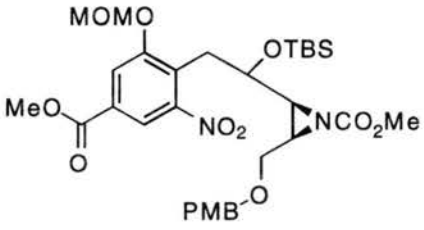
To a 10 mL flask was added 2 mL CH_2Cl_2 and **177** (60 mg mixture of diastereomers, 0.11 mmol, 1.0 eq). The mixture was stirred for 5 min when Dess-Martin periodinane (130 mg, 0.30 mmol, 2.7 eq) was added in one portion. After stirring for 9 h, TLC analysis (2:1 CH_2Cl_2/Et_2O) showed no sign of starting material. The reaction mixture was dissolved in 5 mL Et_2O and poured into a solution of 15 mL sat $NaHCO_{3(aq)}$ with seven fold excess of $Na_2S_2O_3 \cdot 5\ H_2O$ (311 mg). The biphasic mixture was vigorously stirred for 15 min while the milky color of the organic layer slowly disappeared. The two layers were separated. The organic layer was washed 1 x 5 mL sat $NaHCO_{3(aq)}$ and 1 x 5 mL H_2O. The combined aqueous layers were back extracted 2 x 15 mL Et_2O. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude oil was used without further purification to yield 58 mg (98% yield) of **178** as a clear colorless oil.

$\mathbf{R_f}$ = 0.61 (2:1 CH_2Cl_2/Et_2O)

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 3.05 (1H, ddd, J=5.3, 5.8, 6.8 Hz); 3.38 (3H, s); 3.43 (1H, d, J=6.8 Hz); 3.44 (1H, dd, J=5.3, 11.2 Hz); 3.63 (1H, dd, J=5.8, 11.2 Hz); 3.75 (3H, s); 3.76 (3H, s); 3.93 (3H, s); 4.34 (2H, s); 4.47 (1H, d, J=11.6 Hz); 4.50 (1H, d, J=11.6 Hz); 5.17 (1H, d, J=6.9 Hz); 5.20 (1H, d, J=6.9 Hz); 6.83 (2H, d, J=8.7 Hz); 7.23 (2H, d, J=8.7 Hz); 7.98 (1H, d, J=1.5 Hz); 8.28 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 39.2 (t), 43.2 (d); 44.3 (d); 52.7 (q); 54.0 (q); 55.2 (q); 56.5 (q); 66.3 (t); 72.8 (t), 94.9 (t); 113.8 (d), 118.7 (d), 118.8 (d); 123.8 (s), 129.5 (d), 129.6 (s), 130.9 (s), 150.0 (s), 156.1 (s), 159.3 (s), 162.0 (s), 164.7 (s), 198.4 (s).

Silyl ether 183.



To a 10 mL conical flask was added **177** (337 mg, 0.70 mmol, 1.0 eq), and 350 µl of DMF. Once **177** had completely dissolved, imidazole (167 mg, 2.46 mmol, 3.5 eq), and TBSCl (212 mg, 1.41 mmol, 2.0 eq) were added to the flask. After stirring for 24 h, TLC analysis (1:1 Hex/EtOAc) of the crude reaction showed complete loss of starting material, and the reaction was diluted with 15 mL Et_2O. The organic solution was washed with 10 mL water, and the two layers were separated. The aqueous layer was back extracted 6 x 15 mL Et_2O. The combined organic layers were washed with 15 mL sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by column chromatography (1.5:1 Hex/EtOAc) to give 437 mg (96% yield) of **183** as a clear yellow oil (>95% pure).

Major Diastereomer 183

$[\alpha]^{25}_D$ = -27.6 (c=1.6, $CHCl_3$).

R_f = 0.50 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.40 (3H, s); -0.11 (3H, s); 0.69 (9H, s); 2.59 (1H, dd, J=5.5, 6.3 Hz); 2.74 (1H, ddd, J=4.6, 6.3, 6.8 Hz); 3.22 (1H, dd, J=4.8, 13.5 Hz); 3.41 (1H, dd, J=9.0, 13.5 Hz); 3.43 (3H, s); 3.60 (1H, dd, J=6.8, 11.0 Hz); 3.68 (3H, s); 3.68 (1H, dd, J=4.6, 11.0 Hz); 3.77 (3H, s); 3.91 (3H, s); 4.12 (1H, ddd, J=4.8, 5.5, 9.0 Hz); 4.50 (1H, d, J=11.4 Hz); 4.60 (1H, d, J=11.4 Hz); 5.21 (2H, app. sing.); 6.85 (2H, d, J=8.5 Hz); 7.27 (2H, d, J=8.5 Hz); 7.90 (1H, d, J=1.5 Hz); 8.06 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -0.53 (q), -0.48 (q), 17.8 (s), 25.6 (q), 32.2 (t), 41.1 (d), 45.1 (d), 52.6 (q), 53.6 (q), 55.2 (q), 56.6 (q), 67.3 (t), 68.0 (d), 72.5 (t), 94.9 (t), 113.7 (d), 117.6 (d), 118.4 (d), 127.3 (s), 129.5 (d), 129.9 (s), 130.0 (s), 151.7 (s), 156.6 (s), 159.2 (s), 163.5 (s), 164.9 (s).

IR (NaCl/neat): 3001, 2954, 2856, 1731, 1613, 1537, 1514, 1438, 1291, 1248, 1089 cm^{-1}.

Anal. for the mixture of diastereomers:

Calcd for $C_{31}H_{44}N_2O_{11}Si$: C, 57.39; H, 6.84; N, 4.32.

Found: C, 57.50; H, 6.91; N, 4.50.

Minor Diastereomer 183

$[\alpha]^{25}_D = +10.5$ (c=1.1, $CHCl_3$).

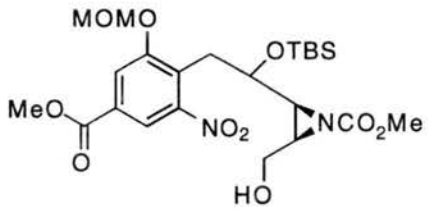
$R_f = 0.5$ (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.31 (3H, s); 0.03 (3H, s); 0.80 (9H, s); 2.61 (1H, ddd, J=4.4, 6.7, 7.1 Hz); 2.69 (1H, dd, J=6.7, 8.5 Hz); 2.98 (1H, dd, J=4.4, 11.1 Hz); 3.11 (1H, dd, J=6.6, 13.2 Hz); 3.19 (1H, dd, J=7.1, 11.1 Hz); 3.23 (1H, dd, J=7.6, 13.2 Hz); 3.39 (3H, s); 3.69 (3H, s); 3.77 (3H, s); 3.79 (1H, m); 3.91 (3H, s); 4.40 (1H, d, J=11.5 Hz); 4.50 (1H, d, J=11.5 Hz); 5.17 (2H, apparent singlet); 6.82 (2H, d, J=8.6 Hz); 7.18 (2H, d, J=8.6 Hz); 7.87 (1H, d, J=1.5 Hz); 8.01 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.3 (q), -4.8 (q), 17.7 (s), 25.6 (q), 31.8 (t), 40.9 (d), 46.0 (d), 52.6 (q), 53.4 (q), 55.1 (q), 56.5 (q), 66.9 (t), 70.3 (d), 72.1 (t), 94.7 (t), 113.6 (d), 117.4 (d), 118.2 (d), 126.1 (s), 129.4 (d), 129.6 (s), 130.1 (s), 151.5 (s), 156.6 (s), 159.1 (s), 163.2 (s), 164.6 (s).

IR (NaCl/neat): 2964, 1732, 1614, 1538, 1514, 1438, 1362, 1291, 1248, 1174, 1157, 1089, 1040 cm^{-1}.

Alcohol 184.



To a 25 mL flask was added **183** (205 mg, 0.32 mmol, 1.0 eq), 2.7 mL of CH_2Cl_2, and 150 μL of H_2O. After stirring for 5 min, DDQ (93 mg, 0.41 mmol, 1.3 eq) was added to the mixture in one portion. The reaction mixture immediately turned dark green, and over the course of the next 1.5 hr, the mixture slowly turned bright orange. After 1.5 h, the crude reaction mixture was passed through a short plug of activated alumina using 10:1 CH_2Cl_2/MeOH as eluant. After concentration *in vacuo*, the crude oil was purified by column chromatography (1:1 Hex/EtOAc) to give 160 mg (93% yield) of **184** as a clear orange oil (>95% pure).

Major Diastereomer 184

$[\alpha]_D^{25} = -55.6$ (c=1.2, CH_2Cl_2).

$\mathbf{R_f} = 0.30$ (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.34 (3H, s); -0.06 (3H, s); 0.73 (9H, s); 1.95 (1H, br, D_2O exch.); 2.61 (1H, dd, J=5.7, 6.2 Hz); 2.72 (1H, ddd, J=4.5, 6.4, 6.4 Hz); 3.26 (1H, dd, J=5.1, 13.4 Hz); 3.42 (1H, dd, J=8.8, 13.4 Hz); 3.49 (3H, s); 3.67 (3H, s); 3.90 (2H, m); 3.92 (3H, s); 4.25 (1H, ddd, J=5.1, 5.7, 8.8 Hz); 5.27 (1H, d, J=6.9 Hz); 5.29 (1H, d, J=6.9 Hz); 7.92 (1H, d, J=1.5 Hz); 8.09 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.2 (q), -4.9 (q), 17.8 (s); 25.5 (q); 32.3 (t); 42.9 (d)45.9 (d); 52.6 (q); 53.7 (q); 56.6 (q); 60.2 (t); 68.1 (d); 95.0 (t); 117.6 (d); 118.4 (d); 127.0 (s); 129.9 (s); 151.6 (s); 156.6 (s); 163.5 (s); 164.8 (s).

IR (NaCl/neat): 3510, 2955, 2856, 1730, 1540, 1438, 1362, 1291, 1224, 1090 cm^{-1}.

Anal. for the mixture of diastereomers:

Calcd for $C_{23}H_{36}N_2O_{10}Si$: C, 52.26; H, 6.86; N, 5.30.

Found: C, 52.22; H, 6.66; N, 5.19.

Minor Diastereomer 184

$[\alpha]_D^{25}$ = +8.8 (c=2.8, CH_2Cl_2).

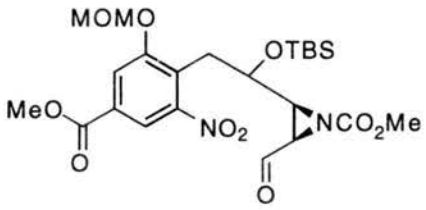
R_f = 0.30 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.24 (3H, s); 0.02 (3H, s); 0.82 (9H, s); 1.78 (1H, br, D_2O exch); 2.56 (1H, ddd, J=4.4, 6.6, 6.7 Hz); 2.74 (1H, dd, J=6.6, 8.7 Hz); 3.13 (3H, m); 3.25 (1H, dd, J=7.2, 13.2 Hz); 3.47 (3H, s); 3.68 (3H, s); 3.90 (1H, m); 3.92 (3H, s); 5.27 (1H, d, J=7.0 Hz); 5.29 (1H, d, J=7.0 Hz); 7.92 (1H, d, J=1.5 Hz); 8.05 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.3 (q), -4.8 (q), 17.7 (s), 25.5 (q), 31.9 (t), 42.8 (d), 46.9 (d), 52.6 (q), 53.4 (q), 56.6 (q), 60.0 (t), 70.0 (d), 94.9 (t), 117.4 (d), 118.1 (d), 125.9 (s), 130.2 (s), 151.5 (s), 156.6 (s), 163.3 (s), 164.6 (s).

IR (NaCl/neat): 3503, 2954, 2857, 1732, 1538, 1438, 1362 1292, 1224, 1158, 1089, 1044, 1012 cm^{-1}.

Aldehyde 185.



To a 25 mL flask was added **184** (98 mg, 0.18 mmol, 1.0 eq), and 1.5 mL of CH_2Cl_2. The mixture was stirred for 5 min when the Dess-Martin reagent (118 mg, 0.32 mmol, 1.8 eq) was added to the flask in one portion. After stirring for 2.5 h, the cloudy white mixture was diluted in 10 mL Et_2O and poured into a solution of 20 mL sat $NaHCO_{3(aq)}$ with 8.0 eq of $Na_2S_2O_3$•5 H_2O (435 mg). The milky biphasic mixture turned clear after 15 min of vigorous stirring. The two layers were separated, and the organic layer was washed 1 x 10 mL $NaHCO_{3(aq)}$, and 1 x 10 mL H_2O. The combined aqueous layers were extracted with 3 x 15 mL Et_2O. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by flash chromatography (2:1 Hex/EtOAc) to give 81 mg (82% yield) of **185** as a clear colorless oil (>95% pure).

Major Diastereomer 185

$[\alpha]_D^{25} = +5.4$ (c=1.1, CH_2Cl_2).

$\mathbf{R_f} = 0.42$ (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.18 (3H, s); 0.00 (3H, s); 0.76 (9H, s); 2.79 (1H, dd, J=3.5, 6.8 Hz); 3.00 (1H, dd, J=4.6, 6.8 Hz); 3.14 (1H, dd, J=6.8, 13.2 Hz); 3.23 (1H, dd, J=7.4, 13.2 Hz); 3.49 (3H, s); 3.68 (3H, s); 3.92 (3H, s); 4.50 (1H, ddd, J=3.5, 6.8, 7.4 Hz); 5.30 (2H, s); 7.94 (1H, d, J=1.5 Hz); 8.12 (1H, d, J=1.5 Hz); 9.50 (1H, d, J=4.6 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.3 (q), -4.7 (q), 17.9 (s), 25.6 (q), 32.1 (t), 45.1 (d), 48.7 (d), 52.7 (q), 53.9 (q), 56.7 (q), 67.7 (d), 94.9 (t), 118.0 (d), 118.4 (d), 125.8 (s), 130.4 (s), 151.2 (s), 156.7 (s), 161.9 (s), 164.6 (s), 196.9 (d).

IR (NaCl/neat): 2962, 2863, 1730, 1537, 1437, 1290, 1217, 1157, 1050, 1014, 838, 779 cm^{-1}.

Anal. for the mixture of diastereomers:

Calcd for $C_{23}H_{34}N_2O_{10}Si$: C, 52.46; H, 6.51; N, 5.32.

Found: C, 52.64; H, 6.61; N, 5.30.

Minor Diastereomer 185

$[\alpha]_D^{25} = +112$ (c=2.0, CH_2Cl_2).

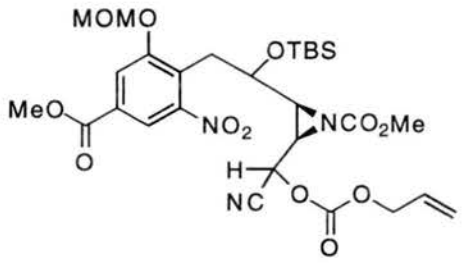
$\mathbf{R_f} = 0.42$ (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.23 (3H, s); 0.08 (3H, s); 0.85 (9H, s); 3.01 (2H, m); 3.05 (1H, dd, J=6.7, 13.3 Hz); 3.23 (1H, dd, J=7.3, 13.3 Hz); 3.47 (3H, s); 3.72 (3H, s); 3.93 (3H, s); 3.96 (1H, m); 5.26 (1H, d, J=7.0 Hz); 5.28 (1H, d, J=7.0 Hz); 7.91 (1H, d, J=1.5 Hz); 8.06 (1H, d, J=1.5 Hz); 8.95 (1H, d, J=5.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.3 (q), -4.8 (q), 17.7 (s), 25.5 (q), 31.7 (t), 45.6 (d), 48.7 (d), 52.6 (q), 53.8 (q), 56.6 (q), 69.7 (d), 94.8 (t), 117.6 (d), 118.3 (d), 125.1 (s), 130.5 (s), 151.4 (s), 156.3 (s), 161.4 (s), 164.5 (s), 195.0 (d).

IR (NaCl/neat): 2956, 2858, 1732, 1538, 1439, 1362, 1290, 1214, 1158, 1091, 1021, 839, 778 cm^{-1}.

Carbonate 187.



To a 25 mL flask was added 1 mL CH_2Cl_2 and **185** (48 mg of the major diastereomer, 88 μmol, 1.0 eq). After stirring for 10 min, allyl chloroformate (9.3 μL, 88 μmol, 1.0 eq) was added in one portion. Next, NaCN (8.6 mg, 1.8 μmol, 2.0 eq) and *n*-Bu_4NBr (1.6 mg, 5 μmol, 0.05 eq) in 0.5 mL of distilled water were added to the stirred mixture dropwise over 30 sec. The reaction was stirred vigorously for 1 h when TLC analysis (1:1 Hex/EtOAc) showed no sign of starting material. The reaction was diluted with 10 mL CH_2Cl_2 and washed 1 x 5 mL H_2O, and dried over Na_2SO_4 for 1 h. The crude reaction mixture was filtered, concentrated, and purified by column chromatography. The diastereomers of **187b** were separated to give 25 mg of each diastereomer as a clear colorless oil (87% total yield).

Higher TLC spot of diastereomer of 187

$\mathbf{R_f}$ = 0.50 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.17 (3H, s); 0.02 (3H, s); 0.76 (9H, s); 2.63 (1H, dd, J=2.2, 6.3 Hz); 3.06 (1H, dd, J=6.3, 8.8 Hz); 3.26 (1H, dd, J=7.1, 13.1 Hz); 3.34 (1H, dd, J=7.6, 13.1 Hz); 3.48 (3H, s); 3.69 (3H, s); 3.93 (3H, s); 4.70 (2H, m); 5.30 (1H, m); 5.38 (2H, s); 5.39 (1H, m); 5.75 (1H, d, J=8.8 Hz); 5.94 (1H, m); 8.00 (1H, d, J=1.6 Hz); 8.16 (1H, d, J=1.6 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.5 (q), -4.7 (q), 18.0 (s), 25.7 (q), 32.2 (t), 40.1 (d), 44.8 (d), 52.8 (q), 54.1 (q), 56.9 (q), 63.3 (d), 68.3 (d), 69.7 (t), 94.9 (t), 115.3 (s), 118.4 (d), 118.6 (d), 119.8 (t), 125.8 (s), 130.7 (d), 130.7 (s), 151.0 (s), 153.1 (s), 157.1 (s), 162.1 (s), 164.7 (s).

IR (NaCl/neat): 2956, 2858, 1762, 1735, 1537, 1439, 1364, 1293, 1233, 1158, 1092, 1020, 838, 781 cm-1.

Lower TLCspot of diastereomer of 187

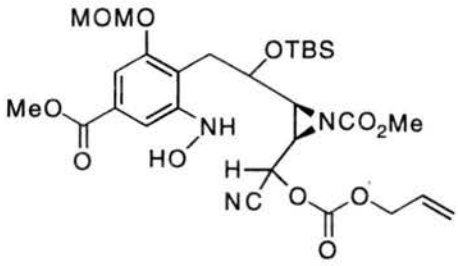
$\mathbf{R_f}$ = 0.35 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.27 (3H, s); 0.02 (3H, s); 0.80 (9H, s); 2.56 (1H, dd, J=2.8, 5.6 Hz); 3.02 (1H, dd, J=5.8, 8.6 Hz); 3.13 (1H, dd, J=6.3, 13.3 Hz); 3.25 (1H, dd, J=7.8, 13.3 Hz); 3.48 (3H, s); 3.74 (3H, s); 3.93 (3H, s); 4.59 (1H, m); 4.76 (2H, dt, J=5.9, 1.3 Hz); 5.28 (1H, m); 5.30 (1H, m); 5.36 (1H, m); 5.40 (1H, m); 5.74 (1H, d, J=8.7 Hz); 5.95 (1H, m); 7.95 (1H, d, J=1.5 Hz); 8.07 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.6 (q), -4.8 (q), 17.9 (s), 25.6 (q), 32.0 (t), 39.6 (d), 44.7 (d), 52.8 (q), 54.2 (q), 56.7 (q), 62.7 (d), 68.0 (d), 70.0 (t), 94.9 (t), 115.3 (s), 117.8 (d), 118.4 (d), 119.9 (t), 125.6 (s), 130.6 (s), 130.7 (d), 151.6 (s), 152.7 (s), 156.4 (s), 162.2 (s), 164.7 (s).

IR (NaCl/neat): 2956, 2858, 1765, 1732, 1538, 1438, 1365, 1292, 1236, 1157, 1089, 1021, 838, 780 cm-1.

Hydroxylamine 188.



To a 25 mL flask was added **187** (30 mg, 47 μmol, 1.0 eq), 2.5 mL Et_2O, and 1 mL H_2O. The mixture was stirred for 5 min when NH_4Cl (10 mg, 180 μmol, 3.8 eq), and Zn (20 mg, 300 μmol, 6.4 eq) were added in one portion. The reaction was vigorously stirred for 3 h when TLC analysis showed complete loss of starting material. The mixture was diluted with 10 mL H_2O and 15 mL Et_2O. The organic layer was separated, and the aqueous layer was extracted with 10 mL Et_2O. The combined organic layers were washed 1 x 10 mL sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, and concentrated. The crude oil was taken on without further purification.

Product from reduction of higher TLC diastereomer of 187

R_f = 0.45 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.11 (3H, s); 0.14 (3H, s); 0.95 (9H, s); 2.45 (1H, dd, J=2.1, 6.4 Hz); 2.87 (1H, dd, J=6.4, 8.7 Hz); 2.97 (1H, d, J=7.1 Hz); 3.20 (3H, s); 3.22 (3H, s); 3.49 (3H, s); 4.22 (2H, m); 4.65 (1H, dt, J=2.1, 7.3 Hz); 4.96 (4H, m); 5.51 (1H, m); 6.04 (1H, d, J=8.7 Hz); 7.79 (1H, d, J=1.5 Hz); 8.05 (1H, d, J=1.5 Hz).

IR (NaCl/neat): 3444, 3311, 2956, 2930, 2857, 1762, 1724, 1587, 1438, 1303, 1235, 1006, 738 cm^{-1}.

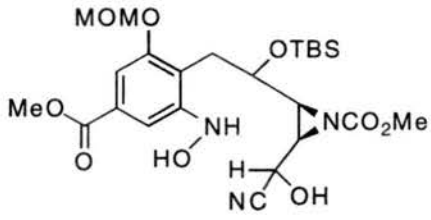
Product from reduction of lower TLC diastereomer of 187

R_f = 0.38 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.33 (3H, s); 0.44 (3H, s); 0.82 (9H, s); 2.68 (2H, m); 2.87 (1H, dd, J=4.5, 13.8 Hz); 3.04 (1H, dd, J=5.7, 8.8 Hz); 3.45 (3H, s); 3.76 (3H, s); 3.86 (3H, s); 4.46 (1H, m); 4.75 (2H, m); 5.23 (2H, s); 5.30 (1H, m); 5.40 (1H, m); 5.74 (1H, d, J=8.8 Hz); 5.93 (1H, m); 7.37 (1H, d, J=1.5 Hz); 7.63 (1H, d, J=1.5 Hz).

IR (NaCl/neat): 3437, 3302, 2955, 2930, 2857, 1763, 1727, 1585, 1439, 1301, 1234, 1006, 781 cm^{-1}.

Cyanohydrin 189.



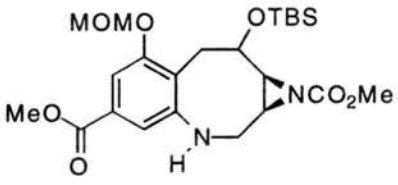
To a 25 mL flask was added 1 mL THF, **188** (29 mg of the diastereomer originating from the higher TLC isomer of **187**, 46 μmol, 1.0 eq), Ph_3P (2.1 mg, 8 μmol, 0.18 eq), and $HN(CH_2C\,H_2OH)_2$ (11μl, 93 μmol, 2.0 eq). The stirred mixture was cooled to -20 °C on a Dry Ice/acetone bath. Next, $Pd(PPh_3)_4$ (1.6 mg, 1.3 μmol, 0.03 eq) was added, and the bright orange reaction was stirred for 1.5 h. The reaction was quenched with 5 mL sat $NH_4Cl_{(aq)}$. The aqueous solution was extracted 3 x 10 mL EtOAc. The combined organic layers were washed 1 x 5 mL sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (3:2 Hex/EtOAc) to yield 13 mg of **189** (51% yield from **187**) as a clear oil.

$\mathbf{R_f}$ = 0.35 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.25 (3H, s); -0.01 (3H, s); 0.81 (9H, s); 2.68 (1H, dd, J=3.2, 6.3 Hz); 2.83 (1H, dd, J=8.6, 13.8 Hz); 2.93 (2H, m); 3.46 (3H, s); 3.68 (1H, br, D_2O exch.); 3.73 (3H, s); 3.87 (3H, s); 4.45 (1H, m); 4.98 (1H, t, J= 6 Hz); 5.24 (2H, s); 5.53 (1H, br, D_2O exch.); 7.15 (1H, br, D_2O exch.); 7.37 (1H, d, J=1.4 Hz); 7.65 (1H, d, J=1.4 Hz).

IR (NaCl/neat): 3428, 3325, 2956, 2932, 2958, 1720, 1587, 1439, 1321, 1236, 1065, 1006, 781 cm^{-1}.

Amine 192.

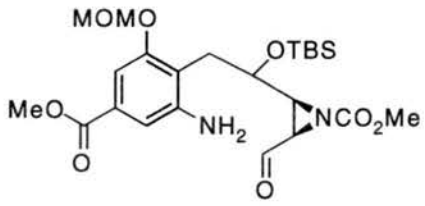


To a 100 mL round bottom flask was added 40 mL of MeOH freshly distilled from CaH_2. The stirred solution was degassed with H_2 for 30 min using a 20 gauge needle connected directly to a H_2 cylinder. The flask was then flushed with argon, and 5% Pd/C (200 mg, 0.095 mmol, 0.25 eq) was added in one portion. The mixture was degassed with H_2 for another 30 min and then kept under H_2 for another 30 minutes.

Nitroaldehyde **185** (200 mg, 0.38 mmol, 1.0 eq) in 2 mL of MeOH was added to the mixture dropwise over one min. After 8 min, TLC analysis (1:1 Hex/EtOAc) of the reaction showed complete loss of **185**. The reaction was diluted with MeOH and passed through a short pad of Celite using MeOH, and the filtrate was concentrated *in vacuo*. The residue was filtered through a short plug of Celite using CH_2Cl_2, and the filtrate was concentrated again. The residue was dissolved in 200 mL of CH_2Cl_2. Activated 4Å molecular sieves (~30 pieces) and $MgSO_4$ (2 gr) were added to the solution. The stirred mixture was heated to reflux for 24 to 36 h. The cooled mixture was filtered through a pad of Celite using 10:1 CH_2Cl_2/MeOH (500 mL). The filtrate was concentrated, and the residue was immediately dissolved in solution of 2:1 CH_2Cl_2/MeOH (12 mL).

After the stirred mixture was cooled on an ice bath for 10 min, $NaCNBH_3$ (23 mg, 0.38 mmol, 1.0 eq) and TFA (29 μL, 0.38 mmol, 1.0 eq) were added in one portion. After 4 min, TLC analysis of the reaction (1:1 Hex/EtOAc) showed no signs of starting material, and the reaction was quenched with 30 mL sat $NaHCO_{3(aq)}$. The two layers were separated, and the aqueous layer was extracted 3 x CH_2Cl_2. The combined organic layers were washed 1 x sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated, and purified by Chromatotron (4 mm plate, 2:1 Hex/EtOAc) to give 110 mg (60% yield from **185**) of **192** as a clear yellow oil (>95% pure).

Spectroscopic Data for the Major Diastereomer of 192 and Intermediates

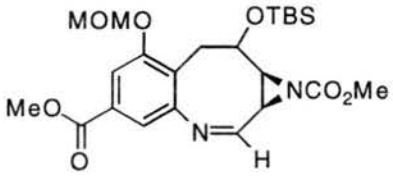


Intermediate aniline 190.

R_f = 0.43 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.13 (3H, s); 0.10 (3H, s); 0.82 (9H, s); 2.69 (1H, dd, J=6.5, 13.8 Hz); 2.82 (1H, dd, J=7.8, 13.7 Hz); 2.88 (1H, dd, J=2.6, 6.9 Hz); 3.05 (1H, dd, J=4.0, 6.9 Hz); 3.47 (3H, s); 3.73 (3H, s); 3.84 (3H, s); 3.89 (2H, br, D_2O exch); 4.44 (1H, m); 5.21 (2H, s); 7.03 (1H, d, J=1.4 Hz); 7.11 (1H, d, J=1.4 Hz); 9.54 (1H, d, J=4.0 Hz).

IR (NaCl)/neat): 3466, 3381, 2954, 2857, 1718, 1586, 1437, 1333, 1286, 1248, 1073, 1009, 838, 779, 724 cm^{-1}.

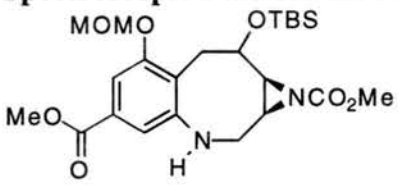


Intermediate imine 193

R_f = 0.49 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 0.17 (3H, s); 0.18 (3H, s); 0.96 (9H, s); 2.29 (1H, dd, J=11.0, 12.9 Hz); 2.56 (1H, dd, J=5.9, 5.9 Hz); 2.86 (1H, d, J=6.0 Hz); 3.00 (1H, dd, J=4.3, 12.9 Hz); 3.46 (3H, s); 3.73 (3H, s); 3.87 (3H, s); 4.28 (1H, ddd, J=4.5, 6.0, 10.7 Hz); 5.22 (1H, d, J=6.9 Hz); 5.28 (1H, d, J=6.9 Hz); 7.36 (1H, d, J=1.4 Hz); 7.50 (1H, d, J=1.4 Hz); 8.09 (1H, s).

Spectroscopic Data for the Major diastereomers of 192 and Intermediates



Amine 192

$[\alpha]^{25}_{D}$ = +48.9 (c=0.9, CH_2Cl_2).

R_f = 0.42 (1:1 Hex/EtOAc)

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 0.15 (3H, s); 0.17 (3H, s); 0.94 (9H, s); 2.52 (2H, m); 2.84 (1H, dd, J=10.5, 13.9 Hz); 3.18 (1H, dd, J=5.3, 13.9 Hz); 3.46 (3H, s); 3.59 (1H, m); 3.67 (3H, s); 3.78 (1H, m); 3.84 (3H, s); 4.06 (1H, br, D_2O exch); 4.47 (1H, ddd, J=5.3, 5.3, 5.3 Hz); 5.19 (1H, d, J=6.6 Hz); 5.23 (1H, d, J=6.6 Hz); 7.04 (1H, d, J=1.5 Hz); 7.18 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.1 (q), -4.9 (q), 18.4 (s), 25.8 (q), 31.0 (t), 41.4 (d), 43.0 (d), 47.3 (t), 52.0 (q), 53.3 (q), 56.3 (q), 69.0 (d), 94.3 (t), 105.3 (d), 114.3 (d), 118.3 (s), 129.4 (s), 148.3 (s), 156.2 (s), 163.9 (s), 166.8 (s).

IR (NaCl)/neat): 3394, 2952, 2855, 1724, 1587, 1438, 1302, 1235, 1085, 1014, 881, 837, 775 cm^{-1}.

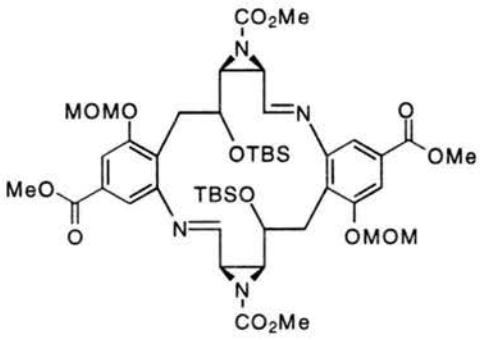
Mass Spectrum (ES^+) m/z: 481 (M+H).

Anal. for the mixture of diastereomers:

Calcd. for $C_{23}H_{36}N_2O_7Si$: C, 57.48; H, 7.55; N, 5.83.

Found: C, 57.77; H, 7.86; N 5.64.

Spectroscopic Data for the Major Diastereomer of 194 and Intermediates



Intermediate imine dimer.

$\mathbf{R_f}$ = 0.37 (1:1 Hex/EtOAc).

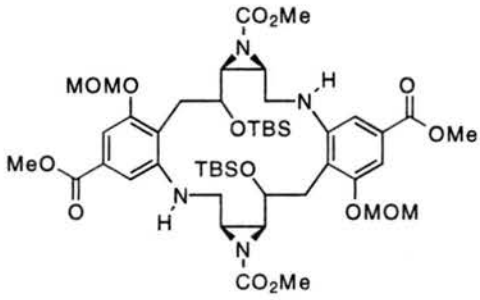
^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 0.03 (3H, s); 0.07 (3H, s); 0.72 (9H, s); 2.75 (1H, dd, J=10.7, 12.2 Hz); 2.78 (1H, dd, J=1.8, 6.8 Hz); 2.88 (1H, dd, J=4.4, 12.2 Hz); 3.40 (1H, dd, J=5.9, 6.7 Hz); 3.46 (3H, s); 3.67 (3H, s); 3.88 (3H, s); 4.55 (1H, ddd, J=1.8, 4.3, 10.7 Hz); 5.23 (1H, d, J=6.9 Hz); 5.32 (1H, d, J=6.9 Hz); 7.15 (1H, d, J=1.2 Hz); 7.52 (1H, d, J=1.2 Hz); 7.71 (1H, d, J=5.7 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.5 (q), -4.6 (q), 17.9 (s), 25.6 (q); 32.5 (t), 44.3 (d), 47.7 (d), 52.2 (q), 53.6 (q), 56.3 (q), 67.3 (d), 94.0 (t), 110.2 (d), 113.3 (d), 121.3 (s), 130.1 (s), 153.5 (s), 155.3 (s), 163.2 (s), 164.4 (d), 166.5 (s).

IR (NaCl)/neat): 2954, 2873, 1731, 1660, 1580, 1439, 1290, 1224, 1062 cm^{-1}.

Mass Spectrum (FAB) m/z: 956(M^+).

Spectroscopic Data for the Major Diastereomer of 194 and Intermediates



Dimer 194.

R_f = 0.28 (1:1 Hex/EtOAc).

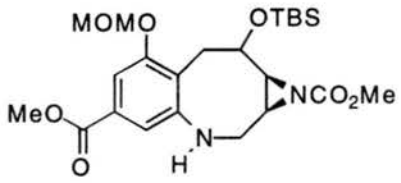
^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.49 (3H, s), -0.05 (3H, s); 0.68 (9H, s); 2.53 (1H, dd, J=2.1, 6.2 Hz); 2.99 (1H, m); 3.31 (1H, t, J=11.7 Hz); 3.46 (3H, s); 3.69 (1H, m); 3.76 (3H, s); 3.88 (3H, s); 3.91 (1H, m); 4.60 (1H, d, J=11.2 Hz); 5.04 (1H, br, D_2O exch); 5.21 (1H, d, J=6.7 Hz); 5.25 (1H, d, J=6.7 Hz); 7.26 (2H, s).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.5 (q), -4.5 (q), 18.3 (s), 26.1 (q), 31.4 (t), 39.6 (d), 42.0 (t), 45.0 (d), 52.4 (q), 54.0 (q), 56.5 (q), 70.4 (d), 94.5 (t), 105.5 (d), 105.7 (d), 119.2 (s), 130.3 (s), 149.1 (s), 156.2 (s), 164.2 (s), 167.7 (s).

IR (NaCl)/neat): 3402, 2955, 2930, 1729, 1585, 1436, 1236, 1157, 1080, 1013 cm^{-1}.

Mass Spectrum (ES^+) m/z: 961 (M+1).

Spectroscopic Data for the Minor Diastereomer of 192



Amine 192

$[\alpha]^{25}_{D}$ = +127 (c=0.9, CH_2Cl_2).

$\mathbf{R_f}$ = 0.50 (1:1 Hex/EtOAc).

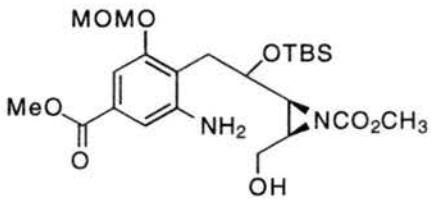
^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 0.10 (3H, s); 0.11 (3H, s); 0.92 (9H, s); 2.58 (1H, m); 2.66 (1H, dd, J=5.1, 6.9 Hz); 3.06 (1H, dd, J=8.7, 15.0 Hz); 3.12 (1H, d, J=4.5 Hz); 3.13 (1H, d, J=4.5 Hz); 3.45 (3H, s); 3.62 (1H, dd, J=3.6, 8.1 Hz); 3.66 (3H, s); 3.83 (1H, br, D_2O exch); 3.84 (3H, s); 4.23 (1H, ddd, J=4.5, 4.5, 4.5 Hz); 5.16 (1H, d, J=6.6 Hz); 5.19 (1H, d, J=6.6 Hz); 7.07 (1H, d, J=1.5 Hz); 7.30 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: -5.0 (q), -4.9 (q), 18.2 (s), 25.7 (q), 33.5 (t), 40.2 (d), 48.2 (t), 48.4 (d), 52.0 (q), 53.4 (q), 56.2 (q), 72.9 (d), 94.6 (t), 107.7 (d), 115.6 (d), 123.3 (s), 129.0 (s), 149.3 (s), 156.4 (s), 163.3 (s), 166.8 (s).

IR (NaCl/neat): 3387, 2953, 2856, 1724, 1585, 1437, 1300, 1229, 1069, 1008, 837, 776 cm^{-1}.

Mass Spectrum (ES^+) m/z: 481 (M+H).

Amino alcohol 195.



To a 10 mL conical flask was added nitro alcohol **184** (60 mg of the major diastereomer, 0.11 mmol, 1.0 eq) and 1 mL of MeOH and CH_2Cl_2. After stirring for 3 min, ammonium formate (68 mg, 1.0 mmol, 9.7 eq) and 5% Pd/C (10 mg) were added to the flask. After 3.5 h, TLC analysis of the reaction (10:1 CH_2Cl_2/MeOH) showed complete loss of starting material. The reaction was diluted with CH_2Cl_2 and passed through a Celite plug using 1:1 CH_2Cl_2/MeOH. The concentrated oil was dissolved in CH_2Cl_2 and washed 1 x H_2O. The aqueous layer was back extracted 1 x CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered, concentrated, and purified by Chromatotron (2 mm plate; 1:1 Hex/EtOAc) to yield 55 mg of amino alcohol **195** (97% yield) as a clear colorless oil.

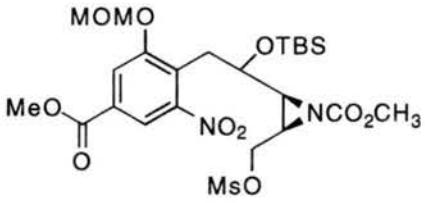
Major Diastereomer

R_f = 0.62 (10:1 CH_2Cl_2/MeOH).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.27 (3H, s); -0.05 (3H, s); 0.78 (9H, s); 2.63 (1H, dd, J=4.8, 6.4 Hz); 2.72 (1H, dd, J=5.7, 11.8 Hz); 2.94 (2H, m); 3.45 (3H, s); 3.71 (3H, s); 3.84 (3H, s); 3.86 (2H, s); 4.28 (1H, m); 5.17 (1H, d, J= 6.5 Hz); 5.20 (1H, d, J=6.5 Hz); 7.04 (1H, d, J=1.2 Hz); 7.13 (1H, d, J=1.2 Hz).

IR (NaCl/neat): 3456, 3377, 2954, 2931 2856, 1722, 1633, 1585, 1436, 1299, 1238, 1154, 1062, 1007 cm^{-1}.

Mesylate 196.



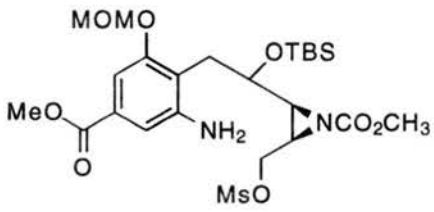
To a 25 mL conical flask was added nitro alcohol **184** (60 mg of the major diastereomer, 0.11 mmol, 1.0 eq), and 500 mL CH_2Cl_2. The stirred solution was placed on an ice bath for 10 min, when Et_3N (30 μL, 0.22 mmol, 2.0 eq), and methanesulfonyl chloride (11 μL, 0.14 mmol, 1.3 eq) were added. After 4 min, TLC analysis (1:1 Hex/EtOAc) showed complete loss of starting material. The reaction was quenched with 10 mL sat $NaHCO_{3(aq)}$ and stirred for 10 min. The solution was extracted 3 x 15 mL EtOAc, and the combined organic layers were dried over Na_2SO_4. The solution was filtered, concentrated, and purified by Chromatotron (2 mm plate, 1:1 Hex/EtOAc) to yield 70 mg (89% yield) of **196** as a clear colorless oil.

Major Diastereomer

R_f = 0.36 1:1 Hex/EtOAc.

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.25 (3H, s); -0.02 (3H, s); 0.76 (9H, s); 2.56 (1H, dd, J=4.0, 6.4 Hz); 2.84 (1H, ddd, J=5.0, 6.6, 6.8 Hz); 3.10 (3H, s); 3.16 (1H, dd, J=6.1, 13.3 Hz); 3.32 (1H, dd, J= 8.1, 13.3 Hz); 3.50 (3H, s); 3.68 (3H, s); 3.93 (3H, s); 4.41 (1H, ddd, J=4.0, 6.1, 8.1 Hz); 4.53 (1H, dd, J=5.1, 11.8 Hz); 4.54 (1H, dd, J=6.9, 11.8 Hz); 5.32 (2H, s); 7.94 (1H, d, J=1.5 Hz); 8.09 (1H, d, J=1.5 Hz).

Amine 197.



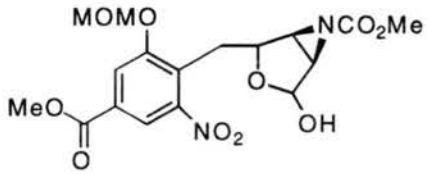
To a 10 mL conical flask was added nitro mesylate **196** (20 mg of the major diastereomer, 33 μmol, 1.0 eq) and 750 μL of MeOH. The solution was stirred for 5 min when 5% Pd/C (15 mg) was added in one portion. Hydrogen gas was bubbled through the reaction for 10 min when TLC analysis (1:1 Hex/EtOAc) showed almost a complete loss of starting material. The reaction was diluted with EtOAc and filtered through Celite using EtOAc. The solution was concentrated to yield amine **197** as a clear yellow oil that was used without further purification.

Major Diastereomer

$\mathbf{R_f}$ = 0.22 (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.21 (3H, s); -0.02 (3H, s); 0.81 (9H, s); 2.65 (1H, dd, J=3.5, 6.4 Hz); 2.84 (3H, m); 3.08 (3H, s); 3.45 (3H, s); 3.71 (3H, s); 3.85 (3H, s); 3.99 (2H, br, D_2O exch.); 4.38 (1H, m); 4.39 (1H, dd, J=5.2, 11.1 Hz); 4.53 (1H, dd, J=7.2, 11.1 Hz); 5.24 (2H, s); 7.06 (1H, d, J=1.5 Hz); 7.13 (1H, d, J=1.5 Hz).

Hemiacetal 198.



To a 10 mL conical flask was **185** (125 mg of the major diastereomer, 0.23 mmol, 1.0 eq) and 2 mL of THF. The stirred solution was cooled on an ice bath for 10 min before 1.0 M TBAF (230 mL, 0.23 mmol, 1.0 eq) in THF was added. After 3 h, the reaction was concentrated, and the residue was diluted in 15 mL sat $NH_4Cl_{(aq)}$. The aqueous solution was extracted 4 x 10 mL EtOAc, and the combined organic layers were dried over Na_2SO_4, filtered, concentrated, and purified by Chromatotron (1:1 Hex/EtOAc, 2 mm plate) to give 70 mg (77% yield) of **198** as a brown oil.

Major Diastereomer

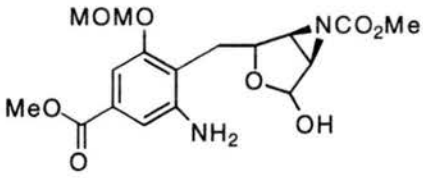
R_f = 0.22 (1:1 Hex/EtOAc)

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 3.21 (1H, br, D_2O exch.); 3.22 (1H, dd, J=4.0, 13.5 Hz); 3.26 (1H, d, J=3.6); 3.29 (1H, d, J=3.6 Hz); 3.33 (1H, dd, J=9.1, 13.5 Hz); 3.51 (3H, s); 3.63 (3H, s); 3.92 (3H, s); 4.55 (1H, dd, J=5.0, 9.0 Hz); 5.34 (2H, s); 5.43 (1H, d, J=3.6 Hz); 7.92 (1H, d, J=1.5 Hz); 8.01 (1H, d, J=1.5 Hz).

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS: 30.8 (t), 42.7 (d), 43.8 (d), 52.7 (q), 53.4 (q), 56.8 (q), 75.6 (d), 94.9 (t), 95.5 (d), 117.6 (d), 118.1 (d), 125.9 (s), 130.5 (s), 151.8 (s), 156.0 (s), 160.0 (s), 164.7 (s).

IR (NaCl/neat): 3444, 2956, 1729, 1538, 14440, 1360, 1293, 1040, 910 cm^{-1}.

Aniline 199.



To a 25 mL flask was added 12 mL of MeOH, and the solution was degassed for 15 min with H_2. The flask was flushed with argon, and 5% Pd/C (50 mg) was added in one portion. The solution was degassed with H_2 for another 15 min after which ketal **198** (45 mg of the major diastereomer, 0.11 mmol, 1.0 eq) in 1 mL of MeOH was added dropwise over 1 min. After 6 min, TLC analysis of the reaction (10:1 CH_2Cl_2/MeOH) showed complete loss of starting material. The reaction was immediately diluted with MeOH and passed through a short plug of Celite using MeOH (~50 mL). The filtrate was concentrated, and the residue (41 mg, 95% crude yield) was used without further purification.

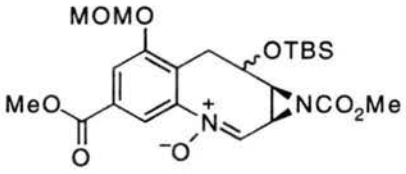
Major Diastereomer

R_f = 0.56 (10:1 CH_2Cl_2/MeOH).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 1.7 (1H, br, D_2O exch.); 2.84 (1H, dd, J=9.6, 13.8 Hz); 2.99 (1H, dd, J=4.5, 13.8 Hz); 3.27 (1H, d, J=3.6 Hz); 3.30 (1H, d, J=3.6 Hz); 3.47 (3H, s); 3.65 (3H, s); 3.85 (3H, s); 4.17 (2H, br, D_2O exch.); 4.54 (1H, dd, J=4.5, 9.6 Hz); 5.24 (2H, s); 5.48 (1H, s); 7.06 (1H, d, J=1.5 Hz); 7.15 (1H, d, J=1.5 Hz).

IR (NaCl)/neat): 3442 (br), 3373 (br), 2954, 1722, 1715, 1585, 1440, 1346, 1243, 1065, 1041, 1006, 917, 769, 732 cm^{-1}.

Nitrone 202.



To a 10 mL flask was added **192** (27 mg, 56 μmol, 1.0 eq) and 500 μl of CH_2Cl_2. Next, *m*-CPBA(50-85%) (32 mg, 140 μmol, 2.5 eq) was added to the mixture in one portion. After 20 min, TLC analysis shown complete loss of starting material. The reaction was quenched with 5 mL 10% aqueous sodium sulfite and stirred for 5 min. The solution was diluted with 15 mL H_2O and extracted 3 x 10 ml CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by Chromatotron (2 mm plate, 2:1 Hex/EtOAc) to yield nitrone **202** as a clear colorless oil.

Major Diastereomer

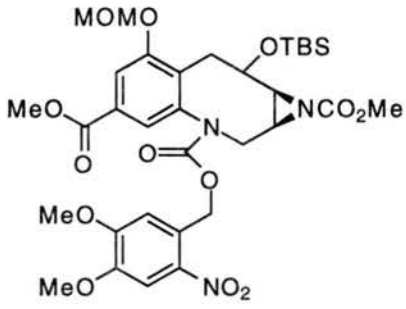
R_f = 0.53 (1:1 Hex/EtOAc)

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: -0.26 (3H, s); -0.02 (3H, s); 0.73 (9H, s); 2.91 (1H, dd, J=3.6, 6.8 Hz); 2.97 (1H, dd, J=4.6, 6.8 Hz); 3.57 (3H, s); 3.65 (3H, s); 3.87 (3H, s); 4.16 (1H, dd, J=6.8, 12.6 Hz); 4.33 (1H, dd, J=7.2, 12.6 Hz); 5.39 (2H, s); 4.66 (1H, ddd, J=3.6, 6.8, 7.2 Hz); 6.47 (1H, d, J=1.5 Hz); 8.08 (1H, d, J=1.5 Hz); 9.50 (1H, d, 4.6 Hz).

IR (NaCl/neat): 2955, 2930, 2857, 1730, 1579, 1502, 1438, 1286, 1214, 1157, 1087, 1014, 912, 838, 778 cm^{-1}.

Mass Spectrum (ES^+) m/z (relative intensity): 527 (M+H+32, 20), 511 (M+H+16, 67), 495 (M+H, 18), 481 (M+H-16, 30), 379 (31), 214 (100).

Nitroveratryl carbamate 209.



To a 25 mL conical flask was added **192** (140 mg, 290 μmol, 1.0 eq) and 3.5 mL of CH_2Cl_2. The solution was stirred for 3 min when *N,N*-diisopropylethylamine (152 μL, 870 μmol, 3.0 eq), 6-nitroveratryl chloroformate (200 mg, 730 μmol, 2.5 eq), and DMAP (36 mg, 290 μmol, 1.0 eq) were added. After 4 h, TLC analysis (1:1 Hex/EtOAc) of the reaction showed no starting material. The reaction was diluted with 15 mL sat $NaHCO_{3(aq)}$ and extracted 3 x EtOAc. The combined organic layers were washed 1 x sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated, and purified using Chromatotron (2:1 Hex/EtOAc, 2 mm plate) to give 185 mg (88% yield) of **209** as a clear yellow oil.

Major Diastereomer

$[\alpha]^{25}_D = +26.9$ (c=1.2, CH_2Cl_2).

$R_f = 0.40$ (1:1 Hex/EtOAc).

^{1}H NMR (300 MHz)(d_6-DMSO)(383 °K) δ TMS: 0.12 (3H, s); 0.14 (3H, s); 0.88 (9H, s); 2.68 (2H, br); 2.88 (2H, s); 2.93 (1H, br); 3.07 (1H, br); 3.46 (3H, s); 3.62 (3H, s); 3.80 (3H, br); 3.86 (3H, s); 3.87 (3H, s); 4.34 (1H, br); 5.29 (2H, s); 5.41 (2H, s); 6.92 (1H, s); 7.46 (1H, d, J=1.5 Hz); 7.63 (1H, d, J=1.5 Hz); 7.65 (1H, s).

IR (NaCl/neat): 2953, 2856, 1726, 1581, 1522, 1440, 1280, 1242, 1070, 1015, 837, 773 cm^{-1}.

Mass Spectrum (ES^+) m/z (relative intesity): 720 (M+H) (100%).

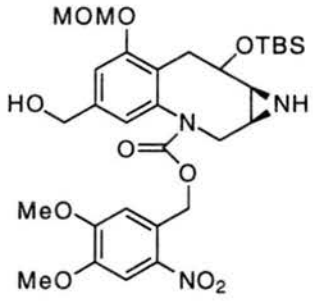
Exact Mass: (FAB) Calcd for $C_{33}H_{46}N_3O_{13}Si_1$: 720.2799.

Found: 720.2786.

Anal. Calcd for $C_{33}H_{45}N_3O_{13}Si_1$: C, 55.06; H, 6.30; N, 5.84.

Found: C, 54.93; H, 6.48; N, 5.66.

Aziridine 216.



To a 25 ml round bottom flask was added **209** (76 mg, 0.105 mmol, 1.0 eq) and 1.5 mL CH_2Cl_2. The stirred solution was cooled to -78 °C on a Dry Ice/acetone bath for 10 min when 1.0 M DIBAL in hexanes (528 mL, 0.528 mmol, 5.5 eq) was added in dropwise portions with 5 min between each addition. After 5 h, the reaction was quenched at -78 °C by the addition of one drop of MeOH and two drops of sat $NaCl_{(aq)}$. After removing from the bath and coming to room temp, the solution was filtered through a short plug of Celite with CH_2Cl_2. The two layers were separated, and the aqueous layer was extracted 3 x CH_2Cl_2. The combined organic layers were dried over Na_2SO_4. The solution was filtered, concentrated, and purified by Chromatotron (2 mm plate, 22:1 CH_2Cl_2/MeOH) to give 39 mg (61% yield) of aziridine **216** as a clear yellow oil.

Major Diastereomer

$[\alpha]^{25}_D = +25.4$ (c=2.6, CH_2Cl_2).

$R_f = 0.41$ (10:1 CH_2Cl_2/MeOH).

^{1}H NMR (300 MHz)(d_6-DMSO)(373 °K) δ TMS: 0.12 (3H, s); 0.13 (3H, s); 0.91 (9H, s); 2.02 (2H, s); 2.86 (2H, s); 2.93 (2H, s); 3.45 (3H, s); 3.83 (3H, s); 3.87 (3H, s); 4.28 (1H, s); 4.46 (2H, s); 4.71 (1H, s, D_2O exch); 5.19 (1H, d, J=6.6 Hz); 5.22 (1H, d, J=6.6Hz); 5.41 (2H, s); 6.81 (1H, s); 7.03 (2H, s); 7.66 (1H, s).

IR (NaCl/neat): 3368, 2954, 2856, 1713, 1582, 1524, 1441, 1324, 1278, 1222, 1068, 908, 837, 732 cm^{-1}.

Mass Spectrum (ES^+) m/z (relative intesity): 634 (M+H, 100%).

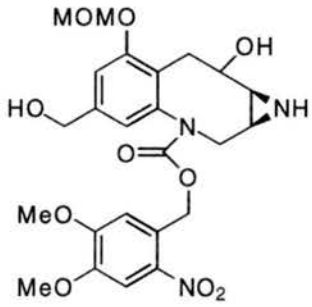
Exact Mass: (FAB) Calcd for $C_{30}H_{44}N_3O_{10}Si_1$: 634.2796.

Found: 634.2760.

Anal. Calcd for $C_{30}H_{43}N_3O_{10}Si_1$: C, 56.85; H, 6.84; N, 6.63.

Found: C, 56.53; H, 7.07; N, 6.37.

Diol 217.



To a 10 mL conical flask was added **216** (50 mg, 0.079 mol, 1.0 eq) and 1 mL THF. The solution was stirred for 5 min on an ice bath when 1.0 M TBAF in THF (135 µl, 0.135 mmol, 1.65 eq) was added dropwise over 1 min. After the addition was complete, the reaction was allowed to warm to room temp. After 4 h, TLC analysis (10:1 CH_2Cl_2/MeOH) showed no sign of starting material. The reaction was diluted with water, and the THF was removed *in vacuo*. The aqueous solution was extracted 3 x EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, concentrated, and purified by Chromatotron (2 mm plate, 10:1 CH_2Cl_2/MeOH) to give 35 mg (85% yield) of diol **217** as a foamy yellow oil. The unstable diol was immediately taken on without full characterization.

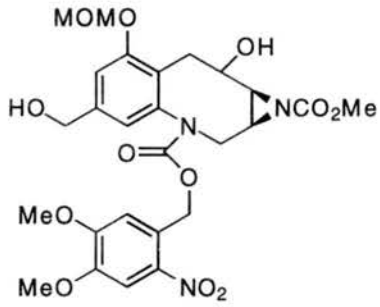
Major Diastereomer

R_f = 0.23 (10:1 CH_2Cl_2/MeOH).

IR (NaCl/neat):3429 br, 3314 br, 2928, 2854, 1704, 1581, 1524, 1440, 1324, 1278, 1221, 1067, 1017, 732 cm^{-1}.

Mass Spectrum (ES^+) m/z (relative intensity): 520 (M+H, 100%).

Diol 218.



To a 10 mL flask was added **217** (15 mg, 29 μmol, 1.0 eq) and 300 μL pyridine. After stirring for 5 min, *N*-((methoxy)carbonyloxy)succinimide (5 mg, 29 μmol, 1.0 eq) was added in one portion. After 2.5 h, TLC analysis (10:1 CH_2Cl_2/MeOH) showed complete loss of starting material, and the reaction was diluted with water and sat $NH_4Cl_{(aq)}$. The aqueous solution was extracted 3 x EtOAc. The combined organic layers were washed 1 x sat $NaHCO_{3(aq)}$ and 1 x sat $NaCl_{(aq)}$, dried over Na_2SO_4, filtered, concentrated, and purified by Chromatotron (10:1 CH_2Cl_2/MeOH, 2 mm plate) to give 14 mg (89% yield from **216**) of **218** as a foamy yellow oil.

Major Diastereomer

$[\alpha]^{25}_D = +30.6$ (c=1.5, CH_2Cl_2).

$R_f = 0.38$ (10:1 CH_2Cl_2/MeOH).

^{1}H NMR (300 MHz)(d_6-DMSO)(378 °K) δ TMS: 2.64 (2H, s); 2.84 (2H, s); 2.91 (2H, s); 3.44 (3H, s); 3.62 (3H, s); 3.78 (3H, s); 3.85 (3H, s); 4.06 (1H, s); 4.41 (1H, br, D_20 exch.); 4.45 (2H, s); 4.72 (1H, D_2O exch.); 5.21 (2H, s); 5.37 (2H, s); 6.81 (1H, s); 6.90 (1H, s); 7.03 (1H, s); 7.65 (1H, s).

IR (NaCl/neat): 3741, 2954, 2852, 1731, 1715, 1614, 1582, 1520, 1442, 1277, 1222, 1067, 1015, 915, 873, 731 cm^{-1}.

Mass Spectrum (ES^+) m/z (relative intensity): 578 (M+H,100%).

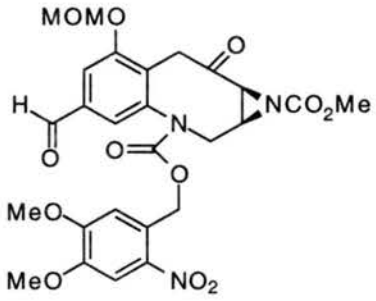
Exact Mass: (FAB) Calcd for $C_{26}H_{32}N_3O_{12}$: 578.1986.

Found: 578.1954.

Anal. Calcd for $C_{26}H_{32}N_3O_{12}$ •0.6 H_2O: C, 53.07; H, 5.51; N, 7.14.

Found: C, 53.39; H, 5.71; N, 6.75.

Ketone 219.



To a 10 mL conical flask was added was added diol **218** (35 mg, 62 μmol, 1.0 eq) and 600 μL CH_2Cl_2. The solution was stirred for 5 min when Dess-Martin periodinane (68 mg, 160 μmol, 2.6 eq) was added in one portion. The reaction immediately became cloudy and white. After 1.5 h, more Dess-Martin reagent (55 mg) and 150 μL CH_2Cl_2 were added to the reaction. After another 0.5 h, TLC analysis (10:1 CH_2Cl_2/MeOH) showed no sign of staring material. The reaction was diluted with Et_2O and added to a solution of sat $NaHCO_{3(aq)}$ and NaS_2O_3•5 H_2O (123 mg, 8 eq). The biphasic mixture was vigorously stirred for 15 min. The organic layer was diluted with EtOAc and separated from the aqueous layer. The organic layer was washed 1 x sat $NaHCO_{3(aq)}$ and 1 x H_2O. The combined aqueous layers were back extracted 2 x EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, concentrated, and purified using Chromatotron (2 mm plate, 2:1 CH_2Cl_2/Et_2O) to give 28 mg (83% yield) of ketone **219** as a clear foamy oil.

Major Diastereomer

$[\alpha]^{25}_D$ = -40.3 (c=1.3, CH_2Cl_2).

R_f = 0.80 (10:1 CH_2Cl_2/MeOH).

^{1}H NMR (300MHz)(d_6-DMSO)(378 °K) δ TMS: 2.91 (4H, s); 3.37 (1H, s); 3.38 (1H, s); 3.44 (3H, s); 3.60 (3H, s); 3.76 (3H, s); 3.85 (3H, s); 5.34 (4H, m); 6.82 (1H, s); 7.27 (1H, s); 7.58 (1H, s); 7.63 (1H, s); 9.93 (1H, s).

IR (NaCl/neat): 2954, 2847, 1729, 1702, 1581, 1521, 1443, 1280, 1151, 1070, 1018, 916, 732 cm^{-1}.

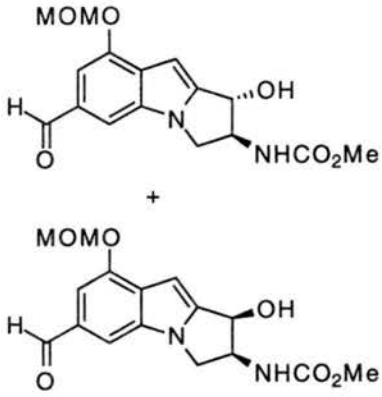
UV λ_{max} (CH_3CN) nm (ε): 345 (6800), 298 (7740), 238 (18500).

Mass Spectrum (FAB) m/z (relative intensity): 574 (M+H, 100%).

Exact Mass: (FAB) Calcd for $C_{26}H_{28}N_3O_{12}$: 574.1673.

Found: 578.1702.

***trans*-Carbamate 221 and *cis*-Carbamate 222.**



To a 5 mL pyrex test tube was added ketone **219** (15 mg, 26 μmol, 1.0 eq), 3 ml CH_3CN, and 1 mL H_2O. The test tube was placed in a 50 mL pyrex test tube. The 50 mL tube was stoppered and placed in a Rayonet™ photochemical reactor and exposed to 350 nm light. Over the course of the reaction, the solution slowly turned dark orange. After 24 h, the reaction mixture was removed from the photo reactor, and the CH_3CN was removed *in vacuo*. The resulting aqueous solution was diluted with water and extracted 3 x EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The orange residue was purified by PTLC to yield 1.5 mg of **221** and **222** (38% yield) as brown solids.

***trans* Diastereomer 221**

$[\alpha]^{25}_D = +15.2$ (c=0.25, CH_2Cl_2).

$\mathbf{R_f} = 0.42$ (20/20/1 CH_2Cl_2/Et_2O/MeOH).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS:2.34 (1H, br, D_2O exch); 3.52 (3H, s); 3.74 (3H, s); 3.92 (1H, dd, J=8.0, 10.0 Hz); 4.55 (1H, dd, J=8.0, 10.0 Hz); 4.88 (1H, m); 5.19 (1H, d, J=4.8 Hz); 5.34 (1H, d, J=6.6 Hz); 5.37 (1H, d, J=6.6 Hz); 5.70 (1H, d, J=7.2 Hz); 6.65 (1H, s); 7.23 (1H, d, J=1.2 Hz); 7.41 (1H, d, J=1.2 Hz); 9.88 (1H, s).

IR (NaCl/neat):3354, 2956, 2923, 1716, 1682, 1558, 1538, 1456, 1374, 1238, 1152, 1076, 1042, 1005 cm^{-1}.

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS:47.9 (t), 52.6 (d), 56.3 (q), 56.5 (q), 66.6 (d), 77.1 (s), 94.1 (d), 94.5 (t), 102.2 (d), 109.4 (d), 127.8 (s), 132.3 (s), 133.5 (s), 144.9 (s), 151.2 (s), 192.0 (d).

Mass Spectrum (ES^+) m/z (relative intensity): 335 (M+H, 100%).

Exact Mass: (FAB) Calcd for $C_{16}H_{19}N_2O_6$: 335.1243.

Found: 335.1229.

***cis*-Diastereomer 222**

$[\alpha]^{25}_{D}$ = -21.6 (c=0.25, CH_2Cl_2).

R_f = 0.27 (20/20/1 CH_2Cl_2/Et_2O/MeOH).

^{1}H NMR (300 MHz)($CDCl_3$) δ TMS: 2.81 (1H, br, D_2O exch.); 3.53 (3H, s); 3.73 (3H, s); 3.93 (1H, m); 4.61 (2H, m); 5.06 (1H, br, D_2O exch); 5.22 (1H, d, J=3.9 Hz); 5.35 (2H, s); 6.64 (1H, s); 7.25 (1H, d, J=1.2 Hz); 7.45 (1H, d, J=1.2 Hz); 9.93 (1H, s).

IR (NaCl/neat):3332, 2923, 2852, 1704, 1682, 1568, 1532, 1455, 1375, 1234, 1150, 1079, 1045, 1002 cm^{-1}.

^{13}C NMR (75 MHz)($CDCl_3$) δ TMS:48.4 (t), 52.7 (d), 56.3 (q), 63.3 (q), 74.2 (d), 77.1 (s), 94.0 (d), 94.9 (t), 103.3 (d), 108.5 (d), 128.7 (s), 132.4 (s), 133.6 (s), 145.2 (s), 151.2 (s), 191.8 (d).

Mass Spectrum (ES^+) m/z (relative intensity): 335(M+H, 100%).

Exact Mass: (FAB) Calcd for $C_{16}H_{19}N_2O_6$: 335.1243.

Found: 335.1244.

References

1) *A New Antitumor Antibiotic, FR-900482 I. Taxonomic Studies on the Producing Strain: A New Species of the Genus Streptomyces*, Iwami, M.; Kiyoto, S.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H., *J. Antibiot.* (1987), **40**, 589.

2) *A New Antitumor Antibiotic, FR-900482 II. Production, Isolation, Characterization, and Biological Activity*, Kiyoto, S.; Shibata, T.; Yamashita, M.; Komori, T.; Okuhara, M.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H., *J. Antibiot.* (1987), **40**, 594.

3) *Structure of FR 900482, a Novel Antitumor Antibiotic from a Streptomyces*, Uchida, I.; Takase, S.; Kayakiri, H.; Kiyoto, S.; Hashimoto, M., *J. Am. Chem. Soc.* (1987), **109**, 4108.

4) *A New Antitumor Antibiotic, FR-900482 III. Antitumor Activity in Transplantable Experimental Tumors*, Shimomura, K.; Hirai, O.; Mizota, T.; Matsumoto, S.; Mori, J.; Shibayama, F.; Kikuchi, H., *J. Antibiot.* (1987), **40**, 600.

5) *A New Antitumor Antibiotic, FR-900482 IV. Hematological Toxicity In Mice*, Hirai, O.; Shimomura, K.; Mizota, T.; Matsumoto, S.; Mori, J.; Kikuchi, H., *J. Antibiot.* (1987), **40**, 607.

6) *A New Antitumor Antibiotic, FR-66979*, Terano, H.; Takase, S.; Hosoda, J.; Kohsaka, M., *J. Antibiot.* (1989), **42**, 145.

7) *Antitumor Activity and Hematotoxicity of a New, Substituted Dihydrobenzoxazine, FK 973, in Mice*, Shimomura, K.; Manda, T.; Mukumoto, S.; Masuda, K.; Nakamura, T.; Mizota, T.; Matsumoto, S.; Nishigaki, F.; Oku, T.; Mori, J.; Shibayama, F., *Cancer Res.* (1988), **48**, 1166.

8) *Effect of FK 973, a New Antitumor Antibiotic, on the Cell Cycle of L1210 Cells In Vitro*, Nakamura, T.; Masuda, K.; Matsumoto, S.; Oku, T.; Manda, T.; Mori, J.; Shimomura, K., *Japan Journal of Pharmacology* (1989), **49**, 317.

9) *A New Antitumor Antibiotic, FR-900482: V. Interstrand DNA-DNA Cross-Links in L1210 Cells*, Masuda, K.; Nakamura, T.; Shimomura, K., *J. Antibiot.* (1988), **41**, 1497.

10) *Interstrand DNA-DNA and DNA-Protein Cross-Links by a New Antitumor Antibiotic, FK 973, in L1210 Cells*, Masuda, K.; Nakamura, T.; Mizota, T.; Mori, J.; Shimomura, K., *Cancer Res.* (1988), **48**, 5172.

11) *Covalent Structure of the DNA-DNA Interstrand Cross-Link Formed by Reductively Activated FR 66979 in Synthetic DNA Duplexes*, Huang, H.; Pratum, T. K.; Hopkins, P. B., *J. Am. Chem. Soc.* (1994), **116**, 2703.

12) *DNA Cross-linking Studies on FR 900482: Observations on the Mode of Activation*, Williams, R. M.; Rajski, S. R., *Tetrahedron Lett.* (1992), **33**, 2929.

13) *Determination of DNA Cross-Linking Sequence Specificity of FR 66979: Observations on the Mode of Action of the FR 900482 Class of Anti-tumor Compounds*, Williams, R. M.; Rajski, S. R., *1993* (1993), **34**, 7023.

14) *DNA Interstrand Cross-Linking by Reductively Activated FR 900482 ans FR 66979*, Woo, J.; Sirurdsson, S. T.; Hopkins, P., *J. Am. Chem. Soc.* (1993), **115**, 1199.

15) *FR 66979 Requires Reductive Activation to Cross-Link DNA Efficiently*, Huang, H.; Rajski, S. R.; Williams, R. M.; Hopkins, P., *Tetrahedron Lett.* (1994), **35**, 9669.

16) *DNA-DNA Interstrand Cross-Linking by FR 66979 and FR 900482: Requirement of Metal Ions During Reductive Activation*, Paz, M. M.; Hopkins, P., *Tetrahedron Lett.* (1997), **38**, 343.

17) *Synthetic Approaches Toward FR-900482. I. Stereoselective Synthesis of a Pentacyclic Model Compound*, Fukuyama, T.; Goto, S., *Tetrahedron Lett.* (1989), **30**, 6491.

18) *Synthetic Studies on FR-900482: Promising Method to Construct the Bicyclic Hydroxylamine Hemi-ketal Ring System*, Yasuda, N.; Williams, R. M., *Tetrahedron Lett.* (1989), **30**, 3397.

19) *Enantiospecific Synthesis of an Aziridinobenzoazocinone, an Advanced Intermediate Containing the Core Nucleus of FR-900482 and FK 973*, Jones, R. J.; Rapoport, H., *J. Org. Chem.* (1990), **55**, 1144.

20) *A Novel Approach to FR-900482 Via Ring Forming Metathesis*, Martin, S. F.; Wagman, A. S., *Tetrahedron Lett.* (1995), **36**, 1169.

21) *Catalytic Ring-Closing Metathesis of Dienes: Application to the Synthesis of Eight-Membered Rings*, Miller, S. J.; Kim, S.-H.; Chen, Z.-R.; Grubbs, R. H., *J. Am. Chem. Soc.* (1995), **117**, 2108.

22) *Synthesis of the Antitumor Antibiotic FR-66979: Dmitrienko Oxidation Expansion of a Fully Functional Core Structure*, Lim, H.-J.; Sulikowski, G. A., *Tetrahedron Lett.* (1996), **37**, 5243.

23) *Enantioselective Synthesis of a 1,2-Disubstituted Mitosene by a Copper-Catalyzed Intramolecular Carbon-Hydrogen Insertion Reaction of a Diazo Ester*, Lim, H.-J.; Sulikowski, G. A., *1995* (1995), **60**, 2326.

24) *An Anomolous Reaction of 2-Benzenesulfonyl-3-aryloxaziridines (Davis Reagents) with Indoles: Evidence for a Stepwise Reaction of the Davis Reagent with a π-bond*, Mithani, S.; Drew, D. M.; Rydberg, E. H.; Taylor, N. J.; Mooibroek, S.; Dmitrienko, G. I., *J. Am. Chem. Soc.* (1997), **119**, 1159.

25) *A Novel Heck Arylation Reaction: Rapid Access to Congeners of FR 900482*, McClure, K. F.; Danishefsky, S. J., *J. Am. Chem. Soc.* (1993), **115**, 6094.

26) *Novel Photochemical Route to Mitomycins and FR-900482 Series*, McClure, K. F.; Benbow, J. W.; Danishefsky, S. J., *J. Am. Chem. Soc.* (1991), **113**, 8185.

27) *Cycloaddition Reactions of Aromatic Nitroso Compounds with Oxygenated Dienes. an Approach to the Synthesis of the FR-900482 Family of Antibiotics*, McClure, K. F.; Danishefsky, S. J., *J. Org. Chem.* (1991), **56**, 850.

28) *A New Approach to the Bicyclic Hydroxylamine Hemiketal ring System of the Antitumor-Antibiotic FR 900482 Via Oxidative Ring Expansion of a Tetrahydropyrrolo[1,2a]indole*, Dmitrienko, G. I.; Denhart, D.; Mithani, S.; Prasad, G. K. B.; Taylor, N. J., *Tetrahedron Lett.* (1992), **33**, 5705.

29) *Total Synthesis of (±)-FR-900482*, Fukuyama, T.; Xu, L.; Goto, S., *J. Am. Chem. Soc.* (1992), **114**, 383.

30) *Total Synthesis of (±)-FR-900482*, Schkeryantz, J. M.; Danishefsky, S. J., *J. Am. Chem. Soc.* (1995), **117**, 4722.

31) *Total Synthesis of Natural (+)-FR900482. 1. Synthetic and End-Game Strategies*, Katoh, T.; Itoh, E.; Yoshino, T.; Terashima, S., *Tetrahedron Lett.* (1996), **37**, 3471.

32) *Total Syntesis of Natural (+)-FR900482. 3. Completion of the Synthesis*, Katoh, T.; Yoshino, T.; Nagata, Y.; Nakatani, S.; Terashima, S., *Tetrahedron Lett.* (1996), **37**, 3479.

33) *Total Synthesis of Narural (+)-FR900482. 2. Efficient Syntheses of the Aromatic and the Optically Active Aliphatic Fragments*, Yoshino, T.; Nagata, Y.; Itoh, E.; Hashimoto, M.; Katoh, T.; Terashima, S., *Tetrahedron Lett.* (1996), **37**, 3475.

34) *Precursors in the Biosynthesis of FR-900482, A Novel Antitumor Antibiotic Produced By Streptomyces Sandaensis*, Fujita, T.; Takase, S.; Otsuka, T.; Terano, H.; Kohsaka, M., *J. Antibiot.* (1988), **41**, 392.

35) *Mitomycin Biosynthesis by Streptomyces verticillatus. Incorporation of the Amino-group of D-[^{15}N]Glucosamine into the Aziridine Ring of Mitomycin B*, Hornemann, U.; Aikman, M. J., *J. Chem. Soc., Chem. Commun.* (1973), 88.

36) *D-Glucosamine and L-Citrulline, Precursors in Mitomycin Biosynthesis by Streptomyces verticillatus*, Hornemann, U.; Kehrer, J. P., *J. Am. Chem. Soc.* (1974), **96**, 320.

37) *Biosynthesis of the Mitomycin Antibiotics from 3-Amino-5-hydroxybenzoic acid*, Anderson, M. G.; Kibby, J. J.; Rickards, R. W.; Rothschild, J. M., *J. Chem. Soc., Chem. Commun.* (1980), 1277.

38) *Studies on the Biosynthesis of the Mitomycin Antibiotics by Streptomyces verticillatus*, Hornemann, U.; Cloyd, J. C., *J. Chem. Soc., Chem. Commun.* (1971), 301.

39) *3-Amino-5-hydroxybenzoic Acid in Antibiotic Biosynthesis. IX. The Status of Reduced Derivatives in Mitomycin Biosynthesis*, Rickards, R. W.; Rukachaisirikul, V., *Aust. J. Chem.* (1987), **40**, 1011.

40) *Revised Absolute Configuration of Mitomycin C. X-ray Analysis of 1-N-(p-Bromobenzoyl)mitomycin C*, Shirahata, K.; Hirayama, N., *J. Am. Chem. Soc.* (1983), **105**, 7199.

41) *Structural Studies on the Mitomycins. I. Absolute Configurations of Mitomycins A and B*, Hirayama, N.; Shirahata, K., *Acta Crystallgr., Sect B* (1987), 555.

42) Gilman, A. G.; Goodman, L. S.; Gilman, A. *The Pharmaceutical Basis of Therapeutics*; Macmillan: New York, 1980.

43) Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. *Molecular Biology of the Cell*; 3 ed.; Garland: New York, 1994.

44) *Mitomycin and Porfiromycin: Chemical Mechanism of Activation and Cross-linking of DNA*, Iyer, V. N.; Szybalski, W., *Science* (1964), **145**, 55.

45) *A Molecular Mechanism of Mitomycin Action: Linking Complementary DNA Strands*, Iyer, V. N.; Szybalski, W., *Proc. Natl. Acad. Sci. U.S.A.* (1963), **50**, 355.

46) *DNA Sequence Specificity of Mitomycin Cross-Linking*, Teng, S. P.; Woodson, S. A.; Crothers, D. M., *Biochemistry* (1989), **28**, 3901.

47) *Isolation and Structure of a Covalent Cross-Link Adduct Between Mitomycin C and DNA*, Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K., *Science* (1987), **235**, 1204.

48) *Mitomycin C: small, fast and deadly (but very selective)*, Tomasz, M., *Chemistry and Biology* (1995), **2**, 575.

49) *Generation of Reactive Oxygen Radicals through Bioactivation of Mitomycin Antibiotics*, Sartorelli, A. C.; Pritos, C. A., *Cancer Res.* (1986), **46**, 3528.

50) *The Total Synthesis of Mitomycins*, Kishi, Y., *Journal of Natural Products* (1979), **42**, 549.

51) *Chiral Aziridinyl Radicals: An Application to the Synthesis of the Core Nucleus of FR-900482*, Ziegler, F. E.; Belema, M., *J. Org. Chem.* (1997), **62**, 1083.

52) *Polonovski Reactions of Mitosane Derivatives*, Danishefsky, S. J.; Feigelson, G. B., *Heterocycles* (1987), **25**, 301.

53) *Total Synthesis of (±)-Mitomycins via Isomytomycin A*, Fukuyama, T.; Yang, L., *J. Am. Chem. Soc.* (1987), **109**, 7781.

54) *Practical Total Synthesis of (±)-Mitomycin C*, Fukuyama, T.; Yang, L., *J. Am. Chem. Soc.* (1989), **111**, 8303.

55) *Catalytic Asymmetric Epoxidation and kinetic Resolution: Modified Procedures Including in Situ Derivatization*, Goa, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B., *J. Am. Chem. Soc.* (1987), **109**, 5765.

56) *Functionalization of Aromatic Systems: A Highly Chemoselective Synthesis of [(Trimethylsilyl)methyl]nitoarenes*, Bartoli, G.; Bosco, M.; Dalpozzo, R.; Todesco, P. E., *J. Org. Chem.* (1986), **51**, 3694.

57) *The Leimgruber-Batcho Indole Synthesis*, Clark, R. D.; Repke, D. B., *Heterocycles* (1984), **22**, 195.

58) *Benzazepinone Calcium Channel Blockers. 2. Structure-Activity and Drug Metabolism Studies Leading to Potent Antihypertensive Agents. Comparison with*

Benzothiaazepinones, Floyd, D. M.; Kimball, S. D.; Krapcho, J.; Das, J.; Turk, C. F.; Moquin, R. V.; Lago, M. W.; Duff, K. J.; Lee, V. G.; White, R. E.; Ridgewell, R. E.; Moreland, S.; Brittain, R. J.; Normandin, D. E.; Hedberg, S. A.; Cucinotta, G. G., *J. Med. Chem.* (1992), **35**, 756.

59) *New Synthesis of Substituted Quinoline N-Oxides via Alkylidene o-Nitroarylacetonitriles*, Wrobel, Z.; Kwast, A.; Makosza, M., *Synthesis* (1993), 31.

60) *Oxidation of Long-Chain and Related Alcohols to Carbonyls by Dimethyl Sulfoxide "Activated" by Oxalyl Chloride*, Mancuso, A. J.; Huang, S.; Swern, D., *J. Org. Chem.* (1978), **43**.

61) *Aminobenzoic Acid Diuretics. 7. 3-Substituted 4-Phenyl-, 4-Arylcarbonyl-, and 4-Arylmethyl-5-sulfamoylbenzoic Acids and Related Compounds*, Nielson, O. B. T.; Bruun, H.; Bretting, C.; Feit, P. W., *J. Med. Chem.* (1975), **18**, 41.

62) *Reserch Report #2* Kim, H.-D. (1990).

63) *Epoxide Migrations with α,β-Epoxy Alcohols*, Payne, G. B., *J. Org. Chem.* (1962), **27**, 3819.

64) *Isomer Selectivity in Stereocontrolled Payne Rearrangement-Epoxide Cleavage of 2,3-Epoxy Alcohols in Aprotic Solvents: Application to an Enantioselective Total Synthesis of (+)-exo-Brevicomin*, Page, P.; Rayner, C.; Sutherland, I., *J. Chem. Soc. Perkin Trans. 1* (1990), 1375.

65) *New Transformations of 2,3-Epoxy Alcohols and Related Derivatives. Easy Rotes to Homochiral Substances*, Behrens, C. H.; Sharpless, K. B., *Aldrichimica Acta* (1983), **16**, 67.

66) *Selective Transformations of 2,3-Epoxy Alcohols and Related Derivatives. Strategies for Nucleophilic Attack at Carbon-3 or Carbon-2*, Behrens, C. H.; Sharpless, K. B., *J. Org. Chem.* (1985), **50**, 5696.

67) Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tables of Spectral Data for Structure Determination of Organic Compounds*; 2 ed.; Springer-Verlag: New York, 1989.

68) *α-Methoxy-α-trifluoromethylphenylacetic Acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines*, Dale, J. A.; Dull, D. L.; Mosher, H. S., *J. Org. Chem.* (1969), **34**, 2543.

69) *Chiral Aziridines-Their Synthesis and Their Use in Stereoselective Transformations*, Tanner, D., *Angew. Chem. Int. Ed. Engl.* (1994), **33**, 533.

70) *Stereospecific Synthesis of a Ribosyl-Diazepanone Derivative; A Synthetic Approach for Elucidation of the Stereochemistry of a Lipid Nucleoside Antibiotic Liposidomycin B*, Spada, M. R.; Ubukata, M.; Isono, K., *Heterocycles* (1992), **34**.

71) *Sixty years of the Staudinger Reaction*, Gololobov, Y. G.; Zhmurova, I. N.; Kasukin, L. F., *Tetrahedron* (1981), **37**, 437.

72) *A New Aziridine Synthesis from 2-Azido Alcohols and Tertiary Phosphines. Preparation of Phenanthrene 9,10-Imine*, Ittah, Y.; Sasson, Y.; Shahak, I.; Tsaroom, S.; Blum, J., *J. Org. Chem.* (1978), **43**, 4271.

73) *Aza-[2,3]-Wittig Rearrangements of Vinylaziridines*, Ahman, J.; Somfai, P., *J. Am. Chem. Soc.* (1994), **116**, 9781.

74) *Acceleration of Dess-Martin Oxidation by Water*, Meyer, S. D.; Schreiber, S. L., *J. Org. Chem.* (1994), **59**, 7549.

75) *An Improved Procedure for the Preparation of the Dess-Martin Periodinane*, Ireland, R. E.; Liu, L., *J. Org. Chem.* (1993), **58**, 2899.

76) *A Useful 12-I-5 Triacetoxyperiodinane (the Dess-Martin Periodinane) for the Selective Oxidation of Primary or Secondary Alcohols and a Variety of Related 12-I-5 Species*, Dess, D. B.; Martin, J. C., *J. Am. Chem. Soc.* (1991), **113**, 7277.

77) *Specific Removal of p-Methoxybenzyl Protection By DDQ Oxidation*, Oikawa, Y.; Yoshioka, T.; Yonemitsu, O., *Tetrahedron Lett.* (1982), **23**, 885.

78) *The Total Synthesis of Steroids*, Woodward, R. B.; Sondheimer, F.; Taub, D.; Heusler, K.; McLamore, W. M., *J. Am. Chem. Soc.* (1952), **74**, 4223.

79) *Palladium Catalyst for Partial Reduction of Acetylenes*, Lindlar, H.; Dubuis, R. *Org. Syn.*; Baumgarten, H. E., Ed.; J. Wiley & Sons: New York, 1973; Vol. V, pp 880.

80) *The Cyanohydridoborate Anion as a Selective Reducing Agent*, Borch, R. F.; Bernstein, M. D.; Durst, H. D., *1971* (1971), **93**, 2897.

81) *The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products*, Mitsunobu, O., *Synthesis* (1981), 1.

82) *The Use of Triphenylphosphine/Diethyl Azodicarboxylate (DEAD) for the Cyclization of 1,4- and 1,5-Amino Alcohols*, Bernotas, R. C.; Cube, R. V., *Tetrahedron Lett.* (1991), **32**, 161.

83) *Chemistry of Oxaziridines. 2. Improved Synthesis of 2-Sulfonyloxaziridines*, Davis, F. A.; Stringer, O. D., *1982* (1982), **47**, 1774.

84) *Oxidation of Amines with 2-Sulfonyloxaziridines (Davis' Reagents)*, Zajac, W. W.; Walters, T. R.; Darcy, M. G., *J. Org. Chem.* (1988), **53**, 5856.

85) *Psoralens as Photoactive Probes of Nucleic Acid Structure and Function: Organic Chemistry, Photochemistry, and Biochemistry*, Cimino, G. D.; Gamper, H. B.; Isaacs, S. T.; Hearst, J. E., *Annu. Rev. Biochem.* (1985), **54**, 1151.

86) *Dimethoxybenzoin Carbonates: Photochemically-Removable Alcohol Protecting Groups Suitable for Phosphoramidite-Based DNA Synthesis*, Pirrung, M. C.; Bradley, J. C., *J. Org. Chem.* (1995), **60**, 1116.

87) *Synthesis of Oligonucleotides Containing 3'-Alkyl Carboxylic Acids Using Universal, Photolabile Solid Phase Synthesis Supports*, Yoo, D. J.; Greenberg, M. M., *J. Org. Chem.* (1995), **60**, 3358.

88) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; 2 ed.; Wiley & Sons: New York, 1991.

89) *Investigation of (Oxodioxolenyl)methyl Carbamates as Nonchiral Bioreversible Prodrug Moieties for Chiral Amines*, Alexander, J.; Bindra, D. S.; Glass, J. D.;

Holahan, M. A.; Reyner, M. L.; Rork, G. S.; Sitko, G. R.; Stranieri, M. T.; Stupienski, R. F.; Veerapanane, H.; Cook, J. J., *J. Med. Chem.* (1996), **39**, 480.

90) *A Total Synthesis of Lasalocid A*, Nakata, T.; Schmid, G.; Vranesic, B.; Okigawa, M.; Smith-Palmer, T.; Kishi, Y., *J. Am. Chem. Soc.* (1978), **100**, 2933.

91) *Experiments on the Synthesis of Tetracycline. Part XI. Oxidation of Ketone Acetals and Ethers by Hydride Transfer*, Barton, D. H. R.; Magnus, P. D.; Smith, G.; Streckert, G.; Zurr, D., *J. Chem. Soc., Perkin Trans. 1* (1972), 542.

92) *Photoremovable Protecting Groups in Organic Synthesis*, Pillai, V. N. R., *Synthesis* (1980), 1.

93) *Part I. Studies Concerning the Reductive Activation of Mitomycins. Part II. A Synthetic Route to the Tricarbonyl Region of FK-506*, Egbertson, M. S., Ph. D. Dissertation, Chemistry Dept., Yale University, 1989.

94) *Enantiopure Aminotriol from D-Isoascorbic Acid. Synthesis of D-Threo-C-18-Sphingosine*, Tuch, A., Sanière, M., Le Merrer, Y., Depezay, J.-C., *Tetrahedron: Asymmetry* (1996), **7**, 897.

Appendix 1

Selected Spectra

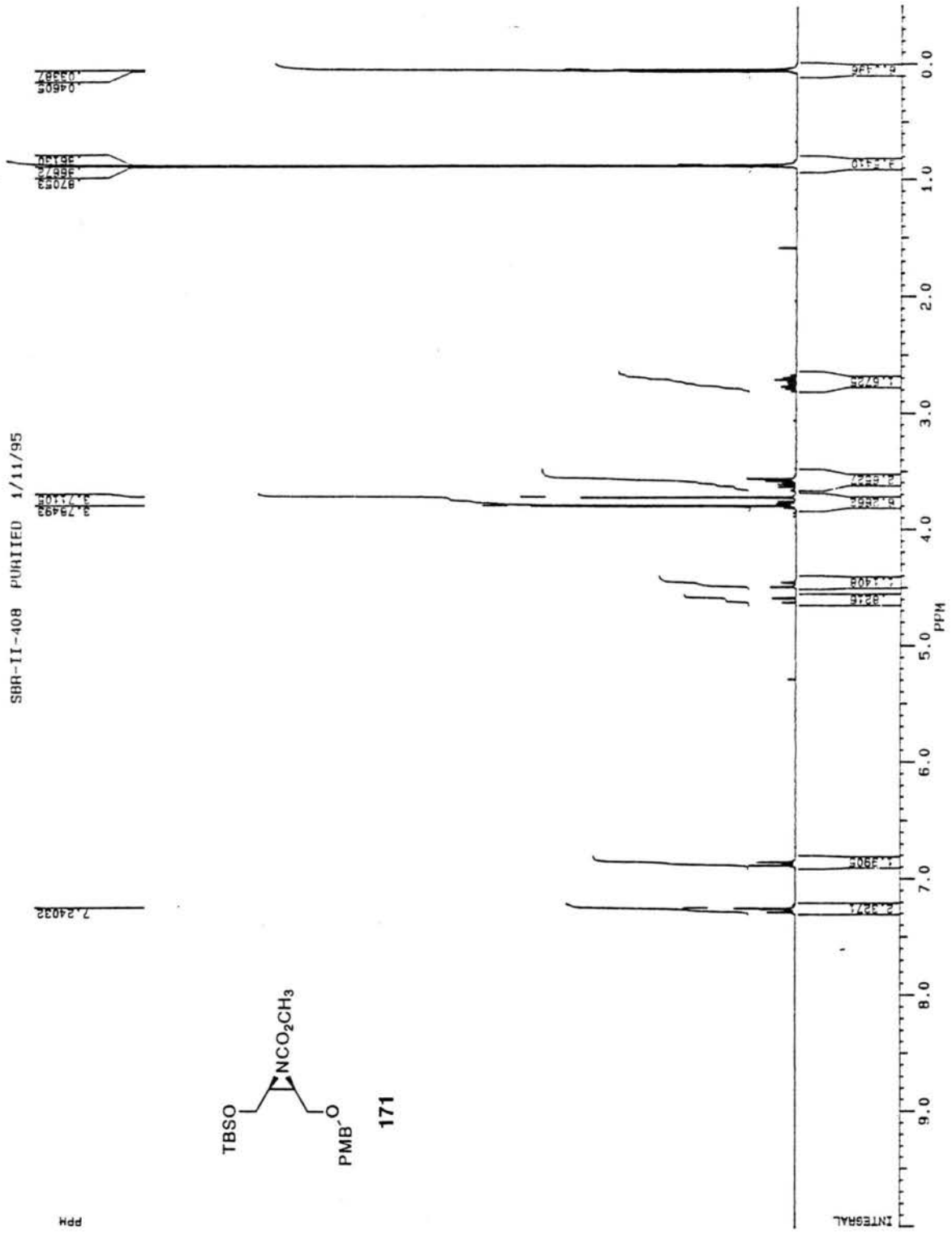
Included are the ^{1}H NMR spectra for the following compounds:

171, 172, 173, 176, 177,

183, 184, 185, 190, 192,

193, 194, 202, 209, 216,

218, 219, 221, 222.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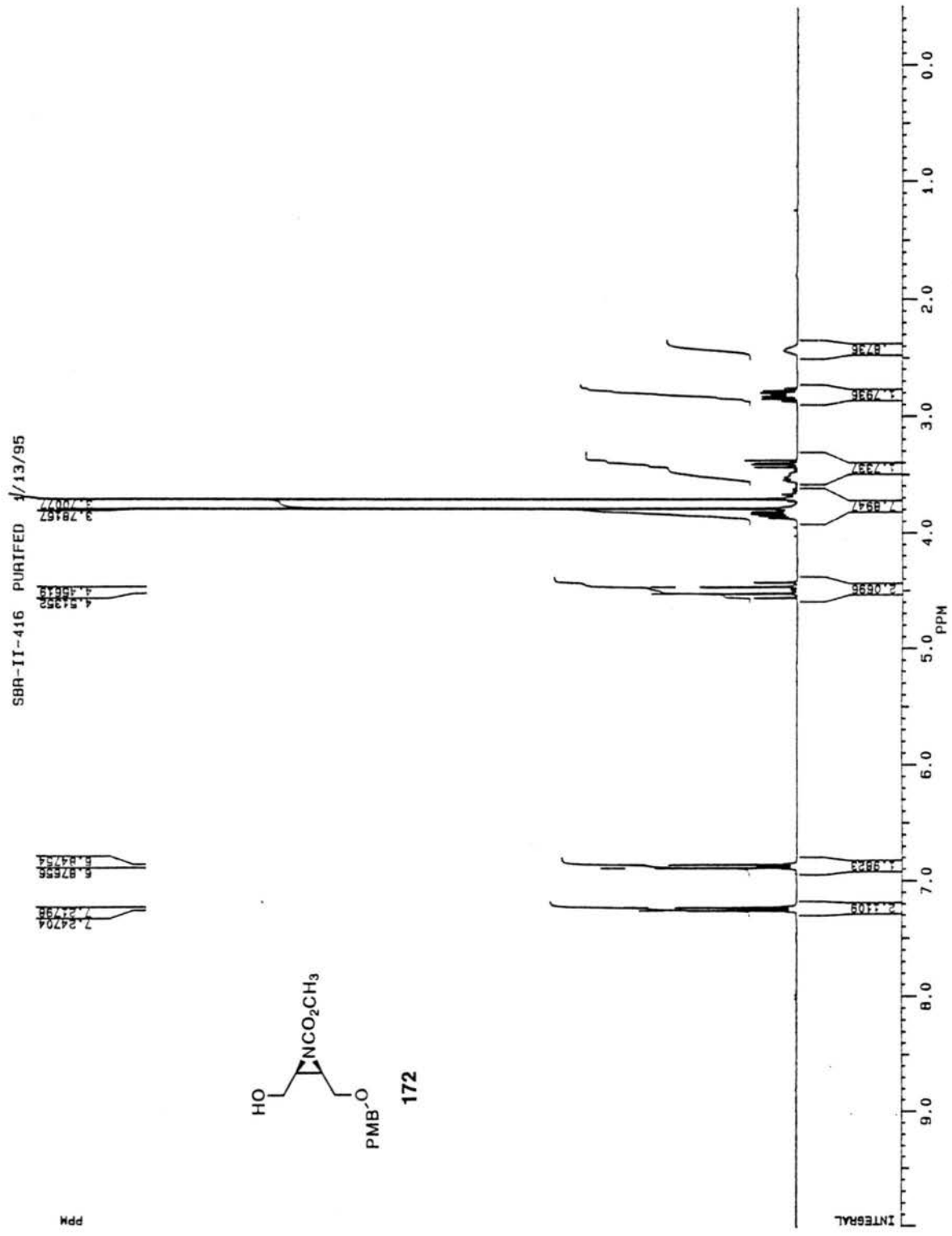
PMB
O
171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PMB-O
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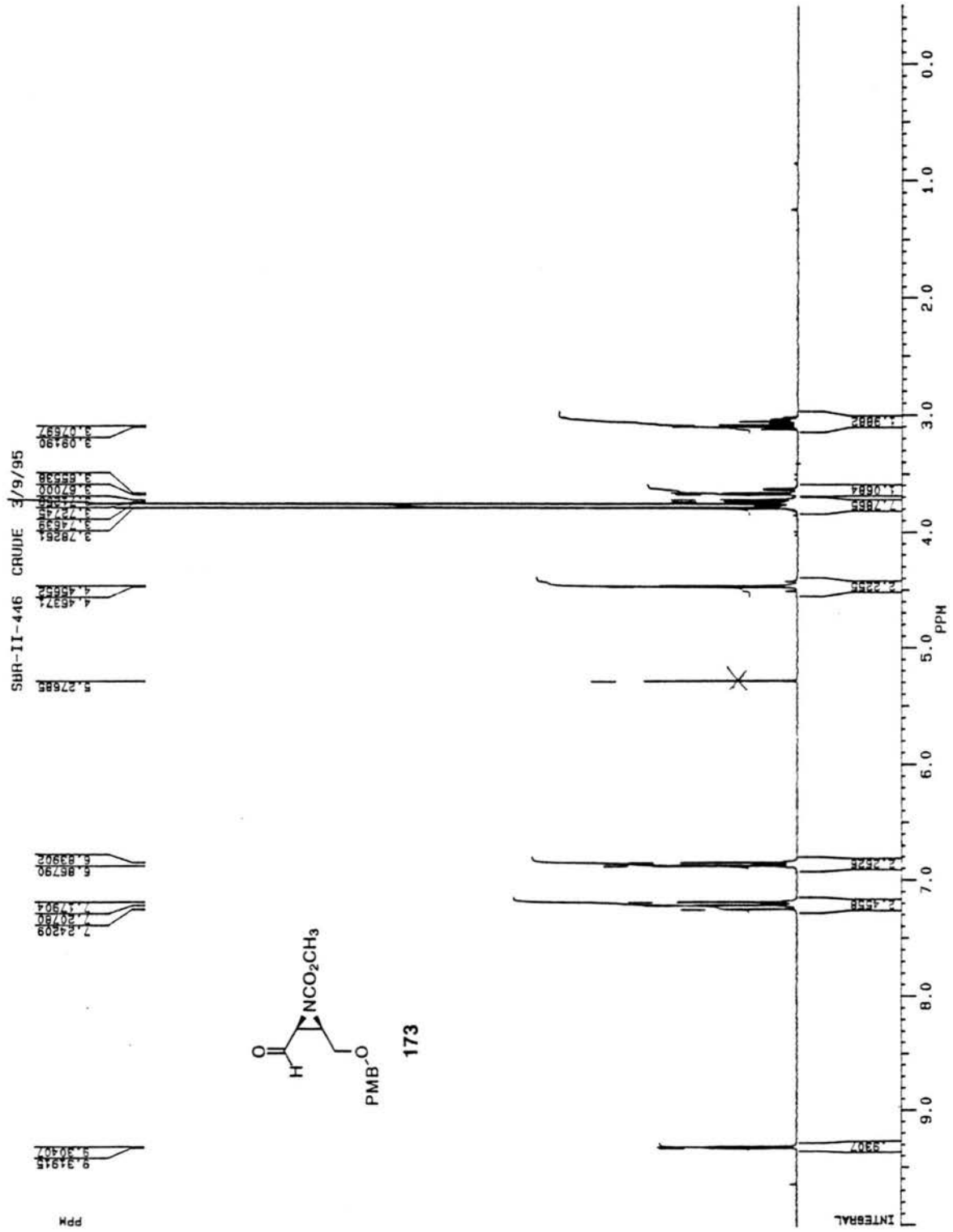

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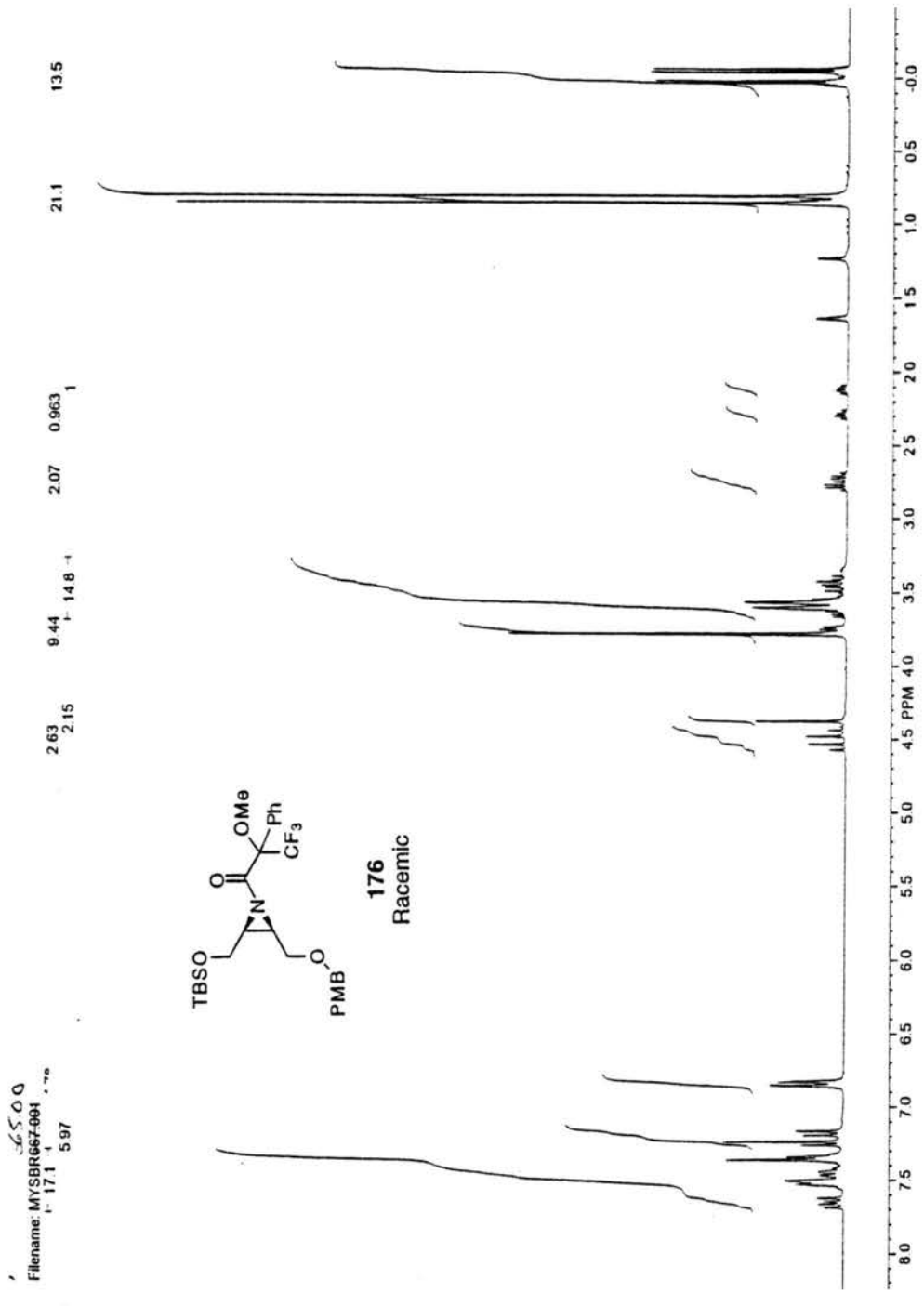
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14.8
2.07
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21.1
13.5
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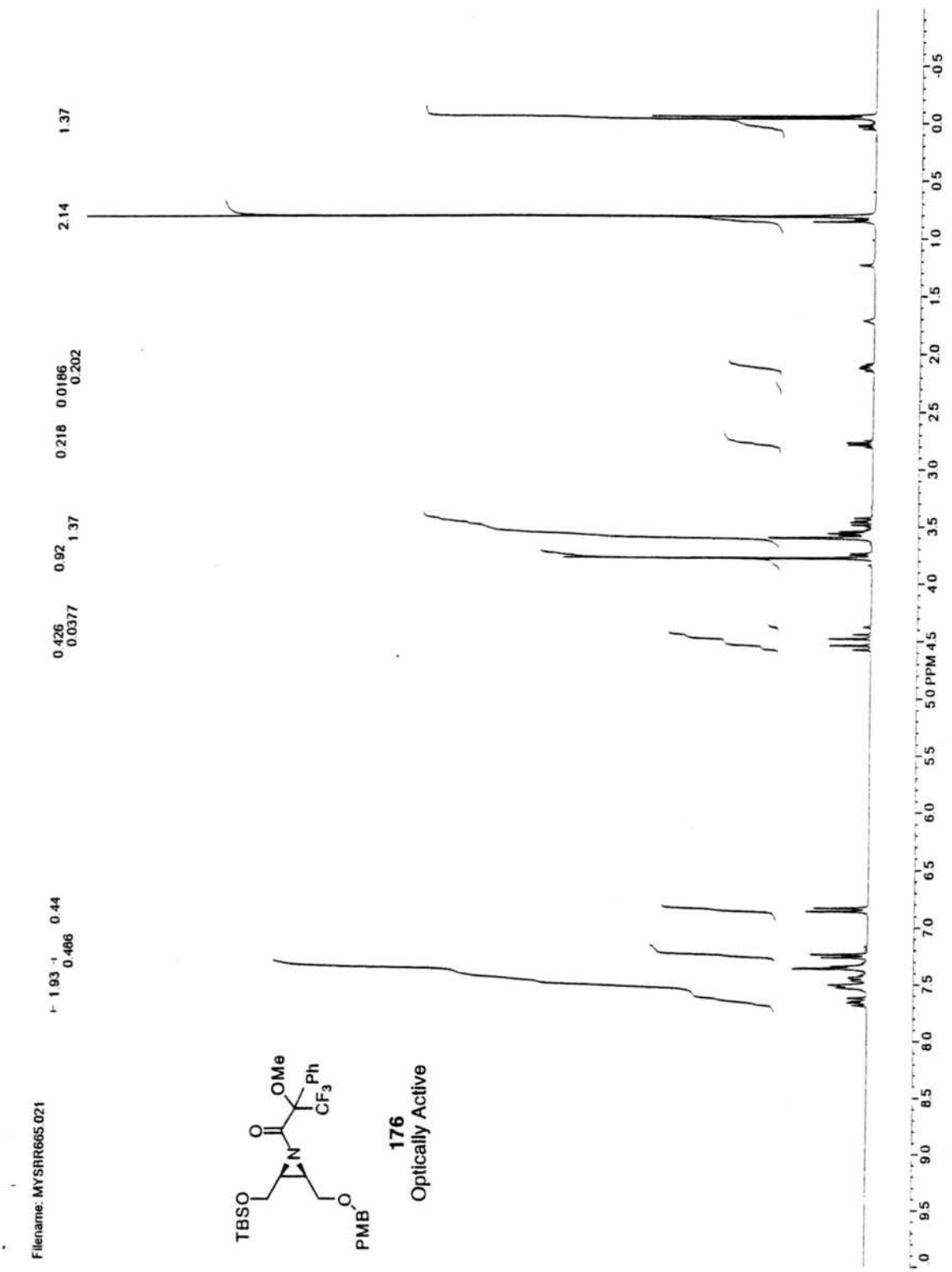
Ph
CF3
N
O
O
PMB
176
Racemic
PPM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0.218
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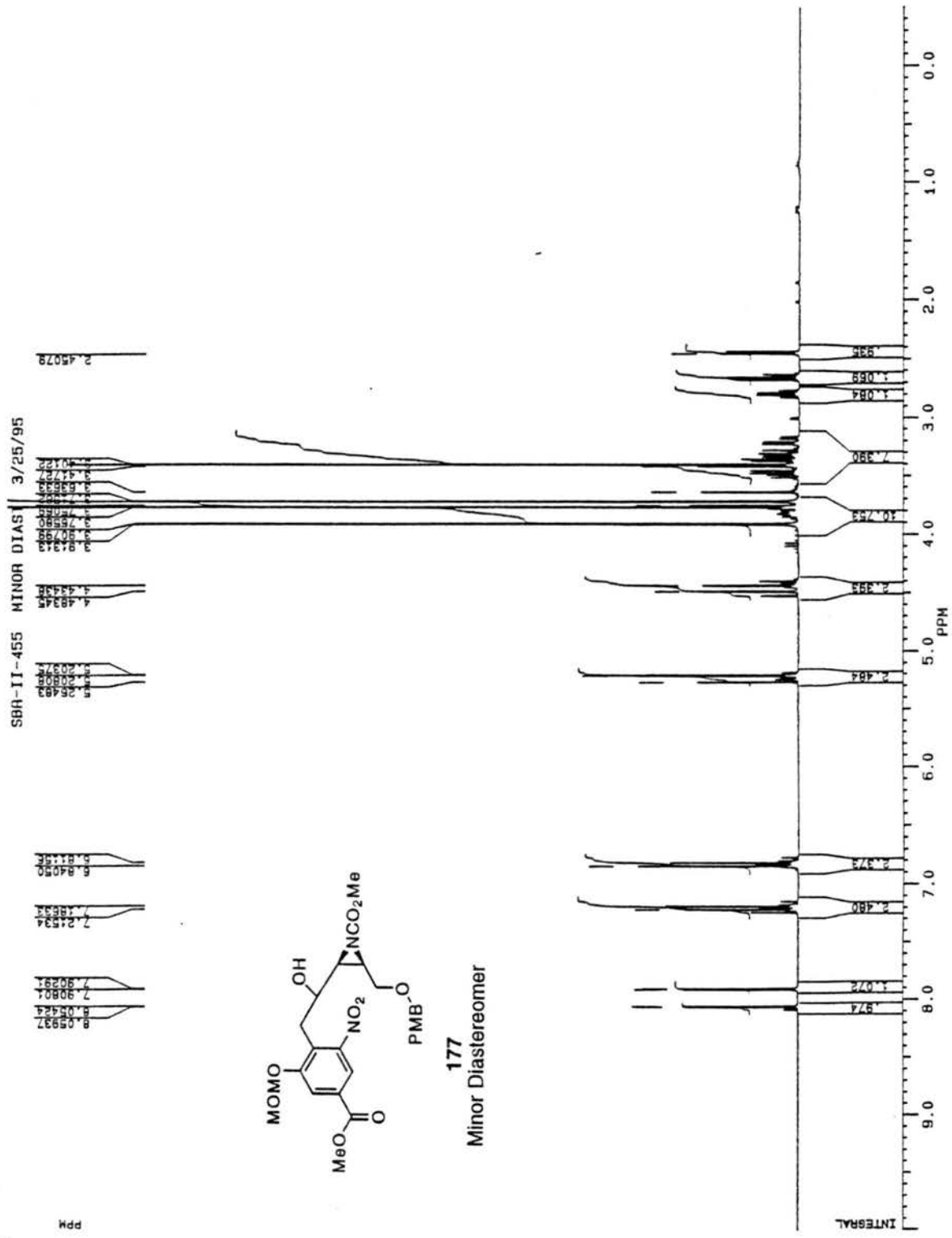
2.14
1.37
TBSO
OMe
Ph
CF3
N
O
PMB
176
Optically Active
PPM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NO2
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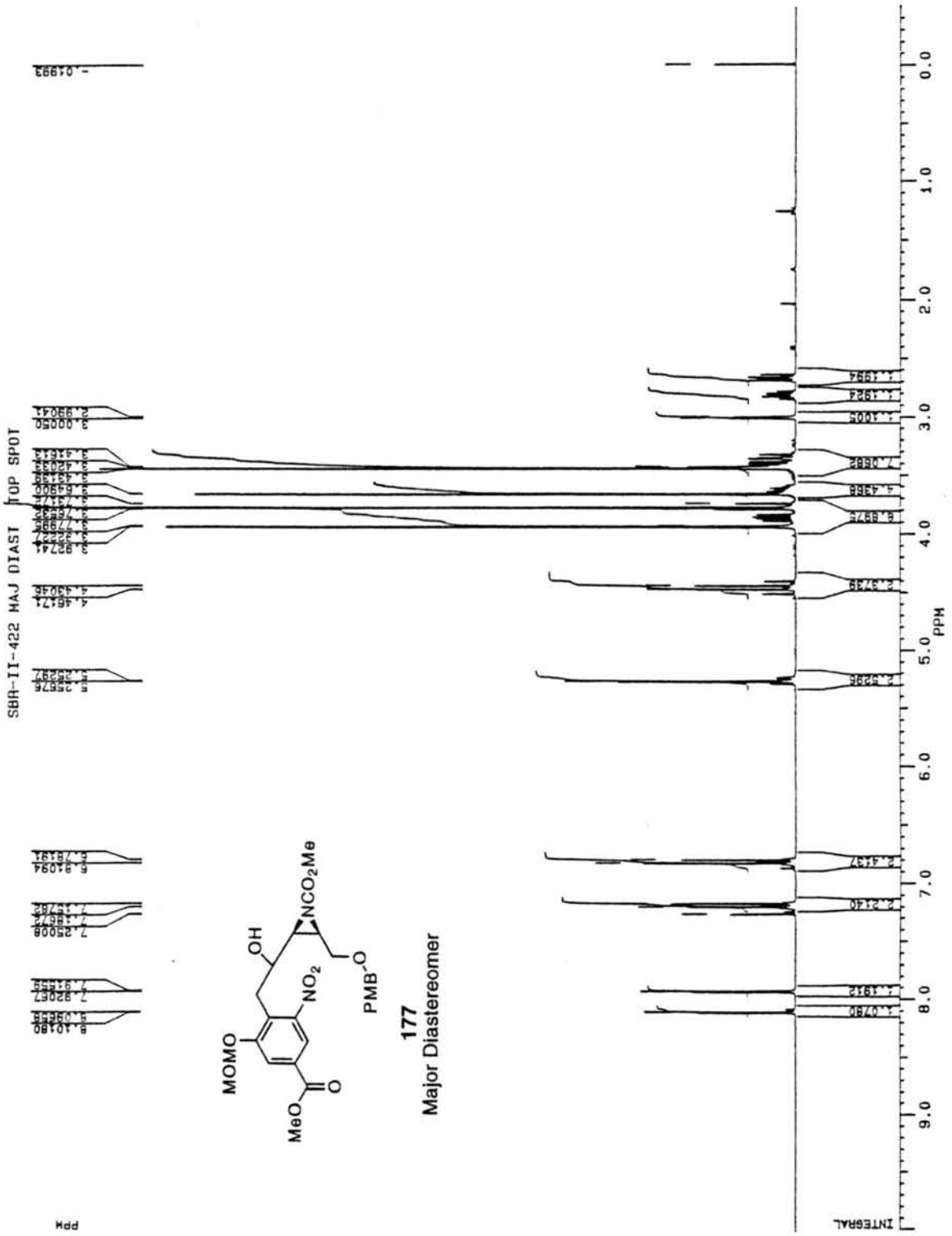
PMB
O
MeO
O
177
Minor Diastereomer
PPM
INTEGRAL


SBR-II-422 MAJ DIAST TOP SPOT
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177
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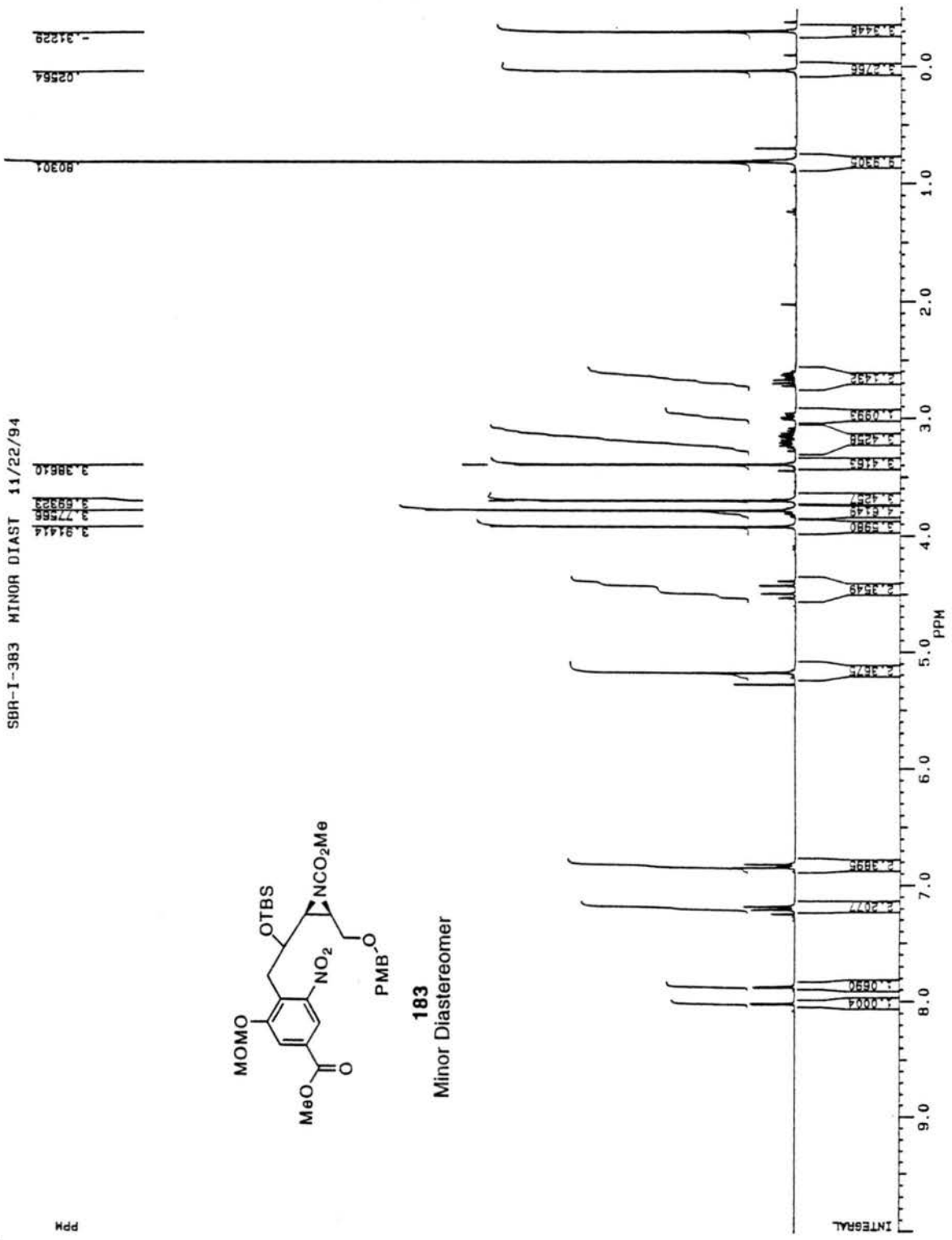
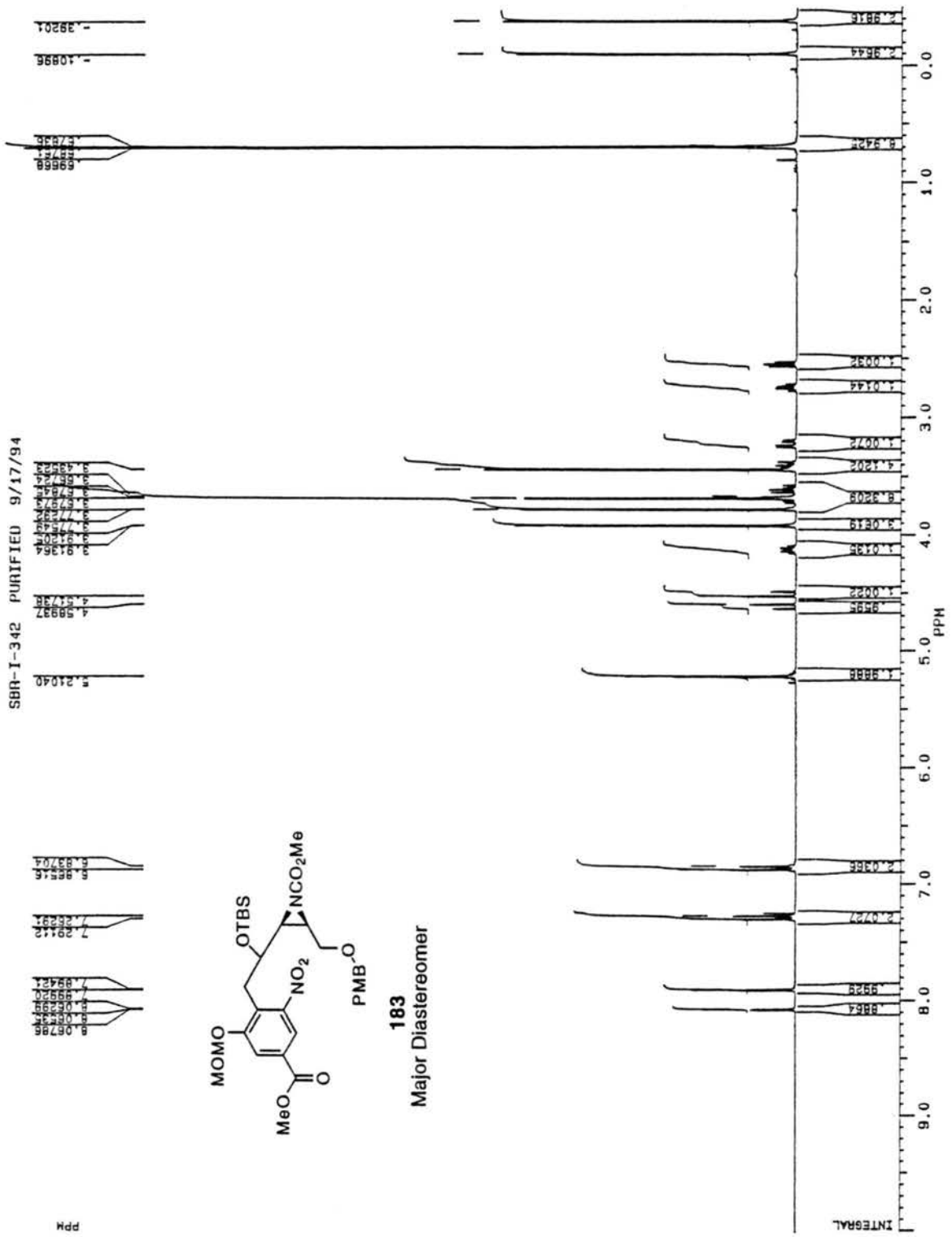


183
Minor Diastereomer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O
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Major Diastereomer
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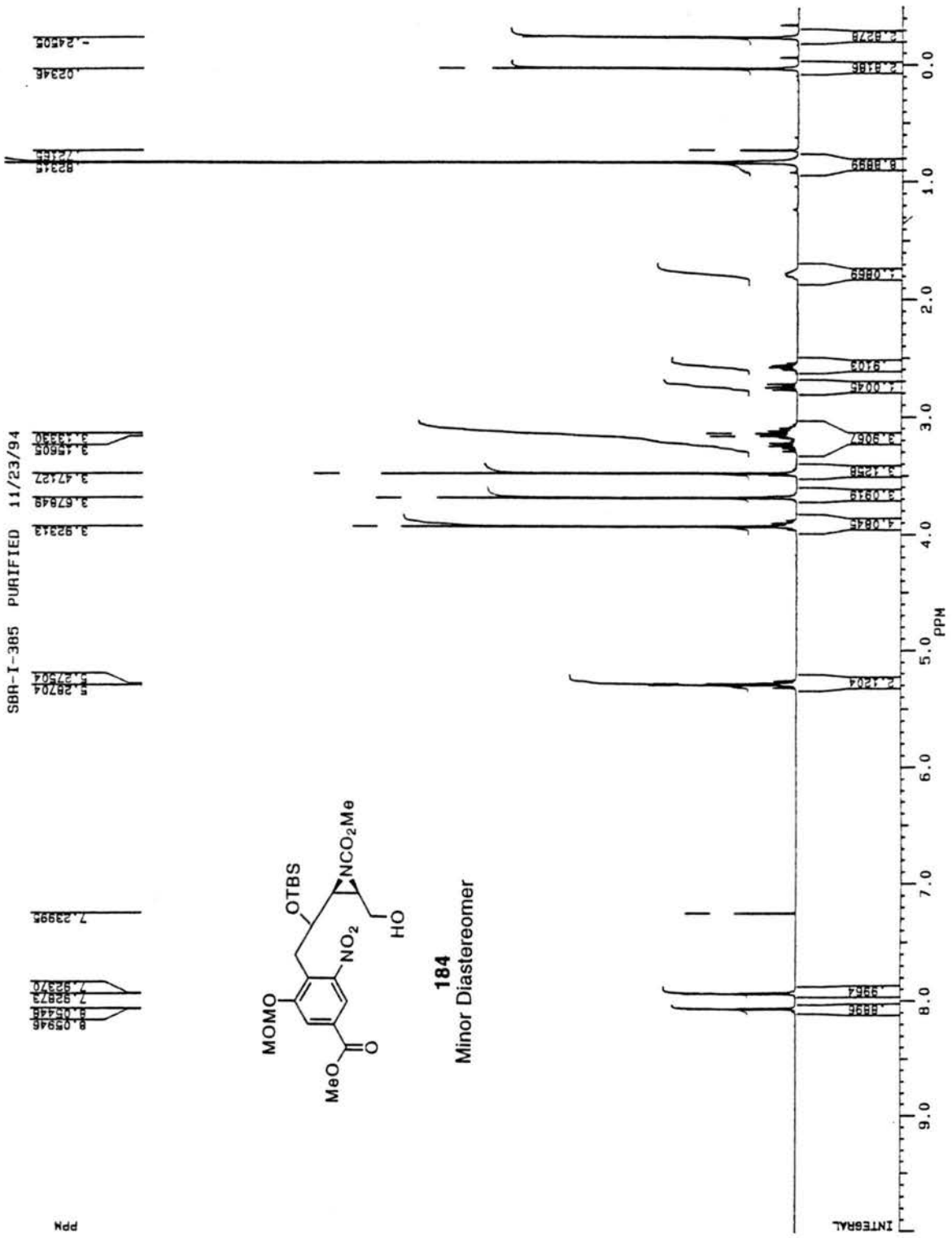
INTEGRAL



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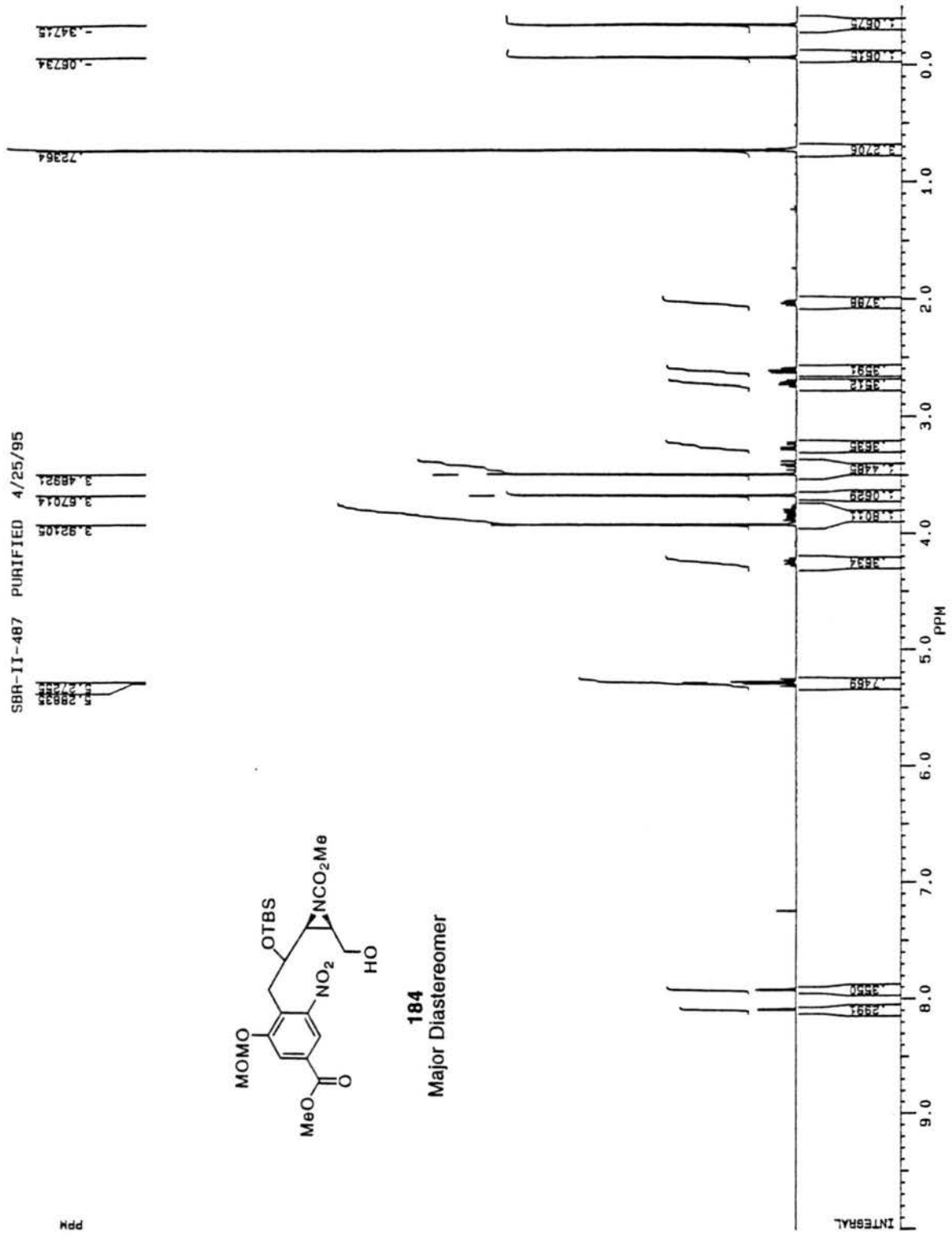

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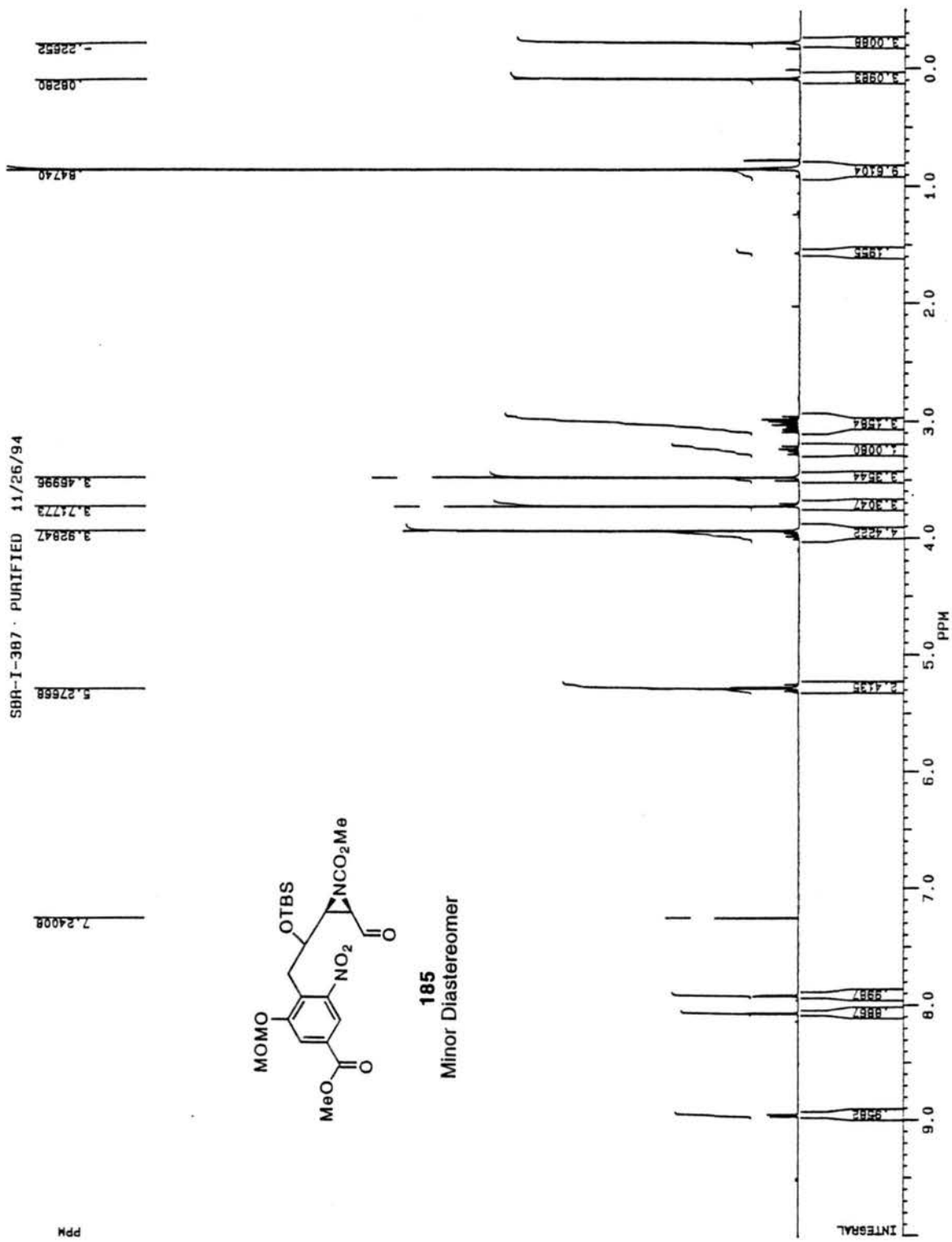
Major Diastereomer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O
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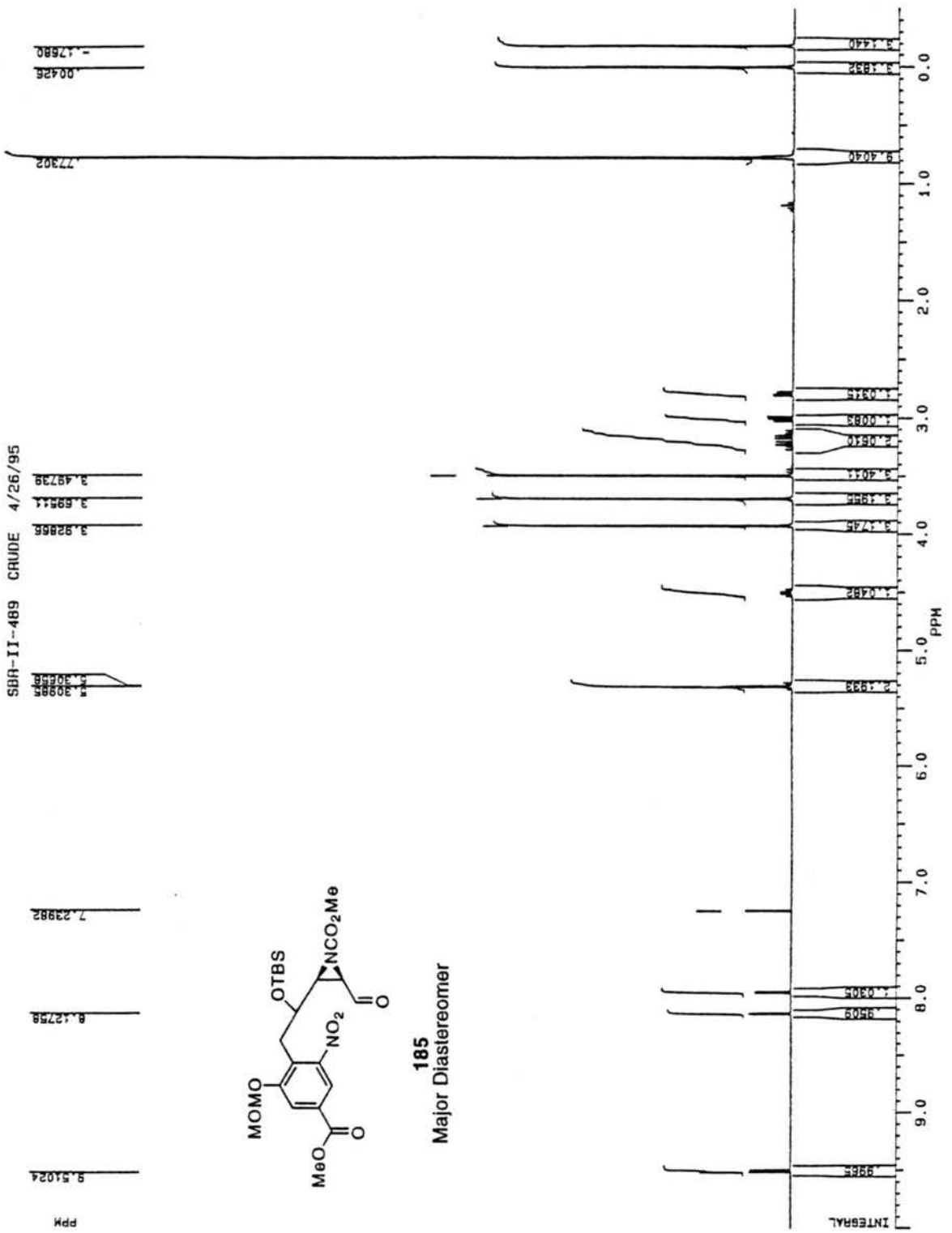
Minor Diastereomer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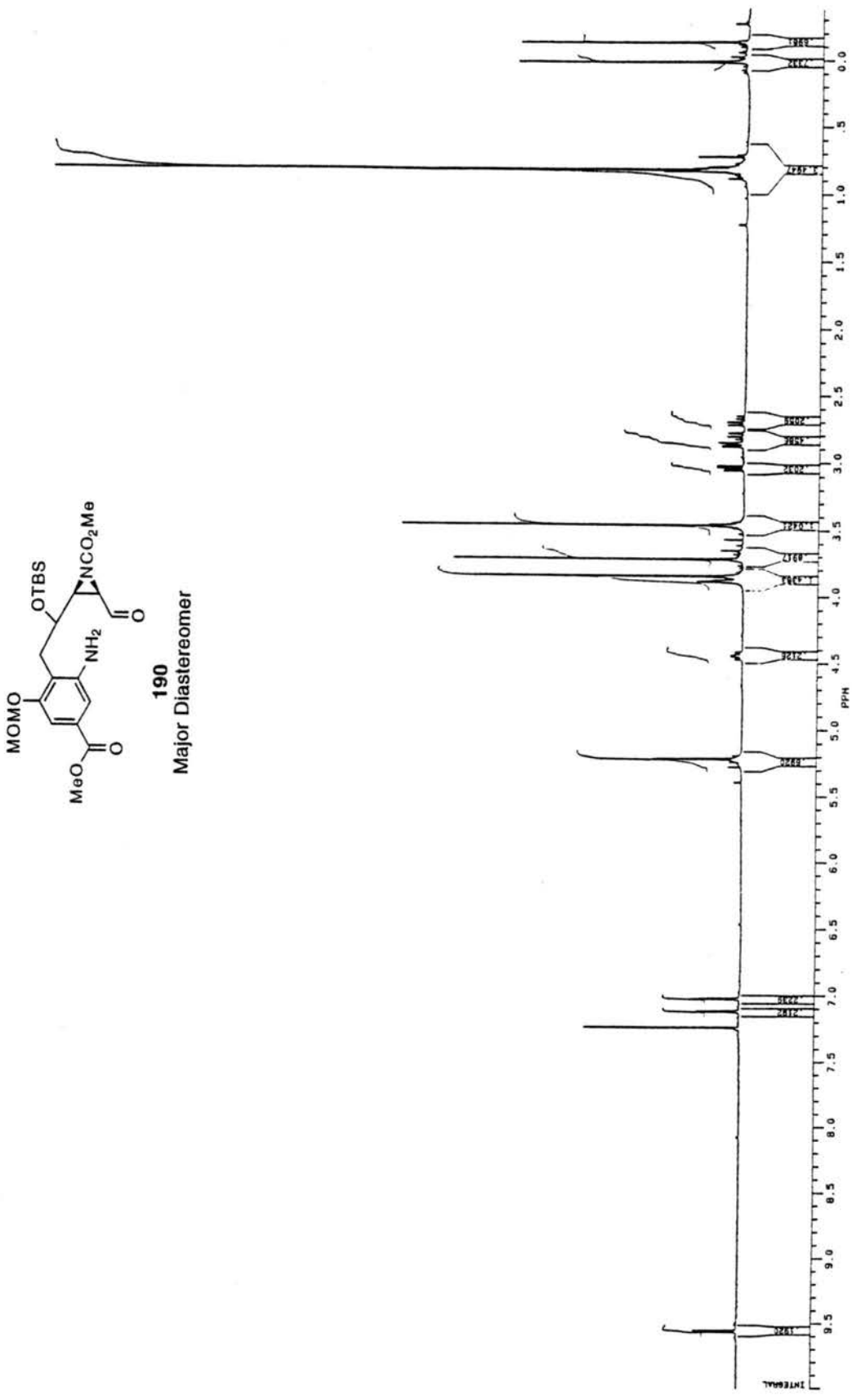
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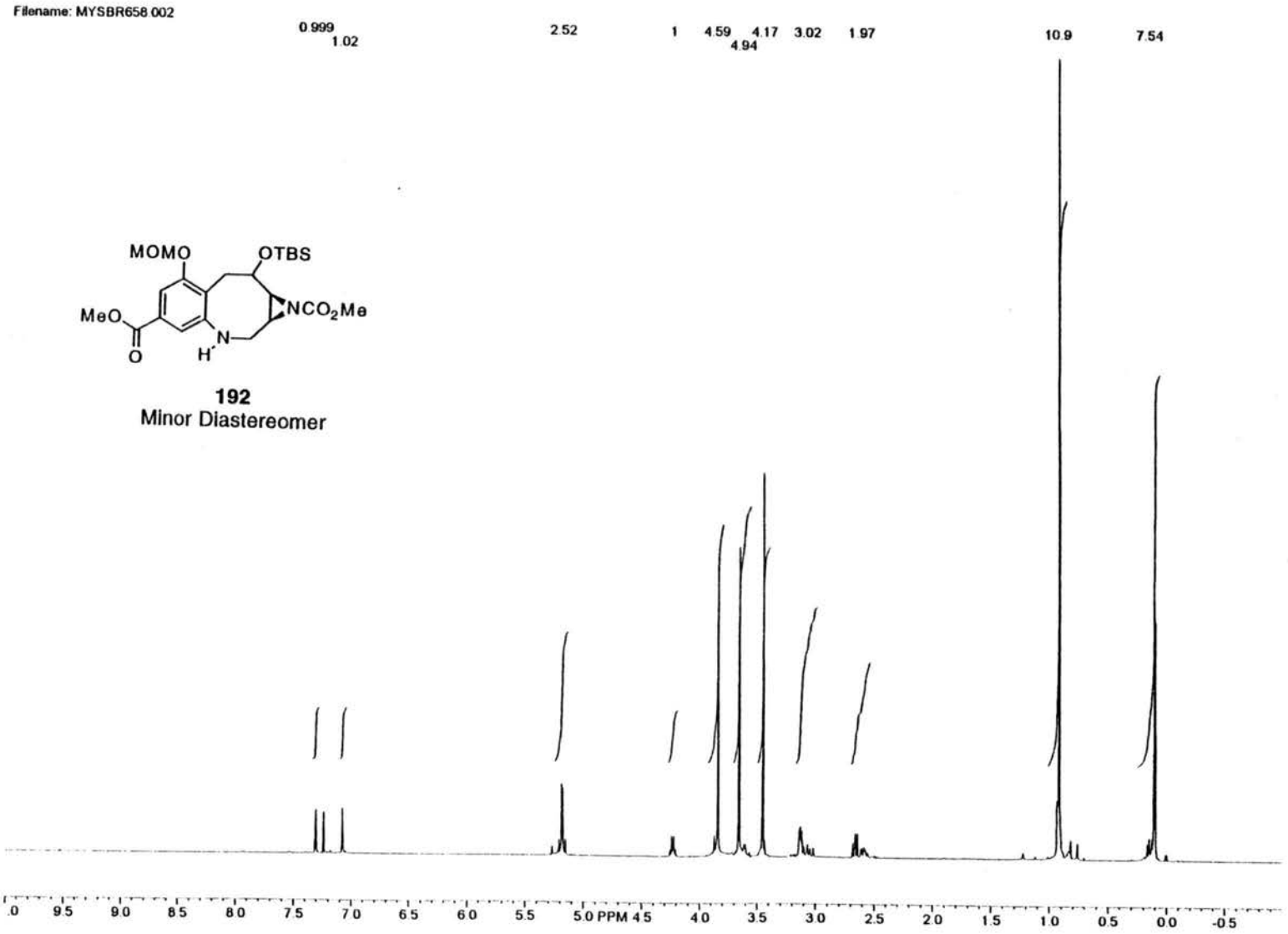
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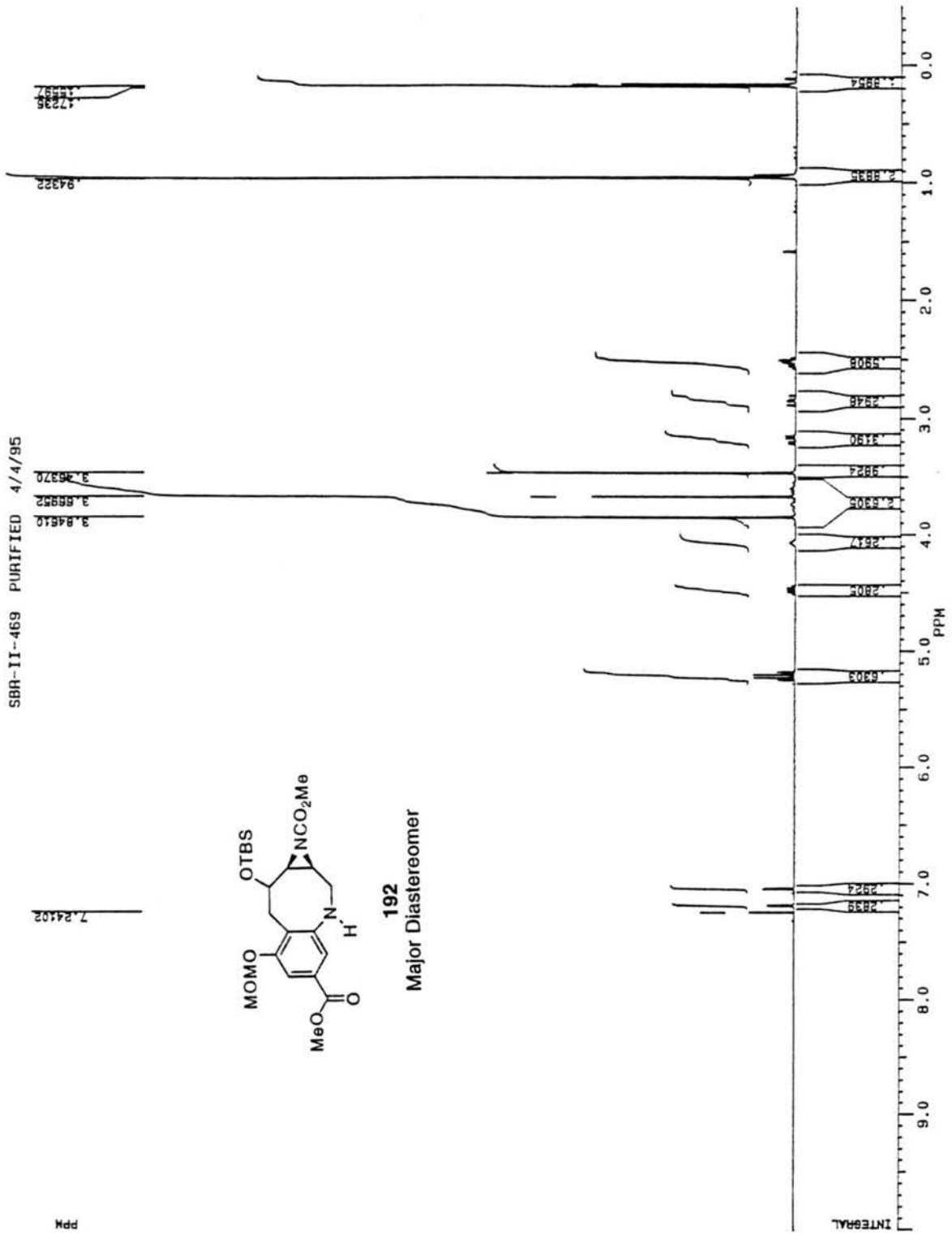
OTBS
MeO
NCO2Me
N
H
O
192
Minor Diastereomer
5.0 PPM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H
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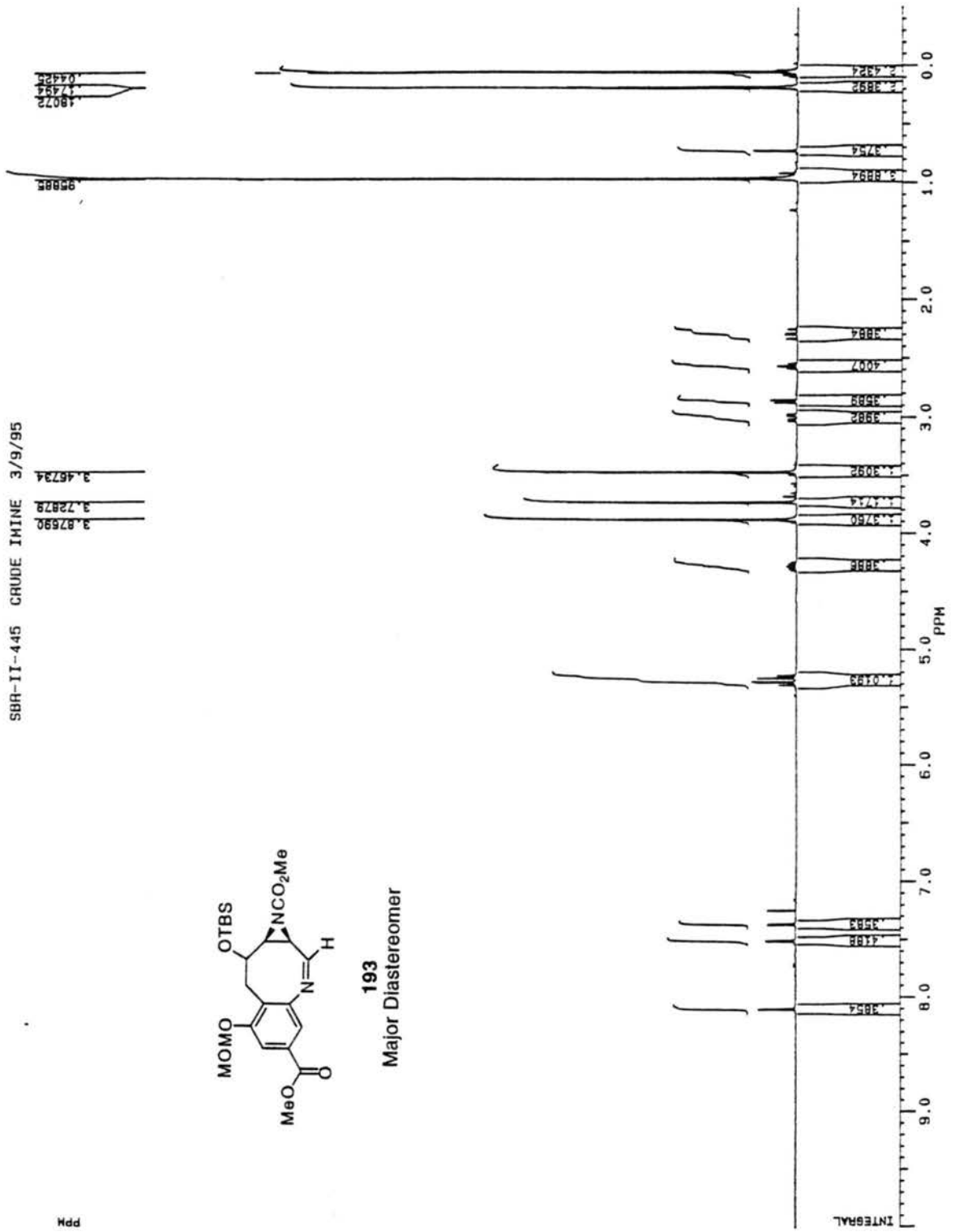
Major Diastereomer



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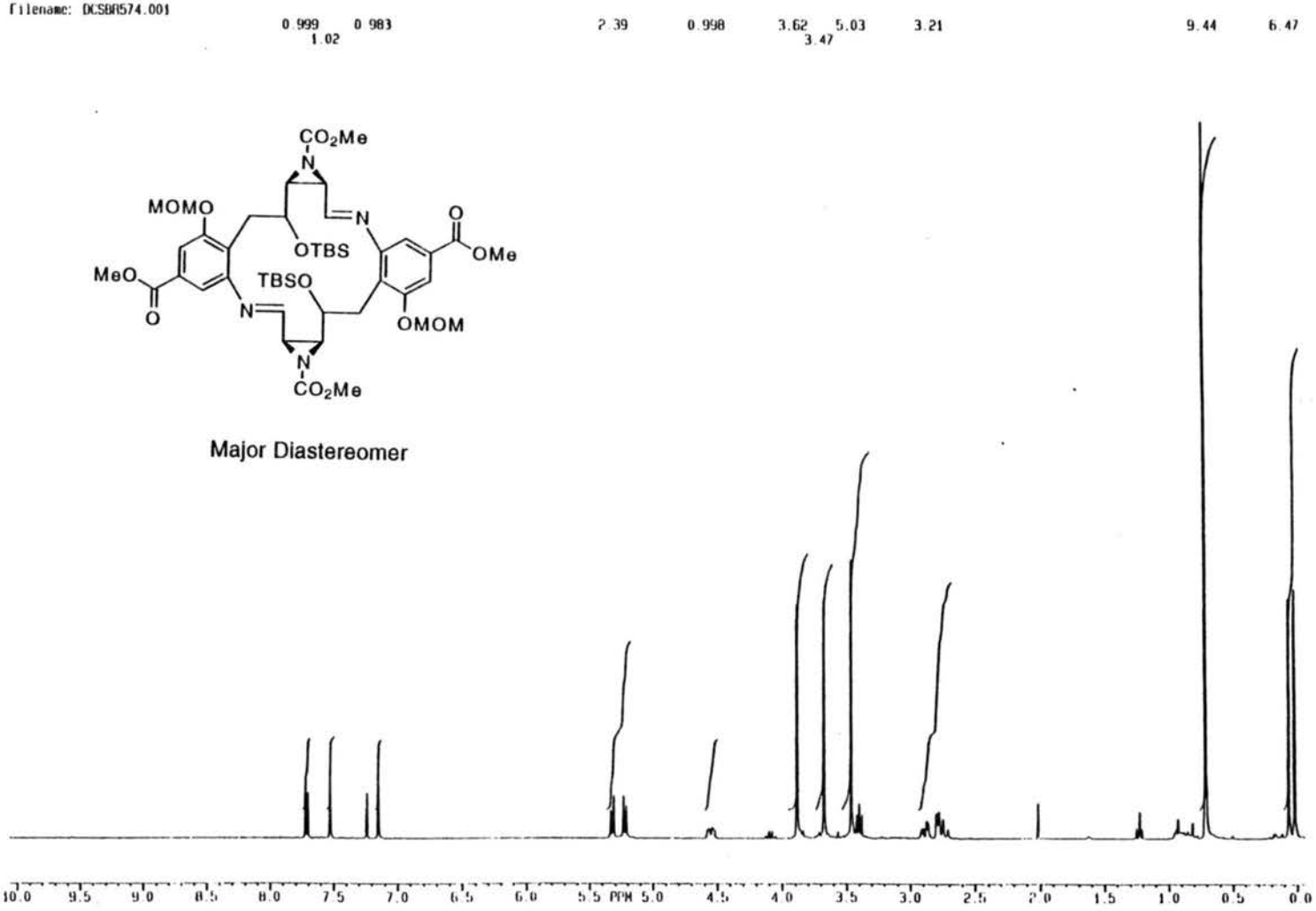
Major Diastereomer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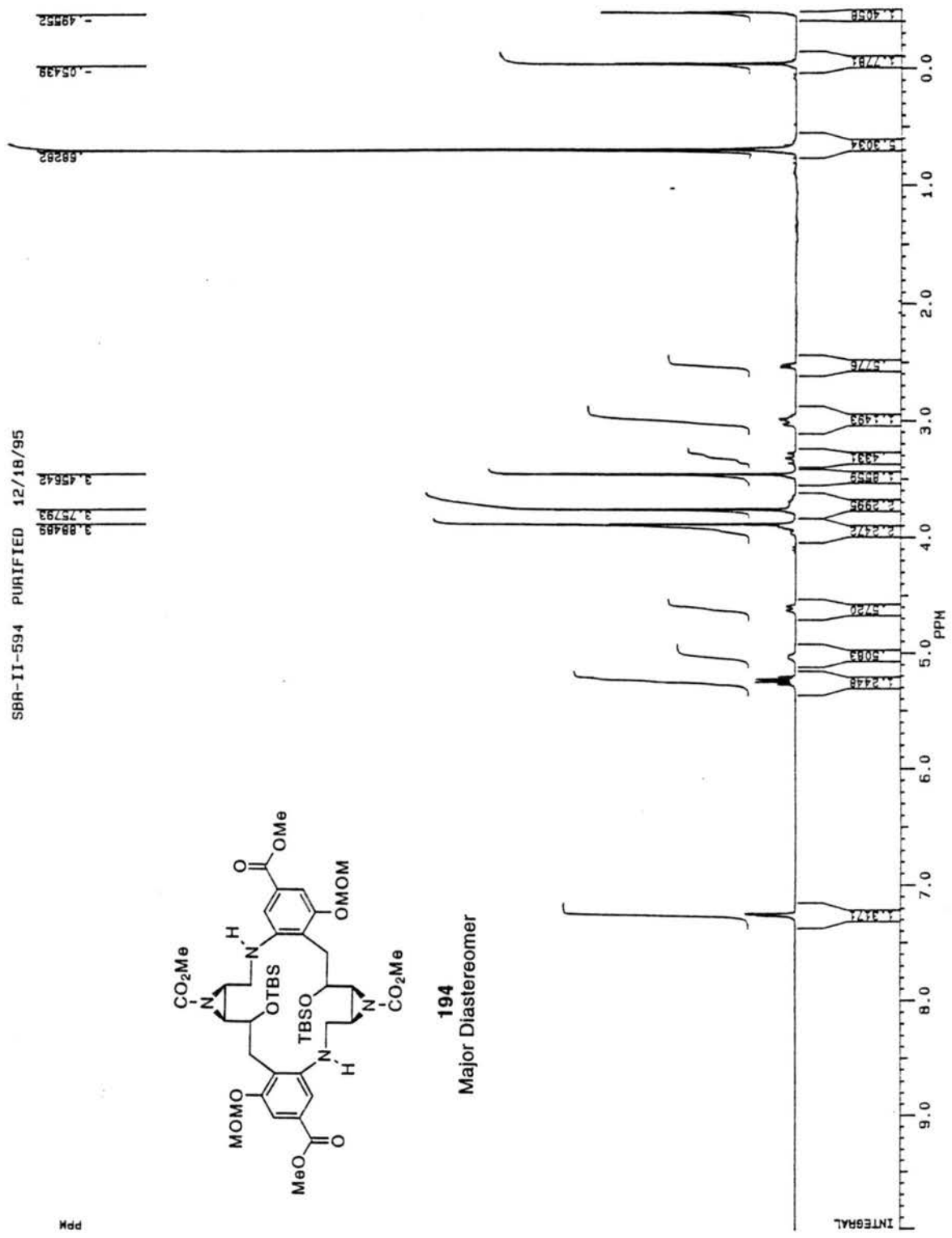

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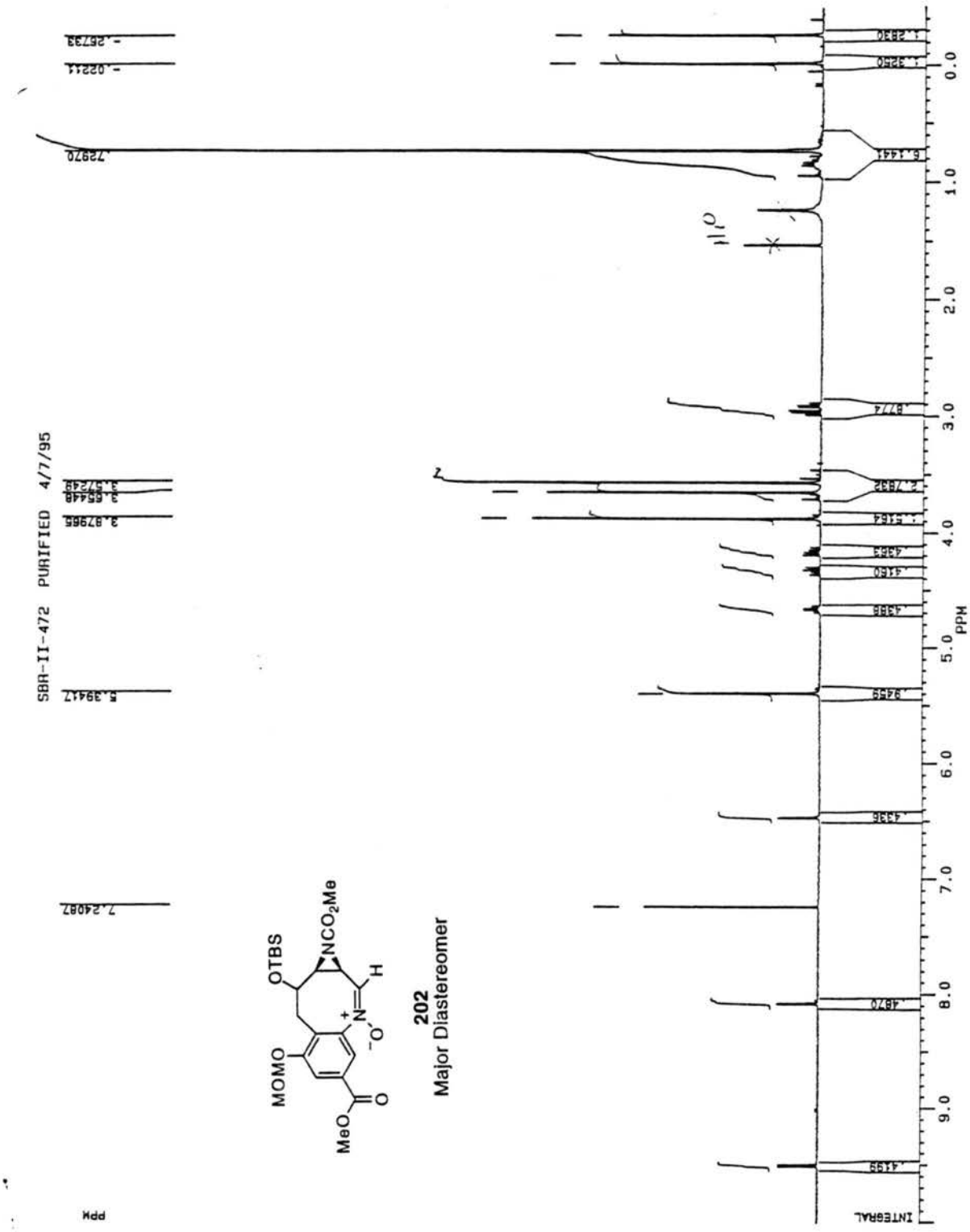
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MeO
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202
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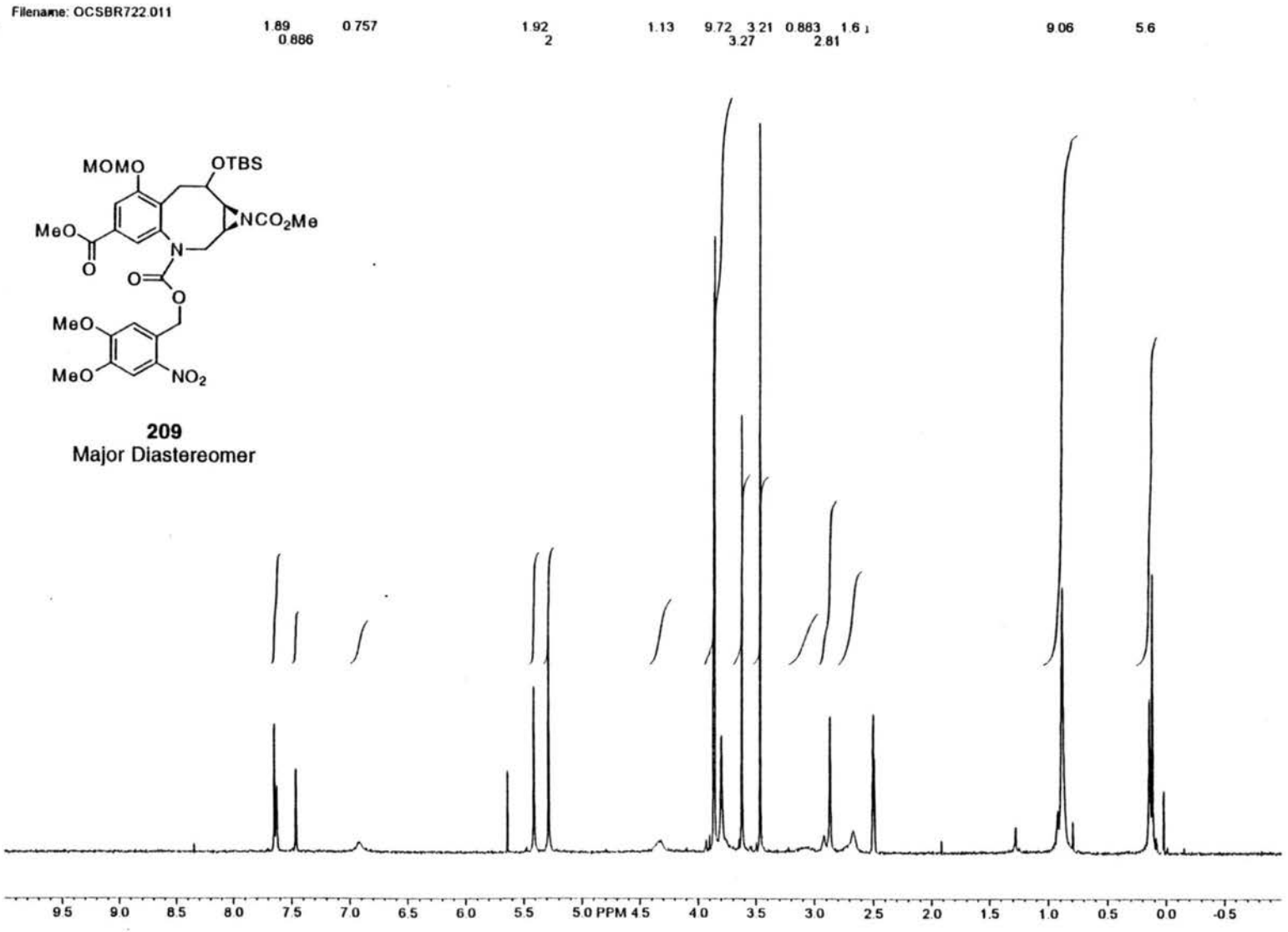

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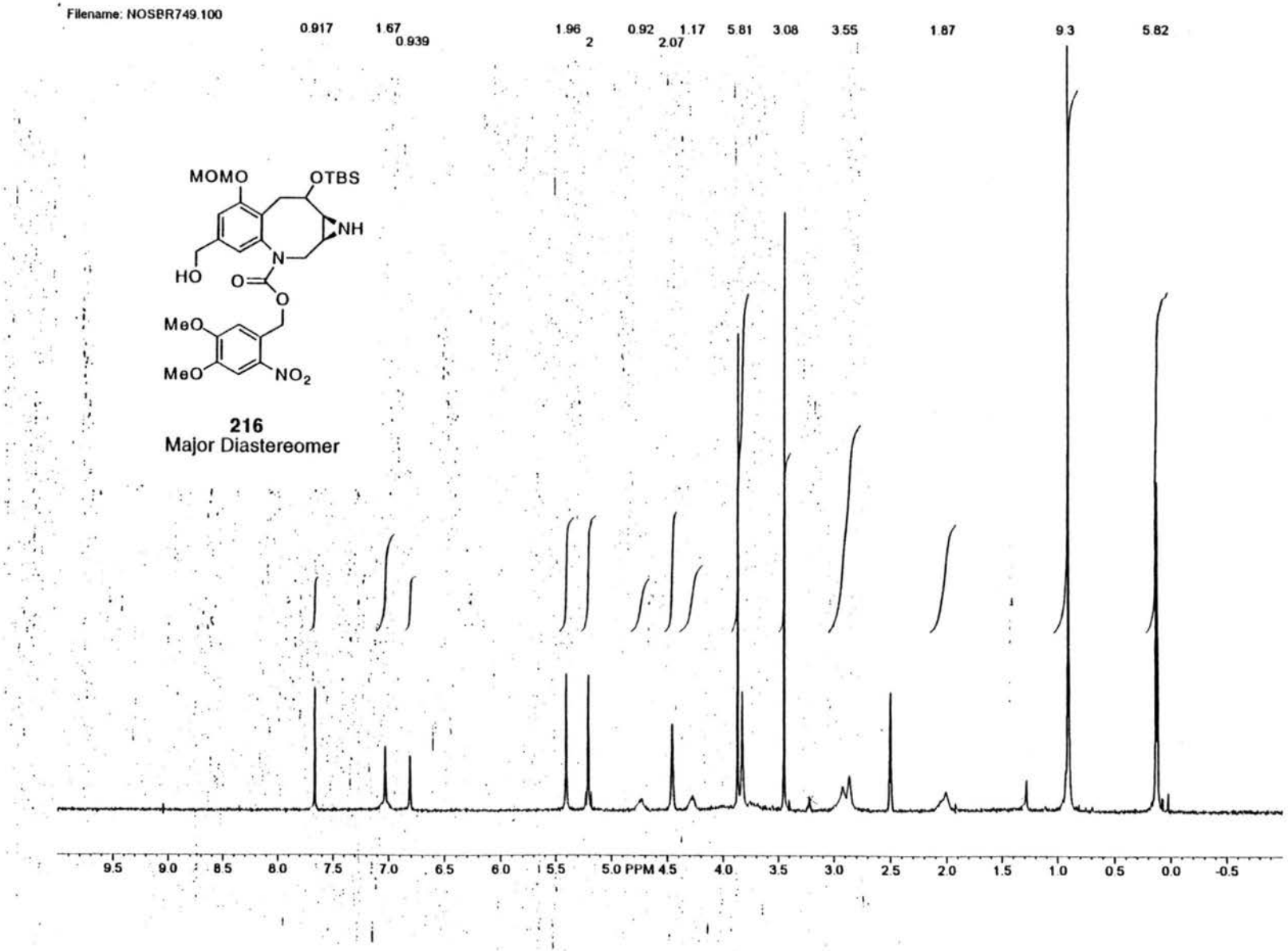
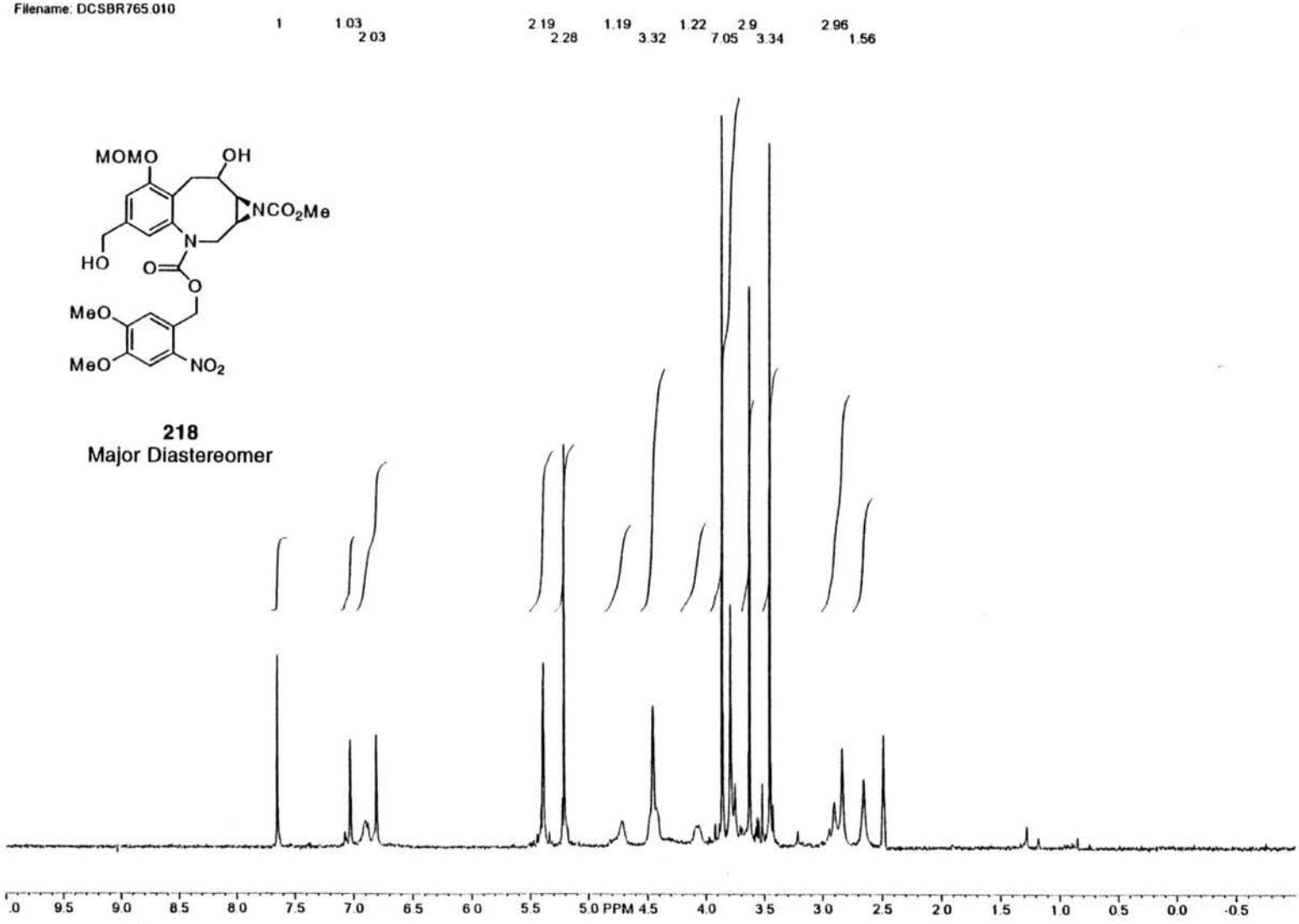


216
Major Diastereomer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3.34
2.96
1.56
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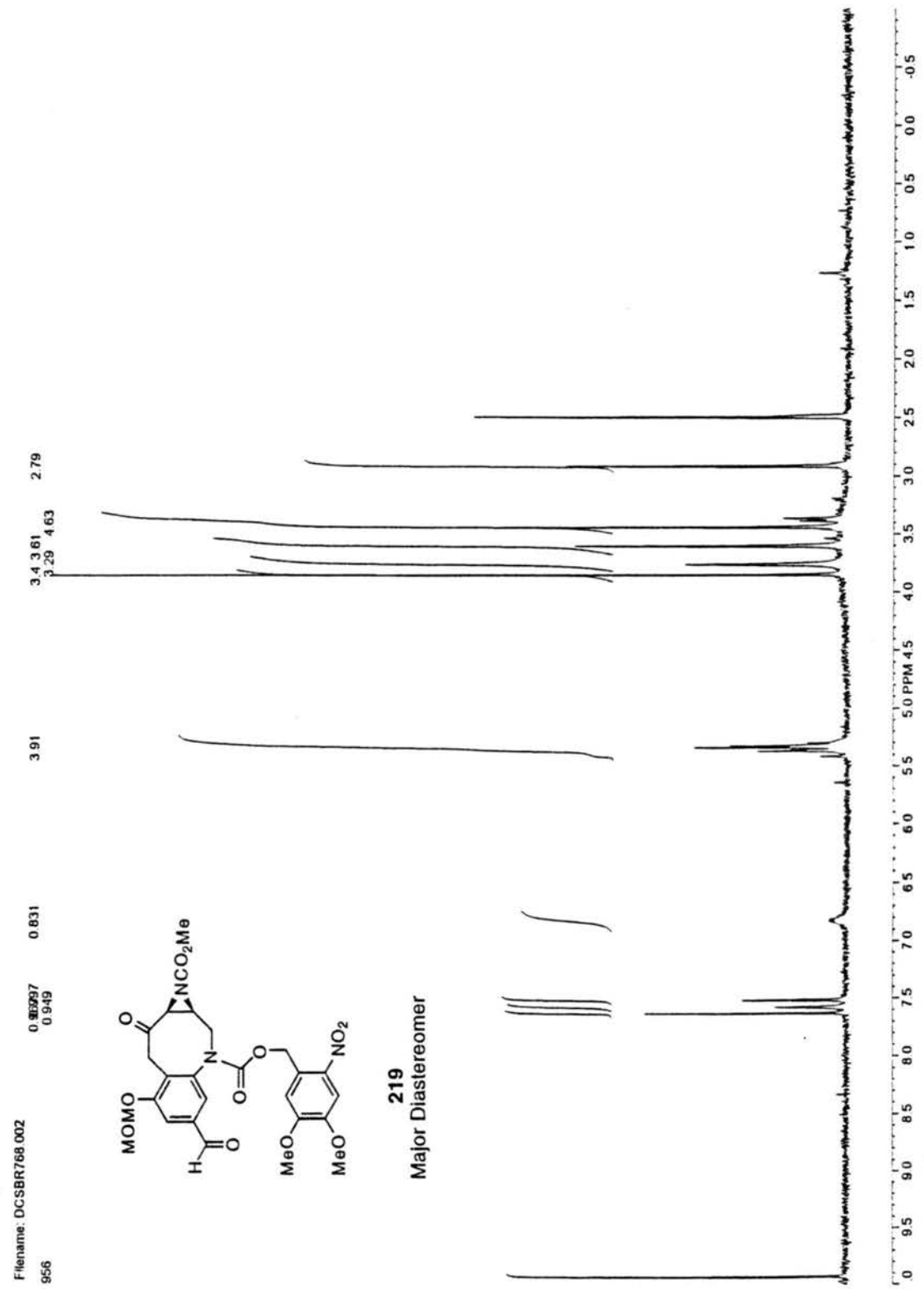
OH
NCO2Me
N
HO
O
O
MeO
MeO
NO2
218
Major Diastereomer
.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 PPM 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5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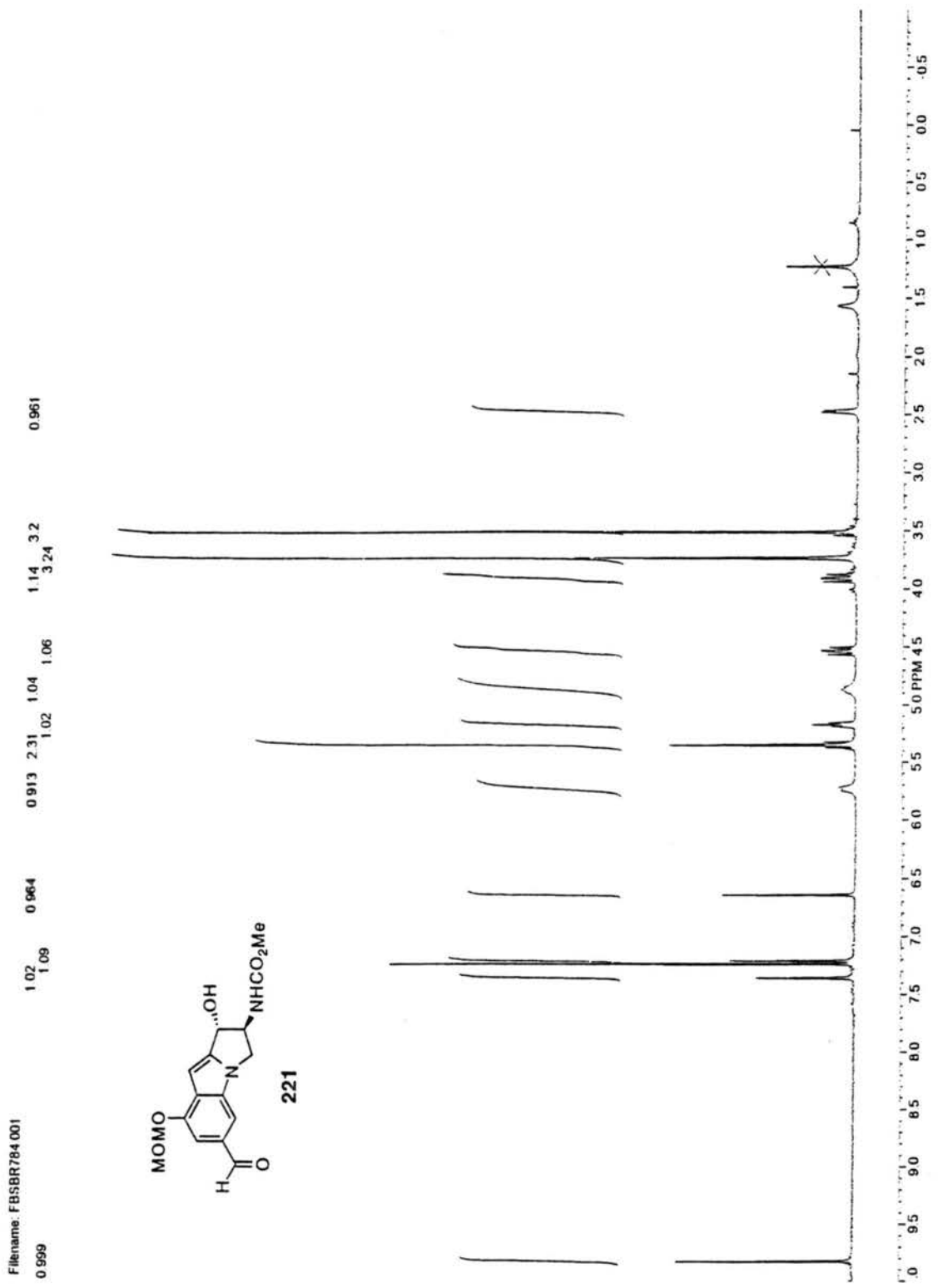
NO2
NCO2Me
219
Major Diastereomer
PPM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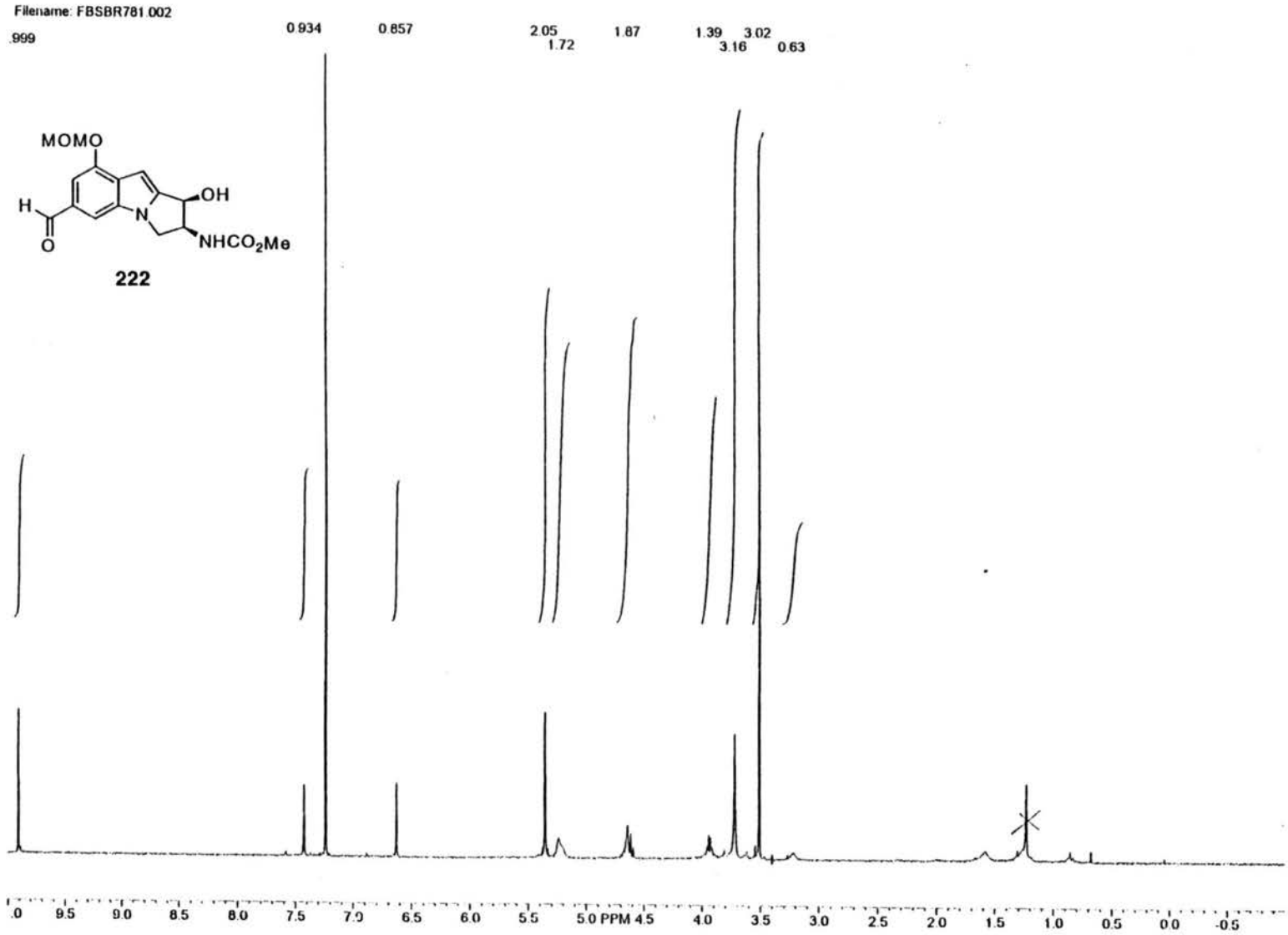
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H
O
N
NHCO2Me
222
.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 PPM 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5

Appendix 2

Publications

1) *Netropsin and Spermine Conjugates of a Water Soluble Quinocarcin Analog: Analysis of Sequence Specific DNA Interactions*. Mark E. Flanagan, Samuel B. Rollins, Robert M. Williams *Chemistry and Biology* (1995), **2**, 147-156.

2) *FR 900482, A Close Cousin of Mitomycin C that Exploits Mitosene-Based DNA Cross-Linking* Robert M. Williams, Scott R. Rajski, and Samuel B. Rollins *Chemistry and Biology* (1997), **4**, 127-137.

3) *Synthesis of a Photoactivated FR 900482 Analog* Samuel B. Rollins, Robert M. Williams *Tetrahedron Lett.* (in press).

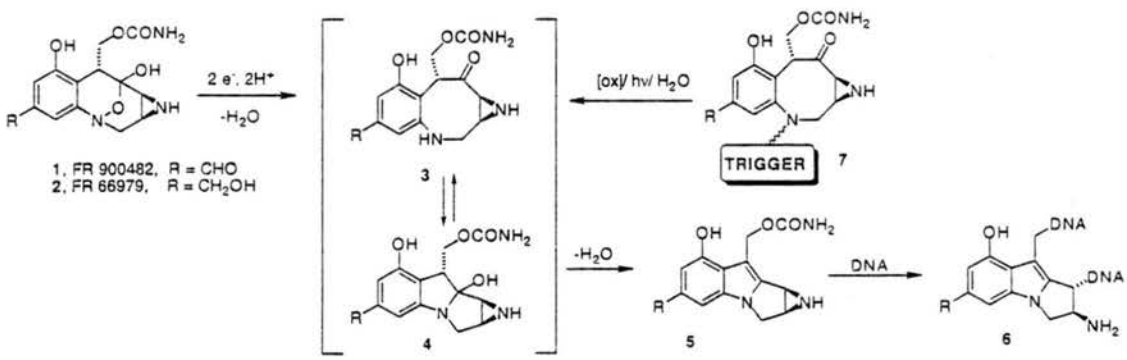
Synthetic Studies on FR 900482. Synthesis of a Photo-triggered Pro-Mitosene‡

Samuel B. Rollins and Robert M. Williams*
Department of Chemistry, Colorado State University
Fort Collins, Colorado 80523

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

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

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



Scheme 1

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

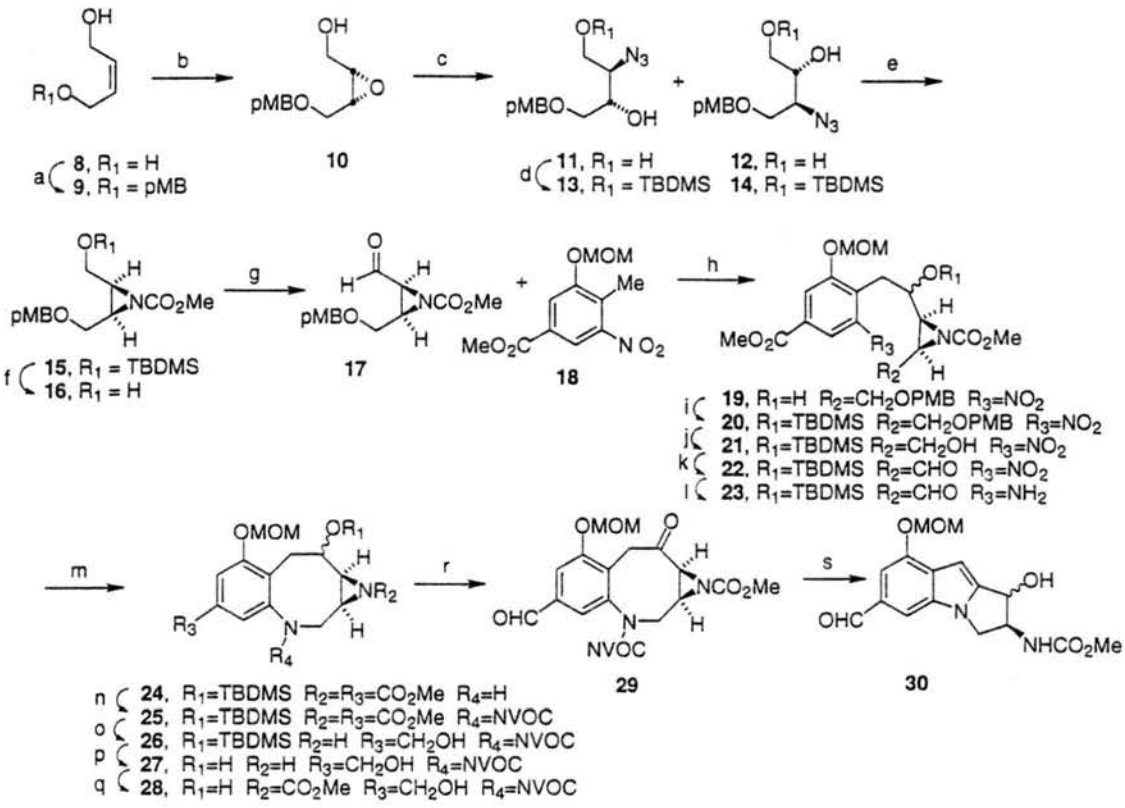
The unique structure of **1** and its extraordinary antitumor activity have made it an attractive synthetic target. Several different approaches to the core nucleus of **1** have been published,[9] and three groups have successfully completed the total synthesis.[10] In an attempt to design and synthesize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

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

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

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

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



Scheme 2[15]

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

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

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References and Footnotes

1. a) Iwami, M.; Kiyoto, S.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiotics*, **1987**, *40*, 589-593; b) Kiyoto, S.; Shibata, T.; Yamashita, M.; Komori, T.; Okuhara, M.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiotics*, **1987**, *40*, 594-599; c) Uchida, I.; Takase, S.; Kayakiri, H.; Kiyoto, S.; Hashimoto, M.; Tada, T.; Koda, S.; Morimoto, Y. *J. Am. Chem. Soc.*, **1987**, *109*, 4108-4109.

2. a) Shimomura, K.; Hirai, O.; Mizota, T.; Matsumoto, S.; Mori, J.; Shibayama, F.; Kikuchi, H., *J. Antibiotics*, **1987**, *40*, 600-606; b) Hirai, O.; Shimomura, K.; Mizota, T.; Matsumoto, S.; Mori, J.; Kikuchi, H., *J. Antibiotics*, **1987**, *40*, 607-611.

3. Terano, H.; Takase, S.; Hosoda, J.; Kohsaka, M. *J. Antibiotics*, **1989**, *42*, 145-148.

4. a) Shimomura, K.; Manda, T.; Mukumoto, S.; Masuda, K.; Nakamura, T.; Mizota, T.; Matsumoto, S.; Nishigaki, F.; Oku, T.; Mori, J.; Shibayama, F., *Cancer Res.*, **1988**, *48*, 1166-1172; b) Nakamura, T.; Masuda, K.; Matsumoto, S.; Oku, T.; Manda, T.; Mori, J.; Shimomura, K. *Japan. J. Pharmacol.*, **1989**, *49*, 317-324.

5. a) Masuda, K.; Nakamura, T.; Mizota, T.; Mori, J.; Shimomura, K. *Cancer Res.*, **1988**, *48*, 5172-5177; b) Masuda, K.; Nakamura, T.; Shimomura, K. *J. Antibiotics*, **1988**, *41*, 1497-1499.

6. a) Williams, R. M.; Rajski, S. R. *Tetrahedron Lett.* **1993**, *34*, 7023-7026; b) Huang, H; Pratum, T. K.; Hopkins, P. B. *J. Am. Chem. Soc.* **1994**, *116*, 2703-2709; c) Williams, R. M.; Rajski, S. R. *Tetrahedron Lett.* **1992**, *33*, 2929-2932; d) Woo, J.; Sigurdsson, S. T.; Hopkins, P. B., *J. Am. Chem. Soc.* **1993**, *115*, 1199-1200; d) Huang, H.; Rajski, S. R.; Williams, R. M.; Hopkins, P. B., *Tetrahedron Lett.*,**1994**, *35*, 9669-9672; e) Paz, M.M.; Hopkins, P. B., *Tetrahedron Lett.* **1997**, *38*, 343-346; f) Williams, R. M.; Rajski, S. R., *Chem. & Biol.*, **1997**, *4*, 127.

7. Fukuyama, T.; Goto, S. *Tetrahedron Lett.*, **1989**, *30*, 6491-6494.

8. a) Tomasz, M; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G.; Nakanishi, K., *Science*, **1987**, *235*, 1204; b) Tomasz, M., *Chem. & Biol.* **1995**, *2*, 575 and references cited therein.

9. a) Yasuda, N.; Williams, R. M. *Tetrahedron Lett.*, **1989**, *30*, 3397-3400; b) Jones, R. J.; Rapoport, H., *J. Org. Chem.*, **1990**, *55*, 1144-1146; c) Martin, S. F.; Wagman, A. S., *Tetrahedron Lett.*, **1995**, *36*, 1169-1170; d) Miller, S.J.; Kim, S.-H.; Chen, Z.-R.; Grubbs, R. H., *J. Am. Chem. Soc.*, **1995**, *117*, 2108-2109; e) Lim, H.-J.; Sulikowski, G. A., *Tetrahedron Lett.*, **1996**, *37*, 5243-5246; f) Ziegler, F. E., Belema, M., *J. Org. Chem.*, **1997**, *62*, 1083-1094; g) Mithani, S.; Drew, D. M.; Rydberg, E. H.; Taylor, N. J., Mootbroek, S., Dmutrienko, G. I., *J. Am. Chem. Soc.*, **1997**, *119*, 1159-1160.

10. a) Fukuyama, T.; Xu, L.; Goto, S., *J. Am. Chem. Soc.*, **1992**, *114*, 383-385; b) Schkeryantz, J. M.; Danishefsky, S. J., *J. Am. Chem. Soc.*, **1995**, *117*, 4722-4723; c) Katoh, T.; Itoh, E.; Yoshino, T.; Terashima, S., *Tetrahedron Lett.*, **1996**, *37*, 3471-3474; d) Yoshino, T.; Nagata, Y.; Itoh, E.; Hashimoto, M.; Katoh, T.; Terashima, S., *Tetrahedron Lett.*, **1996**, *37*, 3475-3478; e) Katoh, T.; Yoshino, T.; Nagata, Y.; Nakatani, S.; Terashima, S., *Tetrahedron Lett.*,**1996**, *37*, 3479-3482.

11. a) Dess, D. B.; Martin, J. C., *J. Org. Chem.*, **1983**, *48*, 4155-4156; b) Dess, D. B.; Martin, J. C., *J. Am. Chem. Soc.*, **1991**, *113*, 7277-7287; c) Ireland, R. E.; Liu, L., *J. Org. Chem..*, **1993**, *58*, 2899.

12. Nielson, O. B. T.; Bruun, H.; Bretting, C.; Feit, P. W., *J. Med. Chem.*, **1975**, *18*, 41-50.

13. Pillai, V. N. R., *Synthesis*, **1980**, 1-26.

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

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

Appendix 3

Research Proposal

I. Abstract

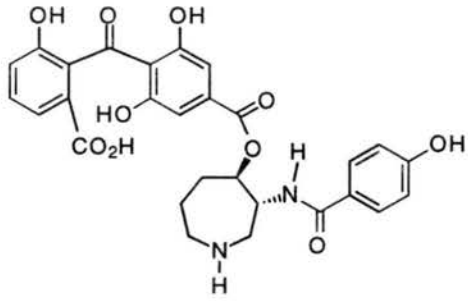
A highly efficient, stereoselective, and unique approach to the hexahydroazepine ring of the natural product balanol is proposed. An intermolecular 1,3-cycloaddition of a resin bound nitrone is employed in this approach. The easily prepared azepine allows for the convenient synthesis of balanol and structural analogs. The application of this approach to the enantiomeric synthesis of these biologically important molecules will be demonstrated.

II. Introduction

As the pursuit of new and more effective treatments for the maladies of mankind continues to tap the resources of bio-organic and organic chemistry, efficient assembly of medicinal agents becomes tantamount to the success of synthetic chemistry. In addition, enantioselective approaches are the ultimate goal of a vast majority of efforts directed towards the assembly of pharmacologically active molecules. With the proliferation of methods for asymmetric induction[1] and the availability of optically pure compounds from the chiral pool,[2] the synthesis of optically active products for biological evaluation is commonplace. However, the synthesis of optically pure complex molecules in an efficient manner still poses the greatest challenge to synthetic chemists. The design of a synthesis or methodology in which a large number of biologically interesting molecules can be synthesized quickly and efficiently is an obvious but seldom realized goal. Currently, particularly intense interest has been directed towards methods for generating libraries of non-polymeric, small organic molecules by solid phase synthesis techniques.[3] Adaptation of versatile solution phase synthetic methods to solid phase synthesis not only serves to expand the synthetic tools required for the preparation of diverse chemical libraries, it also permits efficient construction of increasingly more synthetically challenging targets.

The protein kinase C (PKC) family of enzymes catalyzes the transfer of the γ-phosphate from adenosine triphosphate (ATP) to serine or threonine residues on their

substrate proteins. PKC mediated phosphorylation regulates cellular responses such as proliferation, metabolism, and differentiation.[4] The unregulated activation of PKC has been implicated in a number of disease states including cancer, asthma, inflammation, diabetes, central nervous system dysfunction, and various cardiovascular disorders.[5] Identification of potent and selective PKC inhibitors may result in the development of novel drugs with considerable therapeutic value. Although several PKC inhibitors such as staurosporine,[6] isoquinolinesulfonamides,[7] and K 252a[8] have been reported, they are either highly toxic or not very potent.

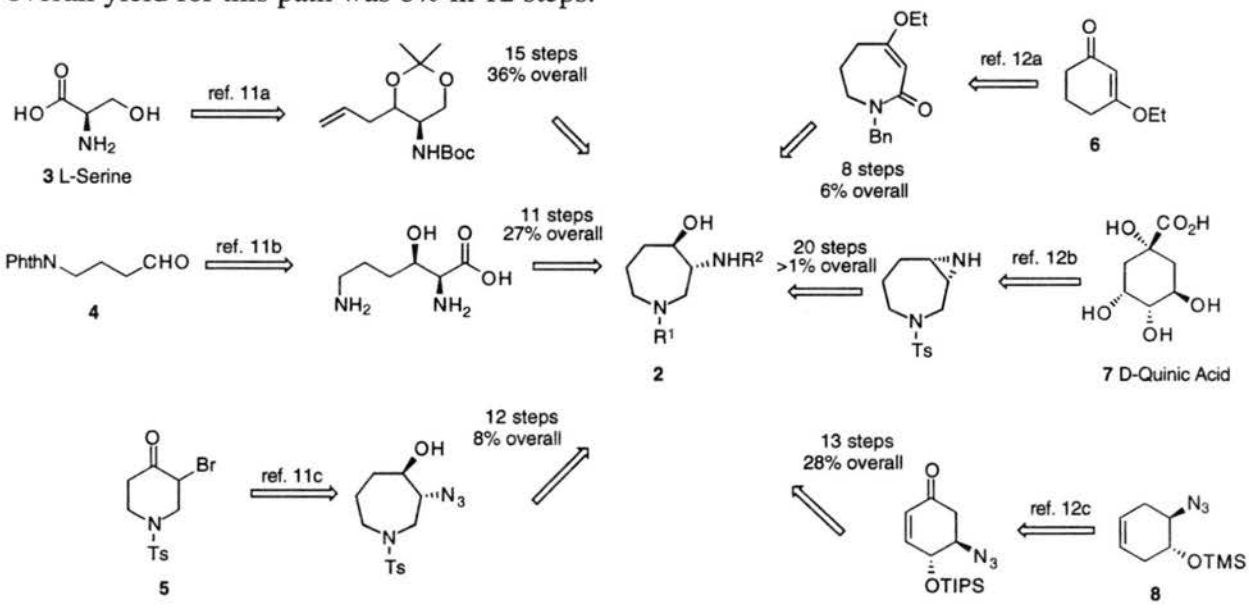


1 (-) Balanol

In 1993, the Sphinx Pharmaceutical Corp. reported the structure of a novel PKC inhibitor isolated from the culture broth of the fungus *Verticillium balanoides*.[9] Oshima *et al.* reported the isolation of the identical compound from the fermentation broth of *Fusarium merismoides* Corda,[10] a different genus than the *Verticillium balanoides*. The 50% inhibitory concentration (IC 50) of balanol was observed to be between 4 and 9 nM in assays against several different human PKC enzymes. Although this is a similar profile to those of staurosporine and K-252a, balanol has a much lower cytotoxicity than staurosporine (*ca.* 450 times less) and K-252a (*ca.* 45 times less).[10] Balanol inhibits PKC activity competitively with ATP. Experimental data suggests balanol does not act as a simple ATP mimetic, which can suppress activity of any enzyme utilizing ATP as a substrate, but acts as an inhibitor which preferentially interacts with protein kinases.[10]

The unique structure of **1** and its extraordinary potential to become an important therapeutic agent have made it an attractive synthetic target.[11,12] Three different groups have published asymmetric total syntheses of balanol (**1**). Nicolaou first published the total synthesis of optically pure balanol (**1**) in 1994.[11a] Shortly after Nicolaou,

researchers at Sphinx Pharmaceutical[11b] and Adams *et al.* [11c] published their own optically pure syntheses of balanol. Others have published unique approaches to the hexahydroazepine fragment of balanol.[12] The various routes to the azepine fragment **2** are outlined in Scheme 1. Nicolaou's synthesis began with L-serine (**3**) and installed the second stereocenter with an asymmetric hydroboration. Cyclization and further elaboration eventually gave **2** (R^1 = Bz, R^2 = 4-(benzyloxy)benzoyl) in 36% overall yield in 15 steps. Sphinx researchers began with achiral aldehyde **4** and installed both stereocenters through an asymmetric dihydroxylation. Cyclization and further elaboration eventually produced **2** (R^1 = Boc, R^2 = 4-(benzyloxy)benzoyl) in 27% overall yield in 11 steps. Adams started with a racemic α-bromoketone **5** and formed the seven membered ring by a regiospecific ring expansion. The enantiomers of azepine **2** (R^1 = Bz, R^2 = 4-(benzyloxy) benzoyl) were resolved by forming their Mosher esters, and the overall yield for this path was 8% in 12 steps.



Scheme 1

Other researchers have focused solely on construction of the hexahydroazepine **2**. An attempt to shorten the route to **2** by starting with ketone **6** accessed racemic azepine **2** (R^1 = Bn, R^2 = H) in 6% overall yield in 8 steps.[12a] Starting with D-quinic acid (**7**),

Albertini *et al.* were able to synthesize **2** (R^1 = H, R^2 = *p*-HOC_6H_4) in 20 steps in an overall yield less than 1%.[12b] Wu and Jacobsen used an asymmetric ring opening reaction catalyzed by a Cr-salen complex to synthesize **8**. Further elaboration gave azepine **2** (R^1 = Bz, R^2 = 4-(benzyloxy)benzoyl) in 28% overall yield in 13 steps.[12c] While all of these syntheses accessed the azepine ring of **1** in an efficient way, none utilized the potentially practical combination of a solid support and a nitrone cyclization.

III. Background

Nitrones undergo cycloadditions with olefins to afford, in principle, two regioisomeric isoxazolidines (Scheme 2). Since the discovery of this [3+2] cycloaddition reaction in the late 1950's, it has been the focus of considerable attention and been incorporated into many synthetic schemes.[13] During the 1970's, attention was concentrated on the controversy of a diradical verses a concerted mechanism for the cycloaddition.[14] The concerted mechanism is supported by many experimental tests and is generally accepted over the diradical mechanism.[13] Upon cycloaddition, the stereochemistry of the olefin is maintained, and substituents *trans* or *cis* on the olefin will remain so on the product. When applied to nitrone cycloaddition, frontier molecular orbital theory has impressive results in rationalizing reactivity, regiochemical, and stereochemical questions.[13] For example, Figure 1 shows how lower HOMO's of dipolarophiles predicts increased interaction of the HOMO of the dipole with the LUMO of the

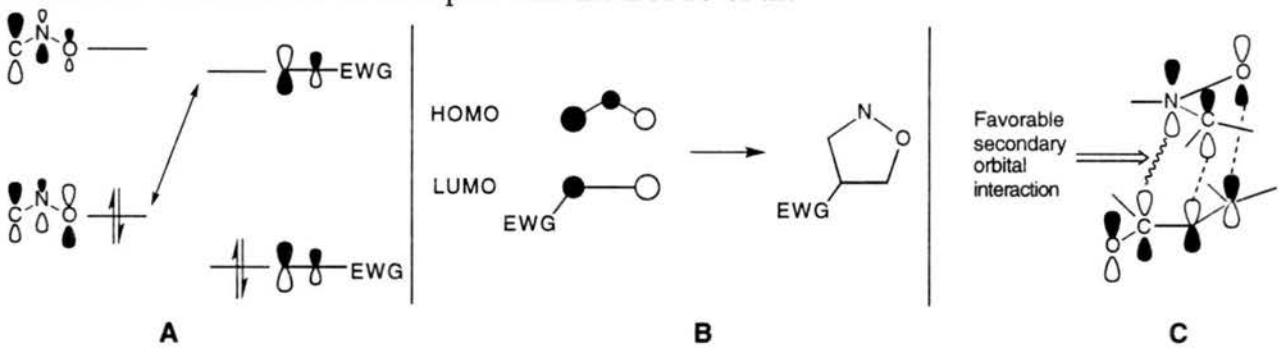
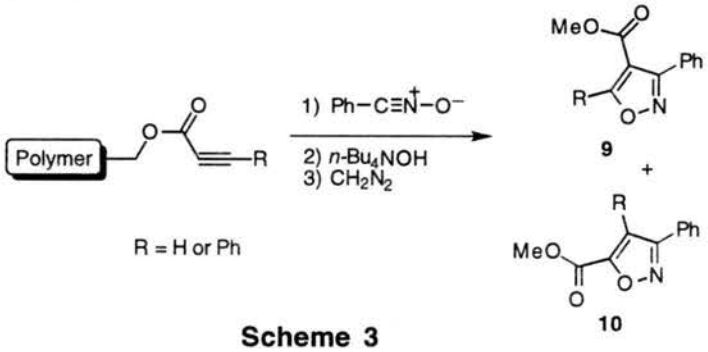


Figure 1

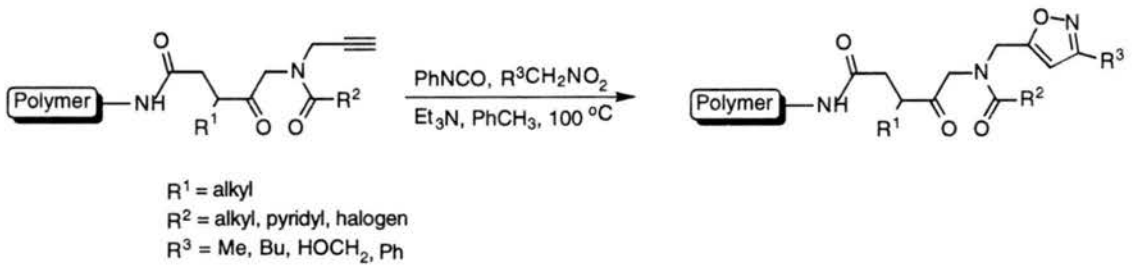
dipolarophile (**A**). This interaction favors 4-substituted adducts over 3-substituted ones and is supported experimentally (**B**).[15] Interpretation of the endo selectivity of reactions of dipolarophiles with conjugated electron withdrawing groups is found by examination of the reaction transition state (TS). It reveals a positive secondary orbital interaction which should favor the endo TS (**C**). Nitrones with chiral substituents on the carbon or

the nitrogen undergo highly diastereoselective cycloadditions with achiral dipolarophiles.[16] The predominant stereochemistry of the products can be explained by assuming a Felkin model TS[16c] is adopted during the cycloaddition. The preferred direction of dipolarophile approach is onto the face of the nitrogen anti to the biggest substituent. Houk[16b] and Anh[17] have concluded that the anti approach is favored due to the lack of unfavorable non-bonded orbital interactions in the TS. Many unrelated examples of asymmetric nitrone cycloadditions are explained using this rational.[18,19] Nitrone-olefin [3+2]-cycloaddition reactions have enjoyed a large popularity in the synthesis of nitrogen containing natural products.[20] The particularly facile additions of electron poor olefins to nitrones make it an attractive sequence to explore using resin supported substrates.

Several research groups have accomplished other types of [3+2] cycloadditions on polymer support. Yedidia *et al.* first published studies of the regio- and stereoselectivity of 1,3-dipolar cycloadditions of nitrile oxides to polymer bound alkynes (Scheme 3).[21] The reactions of support-bound propionic and phenyl propionic acids with benzonitrile oxide yielded exclusively isoxazole carboxylate **9** and isoxazole carboxylate **10**, respectively. These experiments showed that pericyclic reactions could be carried out successfully on polymer supports. The stereoselectivity and regiochemistry was either not affected or only minimally. In addition, the yields and purity of the compounds isolated from the polymer support were considerably improved over the conventional solution phase synthesis. Pei and Moos synthesized isoazoline- and isoxazole- substituted peptoids by

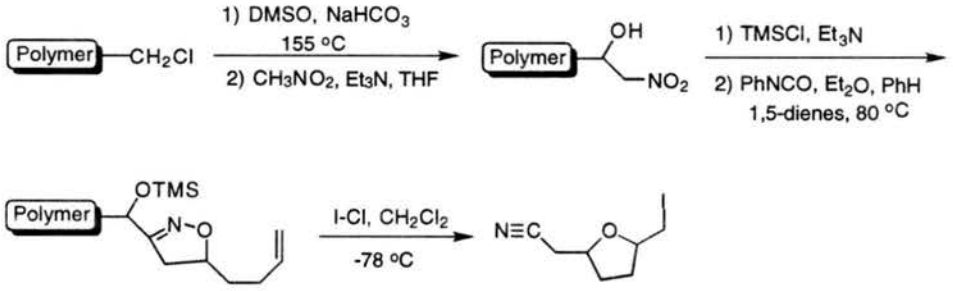


Scheme 3



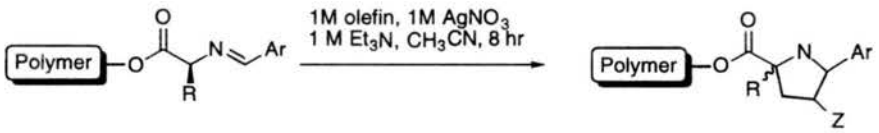
Scheme 4

adding nitrile oxides with *N*-alkenyl and *N*-alkynyl-glycines, respectively (Scheme 4).[22] As in the studies by Yedidia[21], Pei and Moos also used a solution phase 1,3-dipole and polymer bound dipolarophiles. The isoxazoline and isoxazole derivatives were obtained with more than 80% purity. Beebe *et al.* described a polymer supported synthesis of 2,5-disubstituted tetrahydrofurans by a tandem 1,3-dipolar cycloaddition of a nitrile oxide with a 1,5-hexadiene (Scheme 5).[23] Production of a polymer supported 1,3-dipole successfully eliminated the bis



Scheme 5

addition of nitrile oxides to the α,ω-dienes. Reaction conditions were only slightly, if at all, different from those used in conventional solution phase synthesis. The most recent application



Scheme 6

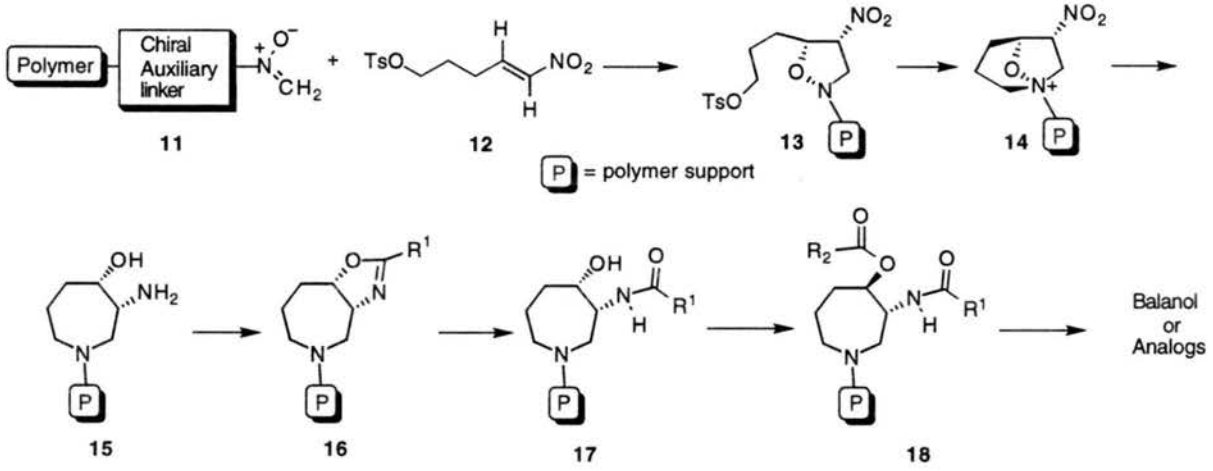
of a [3+2]-cycloaddition on polymer support used the Lewis acid ($AgNO_3$) promoted ionization of α-amino acid aldimines to generate polymer bound azomethine ylides

(Scheme 6).[24] Cyclization with various electron poor olefins produced hundreds of pyrrolidines in satisfactory yields for all but the most sterically hindered coupling partners. As in the solution phase reaction, the cycloaddition reaction on polymer support did not proceed with complete regio- and stereospecificity.

IV. Proposal

This program proposes to develop novel methodology for the 1,3-dipolar cycloaddition of a polymer supported nitrone to a solution phase olefin. To test this methodology, the hexahydroazepine fragment of the natural product balanol (**1**) will be synthesized on a polymer support. The synthesis of the polymer supported hexahydroazepine will allow for the quick and efficient asymmetric synthesis of **1** and structural analogs that vary by the benzophenone and benzoic acid side chains.

As shown in Scheme 7, the nitrone will be connected to the polymer support through a chiral auxiliary linker (**11**). Cyclization with nitroalkene **12** gives the isoxazolidine **13** in a



Scheme 7

regiospecific and stereoselective fashion. After the cycloaddition reaction, the [3.2.1]-bicyclic compound **14** is formed spontaneously. Reduction of the N-O bond and the nitro group produces the *cis*-amino alcohol **15**. Protection of the amino alcohol as oxazoline

16 with acyl chlorides gives variability to the side chains on the amine. Cleavage of the oxazoline forms the free secondary alcohol **17** which is subsequently inverted under Mitsunobu conditions with various carboxylic acids to furnish **18**. The carboxylic acids give variability to the side chains on the alcohol. Cleavage of the molecule from the polymer support and removal of any protecting groups produces the final product. Overall this will be a seven step synthesis on polymer support and should give the final products in highly pure and optically active form.

V. Research Design and Methods

1) Maximize Solution Phase Reactions.

Initially the solution phase chemistry of the proposed synthetic sequence will be investigated and maximized before attempting the polymer supported reactions. The reactions used in the solid supported synthesis must be high yielding, reproducible, and unaffected by excessive amounts of solution phase reagents typically used to drive reactions to completion.[3a] A reaction that relies on the use of stoichiometric reagents is a poor choice for use with polymer supports.

The first reaction to be investigated is the cycloaddition of nitrone **19** with nitroalkene **12**. (Scheme 10). The requisite chiral nitrone **19** will come from the condensation of (*R*)-(-)-*N*-(α-phenyethyl)hydroxylamine[25] with formaldehyde. Nitroalkene **12** will come from the Henry reaction[26] of 4-tosylbutyraldehyde[27] with nitromethane followed by dehydration. Cycloadditions of nitrones with electron deficient olefins are usually spontaneous and exothermic. Typical conditions for cycloadditions of this type are to mix the two compounds at room temperature or below in toluene, benzene, or acetonitrile. The diastereofacial selectivity of the cycloaddition is predicted by assuming a Felkin model transition state[16] is adopted by the nitrone. The diastereoselectivity is also predicted by assuming the dipolarophile approaches in an endo fashion (See Figure 1C).[13] The preferred direction of dipolarophile approach is onto the

face of the nitrone anti to the largest substituent (Ph).[16b,17] Houk[16b] and Anh[17] have concluded that the anti approach, as in A and B (Figure 2), is favored due to the lack of unfavorable nonbonded orbital interactions in the transition state. Using this model, nitrone **19** should adopt and react in the conformation A. Other conformations such as B which allow the dipolarophile to approach anti to the phenyl substituent should be disfavored due to steric repulsion between the methyl and methylene groups. In this case, the *R* absolute configuration of the chiral auxiliary will guide the formation of the desired diastereomer **20**. The diastereoselectivity of similar cycloadditions can be rationalized by using the same assumptions. For example, the cycloaddition of styrene to a nitrone[18] similar to **19** (Scheme 8) shows that the stereoselectivity of the reaction can be predicted if the nitrone reacts in conformation A and the dipolarophile approach is opposite to the phenyl substituent of the chiral auxiliary. The same rational can be used to explain the stereoselectivity of the cycloaddition shown in Scheme 9.[19]

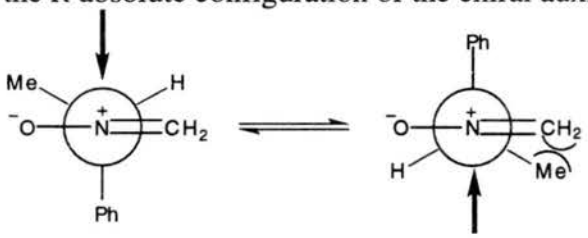
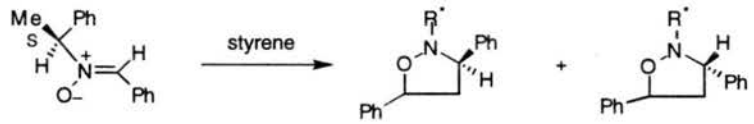
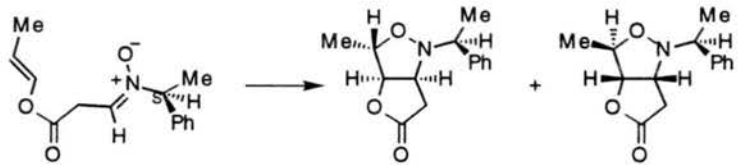


Figure 2



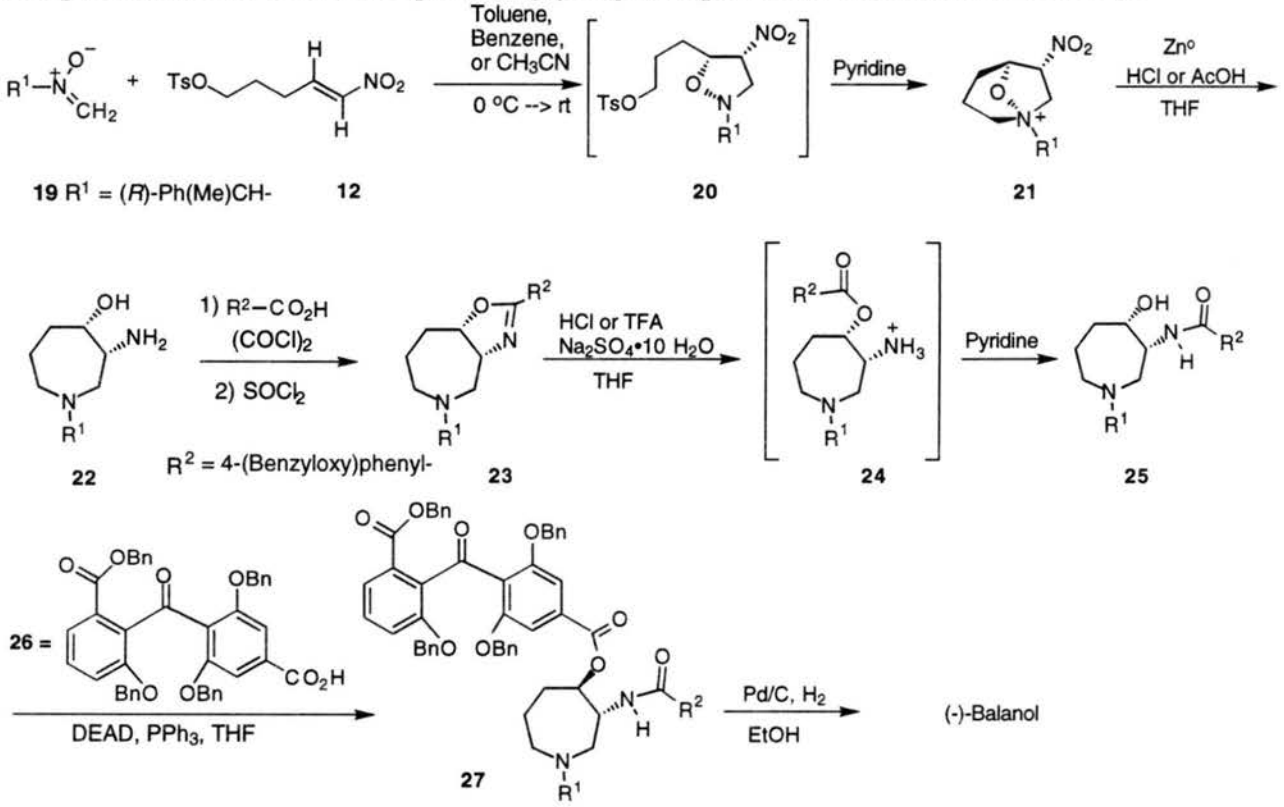
Scheme 8



Scheme 9

Upon cycloaddition, substrates similar to **13** have spontaneously displaced the tosyl group to give bicyclo-[2.2.1] products.[20] If the displacement of the leaving group is

not spontaneous, the reaction can be initiated by the addition of pyridine or other amine base.[20] With bicyclo-[3.2.1] compound **21** in hand, the nitro group and the N-O bond will be reduced with zinc and HCl or acetic acid to construct *cis*-amino alcohol **22**. While the reactivity of a primary amine is usually enough to be selectively acylated in the presence of a primary alcohol, the large excess of solution phase reagents typically used to drive reactions to completion on polymer-supports would eventually acylate the primary alcohol. Construction of oxazoline **23** and cleavage to the amide circumvents this problem without excessive protecting group manipulations. Formation of oxazoline



Scheme 10

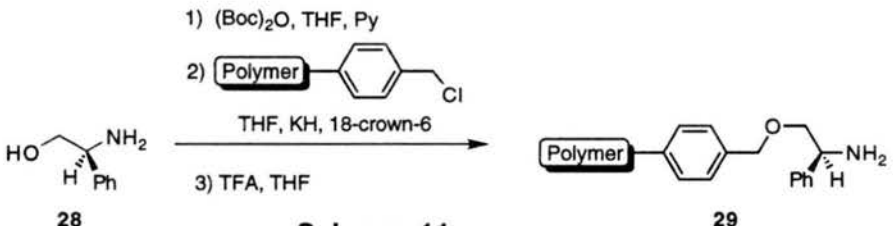
23 using 4-(benzyloxy)benzoyl chloride followed by thionyl chloride protects both the alcohol and amine. Hydrolysis of the oxazoline **23** with HCl or TFA gives the intermediate ester **24**. The benzoyl group migrates to the amine to form amide **25** upon neutralization with pyridine or other amine base. Mitsunobu inversion of the resulting free alcohol with benzophenone acid **26**[11a] produces the fully protected natural product **27**. Cleavage of all the protecting groups and the chiral auxiliary with Pd/C and H_2 gives

(-)-balanol (**1**). Once these reactions have been investigated and maximized, the knowledge gained should facilitate the synthesis of this and other compounds on solid support.

2) Polymer Support and Chiral Auxiliary Linker

Once the asymmetric synthesis of balanol has been demonstrated in solution, attempts will be made to tether a chiral auxiliary to a suitable support and construct a resin bound nitrone. The resins used will be Merrifield's resin and the Tenta Gel resin.[3] Merrifield's resin, commercially available as a polystyrene/ 2% divinylbenzene co-polymer, is insoluble in the proposed reaction solvents in Scheme 8. The resin has been used in a large number of syntheses including [3+2]-cycloadditions.[23] The Tenta Gel resin, commercially available as a polyethylene glycol polystyrene/divinylbenzene copolymer, is soluble in the proposed reaction solvents in Scheme 8 but can be precipitated for purification purposes.

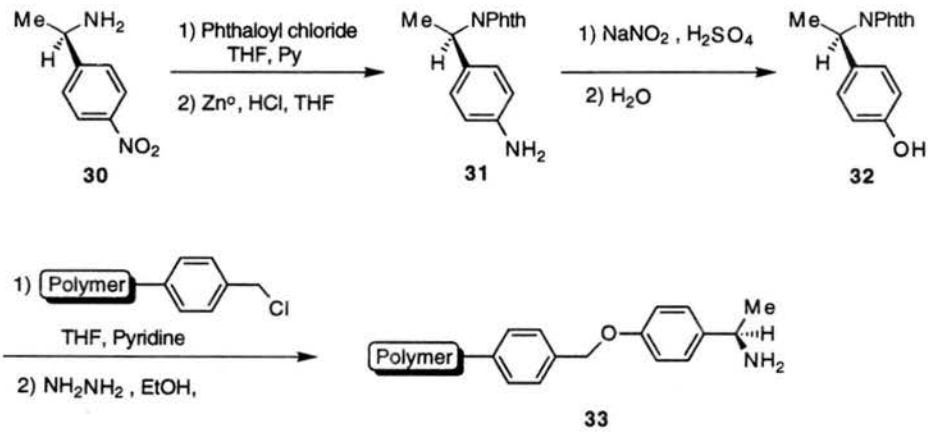
Chiral auxiliaries will be covalently bound to the chloromethylated resins by a nucleophilic substitution reaction. The two auxiliaries to be used are the commercially available (*S*)-phenylglycinol (**28**) and (*R*)-α–(4-nitrophenyl)ethylamine (**30**). These two amines will allow the polymer support to be attached to the chiral linkers through two different groups. Connection of the glycinol **28** to the solid supports is shown in Scheme 11. The amine of **28** will be protected as the *tert*-butyl carbamate and connected to the



28 **29**

Scheme 11

polymer support. Once the Boc group is removed with TFA to give free amine **29**, further elaboration to nitrone **34** will be performed as in the solution phase.[25] Amine **30** needs to be slightly modified before it can be attached to the polymer support (Scheme 12). Protection of **30** with phthaloyl chloride followed by the reduction of the nitro group with zinc and HCl gives aniline **31**. Formation of the diazonium of **31** followed by

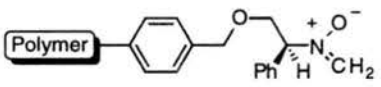


Scheme 12

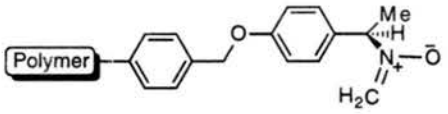
displacement of the diazonium with water effects the conversion of **31** to phenol **32**. Connection of **32** to the chloromethylated polymer support followed by removal of the phthalimide group with hydrazine forms amine **33**. Further elaboration to nitrone **35** will be performed as in the solution phase.[25]

3) Solid Support Reactions

Upon construction of the chiral polymer supported nitrone, the synthesis of balanol (**1**) and analogs on solid support will be investigated. Using the information obtained from the solution phase synthesis, all four possible nitrones **34a**, **34b**, **35a**, **35b** will be used to synthesize **1** (Scheme 7). The effects of the type of support and the type of connection to the chiral



34a Polymer = Merrifield Resin
34b Polymer = Tenta Gel Resin



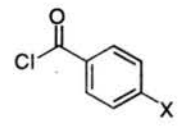
35a Polymer = Merrifield Resin
35b Polymer = Tenta Gel Resin

auxiliary will be investigated. The two types of resins and two types of connections used should allow enough experimental flexibility with the cyclization to find a suitable support and chiral auxiliary linker.

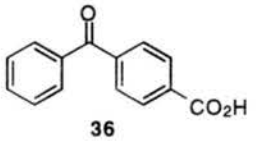
Although the exact consequences of the solid support on the regio- and stereoselectivity of the proposed cyclization are unknown, inferences about its effects can be made from the experiments by Yedida *et al.* some of which are shown in Scheme 3.[21] They investigated the effects of the Merrifield polymer support on the the regioselectivity of cycloaddition reactions (Diels-Alder and [3+2]) and found little or no change between the polymer supported reactions and analogous reactions in solution. Researchers concluded that the Merrifield polymer-support is in some cases no more bulky than a simple benzyl group.[21] As a result of these experiments, it is believed the proposed polymer-supported cycloaddition reaction between **34** or **35** and **12** will show the same regioselectivity as the analogous reaction in solution (See figures 1). The stereoselectivity of the cycloaddition between **35** and **12** should also be the same as the analogous reaction in solution (See figure 2). On the other hand, cycloadditions between **34** and **12** may show the opposite or no stereoselectivity since the polymer support is connected through the methyl group, and the added steric bulk may cause the nitrone to adopt other reactive conformations. If the opposite stereoselectivity is seen for the

cyclization between **34** and **12**, the enantiomer of **34** can be prepared from the commercially available (*R*)-phenylglycinol (See Scheme 9).

Procedures following the [3+2]-cyclization should be the same as the solution phase reactions until oxazoline **16** is formed. Initially, (4-benzyl)benzoyl chloride will be used to form the oxazoline so **1** can be made. Other commercially available acyl chlorides (Figure 4) will also be used to create a small library of natural product analogs.[3]



35 X = H, Me, OMe, F, or Cl



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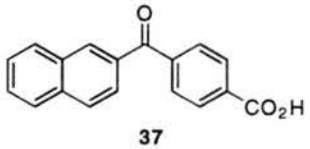

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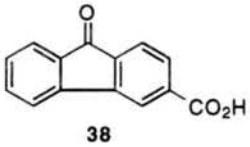

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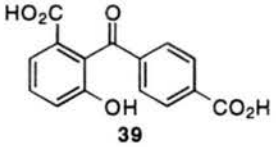

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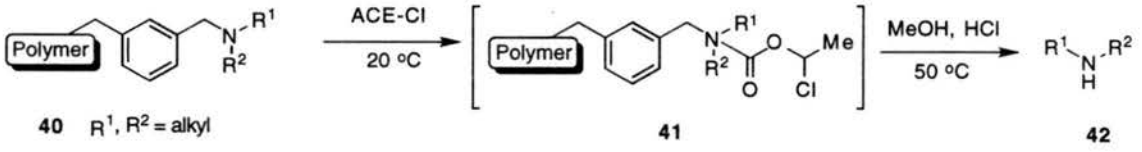
Figure 3

Conditions for oxazoline formation and future synthetic steps will not conflict with the structure of the functional groups on these acyl chlorides. After transforming oxazoline **16** (R^1 = 4-(benzyloxy)phenyl) to amide **17**, Mitsunobu[28] inversion of the free alcohol with **26** gives polymer supported, fully protected natural product **18**. Inversion with other acid chlorides (Figure 3), available through the slight modification of literature procedures used to construct **26**, add a second degree of variability to the types of natural product analogs that can be produced using this method.

Cleavage of the products from the polymer support will depend on the type of resin used. The Tenta-Gel resin is soluble in most organic solvents, and the heterogeneous palladium catalyzed reductive removal of the protecting groups and cleavage of the connection to the solid support will produce the natural product and analogs. On the Merrifield resin, a similar transformation would be impossible using a heterogeneous catalyst since the Merrifield resin is insoluble in most solvents.

Alternatives to effect this transformation on the Merrifield resin exist but require three synthetic steps. In a recently disclosed strategy (Scheme 13), the clean and efficient cleavage of *N*-benzyl linked tertiary amines (**40**) from the Merrifield resin by treatment with α-chloroethyl chloroformate (ACE-Cl) followed by methanolysis yielded the free secondary amine **42**.[29] Although the first step in this procedure should work to cleave the polymer support from the azepine ring, the second step may hydrolyze the ester linkage in the final product. Using either β-trimethylsilylethyl chloroformate[30] or 2,2,2-



Scheme 13

trichloroethyl chloroformate, [31] instead of ACE-Cl, will produce a similar quaternary intermediate to **41**. Treatment of the trimethylsilyl intermediate with *n*-Bu_4NF or the trichloro intermediate with Zn and AcOH will generate the free secondary amine. At this point the benzyl groups on the free amine can be removed by palladium catalyzed hydrogenation.

References

1. Ager, D.J.; East, M.B. *Aymmetric Synthetic Methodology*; CRC, 1996.

2. Hanessian, S. *Total Synthesis of Natural Products: The 'Chiron' Approach*; Baldwin, J.E. Ed. Pergamonn: NY, 1983; *Organic Chemistry Series*, Vol 3.

3. a) *Organic Chemistry on Solid Support* Fruchtel, J.S.; Jung, G. *Angew. Chem. Int. Ed. Eng.* **1996**, *35*, 17; b) *Strategy and Tactics in Combinatorial Organic Synthesis. Applications to Drug Discovery* Gordon, E.M.; Gallop, M.A.; Patel, D.V. *Acc. Chem. Res.* **1996**, *29*, 144.

4. a) *The Molecular Heterogeneity of Protein Kinase C and Its Implications for Cellular Recognition* Nishizuka, Y. *Nature* **1988**, *334*, 661; b) *Studies and Perspectives of Protein Kinase C* Nishizuka, Y. *Science* **1986**, *233*, 305; c) *The Role of Protein Kinase C in Cell Surface Signal Transduction and Tumor Promotion* Nishizuka, Y. *Nature* **1984**, *308*, 693.

5. *Therapeutic Potential of Protein Kinase C Inhibitors* Bradshaw, D.; Hill, C.H.; Nixon, J.S.; Wilkinson, S.E. *Agents Actions* **1993**, *38*, 135.

6. *Staurosporine, A Protein Inhibitor of Phospholipid Calcium Dependent Protein Kinase* Tamoki, T.; Nomot, H.; Takahashi, I.; Kato, Y.; Morimoto, M.; Tomita, F. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397.

7. *Isoquinolinesulfonamides, Novel and Potent Inhibitors of Cyclic Nucleotide Dependent Protein Kinase and Protein Kinase C* Hidaka, H.; Inagaki, M.; Kavamoto, S.; Sasaki, Y. *Biochemistry* **1984**, *23*, 5036.

8. *K-252a, A Potent Inhibitor of Protein Kinase C from Microbial Origin* Kase, H.; Iwahashi, K.; Matsuda, Y. *J. Antibiot.* **1986**, *39*, 1059.

9. *Balanol: a Novel and Potent Inhibitor of Protein Kinase C from Fungus Verticillium Balanoides* Kulanthaivel, P.; Hallock, Y.F.; Boros, C.; Hamilton, S.M.; Janzen, W.P.; Ballas, L.M.; Loomis, C.R.; Jiang, J.B. *J. Am. Chem. Soc.* **1993**, *115*, 6452.

10. *Fusarium Merismoides Corda NR 6356, The Source of the Protein Kinase C Inhibitor, Azepinostatin* Ohshima, S.; Yanagisawa, M.; Katoh, A.; Fujii, T.; Sano, T.; Matsukuma, S.; Furumai, T.; Fujiu, M.; Watanabe, K.; Yokose, K.; Arisawa, M.; Okuda, T. *J. Antibiot.* **1994**, *47*, 639.

11. a)*Total Synthesis of Balanol* Nicolaou, K.C.; Bunnage, M.E.; Koide, K. *J. Am. Chem. Soc.* **1994**, *116*, 8402; b) *Total Synthesis of (-) Balanol* Lampe, J. W.; Hughes, P. F.; Biggers, C. K.; Smith, S. H.; Hu, H. *J. Org. Chem.* **1994**, *59*, 5147; c) *Total Synthesis of Balanol: A Potent Protein Kinase Inhibitor of Fungal Origin* Adams, C.P.; Fairway, S.M.; Hardy, C.J.; Hibbs, D.E.; Hursthouse, M.B.; Morley, A.D.; Sharp, B.W.; Vicker, N.; Warner, I. *J. Chem. Soc. Perkin Trans. I* **1995,** 2355.

12. a)*Two Effiecient Syntheses of (±) anti-N-Benzyl-3-amino-4-hydroxyhexahydroazepine* Hu, H.; Jagdmann, G.E. *Tetrahedron Lett.* **1995**, *36*, 3659; b) *Efficient Synthesis of Chiral N-Tosyl-3,4-Disubstituted Hexahydroazepines from D-(-)-Quinic Acid* Albertini, E.; Barco, A.; Benetti, S.;

De Risi, C.; Pollini, G.P.; Zanirato, V. *Synlett* **1996**, 29; c) *An Efficient Formal Synthesis of Balanol via the Asymmetric Epoxide Ring Opening Reaction* Wu, M.H.; Jacobsen, E.N. *Tetrahedron Lett.* **1997**, *38*, 1693.

13. Tufariello, J.J.; Padwa, A. *1,3-Dipolar Cycloadditon Chemistry*; Taylor, E.C.; Weissberger, A. Eds.; Wiley &Sons: NY, 1984, Vol 2, pp 83-168.

14. a)*The Diradical Mechanism for the 1,3-Dipolar Cycloadditions and Related Thermal Pericyclic Reactions* Firestone, R.A. *Tetrahedron* **1977**, *33*, 3009; b)*The Concerted Nature of 1,3-Dipolar Cycloadditions and the Question of Diradical Mechanism* Huisgen, R. *J. Org. Chem.* **1976**, *41*, 403.

15. *Reversal of Nitrone Cycloaddition Regioselectivity with Electron-Deficient Dipolarophiles* Sims, J.; Houk, K.N. *J. Am. Chem. Soc.* **1973**, *95*, 5798.; b) *Nitrone Ionization Potentials and Cycloaddition Regioselectivities.* Houk, K.N.; Bimanand, A.; Mukherjee, D.; Sims, J.; Chang, Y.-M.; Kaufman, D.C.; Domelsmith, L.N. *Heterocycles* **1977**, *7*, 293.

16. a)*Stereospecific Synthesis of Racemic Daunosamine. Diastereofacial Selectivity in a Nitrone Cycloaddition* Deshong, P.; Leginus, J.M. *J. Am. Chem. Soc.* **1983**, *105*, 1686.; b) *Origin of π-Facial Stereoselectivity in Additions to π-bonds: Generality of the Anit-Periplanar Effect* Caramella, P.; Rondan, N.G.; Paddon-Row, M.N.; Houk, K.N. *J. Am. Chem. Soc.* **1981**, *103*, 2438.; c) Cherest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, *18*, 2199.

17. Anh, N.T.; Eisenstein, O. *Nouv. J. Chim.* **1977**, *1*, 61.

18. *Cycloaddition of Chiral Nitrones. Asymmetric Synthesis of Isoxazolidines* Belzecki, C.; Panfil, I. *J. Org. Chem.* **1979**, *44*, 1212.

19. *A Chiral Synthesis of L-Acosamine and L-Daunosamine via an Enantioselective Intramolecular [3+2] Cycloaddition* Wovkulich, P.M.; Uskokovic, M.R. *J. Am. Chem. Soc.* **1981**, *103*, 3956.

20. *Alkaloids from Nitrones* Tufariello, J. J. *Acc. Chem. Res.* **1979**, *12*, 396.

21. *Regioselectivity in Cycloaddition Reactions on Solid Phases* Yedidia, V.; Leznoff, C.C. *Can. J. Chem.* **1980**, *58*, 1144.

22. *Post-Modification of Peptoid Side Chains: [3+2] Cycloaddition of Nitrile Oxides with Alkenes and Alkynes on the Solid-Phase* Pei, Y.; Moos, W.H. *Tetrahedron Lett.* **1994**, *35*, 5825.

23. *Polymer-Supported Synthesis of 2,5-Disubstituted Tetrahydrofurans* Beebe, X.; Schore, N.E.; Kurth, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 10061.

24. *Combinatorial Organic Synthesis of Highly Functionalized Pyrrolidines: Identification of a Potent Angiotensin Converting Enzyme Inhibitor from a Mercaptoacyl Proline Library* Murphy, M.M.; Schullek, J. R.; Gordon, E.M.; Gallop, M.A. *J. Am. Chem. Soc.* **1995**, *117*, 7029.

25. *Chiral Synthesis of Key Intermediates of (+) and (-)-Thienamycin* Kametani, T.; Nagahara, T.; Honda, T. *J. Org. Chem.* **1985**, *50*, 2327.

26. *Conjugated Nitroalkenes: Versatile Intermediates in Organic Synthesis* Barrett, A.G.M.; Graboski, G.G. *Chem. Rev.* **1986**, *86*, 751.

27. *Preparation of 4,4-Dimethoxybutyl Iodide from 1,4-Butanediol via the Corresponding Tosylate* Wu, Y.; Ahlberg, P. *J. Org. Chem.* **1994**, *59*, 5076.

28. *Solid Phase Synthesis of Aryl Ethers Via the Mitsunobu Reaction* Rano, T.A.; Chapman, K.T. *Tetrahedron Lett.* **1995**, *36*, 3789.

29. *A New Cleavage Strategy for the Solid-Phase Synthesis of Secondary Amines* Conti, P.; Demont, D.; Cals, J.; Ottenheijm, H.C.J.; Leysen, D. *Tetrahedron Lett.* **1997**, *38*, 2919.

30. *The Mild and Selective N-Debenzylation of Tertiary Alkylamines Using β-Trimethylsilylethyl Chloroformate* Campbell, A.L.; Pilipauskas, D.S.; Khanna, I.K.; Rhodes, R.A. *Tetrahedron Lett.* **1987**, *28*, 2331.

31. *Peripheral Synthesis of Secondary Medium Ring Nitrogen Heterocycles* Reinecke, M.G.; Daubert, R.G. *J. Org. Chem.* **1973**, *38*, 3281.