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# Synthetic Enantiopure Aziridinomitosenes: Preparation, Reactivity, and DNA Alkylation Studies

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***Abstract:*** An enantiocontrolled route to aziridinomitosenes had been developed from L-serine methyl ester hydrochloride. The tetracyclic target ring system was assembled by an internal azomethine ylide cycloaddition reaction based on silver ion-assisted intramolecular oxazole alkylation and cyanide-induced ylide generation via a labile oxazoline intermediate (**62** to **66**). Other key steps include reductive detritylation of **26**, methylation of the N-H aziridine **56**, oxidation of the sensitive cyclohexenedione **68** to quinone **70**, and carbamoylation using Fmoc-NCO. Although the aziridinomitosene tetracycle is sensitive, a range of protecting group manipulations and redox chemistry can be performed if suitable precautions are taken. A study of DNA alkylation by the first C-6,C-7-unsubstituted aziridinomitosene **11a** has been carried out, and evidence for DNA cross-link formation involving nucleophilic addition to the quinone subunit is described.

Mitomycins A (**1**), B (**2**), and C (**3**)[1−3] have been the focus of intensive study due to their fascinating structures, unusual metabolic activation pathways, and clinical antitumor activity.[4] Thus, **1**, **3**, and mitomycin K (**4**) have served as targets for total synthesis,[5−8] while **1** and **3** have been investigated in detail to clarify the mechanisms of DNA alkylation (Scheme 1).[1,9−12]

Reductive activation of mitomycins generates leucoaziridinomitosenes such as **5a**, the intermediates responsible for DNA cross-link formation.[11] Leucoaziridinomitosenes are too reactive for isolation, but **5a** can be observed in solution using NMR techniques,[12] and the bis-silyl derivative **5b** can be purified by chromatography.[13] Aziridinomitosenes **6**, **7**, **8**, and **9** are relatively stable, although they are more sensitive than the parent mitomycins due to the activating effect of the indole nitrogen on heterolysis of the aziridine C−N bond.[1c,11,14,15] Aziridinomitosenes do not require reductive activation to alkylate DNA, in contrast to the mitomycins. Thus, **7** has been shown to monoalkylate DNA,[15] while **9** has in vivo activity similar to that of mitomycin C.[16]

Several total syntheses of racemic mitomycins have been reported,[5−8] but there has been no enantioselective synthesis.[17,18] Only one synthesis of a fully functional aziridinomitosene (racemic **6**) has been reported to date (Jimenez and Dong).[19] The highly sensitive aziridine ring was installed in the final step, a strategy that minimizes problems with reactivity but that somewhat limits options for enantioselective synthesis.

Efforts in our laboratory have been focused on previously unknown aziridinomitosenes such as **10**, **11a**, and **11b**. These structures contain potentially electrophilic sites at C-6 or C-7

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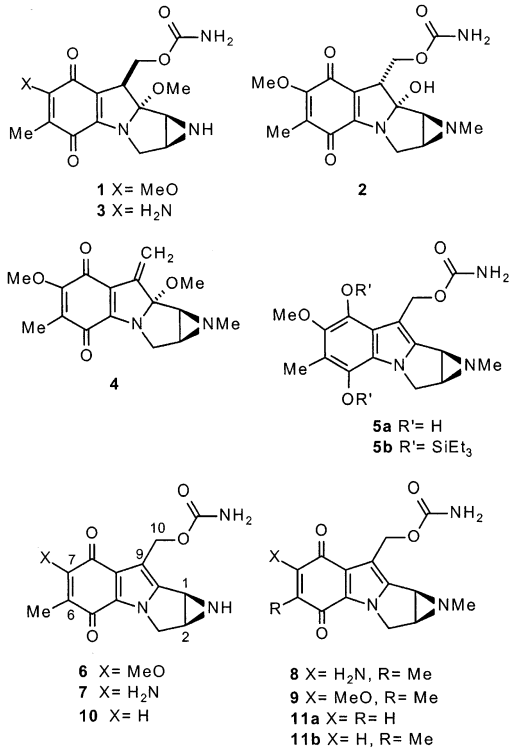
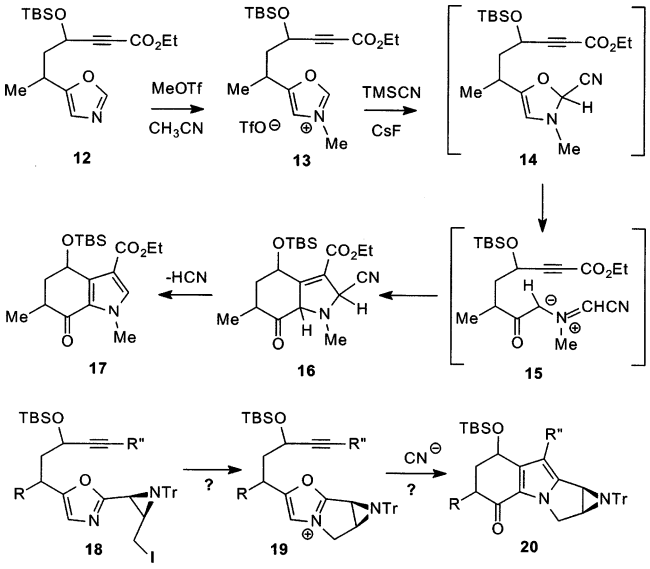
***Scheme 1***




***Scheme 2***




(mitomycin numbering) in addition to the electrophilic C-1 and C-10 carbons and may have new options for DNA alkylation as well as additional pathways for activation. Our approach is based on azomethine ylide generation from oxazolium salts,[20] a methodology developed in our laboratory for rapid access to indoloquinones (Scheme 2).[21] The key sequence is triggered by the nucleophilic addition of cyanide ion to oxazolium salt **13** to afford the transient 4-oxazoline **14**, followed by electrocyclic ring opening to the azomethine ylide **15**. Internal cycloaddition to a tethered alkyne then produces pyrroline **16** that aromatizes by loss of HCN to generate the pyrrole **17**. All of these events occur at room temperature or below. Under such mild conditions, it seemed possible that an aziridine subunit might survive the steps from bicyclic oxazolium salt **19** to the internal cycloadduct **20**. The potential for participation by aziridine nitrogen during the internal alkylation from **18** to **19** was a concern in view of prior reports describing generation of bicyclic azetidinium intermediates from related substrates.[22] However, model studies indicated that the oxazole nitrogen is more nucleophilic than the *N*-tritylaziridine in reactions involving intermolecular *N*-alkylation.[21a] Thus, intramolecular oxazolium salt formation might be faster than participation by aziridine nitrogen.

The overall strategy goes against the conventional wisdom that the most labile functionality (the solvolytically reactive aziridine) should be introduced at the end of the synthesis. To the contrary, our approach incorporates an aziridine subunit early on. The challenge of handling sensitive aziridines at several stages was expected to stimulate development of methodology and to add to our understanding of aziridinomitosene chemistry. Another advantage of using an intact aziridine early in the sequence is that enantioselective synthesis is easily achieved starting from simple amino acid precursors, as discussed below in the context of target structures **11** (**a** series, R= H; **b** series, R= methyl).[23]

## Results and Discussion

The above strategy requires the enantiocontrolled synthesis of a 2,5-disubstituted aziridinyloxazole related to **18**. Our plan was to assemble the aziridine subunit from amino alcohol **25** (Scheme 3). This structure should be accessible by the coupling of aldehyde **21** (prepared by *O*-allylation and DIBAL reduction of methyl *N*-tritylserinate[24]) with the lithiated oxazole **24** if control for the desired relative stereochemistry can be achieved. Earlier studies in our laboratory had shown that electrophiles react cleanly with **24a** at oxazole C-2, without interference by electrocyclic ring opening.[25,26] We had also shown that the precursor oxazole **23a**[27] can be prepared by the reaction of lithiated methyl isocyanide[28,29] with butyrolactone. The corresponding reaction with 2-methylbutyrolactone proved difficult to control, but **23b** could be made using the analogous Schöllkopf oxazole synthesis[28] from the ester **22**.

Oxazole **23a** was converted into the lithiated borane complex **24a** by treatment with THF−borane followed by deprotonation with *n*-BuLi, and reaction with the aldehyde **21** in THF gave a 6:1 diastereomer mixture of amino alcohols **25a** (93%). Similar diastereomer ratios were obtained in toluene or ether, although the reactions were not as clean. The stereochemistry of the major product could not be established at this stage, but treatment of the mixture with diethyl azodicarboxylate and triphenylphosphine under Mitsunobu conditions[30] afforded the single aziridine

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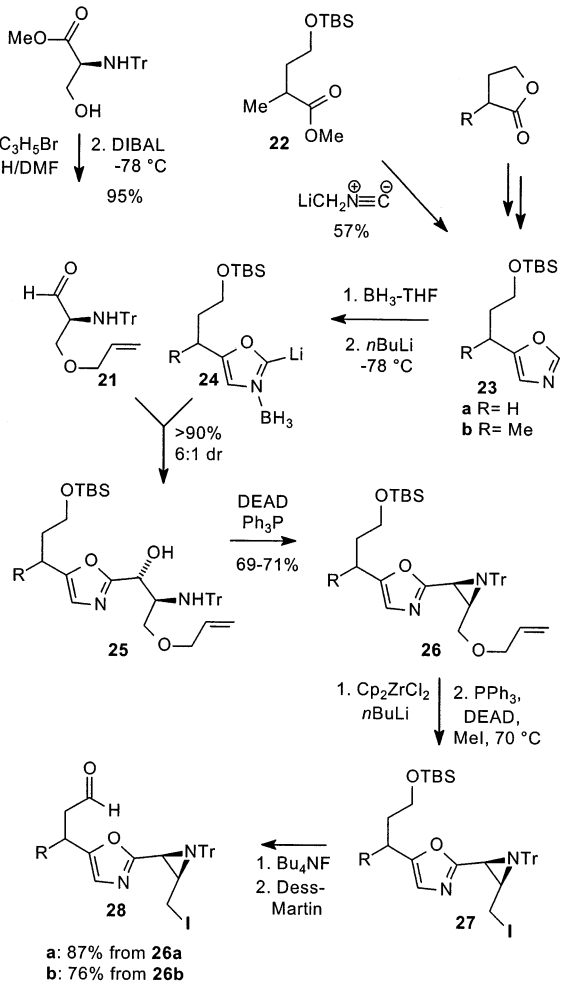
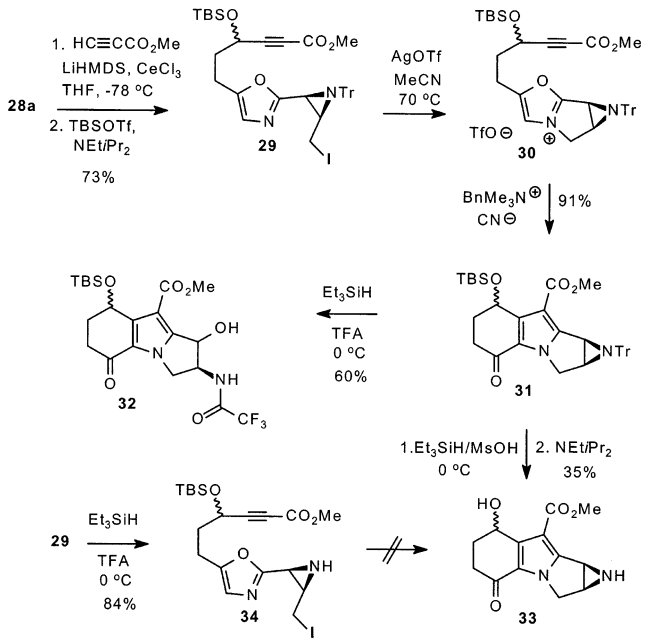
***Scheme 3***




***Scheme 4***




**26a** after chromatographic purification (71% isolated). The desired *cis* stereochemistry was confirmed by NMR, based on the characteristic 6.1 Hz vicinal coupling constant for the aziridine protons.[31,32] Furthermore, the product was shown to have 98.1% ee by hplc assay comparison with a sample prepared from racemic **21**. Thus, addition of **24a** to **21** occurs without significant racemization, and the desired stereoisomer **25a** is the major product.

The same methods were used to convert **23b** into **26b** (65% overall). As with **26a**, the minor diastereomers from the aldehyde coupling step were removed during purification of **26b**, but the presence of the remote methyl substituent resulted in a 1:1 diastereomer mixture of *cis*-aziridines (5.9 Hz coupling between the aziridine protons). Because the added *C*-methyl stereocenter disappears later in the synthesis, the diastereomers were taken through the next steps without separation.[33]

Removal of the allyl protecting group from **26** using an in situ generated zirconium(II) reagent[34] provided the corresponding alcohol in excellent yield, but conversion to the iodide **27** required for intramolecular oxazole *N*-alkylation was challenging. The triphenylphosphine−iodine adduct was initially used,[35] and product **27a** was obtained in 50% yield. A byproduct derived from aziridine rearrangement and cleavage was also isolated.[36] Several other phosphorus-based reagents proved ineffective,[37] but Mitsunobu conditions gave promising results.[38] The combination of triphenylphosphine, diethyl azodicarboxylate, and methyl iodide in THF at 70 °C effected ca. 50% conversion to the desired iodide. Attempts to improve the conversion by varying reaction time or stoichiometry were not successful, but use of toluene in place of THF had a remarkable effect on rate as well as yield. Starting material was completely consumed in 2 h, and **27a** was obtained in >95% yield. The dramatic solvent effect may be the result of tight ion pairing in the nonpolar toluene between the alkoxyphosphonium salt and the iodide, thus ensuring proximity of the reactive ions.[39] Once formed, the iodides **27a** and **27b** were relatively stable despite the presence of neighboring nitrogen nucleophiles at the aziridine and oxazole subunits. Thus, deprotection with $Bu_4NF$ (TBAF) followed by Dess−Martin oxidation proceeded smoothly to the aldehydes **28** (87−89% from **27**).

In preparation for the crucial intramolecular 2 + 3 cycloaddition, it was now necessary to incorporate a suitable dipolarophile (Scheme 4). Model studies had shown that ester-activated tethered alkynes are effective for this purpose,[21a] so the initial experiments were conducted with an ynoate **29** as the dipolarophile. Following the earlier study, methyl propiolate was treated with LiHMDS in the presence of cerium(III) chloride

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(32) Brois, S. J.; Beardsley, G. P. *Tetrahedron Lett.* **1966**, *7*, 5113.
(33) Amino alcohol **25b** is formed as a 6:6:1:1 mixture of four diastereomers.
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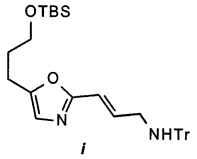
(36) The allylic amine structure *i* is supported by $^1$H NMR, $^{13}$C NMR, and high-resolution mass spectrometry, possibly resulting from a bicyclic azetidinium cation.




*i*

(37) Other conditions include the following: (a) $Ph_3P-I_2$, $Et_3N$ or DBU, with or without propylene oxide. (b) $(PhO)_3P$, MeI.; Landauer, S. R.; Rydon, H. N. *J. Chem. Soc.* **1953**, 2224. (c) $(PhO)_3P$-MeI Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1970**, *35*, 2319.
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(39) See Hughes, D. L. *Org. React.* **1992**, *42*, 335.

and was reacted with **28a**. The resulting alcohol was protected with TBSOTf/*i*$Pr_2$NEt to provide the silyl ether **29** in 73% overall yield.

Attempts to induce the intramolecular alkylation from **29** to oxazolium salt **30** by heating in various solvents were not successful. However, when the experiment was conducted using commercial silver triflate to activate the iodide in acetonitrile, downfield shifts were observed in the NMR spectrum consistent with the formation of **30**. When **30** was added dropwise to $BnMe_3N^+CN^-$ in acetonitrile,[21b] each drop produced a transient yellow color, tentatively attributed to an azomethine ylide intermediate. The color faded within a second as each drop of **30** was added, and the desired tetracyclic pyrrole **31** was isolated in a remarkable 91% yield. Similar experiments conducted in the course of our model studies had rarely exceeded 60% yield. Evidently, the *N*-protected aziridine does not interfere with either the intramolecular oxazole alkylation step, nor with the multistage 2 + 3 cycloaddition−aromatization sequence.

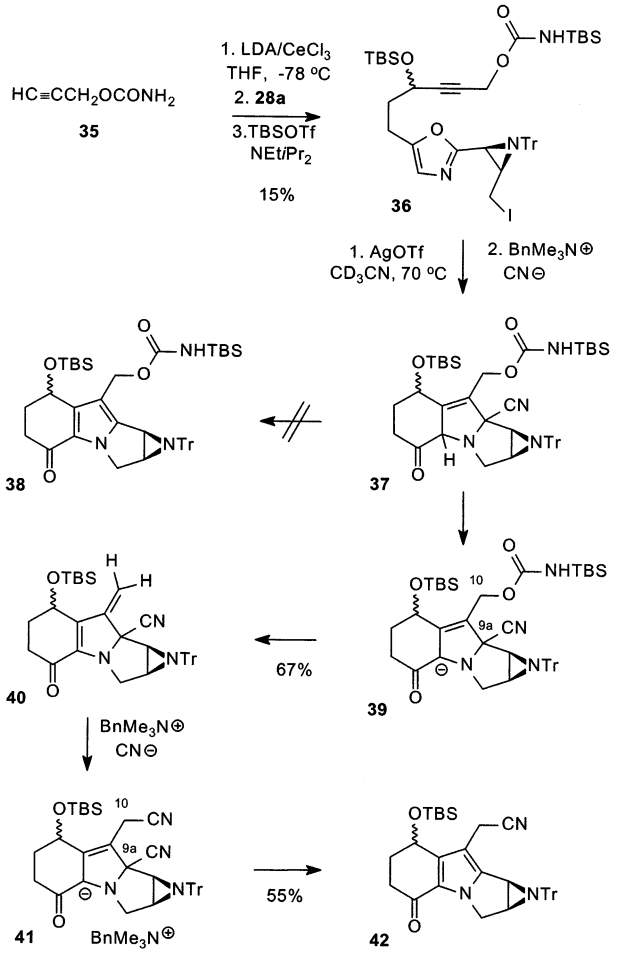
To demonstrate the viability of the approach, removal of the *N*-trityl protecting group was investigated using variations of our recently optimized reductive detritylation methodology.[40] Attempted deprotection of **31** with the TFA−triethylsilane reagent effected trityl cleavage but also opened the aziridine ring to afford an amide **32**, the result of aziridine trifluoroacetolysis followed by *O*- to *N*-migration of the trifluoroacetyl group. The same complication has been encountered with other solvolytically sensitive aziridines under these conditions.[40]

In a second experiment, **31** was treated with methanesulfonic acid−triethylsilane (Scheme 4), followed by quenching with *i*$Pr_2$NEt. The aziridine **33**, derived from cleavage of *O*-silyl as well as *N*-trityl groups, was isolated in 35% yield along with unknown decomposition products. The NMR spectrum of **33** was complicated by slow inversion at the aziridine nitrogen resulting in signal broadening and two sets of signals due to a ca.1:1.5 mixture of the N−H invertomers. A similar phenomenon has been reported by Kohn and Han in their studies of the naturally derived aziridinomitosenes.[15a,41] The line shapes could be improved dramatically by addition of activated powdered molecular sieves to the NMR sample, suggesting that broadening is due to proton exchange catalyzed by traces of water in the sample.

Before proceeding further, control experiments were performed to learn whether aziridine nitrogen protection is required in the ylide generation/2 + 3 cycloaddition step. Thus, **29** was treated with TFA−triethylsilane to remove the trityl group. This procedure worked well and afforded **34** in 84% yield, a result that can be contrasted with the conversion from **31** to **32** under similar conditions. The electron-withdrawing oxazole substituent in **34** probably retards aziridine ring cleavage, thereby improving the yield in the detritylation step. However, treatment of **34** with AgOTf in $CD_3CN$ resulted in a complex mixture of decomposition products, and conversion to the oxazolium salt could not be achieved in the presence of the unprotected N-H aziridine.

The next challenge was to determine whether the ester activating group is essential for the intramolecular cycloaddition, or whether the reaction can also be performed at the correct

***Scheme 5***

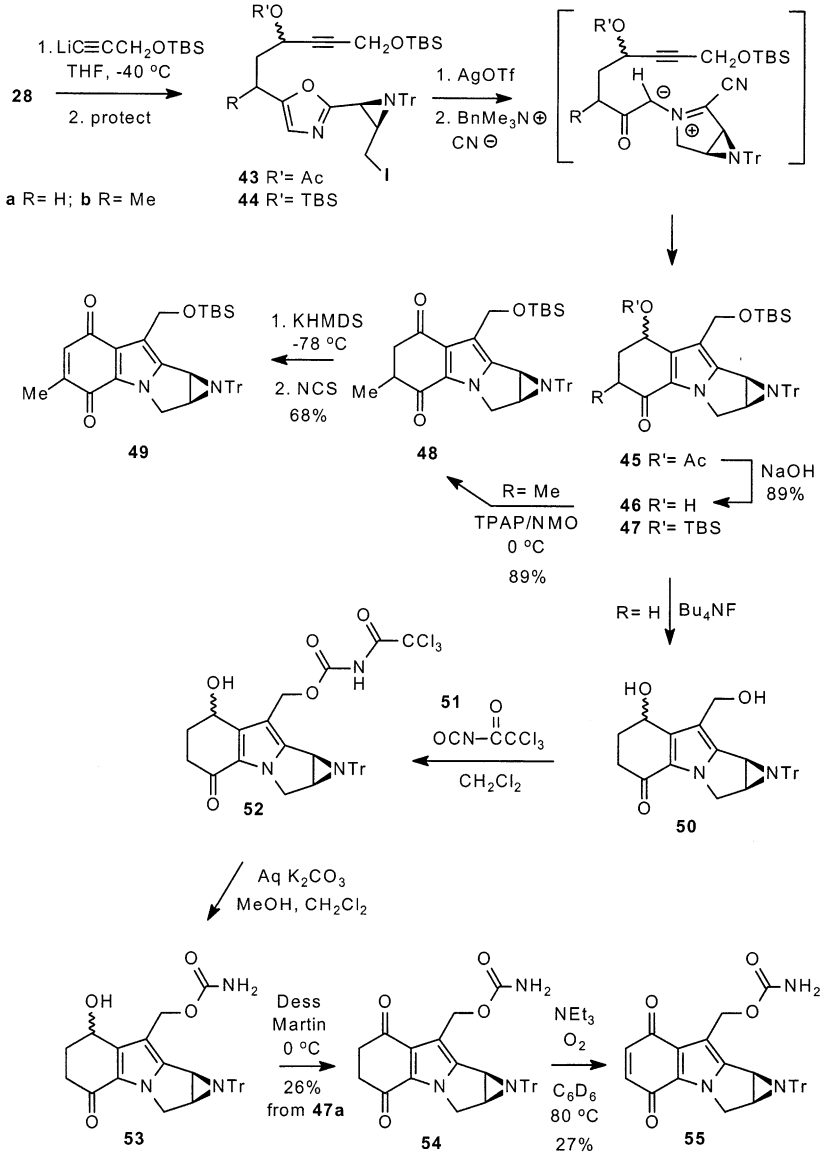



oxidation state and natural substitution pattern corresponding to the carbamate ester of an unactivated propargyl alcohol derivative (Scheme 5). Addition of lithiated propargyl carbamate to the aldehyde **28a** in the presence of $CeCl_3$ followed by silylation gave a disappointing 15% yield of **36**, but sufficient material was obtained to test the cycloaddition. The intramolecular alkylation of the oxazole **36** induced by silver triflate cleanly provided the oxazolium salt according to NMR assay, and addition of the crude salt solution to $BnMe_3N^+CN^-$ in acetonitrile afforded cycloadducts within 5 min at room temperature. However, none of the desired product **38** was detected. Instead, the nitrile assigned structure **40** was obtained in 67% yield. Only two of the four possible diastereomers of **40** were present (ca.1:1 ratio) and were readily separated by chromatography (relative configuration of diastereomers not assigned).

The structure of **40** was deduced from the spectroscopic data. The $^1H$ NMR spectra of both diastereomers displayed two characteristic signals for the exocyclic methylene protons, 5.6 (d, $J = 1.4$ Hz) ppm and 5.4 (d, $J = 1.4$ Hz) ppm. The presence of a nitrile was indicated by the characteristic $^{13}C$ chemical shift (an additional signal between $\delta$ 111−120 ppm), as well as an infrared absorption at 2243 $cm^{-1}$. The $^{13}C$ signals usually observed for the pyrrole ring in structures similar to **38** (ca. $\delta$ 142, 138, 124, 118 ppm) were replaced by modified olefinic signals in the range of $\delta$ 148−111 ppm. Finally, the UV spectrum revealed a dramatic change in the chromophore, $\lambda_{max}$ = 353 nm, compared to pyrrole analogues of **38** (typically $\lambda_{max}$ = 300 nm).

(40) Vedejs, E.; Klapars, A.; Warner, D. L.; Weiss, A. H. *J. Org. Chem.* **2001**, *66*, 7542.
(41) Solvent-dependent aziridine invertomer ratios of 1.3−1.7:1 were observed for the aziridinomitosene **7** in ref 15a, while **8** was reported as a single isomer.

***Scheme 6***




The formation of **40** indicates that the azomethine ylide was generated normally and that intramolecular [3 + 2]-cycloaddition had probably occurred to give the expected intermediate **37**. At this stage, enolization of **37** probably generates **39**, an intermediate having two potential leaving groups (C-9a cyanide; C-10 carbamate). Carbamate is the better leaving group, and its departure apparently is favored relative to aromatization via loss of the cyanide. Thus, **40** is formed instead of the desired product **38**.

Nitrile **40** was quite stable after isolation, but further transformations took place if the crude reaction mixture was stirred an additional hour in the presence of excess $BnMe_3N^+CN^-$. In this case, isomeric, aromatized nitrile **42** was isolated in 55% yield as a ca. 1:1.5 mixture of two diastereomers. The most likely sequence of events leading to **42** involves conjugate addition of cyanide anion to the exocyclic double bond in **40** followed by elimination of the C-9a cyanide. The structure of **42** was readily deduced because the spectroscopic data correlate well with other pyrrole-containing cycloaddition products while also containing the signals corresponding to the nitrile ($^{13}C$ NMR $\delta$ 118 ppm; IR 2250 $cm^{-1}$).

Although cycloadduct **38** could not be prepared in the above experiments, the isolation of **40** and **42** suggested that the unactivated dipolarophile had intercepted the azomethine ylide with reasonable efficiency. The approach was therefore modified by using a tethered propargyl ether as the dipolarophile in the hope that the undesired elimination could be avoided (Scheme 6). Thus, lithiated *tert*-butyldimethylsilyl propargyl ether was added to the aldehyde **28a** at −40 °C, and the resulting alcohol was protected as the acetate **43a** (87%) or the TBS ether **44a** (93%). Either derivative proved suitable for intramolecular oxazolium salt formation promoted by silver triflate. When the resulting oxazolium salts were treated with $BnMe_3N^+CN^-$ at room temperature as before, the desired cycloadducts **45a** or **47a** were obtained in 59% or 70% yield, respectively. The same procedures were also effective starting from **28b** to **43b** (78% overall) and on to **45b** (66%). These exciting results confirmed the feasibility of the internal cycloaddition strategy employing an unactivated dipolarophile, although the yields were significantly lower than with the activated ynoate dipolarophile. Qualitative rate differences in the cycloaddition step were also noted, as indicated by characteristic color changes. Thus, the

transient yellow color attributed to the azomethine ylide precursor of **47a** faded over several seconds at room temperature, while the color due to the ylide precursor of ester **31** (Scheme 4) disappeared in <1 s as each drop of the oxazolium salt was added to a stirred solution of $BnMe_3N^+CN^-$.

Selective saponification of the acetate ester **45b** was easily achieved using 1% NaOH without interference by the sensitive aziridine. This provided an opportunity to explore the oxidative conversion of **46b** to the quinone substitution pattern that is characteristic of the aziridinomitosenes. One added reason for pursuing this route was that **45b** and its precursors had been prepared as mixtures of diastereomers, while the quinone **49** should be a single isomer that would be easier to characterize. When alcohol **46b** was oxidized under Dess−Martin conditions, a complex mixture was obtained containing quinone **49** as well as the diketone **48** and unknown side products. Bis-enolization of **48** may have occurred to generate a hydroquinone intermediate having properties similar to those expected for a labile leucoaziridinomitosene such as **5a** (Scheme 1). Better results were obtained using the mildly basic tetrapropylammonium perruthenate in the presence of *N*-methylmorpholine *N*-oxide (TPAP/NMO), a reagent combination that cleanly provided diketone **48** in 89% yield.[42]

The final conversion of **48** to the quinone **49** was performed on the basis of a double enolization−oxidation approach. The strong base version of this transformation is unprecedented for aziridinomitosenes, but potassium (bistrimethylsilyl)amide (KHMDS) is known to convert simpler cyclohexenediones to the corresponding hydroquinone anions at low temperatures.[43] Thus, treatment of a THF solution of diketone **48** at −78 °C with KHMDS instantaneously produced a color change to deep, dark green. Passing oxygen into the solution rapidly discharged the green color and produced the yellow-orange quinone **49** in 64% yield. Alternatively, addition of *N*-chlorosuccinimide to the dienolate at −78 °C also gave **49** (68%). As expected, **49** was formed as a single isomer.

Selective deprotection of the primary TBS ether in **47a** was attempted, but the addition of 1 equiv of TBAF to the bis-silyl ether **47a** gave a 1:1 mixture of the diol **50** and the starting material **47a**. No intermediates could be detected by TLC, suggesting that deprotection of the first silyl group facilitates the deprotection of the second. The use of excess TBAF provided the crude diol **50** in ca. 80% yield after quick filtration chromatography over buffered silica gel (triethylamine), but **50** was too sensitive for more extensive purification and was used directly in the next step. Selective carbamoylation of the primary alcohol with trichloroacetyl isocyanate[44] gave the unstable imide **52**. Attempted purification resulted in decomposition, so the crude material was stirred with aqueous $K_2CO_3$ to remove the trichloroacetyl group, followed by oxidation of crude **53** with pyridine-buffered Dess−Martin periodinane to afford the sensitive, but isolable diketone **54** in 26% yield over four steps.

Despite the imminent threats of aziridine solvolysis and aromatization by double enolization, the diketone **54** proved to be reasonably stable at neutral pH and survived chromatography even on unbuffered silica gel. Aziridine solvolysis is probably retarded in **54** compared to **50**, **52**, or **53** due to the presence of an additional electron-withdrawing carbonyl group. However, the final oxidation from diketone **54** to the quinone **55** proved very difficult to control. The best result was obtained using triethylamine as the base in $C_6D_6$ at 70 °C under an oxygen atmosphere; conditions that afforded **55** in 27% yield.

The oxidation to **55** was not optimized due to another complication that was encountered once sufficient material had been prepared. Our most effective reductive detritylation conditions with MsOH/triethylsilane proved to be too harsh for the fully intact aziridinomitosene skeleton of **55**, and several attempts resulted in complex product mixtures. Detritylation occurred, as evidenced by the formation of triphenylmethane, but disappearance of the characteristic quinone color suggested that reduction of the quinone had also taken place. This presumably leads to a leucoaziridinomitosene and to rapid destruction via facile aziridine ring opening in the electron-rich environment. Attempted deprotection of ketol **53** or diketone **54**, substrates that lack the quinone moiety, also failed to produce any of the desired deprotected aziridines. It became clear that the trityl protecting group would have to be removed earlier in the synthetic sequence. This was a disappointing outcome, but it was not unexpected in view of the difficulties already encountered in the case of tetracyclic keto ester **31**. Furthermore, the successful deprotection of an advanced intermediate **29** (Scheme 4) suggested alternative approaches that eventually were successful, as described in the next section.

The need to remove *N*-trityl at an early stage focused attention on the oxazole aziridines **26** (Scheme 7). The same triethylsilane−trifluoroacetic acid procedure (2 h at 0 °C; NE*t*i$Pr_2$ quench) was attempted that had worked well for deprotection of **29**. Unexpectedly, this gave an inseparable mixture of the desired product **56a** and the triethylsilyl ether **57a**, resulting from partial cleavage of the *tert*-butyldimethylsilyl (TBS) group under the acidic reaction conditions. Fortunately, the reaction of **26a** with trimethylamine−borane and trifluoroacetic acid cleanly produced the deprotected aziridine **56a** in 82% yield. Monitoring the reaction by TLC indicated that deprotection was complete within 5 min at 0 °C, while the triethylsilane conditions required 2 h for complete conversion.

The *N*-methylation of the deprotected aziridine **56a** was performed next. Competition between the aziridine and the oxazole nitrogen was observed in model exeriments using methyl iodide or methyl triflate, but the selectivity was dramatically improved if the aziridine was lithiated. Thus, **56a** was treated with *n*BuLi followed by methyl iodide to produce the desired *N*-methylaziridine **58a** in 91% yield. Following the precedents of Scheme 3, **58a** was then converted into the iodide **59a** (86%). Removal of the silyl protecting group in **59a** using TBAF encountered purification difficulties due to the highly polar nature of the resulting alcohol **60a**, but good results were obtained with HF−pyridine, and **60a** was produced in 94% yield. Oxidation of **60a** with the Dess−Martin periodinane followed by addition of lithiated *tert*-butyldimethylsilyl propargyl ether to the resulting aldehyde at −40 °C then gave the alcohol **61a** as a ca. 1:1 mixture of diastereomers. The same optimized procedures were applied to the conversion of **26b** into **61b**, and generally similar results were obtained (26% overall from **26b**).

The key [3 + 2]-cycloaddition reactions could now be explored. Attempts to carry out the AgOTf−$BnMe_3N^+CN^-$

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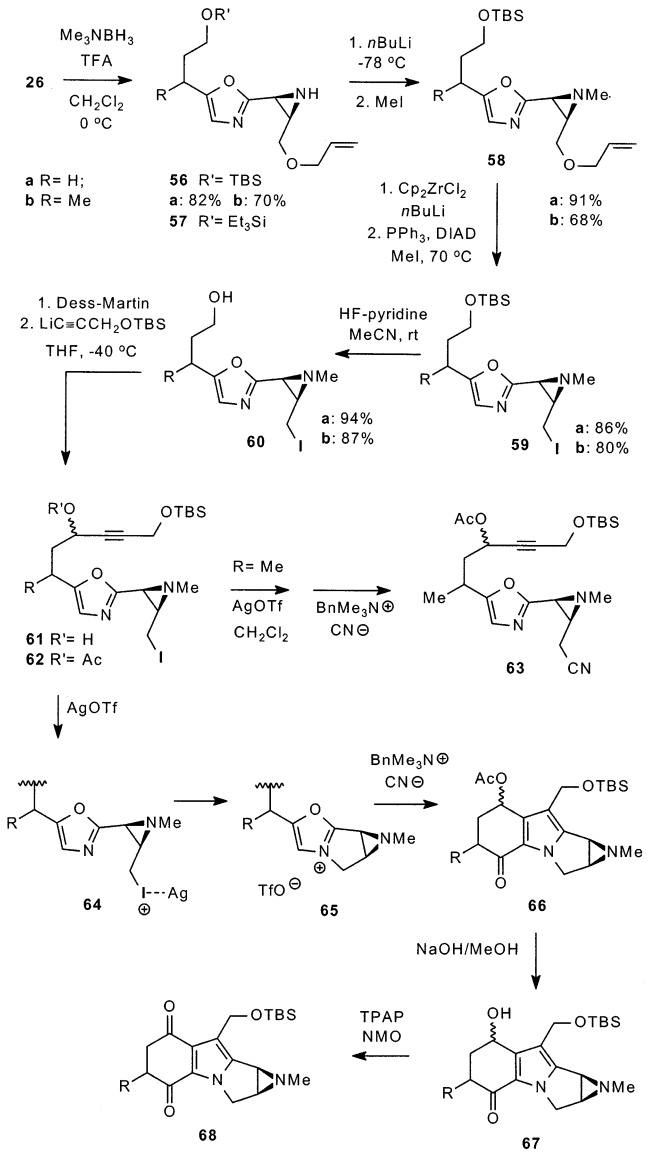
***Scheme 7***




sequence with the unprotected alcohol **61a** failed, so the acetate **62a** was prepared. The usual conditions for AgOTf-promoted intramolecular alkylation (MeCN; 70 °C) to form the crucial oxazolium salt **65a** were not very effective according to NMR assay, and significant decomposition was apparent. Nevertheless, addition of crude **65a** to a solution of $BnMe_3N^+CN^-$ in acetonitrile gave the desired cycloadduct **66a** in a respectable 40% yield. Interestingly, the $^1H$ NMR spectrum of **66a** and all of the subsequent tetracyclic intermediates displayed an additional minor set of signals. Both sets of NMR signals exhibited similar features except for a marked difference (0.5−1 ppm) in the chemical shifts of the *N*-methylaziridine signals, thereby suggesting invertomers at the aziridine nitrogen. The invertomer ratio varied from 1:10 to 1:30 depending on the substitution pattern of the aziridinomitosene ring system. Notably, no invertomers could be detected in the NMR spectrum of the *N*-methylaziridine **62a** or other intermediates preceding the cycloaddition step.

When the internal alkylation−cycloaddition sequence was applied to **62b**, the cycloadduct **66b** was obtained in 37% yield from the AgOTf-induced oxazolium salt formation in MeCN (70 °C) followed by ylide generation with $BnMe_3N^+CN^-$. Although this result was similar to that from **62a**, the erosion in yield prompted additional attempts to re-optimize the reaction. Promising room-temperature conditions for the internal *N*-alkylation from **62b** to **65b** were found using purified AgOTf·$C_6H_6$[45] as the activating agent, but the efficiency (30% yield of **66b**, 43% based on recovered **62b**) at partial conversion was not sufficiently improved to warrant the effort needed to prepare the pure silver complex or to recover and recycle **62b**.

Another experiment using AgOTf·$C_6H_6$ was conducted starting with **62b** in dichloromethane at room temperature. Although no precipitated AgI was observed, the starting halide **62b** disappeared according to TLC analysis, so the solution was treated with $BnMe_3N^+CN^-$ in acetonitrile in an attempt to induce internal cycloaddition. The resulting mixture proved to be complex and contained little if any (<5%) of the cycloadduct **66b**. After chromatography, one zone could be separated sufficiently to allow a tentative assignment of structure **63** (19% isolated). Although the material was obtained as a mixture of diastereomers, incorporation of cyanide at the primary carbon was easily deduced from replacement of the downfield (3.29−3.48 ppm) signals of **62b** by new signals at 2.65−2.90 ppm and from characteristic ESMS ($m/z$ = M + Na) and IR (2250 $cm^{-1}$) data. The nitrile product **63** was never observed using the standard acetonitrile conditions for oxazolium salt formation and ylide generation with $BnMe_3N^+CN^-$. Indeed, the unreacted starting material **62b** could be recovered from incomplete reactions in acetonitrile even though excess $BnMe_3N^+CN^-$ was always used. These observations indicate that substrate complexation by silver ion is different in dichloromethane compared to acetonitrile and that the resulting activation of iodine leads to a distinct pathway from **62b** to **63**.

In any event, both **66a** and **66b** were available in reasonable yield using the ylide cycloaddition strategy, and further steps would now have to contend with the solvolytically labile teracyclic ring system. Cleavage of the acetate protecting group in the cycloadduct **66a** with NaOH in methanol provided an alcohol **67a** that was unstable on unbuffered silica gel. Fortunately, purification of **67a** was possible if the silica gel was pretreated with triethylamine. As expected from the experience with **53** (Scheme 6), oxidation of the sensitive alcohol **67a** to the corresponding ketone proved to be exceptionally troublesome. Performing the oxidation with pyridine-buffered Dess−Martin reagent[46] provided the impure diketone **68a** in a less than 30% yield. Because the result was not reproducible, the reaction was investigated in more detail. Higher purity samples of the Dess−Martin periodinane[47,48] gave only a small improvement (to 36% of **68a**) after much optimization (2 equiv of the reagent, 7 equiv of pyridine, 2 equiv of water, $CH_2Cl_2$, room temperature).[49] Given the acid sensitivity of the alcohol **67a**, the nonacidic tetrapropylammonium perruthenate (TPAP) reagent was tried.[42] Indeed, catalytic TPAP and *N*-methylmorpholine *N*-oxide as the stoichiometric oxidant (molecular sieves added) provided the diketone **68a** in an improved 73% yield. Once again, chromatographic purification of **68a** was difficult due to decomposition on silica gel. In

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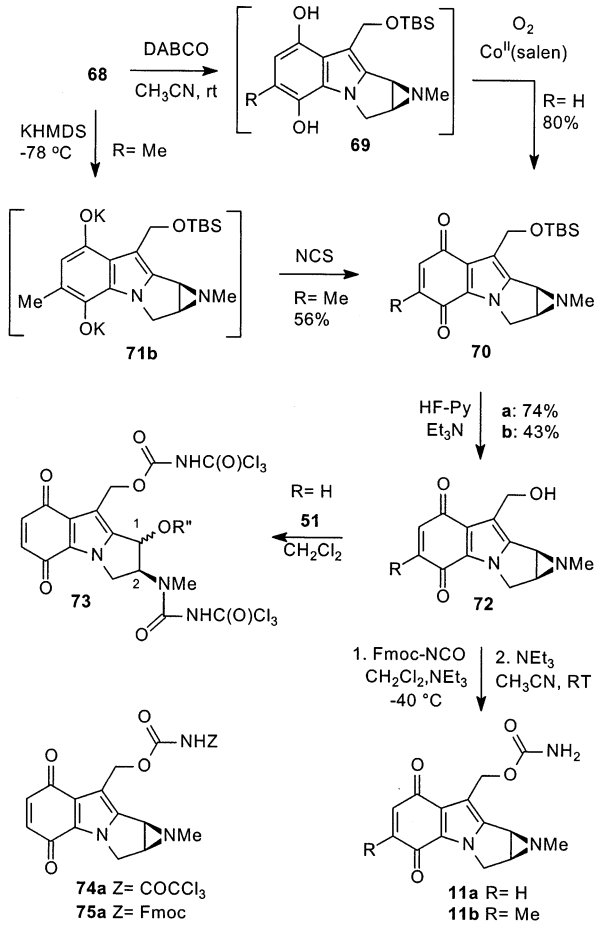
***Scheme 8***




contrast to the other tetracyclic intermediates mentioned earlier, the decomposition of **68a** was *accelerated* by buffering the silica gel with triethylamine, presumably via base-catalyzed enolization to the highly reactive hydroquinone intermediate **69a** (Scheme 8). We therefore explored ways to conduct the conversion of crude **68a** directly to quinone **70a** in the hope that the latter would be more stable.

Initially, the oxidation of **68a** was performed using diazabicyclo[2.2.2]octane (DABCO) in acetonitrile under oxygen (Scheme 8). The reaction required heating at 70 °C, and only ca. 40% yield of the desired product **70a** was obtained. In an attempt to lower the reaction temperature, a catalytic amount of cobalt(II) salen complex was added.[50] This improved the oxidation from **68a** significantly and provided the quinone **70a** in a satisfactory 80% yield after 2 h at room temperature. Although **70a** is a sensitive molecule that must be handled with considerable care, it proved to be significantly more stable than **68a** and could be purified as well as fully characterized.

The optimized procedures were then applied to the **b** series. Thus, **68b** was obtained in 68% yield after saponification, TPAP oxidation, and carefully controlled purification and workup. Initial attempts to conduct the cobalt-catalyzed oxidation to quinone **70b** proved difficult on small scale, so the alternative of oxidation via the dienolate was tested, based on the precedent of **48** to **49** (Scheme 6). Although the sequence of KHMDS deprotonation and NCS oxidation gave **70b** in a modest 56% yield, the reaction was relatively clean and repeatable.

Attempted deprotection of the silyl group in **70a** with TBAF gave a complex mixture. The alternative of using the HF−pyridine reagent was suspect because the reagent is acidic (commercial HF−pyridine contains ca. 90 mol % of HF and 10 mol % of pyridine) and would cleave the aziridine. However, when the HF−pyridine reagent was buffered with triethylamine,[51] deprotection of **70a** provided the desired alcohol **72a** in 74% yield (88% based on recovered **70a**).

Finally, introduction of the carbamate group in the alcohol **72a** was attempted using the trichloroacetyl isocyanate reagent **51**.[5c,6a,44] However, the desired carbamoylated product **74a** was not obtained. New doublets were observed in the NMR spectrum at $\delta$ 6.37, 6.28, 6.00, and 5.90 ppm, some of which may be due to the C-1 proton of tentative structure **73a**, derived from aziridine ring opening. Apparently, the strongly electrophilic isocyanate regent activates the *N*-methylaziridine moiety for solvolytic ring cleavage.

The exceptional sensitivity of the aziridinomitosene alcohol **72a** dictated the use of a milder carbamoylating agent. Replacing the trichloroacetyl group of **51** with an alkoxycarbonyl group was expected to moderate reagent electrophilicity. The previously unreported Fmoc-N=C=O (Fmoc = 9-fluorenylmethoxycarbonyl) was attractive because Fmoc is removed under mildly basic conditions[52] that might be compatible with the sensitive aziridinomitosene. Gratifyingly, the reaction of alcohol **72a** with Fmoc-N=C=O cleanly provided the desired product **75a** in an excellent 89% yield.[52c] Furthermore, treatment of **75a** with triethylamine at room temperature in acetonitrile provided the aziridinomitosene **11a** (81%). The same techiques were then used to convert **70b** to **11b** to establish generality. These studies were limited to small scale experiments, resulting in a relatively low overall yield (ca. 15%). However, the formation of **11b** was confirmed by comparison of spectroscopic data with those of **11a**.

After several synthetic stages involving sensitive synthetic intermediates, the target aziridinomitosene **11a** was now in hand. Its stability proved comparable to that of the precursor quinones. Furthermore, **11a** was highly crystalline and the pure material could be stored with relative ease. On the other hand, solutions of the substance in protic solvents were sensitive to decomposition as expected from the precedents with naturally derived aziridinomitosenes.[15] Unlike any of these precedents, **11a** has no substituents at the quinone carbons C-6 and C-7. Therefore, the stability profile of this unusual aziridinomitosene was investigated to learn about the limits for its survival.

The solvolytic stability of **11a** was evaluated in methanolic solutions buffered with the Tris and Bis-Tris amine hydrochlorides. Solvolysis was conveniently monitored by UV spectroscopy at "pH" 6.0 ("pH" refers to methanol conditions), and a well-defined isosbestic point at 424 nm was observed. A similar UV spectrum was obtained if the solvolysis of **11a** was performed at "pH" 7.0 although no isosbestic points were seen, suggesting interference from unidentified minor decomposition

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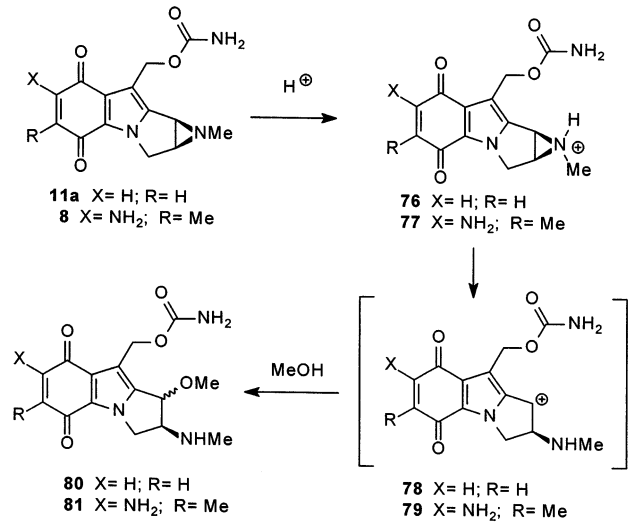
***Table 1.*** Observed First-Order Rate Constants and Half-Lives for Methanolysis of Aziridinomitosenes **11a**, **7**, and **8** at 20 °C

| | **11a** | | **8**[15a] | | **7**[15a] | |
|---|---|---|---|---|---|---|
| "pH" | $k_1$ ($s^{-1}$) | $t_{1/2}$ (min) | $k_1$ ($s^{-1}$) | $t_{1/2}$ (min) | $k_1$ ($s^{-1}$) | $t_{1/2}$ (min) |
| 6.0 | $3 \times 10^{-5}$ | 400 | | | | |
| 7.0 | $5 \times 10^{-6}$ | 2000 | $8 \times 10^{-4}$ | 14 | $4.1 \times 10^{-3}$ | 3 |
| 8.5 | decomp. | decomp. | $3.6 \times 10^{-5}$ | 324 | $5.1 \times 10^{-5}$ | 228 |

***Scheme 9***




pathways. The reaction rate was followed by measuring the time-dependent decrease in UV absorption at 480 nm, and first-order rate constants were obtained (Table 1). For comparison, Table 1 also includes the first-order rate constants reported by Kohn and Han[15a] for methanolysis of the aziridinomitosenes **7** and **8** under similar conditions. Interestingly, methanolysis of the synthetic aziridinomitosene **11a** at "pH" 7.0 was ca. 160 times slower compared to that of **8**. The reactivity difference may be a result of electron donation from the C(7) amino group of **8** into the quinone $\pi$-system. If this delocalization effect increases electron density in the carbocyclic subunit of **8** compared to **11a**, then the indole nitrogen in **79** would be better able to stabilize the carbocation intermediate compared to the situation in **78** (Scheme 9). The result would be to facilitate the $S_N1$ aziridine ring opening in **77** compared to **76**.

When the methanolysis of **11a** was performed on a preparative scale at "pH" 5.8, product **80** was isolated in 41% yield as a ca. 1:1.2 mixture of the cis/trans isomers. The low yield reflects the instability of the initially formed **80**. Although methanolysis proceeds cleanly at "pH" <7 according to TLC analysis, significant decomposition occurs as the sample is concentrated.

In contrast to the aziridinomitosenes **7** and **8**, synthetic **11a** is sensitive to decomposition at "pH" 8.5 in a methanolic buffer solution over ca. 10 h at room temperature. No individual component could be isolated from the complex mixture of polar products. A strong UV absorption band at $\lambda$ 291 nm emerged in the course of the reaction, reminiscent of the $\lambda$ 286 nm maximum reported for 7-methoxyaziridinomitosene **6**.[53] If this analogy holds, then base-catalyzed decomposition of the aziridinomitosene **11a** may be initiated by 1,4-addition of an oxygen

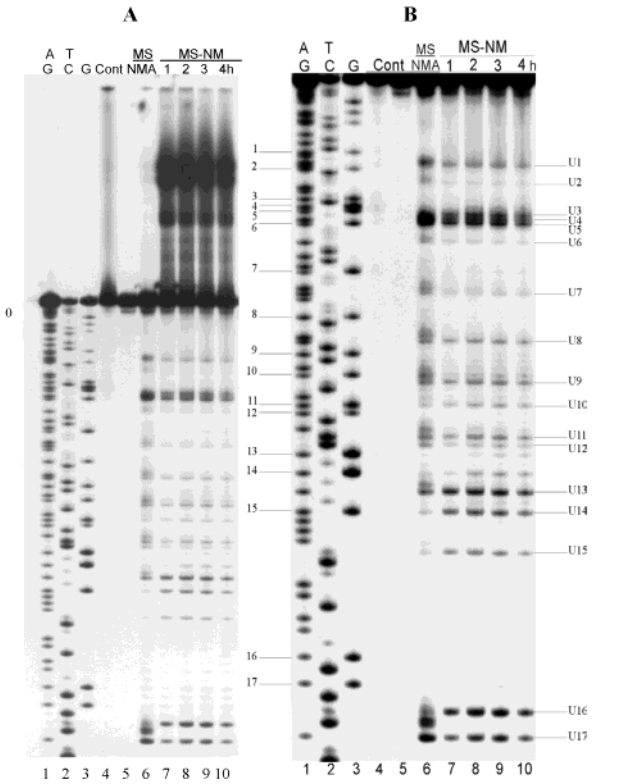



***Figure 1.*** (**A**) Autoradiogram of UvrABC nuclease cutting of *N*-methyl-7-methoxyaziridinomitosene (**9**; MS−NMA)-modified and synthetic *N*-methylaziridinomitosene (**11a**; MS−NM)-modified 3′ end $^{32}$P-labeled *Bst*NI-*Eco*RI 129-bp fragment from pBR322 plasmid. Lanes 1−3, Maxam−Gilbert chemical sequencing reactions of AG, CT, and G, respectively; lane 4, DNA treated with **11a** (MS−NM) alone (control); lane 5, unmodified DNA treated with UvrABC (control); lane 6, DNA modified with 1.5 mM **9** (MS−NMA) after incubation at 37 °C (2 h); lanes 7−10, DNA modified with 1.5 mM **11a** (MS−NM) after incubation at 37 °C (1 h), 37 °C (2 h), 37 °C (3 h), and 37 °C (4 h). The band corresponding to 3′-end labeled *Bst*NI-*Eco*RI 129-base fragment is labeled O. Bands above O have been attributed to higher molecular weight DNA products and those below corresponded to UvrABC nuclease incision adducts. (**B**) Same as Figure 1A except only the central portion of the 129-base fragment is shown. The drug modification induced UvrABC nuclease incision bands (U1−U17) are labeled on the right side of the panel, and the numbers (1−17) corresponding to the guanine modification sites are provided on the left side of the panel.

nucleophile at C-6 or C-7 of the quinone. This decomposition pathway is blocked by substituents in the naturally derived aziridinomitosenes, but not in the synthetic analogue.

The ability of **11a** (MS−NM in Figures 1A, 1B, and 2) to modify DNA was also explored. The key experiment was conducted under *nonreductive* conditions using the 3′ end $^{32}$P-labeled *Bst*NI-*Eco*RI 129-bp fragment from pBR322 plasmid.[54] In these initial studies, we employed **9** (*N*-methyl-7-methoxyaziridinomitosene; MS−NMA in Figures 1A, 1B, and 2) as a control substrate because **9** efficiently and selectively modifies DNA to give only mitosene−DNA monoadducts under these conditions.[15] The site of DNA alkylation was determined using the UvrABC nuclease assay.[54b,55]

The aziridinomitosenes were incubated (37 °C, 1−4 h) with the radiolabeled DNA, and then the DNA was separated from the reaction mixture and treated with UvrABC nuclease. Figure

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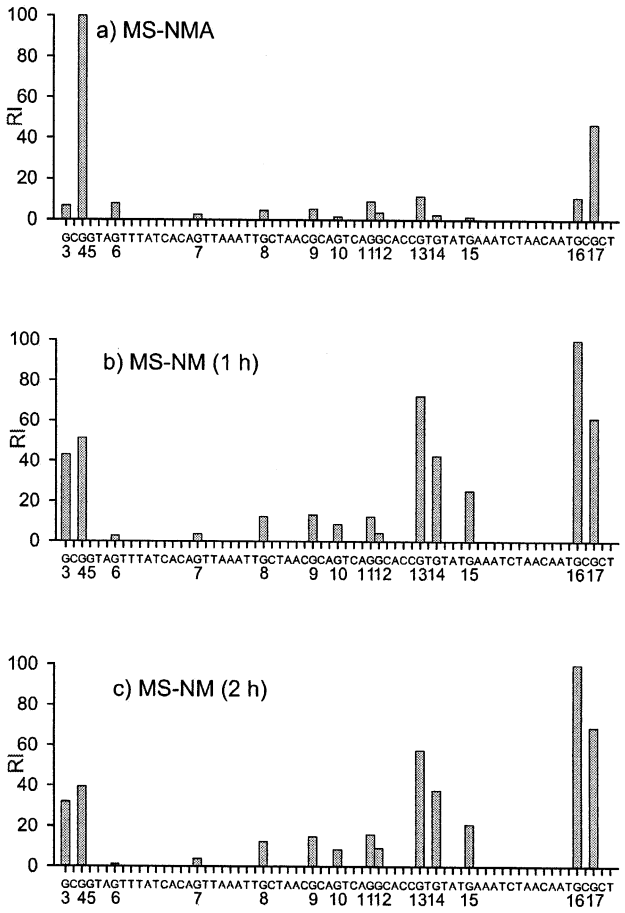
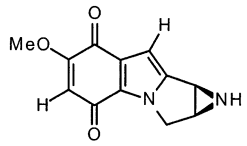



***Figure 2.*** Relative intensities (RI) of UvrABC nuclease incision sites of the 81-base region within 3′ end $^{32}$P-labeled *Bst*NI-*Eco*RI 129-bp sequence from pBR322 plasmid spanning G3-G17. Panels a (**9**; MS−NMA) and b, c (**11a**; MS−NM) correspond to Figure 1B lanes 6, 7, and 8, respectively. The intensity of UvrABC nuclease incision bands in Figure 1, lanes 6, 7, and 8 were scanned in a Bio-Image Analyzer. The intensities were normalized to 100% for the most intense band (100) within each experiment.

1A shows the autoradiogram for the full-length gel and Figure 1B provides a blow-up of the central region of the 129-bp fragment. The drug−DNA bonding-induced UvrABC nuclease incision bands are labeled U1−U17, which corresponds to modification at guanines 1−17, respectively.

Mitosene−DNA adduction was observed for both compounds at 37 °C within 2 h. For the naturally derived **9**, we detected no appreciable amounts of DNA products that correspond to adduct molecular weights higher than the starting 129-base DNA radiolabeled fragment (Figure 1A, lane 6). UvrABC nuclease treatment of the **9**-DNA modified sample provided a DNA bonding profile in agreement with earlier findings (Figure 2, panel a). Only the guanine (G*) bases were modified and DNA adduction had occurred preferentially at 5′CG* sites. By comparison, the UvrABC-treated samples of synthesis-derived **11a** showed extensive amounts of high molecular weight DNA products along with bands associated with UvrABC-incised adducts (Figure 1A, lanes 7−10). Increased incubation times (1−4 h) for **11a** gave a greater percentage of the radiolabeled DNA adhered to the siliconized Eppendorf tubes (1 h, ∼ 13%; 2 h, ∼ 20%; 3 h, ∼48%; 4 h, ∼ 55%). Reduction of the reaction temperature from 37 to 22 °C led to lower amounts of the higher molecular weight products. Furthermore, we observed that one of the higher molecular weight bands increased with UvrABC




**82**

***Figure 3.*** Synthetic aziridinomitosene **82**.

nuclease treatment (Supporting Information, Figure 1, lanes 8−16). We have tentatively attributed the slower moving bands to mitosene−DNA cross-linked and/or mitosene−DNA−protein adducts. The histogram for the UvrABC nuclease incision products for **11a** (MS−NM; Figure 1B, lanes 7, 8) is provided in Figure 2 (panels b and c). We observed that DNA adduction occurred only at guanine (G*) and that the reaction proceeded at both 5′-CG* and 5′-TG* sites to a greater extent than 5′-GG* and 5′-AG* loci. Moreover, upon comparing the DNA profiles of **9**, **11a**, and **82** (Figure 3), a mitosene lacking the C(10) substituent,[56] we found that both **9** and **11a** showed increased DNA selectivity compared with **82**. This finding supports earlier results demonstrating that the mitosene C(10) oxygen substituent facilitates preferential mitomycin−DNA bonding.[57]

Our preliminary studies document that **11a** readily modifies DNA under nonreductive conditions. Similar to mitomycin C (**3**), and other mitomycins and mitosenes, **11a**−DNA adduction occurred at guanine (G*) sites. Unlike previous mitomycins and mitosenes, higher molecular weight adducts were observed under nonreductive conditions. These products appeared both with and without UvrABC nuclease treatment, and one of these bands increased upon incubation with the nuclease. Higher molecular weight products have been associated with drug−DNA cross-linked adducts and drug−DNA−protein conjugates.[58] This finding is consistent with the structure of **11a** because drug activation can proceed by a solvolytic pathway, and adduction may proceed at multiple sites (C(1), C(6), C(7), C(10)).

## Summary

An enantiocontrolled route to aziridinomitosenes has been developed. The longest linear sequence is 20 steps (2.9% overall yield) starting from L-serine methyl ester hydrochloride. Several unusual techniques were developed in the course of this project; most importantly, the internal azomethine ylide cycloaddition reaction based on silver ion-assisted intramolecular oxazole alkylation. Other important developments include the *N*-methylation of N-H aziridines in the aziridinomitosene environment,[59] deprotection of *N*-trityl aziridines under reductive conditions, oxidation of sensitive cyclohexenediones to quino-

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nes, and a mild procedure for carbamoylation using Fmoc-NCO. Diverse experimental conditions, including protecting group manipulations and redox chemistry, have been developed that tolerate the presence of the sensitive aziridinomitosene tetracycle. Finally, a preliminary exploration of DNA alkylation by the first C-6,C-7-unsubstituted aziridinomitosene **11a** has been carried out. This study suggests a new pathway for DNA cross-link formation involving nucleophilic addition to the quinone subunit.

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**Supporting Information Available:** Experimental details and spectra of compounds studied (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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