

## A Synthetic Approach toward the Proposed Tetracyclic Aziridinomitosene Derived from FK317

Musong Kim and Edwin Vedejs\*

Department of Chemistry, University of Michigan, 930 North University Avenue, Ann Arbor, Michigan 48109

edved@umich.edu

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A synthesis of the FK317 derivative 25 is described using internal Michael addition. Tin-lithium exchange of the deuterated stannylaziridine 18 generated the key lithioaziridine intermediate, followed by cyclization and aromatization of the pyrrole ring to give 7. Ester reduction from 7 to 23 was effected via temporary aldehyde protection as the silylimidazole adduct 22, and conversion to the carbamate 25 was carried out using FmocNCO and FMOC cleavage. Structure 25 is the *N*-trityl-protected derivative of the proposed intermediate from bioactivation of FK317 that is responsible for DNA cross-linking. Attempted nitrogen deprotection of 25 using MsOH/i-Pr<sub>3</sub>SiH resulted in replacement of the C(10) carbamate by hydride. Deprotection of the more stable 21 gave the desired aziridine 26.

Mitomycin C (1; Figure 1) has long been used against a variety of solid tumors despite significant side effects.<sup>1</sup> The related aziridine-containing antitumor agents 2 and 3 were isolated more recently from Streptomyces sandaensis, and the semisynthetic derivative FK973 (4) was found to have potent antitumor activity.<sup>2,3</sup> However, attempts to develop FK973 were terminated due to toxicity from vascular leak syndrome (VLS).<sup>4</sup> In 1998, FK317  $(5)^5$  was shown to have improved antitumor activity compared to FK973 or mitomycin C, but without the VLS side effects. The mode of action of FK317 is believed to involve a sequence of metabolic activation steps, including deacylation, reductive N-O bond cleavage, and cyclization to give the mitosene-like intermediate 6 as the activated form responsible for DNA-DNA cross-linking.6

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Although FK317 has not yet lived up to its early promise,<sup>7</sup> it has attracted considerable interest due to the structural similarity between the proposed intermediate 6 and the aziridinomitosene intermediates responsible for the antitumor activity of mitomycins. We have therefore initiated a synthetic effort to prepare 6 and to learn whether structures containing the sensitive substitution pattern can be isolated. Here we report our first attempts to synthesize the fully functionalized 6 using an internal Michael addition approach based on lithiated aziridines.8

## **Results and Discussion**

Our strategy involves the convergent synthesis of 8 via the intermediates 9 and the known 10<sup>8</sup> (Scheme 1). In

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FIGURE 1.

**SCHEME 1** 



earlier studies, we had demonstrated the key bond formation between C(1) and C(9a) (mitomycin numbering) using a model substrate **11**. Treatment of **11** with methyllithium generated an aziridinyllithium intermediate **12**, followed by cyclization via internal Michael addition to give the tetracyclic enolate **13**. Enolate trapping with PhSeCl then gave the desired tetracyclic indole **14** in good yield. The presence of C(9a) deuterium at the stage of tin-lithium exchange was essential. Without the kinetic isotope effect of a deuterium blocking group, competing lithiation at C(9a) was the major pathway and effectively prevented the internal Michael addition step.

The more highly functionalized indole **15**<sup>9</sup> (Scheme 2) required to access aziridinomitosene-like structures related to **6** was prepared in four steps from pyrrole-2-carboxaldehyde using a recently optimized procedure as described elsewhere.<sup>10,11</sup> The ester side chain of **15** was reduced with LiAlH<sub>4</sub> and subsequent silylation afforded **16** in 91% yield. Carboxylation at the C(9) position of indole **16** was carried out by bromination, lithium halogen exchange, and quenching of the resulting anion with ethyl chloroformate.<sup>12</sup> Selective N-desilylation with

**SCHEME 2** 



potassium carbonate in ethanol/THF/water (3:1:1) then afforded the desired indole 9 (78% from 17).<sup>13</sup>

Indole **9** was deprotonated with NaHMDS and coupled with the aziridine mesylate **10**. To force the reaction of the mesylate **10** to completion, it was necessary to use indole **9** in excess. When 3 equiv of **9** was used, **8** was obtained in 95% yield based on **10**, and 64% of **9** was recovered (97% material balance) and could be reused in subsequent experiments.

In preparation for the key internal Michael cyclization, the C(9a) position of indole **8** was deprotonated using freshly prepared mesityllithium (MesLi),<sup>14</sup> and the resulting lithiated indole was quenched with EtOD to give **18** in excellent yield. In our model study where there were no substituents at C(6) or C(8), PhLi had been used as the base to prepare **11** with no complications.<sup>8</sup> However, in the case of **8**, significant addition of PhLi to the ethyl ester was observed under the same conditions. Although TMEDA<sup>15</sup> suppressed the undesired addition of PhLi to the ester, MesLi gave the best result (98% yield with >95% D<sub>1</sub> by <sup>1</sup>H NMR). The deuterated indole **18** 

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## SCHEME 3



was then reacted with MeLi followed by PhSeCl to give tetracycle **7** in an overall yield of 79% via the same sequence of tin–lithium exchange, internal Michael addition, and aromatization steps as in the model study.<sup>8</sup>

The most direct strategy at this point would be to attach the C(10) carbamate and then to deprotect and modify the C(6) hydroxymethyl ether group of tetracycle 7. Ester 7 was reduced with LiAlH<sub>4</sub> to give hydroxy indole 19 with relative ease (Scheme 3). However, all attempts to install the carbamate moiety from 19 using precedented methods<sup>16</sup> failed due to the sensitivity of the desired product 20. This was no surprise because 20 contains potential leaving groups at C(1) (aziridine C-N) and C(10) (carbamate C-O) that are activated by donation from the electron-rich indole nitrogen. To suppress leaving group reactivity, an alternative sequence was followed from 7 via initial deprotection of the C(6) silyl ether with TBAF followed by TPAP oxidation to the aldehyde 21 (65% from 7). The electron-withdrawing CHO group in **21** was expected to improve stability by a delocalization effect due to the conjugated, vinylogous formamide subunit, resulting in lower electron density in the indole and a higher barrier for leaving group departure. On the other hand, the presence of a C(6)aldehyde would not be compatible with reduction of the C(10)-ester. Fortunately, temporary protection of the CHO group was possible using TMS-imidazole<sup>17</sup> in refluxing  $CH_2Cl_2$  to give the N,O acetal **22**. Without purification of 22, the ester group was reduced with LiAlH<sub>4</sub>, followed by workup using solid Na<sub>2</sub>SO<sub>4</sub>·10H<sub>2</sub>O.

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This resulted in the neutralization of alkoxides as well as hydrolysis of the N,O acetal to give **23** (82% from **21**). The indole **23** was then treated with freshly prepared Fmoc-NCO<sup>16c</sup> to give the Fmoc-carbamate **24**, and cleavage of Fmoc with Et<sub>3</sub>N in a one-pot procedure afforded the desired **25** (55% from **23**).<sup>16e</sup>

The challenging removal of the N-trityl group could now be explored in the highly sensitive environment. Preliminary attempts to deprotect the most sensitive substrate 25 were not promising, so deprotection of the ester aldehyde 21 was studied in the expectation that the electron-withdrawing substituents would help to stabilize the product **26** (Scheme 4). Electrospray mass spectroscopy (ES/MS) was used to monitor events in small scale experiments based on the observation that all of the tetracyclic structures prepared up to this point had given distinct peaks for the  $M + Na^+$  ions, characteristic of the expected structures. We anticipated the same behavior for **26** because removal of the trityl group was not expected to adversely affect the stability of the aziridine. However, the recently optimized deprotection conditions (MsOH/Et<sub>3</sub>SiH at 0 °C)<sup>18</sup> gave no mass peak for the desired aziridine 26, and identifiable components could not be separated from the complex product mixture.

Attempts to control the deprotection of **21** with the Et<sub>3</sub>-SiH/MsOH reagent at lower temperatures were not successful, but a similar experiment using the bulkier triisopropylsilane at -42 °C (10 min) gave the desired aziridine **26** (41%) together with recovered **21** (16%). The structure of **26** was supported by ES/MS and NMR data and by comparisons with the simpler structure **27** prepared earlier in our laboratory.<sup>8</sup>

The reoptimized detritylation procedure (-42 °C) was then applied to **25** with monitoring by ES/MS. However, no clear evidence to support the formation of the deprotected aziridine **6** was obtained, and an alternative reductive pathway was encountered. The major product (65%) still contained an *N*-trityl group, as well as the characteristic <sup>1</sup>H NMR signals for the bridgehead protons of an intact aziridine and the adjacent CH<sub>2</sub> protons in the five-membered ring. Structure **29** was assigned, based on the presence of a new methyl singlet at  $\delta$  2.52 ppm, together with the disappearance of the characteristic AB pattern for the C(10) protons. In contrast to the tetracyclic structures **21**, **23**, or **25**, no M + Na<sup>+</sup> ion was observed in the ES/MS output for **29**. Instead, a strong

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peak corresponding to  $M + MeOH + Na^+$  was found, suggesting that **29** undergoes solvolytic aziridine ring opening under electrospray sampling conditions due to increased electron density in the pyrrole ring of **29** compared to **26**.

Analysis of the minor chromatography fractions by NMR was uninformative, but ES/MS assay revealed the characteristic mass of an iminium ion **28**. The same ion was observed in the electrospray mass spectrum of the carbamate **25** as well as the precursor alcohol **23**, so this ion can be taken as evidence for the presence of a C(10)heteroatom that is capable of heterolysis, as well as for the survival of the *N*-trityl group. Weak ions were also detected in several fractions corresponding to the adduct of **28** + Na + OMe or **28** + MeOH, but these peaks were always small compared to that of **28**.

Detritylated products were detected in trace amounts based on a weak ion corresponding to **30**, but no products were found having the characteristic mass of the deprotected carbamate **6**. When detritylation was attempted on the alcohol **23** in small scale test experiments, the formation of the same over-reduction product **29** was indicated by ES/MS. Evidently, the C(10) hydroxyl group of **23** is also easily activated for heterolysis and reduction, presumably via the acid-induced formation of the iminium ion **28**. We conclude that the reductive detritylation of **25** via *N*-protonation and cleavage to give the trityl cation cannot compete with the undesired conversion into the iminium ion **28**.

From the above evidence, it is likely that the late stage deprotection of **25** will not be feasible. On the other hand, detritylation is possible earlier, prior to reduction of the ester group, as demonstrated in the conversion from **21** to **26**. Although the earlier deprotection would require adjustments in the carbamoylation chemistry and the timing of redox events, the stability of **25** under carefully

controlled conditions suggests that **6** will also prove to be an isolable substance. Prior synthetic efforts have accessed tetracyclic aziridinomitosene derivatives stabilized by the presence of the quinone ring,<sup>16b,c,19</sup> but reports describing the isolation of solvolytically sensitive analogues of **6** or **25** have been rare.<sup>8c,20</sup> Further studies toward this goal are underway.

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**Supporting Information Available:** Experimental details, characterization, and NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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