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Brady Marburger
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A Novel Synthetic Pathway for Aspernigrin A

Brady Marburger* and Anne Reeve, Ph.D.†

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Messiah College, One College Ave, Mechanicsburg, PA 17055

*Department of Biological Sciences
† Department of Chemistry and Biochemistry
ABSTRACT

Aspernigrin A (6-benzyl-4-oxo-1,4-dihydropyridine-3-carboxamide) is a natural product isolated from the fungus Aspergillus niger found in the Mediterranean sea sponge, Axinella damicornis. This secondary metabolite has proven to be cytotoxic to certain colon cancer cell lines. The synthesis of aspernigrin A is required to obtain enough material to study its bioactivity and potentially optimize it for clinical drug use. The new synthetic pathway being tested utilizes ethyl acetoacetate to create an enaminone capable of being directly converted to the pyridinone required for aspernigrin A. Through reaction in a DMF-Me$_2$SO$_4$ adduct, ethyl acetoacetate was successfully reacted to give the enaminone product, ethyl-2-[(dimethylamino)methylene]-3-oxobutanoate, at an average yield of 79%. Subsequent reaction of the enaminone with n-BuLi, LiHMDS, and phenylacetyl chloride showed promise for the production of the pyridinone ethyl ester, 3-carbethoxy-6-phenyl-4(1H)-pyridinone. Continued optimization of this reaction scheme will allow greater production of the enaminone and subsequent reaction to form the pyridinone ethyl ester that could eventually yield aspernigrin A.
INTRODUCTION

Natural products, historically and in recent years, have played an invaluable role in both primitive and novel drug development. Natural products, or secondary metabolites, are organic compounds primarily produced in plants or microbes (fungi, other endophytic organisms, etc.). More specifically, secondary metabolites are compounds produced by an organism’s secondary metabolism. As such, these compounds are not strictly necessary for the organism’s survival, but do impart some advantageous characteristics. Given the nonessential nature of these secondary metabolites, organisms often stop producing them when taken out of the wild, making them difficult to harvest in a lab setting. Nonetheless, these compounds have been utilized by humans for medicinal purposes for thousands of years and are the basis for some of the most widely used drugs today, including aspirin, morphine, and penicillin. In addition, the great diversity of these secondary metabolites provides a wealth of potential research in the future. The structures and activities of thousands of secondary metabolites have already been characterized, but new compounds are continually being discovered. In fact, less than 10% of nature’s biodiversity has been explored for natural products that are biologically active, so the possibilities seem endless.

Since much of nature has yet to be explored, this area of development remains very promising. In recent years, organisms from marine sources have proven to be a very valuable resource in the screening of natural products. Considering that two-thirds of the earth’s surface is covered in water, marine life provides an immense pool of potentially biologically-active natural products. Research into such natural products has provided the basis for a lot drug development. Such drug development usually follows these general steps:
1. Screening of natural products for biological activity
2. Isolation and purification of the active molecule
3. Determination of structure
4. Synthesis of analogues
5. Determination of receptor-binding mechanisms
6. Design and synthesis of the novel drug structures

The exploration of marine natural products has followed this pattern of novel drug development in recent years. In fact, the secondary metabolite of interest for this study, aspernigrin A, is isolated from the marine fungus, *Aspergillus niger*, and is currently at the analog synthesis step of this process.\(^5\)

*A. niger* has previously been investigated as a terrestrial fungus, but the study of its marine-derived endophytic strain in the Mediterranean sea sponge, *Axinella damicornis*, revealed seven previously unknown secondary metabolites. Isolation and analysis by Hiort via NMR spectroscopy and mass spectrometry identified the molecular formula and structures of these new metabolites\(^5\). All isolated compounds were then exposed to a series of bioassays, including a test for insecticidal qualities and a cytotoxicity assay involving human cancer cells. Only one of the compounds exhibited insecticidal qualities, while two of the metabolites, aspernigrins A and B, proved to be cytotoxic toward various carcinoma cell lines at 50 \(\mu\)g/mL. Aspernigrin B was also found to exhibit a neuroprotective effect, decreasing the intracellular \([Ca^{2+}]\) level in neuronal cells previously heightened by L-glutamic acid.\(^5\)
However, as aforementioned, aspernigrin A and not aspernigrin B, is the subject of this research study. This is largely because it has less structural complexity than aspernigrin B, presumably making it, and any analogues, easier to synthesize in order to study their cytotoxicity towards various cancer cell lines.

The molecular formula of aspernigrin A, as isolated by Hiort in 2004, was determined by HREIMS (high-resolution electron impact mass spectroscopy) to be C$_{13}$H$_{12}$N$_{2}$O$_{2}$. Through the use of $^1$H and $^{13}$C-NMR spectroscopy and confirmed through HMBC (heteronuclear multiple bond correlation) its structure (shown in Figure 1) was found to be 4-benzyl-6-oxo-1,6-dihydropyridine). Aspernigrin A was isolated again by Ye in 2005, but this time from the endophytic fungus *Cladosporium herbarum* found in *Cynodon dactylon* leaves. Although the $^1$H and $^{13}$C-NMR spectra and the HMBC were nearly identical between these separate isolations of aspernigrin A, Ye proposed a slightly different structure for the pyridone (shown in Figure 2). This alternative structure switched the position of the pyridone from C-4 to C-2, giving aspernigrin A the official name 6-benzyl-4-oxo-1,4-dihydropyridine-3-carboxamide. X-ray crystallography confirmed this revised structure to be correct, and Hiort and his research group revised their originally proposed structure to reflect the new results. Activity assays performed after this isolation also found aspernigrin A to be cytotoxic to the colon tumor cell line SW1116 at a concentration of 15.7 µmole/ L.
With its isolation and purification completed, as well as its structure and biological activity determined, the next step is to acquire enough natural product to make additional study possible. The synthesis of the parent molecule is often required to obtain enough material to actually study the product and to optimize it for clinical drug use. This is perhaps the biggest challenge in working with natural products, especially those with fairly complex chemical structures like aspernigrin A. Finding a successful pathway for its synthesis would be useful for elucidating the structural features responsible for its cytotoxicity as well as for determining different analogs that could produce a similar or improved biological activity.

Multiple synthetic schemes have been developed to date in an attempt to synthesize aspernigrin A in vitro. Most synthetic approaches in the Reeve lab are predicated on the construction of a pyridone from a pyrone. Kilbourn and Seidel have previously found a high-yielding pathway for the conversion of pyrone 3, 4-hydroxy-6-methyl-2-pyrone, to a model pyridone. If this is true, and a pyrone may be easily converted to its respective pyridone, then the benzyl pyrone 2 could possibly be converted to the benzyl pyridone 1. In other words, it would be possible to hinge the synthesis of aspernigrin A upon the phenyl pyrone precursor 2. Any such synthesis would then follow the general retrosynthetic pathway shown in Figure 3; starting with a pyrone, converting it to a benzyl pyrone, and finally converting the benzyl pyrone to a benzyl pyridone (aspernigrin A).
Hoeckle successfully confirmed the possibility of forming a pyridone from a model pyrone as shown in Scheme 1.\textsuperscript{10} In the total synthesis, this scheme would be applied to benzyl pyrone 2 after the addition of the benzyl group at the 6-methyl position of 3.

Scheme 1

Upon enacting this scheme, Hoeckle was able to synthesize methyl pyridones 5 and 6 with fairly good yield and purity. However, some solubility issues were encountered, and as such the use of different R groups were investigated in the transformation of 4 to 5 in order to increase yield and purity. Some moderate success was found in using a benzyl as the R group for 5b. This gave the product greater organic solubility than was the case for 5a, resulting in a yield increase from 61\% to 80\%. The carboxylic acid in 5b could also be converted to several different amides in 6 with variable yield. However, while the benzyl group could be removed from the pyridone of 6a and 6b, both benzyl and t-butyl still remained at the amide position despite efforts to remove them, and the reaction forming 6c was not reproducible. This reflects
the need for another potential R group or deprotection method that could be implemented to produce pyridone 7. Nonetheless, Hoeckle’s work did show that pyridones could be synthesized from pyrone precursors.\textsuperscript{10}

Given that it is possible to form a pyridone from a relevant pyrone, the next step in the retrosynthetic pathway for aspernigrin A would be the addition of a benzyl group at position 6 at some point in the synthesis. Buhler’s work focused on attempting to add this benzyl group by first brominating the 6-methyl of the pyrone precursor.

**Scheme 2**

The first synthesis attempted to use photo-activation in order to generate an allylic bromine at the 6-methyl position of 3 to produce 8.\textsuperscript{11} If successful, the brominated pyrone could then undergo a Stille palladium catalyzed cross-coupling which would substitute in a phenyl group and produce 2.\textsuperscript{12} Unfortunately, when the photo-activated allylic bromination was performed, 3 was brominated at ring position 5 instead of at the 6-methyl position. As a result, 8 was never synthesized.

**Scheme 3**
Buhler, however, attempted another synthesis using the less reactive dehydroacetic acid 9 instead of 3. This method still employed a photo-activated allylic bromination, but Buhler also utilized a variety of other reagents in attempting this direct bromination. Through this synthesis, 56% bromination at the 6-methyl position was achieved by using carbon tetrachloride. However, 9 was inseparable from its brominated products, making further synthesis infeasible. As such, attempts to directly brominate 3 and 9 were abandoned.\(^\text{13}\)

Although direct bromination of a pyrone precursor was unsuccessful, Eldredge sought to implement another scheme that would act as a workaround of direct bromination. This scheme utilizes a series of oxidations and reductions to prepare an allylic alcohol at the 6-methyl position.\(^\text{14}\) This alcohol can then be converted to a bromine through an Appel allylic substitution, thus achieving the necessary precursor for 2.\(^\text{12}\)

**Scheme 4**

![Chemical Diagram](image)

This scheme is not limited to allylic brominations either. The allylic alcohol can also participate in a variety of other halogenations or the preparation of a triflate or tosylate, all of which would be satisfactory leaving groups for Stille coupling reactions. Such variety would provide more opportunity to overcome potential solubility and purity issues. Eldredge’s work on this pathway did show evidence of the allylic alcohol 13, but was largely inhibited due to solubility and purity issues when oxidizing 11 to 12.\(^\text{14}\)
Nonetheless, this pathway still showed much promise, and as such Sharber’s work focused on ways to optimize it. One of the main steps taken to optimize the pathway was using potassium carbonate (K$_2$CO$_3$) instead of potassium hydroxide (KOH) as a catalyst in the production of a methyl ether at the 4-hydroxyl position.$^{12}$

**Scheme 5 (optimization of Scheme 4 by Sharber)**

![Scheme 5](image)

Previously, methylation using KOH in dimethylformamide (DMF) resulted in fairly good product purity with yields approaching 60%. However, the use of K$_2$CO$_3$ in acetone proceeded with much higher purity and improved yields to 61% on average. The subsequent oxidation step to the aldehyde of 12 also had improved purity from the Eldredge trials. However, the yield of 43% could be improved upon. Reduction to the alcohol of 13 had similar yields, but purity suffered significantly when the aldehyde 12 was at any less than 60% purity. Despite these issues of yield and purity, the eventual production of the allylic bromide 14 was confirmed through GC-MS. Yield and purity of 14 were very low, however, and Sharber recommended tosylation rather than bromination of 13 as a way to improve upon this in future experiments.$^{12}$

Despite all of these efforts, the desired benzyl pyrone 2 has remained elusive, and aspernigrin A is yet to be synthesized. Given the past troubles encountered with approaches based on the retrosynthetic pathway, this experiment focused on a synthetic pathway for
aspernigrin A that does not hinge upon the benzyl pyrone 2. Previous work by McCombie, et al. indicates that commercially-available ethyl acetoacetate 16 can be used to synthesize the enaminone 17, ethyl-2-[(dimethylamino)methylene]-3-oxobutan-2-one, when reacted with a DMF-Me$_2$SO$_4$ adduct. The enaminone can then be directly converted to the pyridinone ethyl ester 18 through a two-step reaction in which it is first reacted with phenylacetyl chloride and LiHMDS at -70°C, and then worked up in acetic acid and ammonium acetate.\textsuperscript{15} From the pyridinone ethyl ester 18, methods used by Bakali, et al. will be utilized to convert the ester to the carboxylic acid through reaction with NaOH. Finally, the resulting pyridone carboxylic acid 19 will be reacted with ethyl chloroformate and triethylamine followed by ammonium carbonate to give aspernigrin A (Scheme 6).\textsuperscript{16}

\textbf{Scheme 6}
RESULTS AND DISCUSSION

Synthesis of Ethyl-2-[(dimethylamino)methylene]-3-oxobutanoate (17)

Following the methods developed by McCombie, et. al., synthesis of the enaminone 17, ethyl-2-[(dimethylamino)methylene]-3-oxobutanoate, was attempted through reaction of ethyl acetoacetate with \( \text{Et}_3\text{N} \) and a DMF-Me\(_2\)SO\(_4\) adduct in CH\(_2\)Cl\(_2\). Before beginning the reaction, ethyl acetoacetate 16 and DMF were distilled over anhydrous CaSO\(_4\) into oven-dried glassware to ensure that all reagents remained completely anhydrous. The reaction was run, and the resulting product was washed with water and 10% w:v tartaric acid and dried over anhydrous MgSO\(_4\). Excess solvent was removed from the extracted organic layer by rotary evaporation, and the resulting pale, yellow oil was analyzed via TLC and \(^1\)H-NMR. The \(^1\)H-NMR spectrum did not show peaks that were anticipated for the enaminone 17. Through \(^1\)H-NMR of the starting materials, it was found that N,N-dimethylformamide dimethyl acetal was used rather than DMF, resulting in the wrong product.

The reaction was repeated, this time using a fresh stock of DMF that did not need to be distilled. Upon completion, the product was washed as previously described. After rotary evaporation, the resulting pale, yellow oil (which became orange upon standing for a day) was then analyzed via TLC, which confirmed new product formation and indicated slight presence of starting material. \(^1\)H-NMR of the product matched that of the predicted spectrum in the literature, confirming the successful formation of the enaminone 17.\(^{15}\)

In order to ensure better purity of the enaminone 17 for subsequent reactions, the product was subjected to silica gel flash chromatography, using 2:3 v:v ethyl acetate:hexanes. Fractions of approximately 15 mL were taken, and TLC indicated the presence of starting material and/or product. All of the starting material eluted out in the 2\(^{nd}\) fraction, and the product first appeared
in the 16\textsuperscript{th} fraction. However, product was still being eluted by the 30\textsuperscript{th} fraction, so the polarity of the eluent was increased to 2:1 ethyl acetate:hexanes. Ten additional fractions were taken, but product was still present in all of them. In order to ensure removal of all product from the column, a whole 125 mL fraction using pure ethyl acetate was taken. All product-containing fractions were combined and rotary evaporation gave high purity product at an 86\% yield.

Upon running out of the initially-synthesized enaminone 17 after multiple attempts to synthesize the pyridinone 18, this reaction was repeated. After following the reaction and work up methods as before, analysis of the initial product via TLC showed the presence of product and two contaminating spots. Once again, flash column chromatography was utilized in order to further purify the product. Given the issues with slow product elution using 2:3 v:v ethyl acetate:hexanes in the last column, an eluent with slightly higher polarity was chosen for this column. As such, a total of 135 mL of 1:1 v:v ethyl acetate:hexanes was used as an eluent and collected in 15 mL fractions. Analysis of all fractions via TLC indicated the presence of starting material and/or product. Both of the contaminating spots were completely eluted in the 2\textsuperscript{nd} and 3\textsuperscript{rd} fractions. After eluting all 135 mL of 1:1 v:v ethyl acetate:hexanes, product still remained in the 9\textsuperscript{th} fraction. Pure ethyl acetate was then used as an eluent to remove the remainder of the product from the column. Again, 15 mL fractions were collected, but by the 15\textsuperscript{th} fraction product was still being eluted. To ensure removal of all product from the column, two whole 100 mL fractions were taken. All product-containing fractions were combined and rotary evaporation gave high purity product at a 72\% yield.

In the end, this reaction gave the enaminone 17 at an average yield of 79\%. These results show promise for the reaction scheme as whole. The competency of this reaction will allow
continued production of the enaminone 17 at high yields for use in the subsequent reaction to produce pyridinone 18.

**Synthesis of 3-Carbethoxy-6-phenyl-4(1H)-pyridinone (18)**

Again, following the methods developed by McCombie, et. al., synthesis of the 3-carboxy-6-phenyl-4(1H)-pyridinone 18 was attempted. First, a solution of LiHMDS and n-BuLi in hexanes was combined with a solution of the enaminone 17 and phenylacetyl chloride at -78°C using THF as a solvent. This initial step in the reaction gave the solution a pale yellow hue. Acetic acid and ammonium acetate were added at room temperature, and a white precipitate formed within the pale yellow solution. Excess THF was removed via rotary evaporation, and the remaining solution was heated for 1.5 hours at 60°C.16 While heating, the solution became a dark orange, but the precipitate remained white. Unfortunately, during rotary evaporation, contamination from the water bath was introduced to the reaction mixture. Further rotary evaporation was utilized in an attempt to remove the contaminating water before heating the reaction mixture. Upon completion of the reaction, TLC plating revealed the presence of multiple products and contaminants as well as remaining starting material. 1H-NMR of the product produced complex peaks that could not be used to verify the presence of the pyridinone product 18. Given these inconclusive results, the reaction had to be repeated.

The second attempt of the reaction was met with similar issues. At the rotary evaporation step after addition of acetic acid and ammonium acetate, contamination from the water bath was once again introduced to the reaction mix. This time a significant amount of product was lost to the water bath. As such, no further work up or purification steps were performed and it was determined that the reaction should be run a third time.
The third attempt of the reaction was more successful. Addition of the acetic acid and ammonium acetate produced a white precipitate as expected, and subsequent rotary evaporation and heating gave the solution a dark orange hue. The mixture was worked up in water and CH\(_2\)Cl\(_2\), and the organic phase was washed with water and aqueous NaHCO\(_3\). Excess CH\(_2\)Cl\(_2\) was then removed by rotary evaporation, resulting in a dark orange oil with no precipitate. A TLC plate of the product was eluted with 100:1 v:v CH\(_2\)Cl\(_2\):Et\(_2\)O to confirm product formation. The TLC plate revealed five potential product spots (Spot 1, \(R_f = 0.88\); Spot 2, \(R_f = 0.76\); Spot 3, \(R_f = 0.70\); Spot 4, \(R_f = 0.58\); Spot 5, \(R_f = 0.43\)) and unreacted enaminone (Spot 6, \(R_f = 0.16\)).

In order to separate all of the potential products, silica gel flash column chromatography was utilized. The column was eluted with 100:1 v:v CH\(_2\)Cl\(_2\):Et\(_2\)O. TLC plating of each fraction indicated full separation of Spots 1 & 5, partial separation of Spots 2 & 4, two fractions containing both Spots 2 & 3, and two fractions containing both Spots 3 & 4. Spot 6 did not elute with the 100:1 v:v CH\(_2\)Cl\(_2\):Et\(_2\)O, so the polarity of the eluent was increased by using 100% ethyl acetate. TLC plating of the fractions indicated full removal of Spot 6 from the column. All fractions containing fully or partially separated spots had excess solvent removed by rotary evaporation. \(^1\)H-NMR spectra from Spots 1, 2, 4, and 5 showed complex peaks that did not match with the predicted spectrum for the pyridinone 18. The \(^1\)H-NMR spectrum for Spot 6 confirmed it to be unreacted enaminone 17. Unfortunately, Spot 3 could not be further analyzed due to lack of separation from other products in the column. In the end, formation of the pyridinone 18 could not be verified.

Fortunately, our failure to produce the pyridinone 18 through this reaction can be explained. Upon reviewing reaction conditions, it was discovered that the THF used as a solvent was not truly anhydrous. The SureSeal™ cap of the bottle in which the THF was kept had
degraded, allowing atmospheric moisture to contaminate it. Thus, the reaction was not kept anhydrous and this likely caused degradation of the starting materials, resulting in a failed overall reaction. Given these results, the reaction should be repeated using distilled THF to ensure entirely anhydrous reaction conditions.
CONCLUSION

The first step of the novel synthetic pathway toward aspernigrin A (Scheme 6) was successful. Ethyl acetoacetate was successfully reacted with Et$_3$N and a DMF-Me$_2$SO$_4$ adduct to give the enaminone 17, ethyl-2-[(dimethylamino)methylene]-3-oxobutanoate, at an average yield of 79% in good purity. However, the subsequent reaction of the enaminone 17 with phenylacetyl chloride and LiHMDS did not conclusively yield the pyridinone 18, 3-carboxy-6-phenyl-4(1H)-pyridinone. Further analysis of the reaction conditions indicated that the THF used as a solvent for the reaction was not truly anhydrous, and this likely caused the failure of the reaction. Thus, it was determined that the reaction should be repeated using distilled THF.

However, before repeating the reaction, a test reaction should be performed that follows the exact procedures outlined by McCombie, et al. Our reaction to produce pyridinone 18 utilizes phenylacetyl chloride, while McCombie, et al. use benzoyl chloride. Thus, a reaction should first be performed using benzoyl chloride in order to prove that the literature reaction can be replicated. Should this reaction prove successful, our reaction with phenylacetyl chloride can then be repeated. Overall, this novel synthetic scheme still shows much promise, and continued success would be a major step toward the synthesis of aspernigrin A.
EXPERIMENTAL SECTION

All glassware used for anhydrous reactions was oven dried. In addition, all anhydrous reactions were carried out in an atmosphere of dry, oxygen-free nitrogen. Flash column chromatography was performed on Baker silica gel (SG60-200 mesh). TLC was conducted according to standard procedures using 2.5 x 7.5 cm Bakerflex silica gel IB-F plates. All solvents and reagents were used as received from commercial vendors (Sigma-Aldrich) except for ethylacetoacetate, which was distilled over anhydrous CaSO₄. ¹H-NMR spectra were recorded at 60 MHz on a Varian EM360L permanent magnet interfaced to an Anasazi Instruments, Inc. Eft-60 NMR spectrometer. ¹H-NMR chemical shifts recorded in ppm. s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Data represent pure, representative spectra for each compound.

Synthesis of Ethyl-2-[(dimethylamino)methylene]-3-oxobutanoate (17). Ethyl acetoacetate (1 mL) and the DMF-Me₂SO₄ adduct (Obtained by keeping 2.8 mL of DMF and 3.3 mL Me₂SO₄ at 40°C/4 hours, then room temperature/48 hours) were stirred in CH₂Cl₂ (12.2 mL) in an ice bath, and Et₃N (1.6 mL) was added over 5 minutes. The reaction mixture was stirred for 2 hours at room temperature and then washed with aqueous tartaric acid (10% w/v, 30 mL) and water (30 mL), and dried over anhydrous MgSO₄. The reaction resulted in a pale yellow oil that became orange upon standing. TLC plates were eluted with 1:1 v:v ethyl acetate:hexanes to confirm product formation. Product was purified via silica gel flash chromatography (20 g SG) and eluted most successfully with 1:1 v:v ethyl acetate:hexanes (135 mL) followed by 100% ethyl acetate (200 mL). TLC was done on each fraction to confirm presence of product and/or reagents. Fractions containing only the product were combined and rotary evaporation gave the
enaminone 18 (1.15 g, 79%). Product structure was confirmed via $^1$H-NMR: (Chloroform-d) $\delta = 7.7$ (s, 1H), 4.2 (q, 2H), 3.0 (s, 6H), 2.3 (s, 3H), 1.3 (t, 3H).

**Synthesis of 3-Carbethoxy-6-phenyl-4(1H)-pyridinone (18).** A solution of HMDS (0.85 mL) in THF (5 ml) was stirred at 0°C, and $n$-BuLi in hexanes (1.58 mL of a 2.5 M solution) was added dropwise over 5 minutes. The mixture was cooled to -78°C in a dry ice bath and a solution of enaminone (0.308 g) and phenylacetyl chloride (0.262 mL) all in THF (5 mL) was added dropwise over 3 minutes. The reaction mixture was removed from the dry-ice bath and stirred for 5 minutes, resulting in a pale yellow solution. Acetic acid (3.33 mL) and ammonium acetate (0.166 g) were added, and a white precipitate was produced. Excess THF was removed by rotary evaporation at 60°C. The remaining residue was heated for 1.5 hours at 60-65°C and then cooled, resulting in a dark orange oil with white precipitate. The mixture was worked up in water (30 mL) and CH$_2$Cl$_2$ (30 mL). The organic phase was washed with three 10 mL portions of water and aqueous NaHCO$_3$ (10% w:v, 30 mL) and dried over anhydrous MgSO$_4$. Rotary evaporation gave a dark orange oil (1.2 g).

A TLC plate was eluted with 100:1 v:v CH$_2$Cl$_2$:Et$_2$O to confirm product formation. The TLC plate revealed five potential product spots (Spot 1, $R_f = 0.88$; Spot 2, $R_f = 0.76$; Spot 3, $R_f = 0.70$; Spot 4, $R_f = 0.58$; Spot 5, $R_f = 0.43$) and unreacted enaminone (Spot 6, $R_f = 0.16$). Silica gel flash column chromatography was utilized (30 g SG), eluted with 300 mL 100:1 v:v CH$_2$Cl$_2$:Et$_2$O, and collected in 7 mL fractions. TLC plating of each fraction indicated full separation of Spots 1 & 5, partial separation of Spots 2 & 4, two fractions containing both Spots 2 & 3, and two fractions containing both Spots 3 & 4. Spot 6 did not elute, so 150 mL of 100% ethyl acetate were used as an eluent and collected in 15 mL fractions. TLC plating of each fraction indicated full removal of Spot 6 from the column. All fractions containing fully or
partially separated spots had solvent removed by rotary evaporation. $^1$H-NMR spectra from Spots 1, 2, 4, and 5 showed complex peaks that did not match with the predicted spectrum for the pyridinone 18. The $^1$H-NMR spectrum for Spot 6 confirmed it to be unreacted enaminone 17. Spot 3 could not be further analyzed as it could not be separated individually through the column. Therefore, formation of the pyridinone 18 was not verified.
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