A Novel Synthetic Pathway for Aspernigrin A

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A novel synthetic pathway to aspernigrin A

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ABSTRACT: Many natural products have been found to be beneficial in medicinal applications. Aspernigrin A, a secondary metabolite from a strain of Aspergillus niger, was isolated from the Mediterranean sea sponge Axinella damicornis. Preliminary assays of aspernigrin A showed moderate cytotoxicity to the colon cancer cell line SW1116. Due to its limited availability from the producer organisms and relatively simple structure, it was hypothesized that the compound and structural analogs could be synthesized. Several synthetic strategies have been explored in the past years. The current pathway begins with installing a benzyl ether protecting group on the commercially available 4-hydroxy-6-methyl-2-pyrene followed by SeO₂ oxidation to the 6-oxo derivative in greater than 50% yield over two steps. The subsequent reactions, reduction of the aldehyde to the corresponding alcohol, conversion of the alcohol to a suitable leaving group, and a Suzuki coupling reaction with phenylboronic acid, are well established in organic chemistry and will be pursued to synthesize aspernigrin A.

INTRODUCTION: Natural products are chemical compounds that are naturally occurring and are harvested from biological organisms, such as microbes, plants, or animals. These can either be primary or secondary metabolites; the difference being that primary metabolites are involved in necessary biological processes like growth and reproduction, while secondary metabolites are not. Certain natural products have been found to be of particular interest in the medical field, having desirable effects against a wide variety of diseases. This is no recent discovery—evidence suggests that Neanderthals more than 60,000 years ago may have known that certain plants held medicinal benefits and since then, countless natural products have been used by humankind for their positive effects.¹ Over time, scientists have developed the modern process for synthesizing a novel drug from a natural product. This process includes the following: screening of natural compounds for biological activity, isolating and purifying of the active principle, determining the structure, developing structure-activity relationships, synthesizing analogues, developing receptor theories, and designing and synthesizing novel drug structures. Through these steps, it has been possible for scientists to discover large numbers of compounds along with their structures and benefits, as well as creating thousands of analogues of these compounds to see if any of them will be more effective than the natural product itself.²

Out of all of the potential sources for the discovery of natural products, microorganisms present a promising source of many yet-undiscovered secondary metabolites. Since microorganisms are found throughout the Earth in every single niche, an extremely low number of these microbes have been discovered and identified. Much fewer of those organisms have actually been analyzed for medicinal benefits. This has been partly due to a lack of techniques that can successfully cultivate these microorganisms in the lab. New techniques have been recently developed and more are on the way that allow for further analysis of many more of these microbes. These include parallel cultivation of gel-encapsulated single cells and screening cosmids libraries of environmental DNA. Actinomycetes and fungi have more recently been found to produce many secondary metabolites that are of biological interest, but these fungi have not been studied intensively.³ Endophytic fungi, which live on the internal tissue of a host, show promise to contain useful secondary metabolites.⁴ The secondary metabolite of interest in this study is produced by an endophytic fungus.

The organism of interest for this study is the fungus Aspergillus niger, isolated from the Mediterranean sea sponge Axinella damicornis. A 2004 experiment by Hiort used fractionation to discover seven new secondary metabolites from this strain of A. niger. These products were then analyzed with NMR spectroscopy and mass spectrometry to find their molecular formulas and structures. Four bioassays were performed on these secondary metabolites: a test for insecticidal properties, a brine shrimp assay, an agar plate diffusion assay, and a cytotoxicity testing assay. Notable results of this experiment were that one product, pyranonigrin A inhibited some insecticidal growth, and two of the products, aspernigrin A and B showed moderate cytotoxicity toward various leukemia and carcinoma cell lines at a concentration of 50 μg/mL. Further testing found that aspernigrin B could potentially be effective against neurodegenerative diseases.⁵ This study, however, will focus on aspernigrin A as it has a much simpler structure that is more plausible to synthesize and study in the lab.

Figure 1. Structures of aspernigrin A and aspernigrin B

The Hiort lab performed preliminary tests in order to determine the formula and structure of the analog called aspernigrin A. The molecular formula of this secondary metabolite was found to be C₁₃H₁₁N₂O₂ though high-
resolution electron impact mass spectrometry. $^1$H and $^{13}$C-NMR spectroscopy were then used to determine a preliminary structure. Five hydrogens with a chemical shift of between 7.2–7.4 ppm indicated the presence of a phenyl ring. The $^1$H-NMR spectra also showed that the molecule should contain two additional double bonds, which were identified as an amide and a lactam carbonyl with the help of the $^{13}$C-NMR spectra. Heteronuclear multiple bond correlation suggested that a 2-pyridone was present in the compound, and allowed the researchers to determine the placement of all of the substituents on the rings. The compound was therefore assigned the name 4-benzyl-6-oxo-1,6-dihydropyridine-3-carboxamide, or aspermigrin A.$^5$

In 2005, the Ye lab performed tests on Cladosporium herbarum, in which aspermigrin A was present, and it was found to inhibit the growth of the colon cancer cell line SW1116.$^6$ Further analysis of the aspermigrin A strain revealed a discrepancy in the structure. The $^{13}$C-NMR signal at 177.6 ppm assigned to the amide was more deshielded than it was expected to be.

![Figure 2. Revised structure of aspermigrin A](image)

Nuclear overhauser enhanced differential spectroscopy and correlation spectroscopy suggested that a 4-pyridone was present instead of a 2-pyridone, meaning that the carbonyl was likely located next to the amide. (Figure 2) These results were confirmed using an X-ray diffraction study.$^6$

Since this secondary metabolite is not easily harvested in large quantities directly from nature, it is necessary to synthetically prepare it in order to further test its potential medicinal benefits. There are many different pathways that could be hypothetically followed in order to obtain aspermigrin A.

![Figure 3. A retrosynthetic pathway for aspermigrin A](image)

Figure 3 above shows a possible plan to synthesize aspermigrin A by taking the pyrone 3, converting it to the benzyl pyrone 2, which then could be converted to a pyridone to make aspermigrin A 1. Kilbourn and Seidel have used a reaction of a 4-hydroxy-2-pyridone with the dimethyl acetal of dimethylformamide to produce N-alkyl-3-carboxy-4-pyridones with a respectable yield.$^7$ McCombie et al. showed that a benzyl pyrone could be synthesized from pyridinones and 4-pyrones.$^8$ From here, it is reasonable to believe that the benzyl pyrone could be converted to a benzyl pyridone, much like the 4-hydroxy-2-pyridone was converted to a pyridone, which would then allow for aspermigrin A to be synthesized.

**Scheme 1.**

The above scheme was performed primarily by Hoeckle in order to reproduce the results of Kilbourn and Seidel.$^{7,9}$ It was found that a pyridone could indeed be produced from a pyrone. Different R groups were used in this reaction, including a benzyl group, hydrogen, and tert-butyl in order to get a higher yield and to get more favorable solubility. Product 7 was not able to be produced by Hoeckle because the R groups attached to the nitrogens on the pyridone 6 were unable to be removed. If an effective R group is found that will allow the reaction to run while still being able to be removed in order to synthesize product 7, this will prove to be a very effective scheme.

**Scheme 2.** Allylic bromination of 6-methyl-4-hydroxy-2-pyrene

This scheme was proposed by Buhler.$^{10}$ It involved brominating the pyrone so that the benzyl group could be added. This pyrone could then be converted to a pyridone as discussed above. This scheme did not work as planned, however. The
ring was only brominated at ring position 5 next to the hydroxyl group rather than at the allylic methyl, so product 2 was not able to be produced.

Scheme 3. Allylic bromination of acetyl pyrone

Buhler attempted to use a different reactant 9 rather than 3, as well as using different reagents in order to brominate the pyrone at the methyl group. While the bromination proved to be successful, the reaction could not be finished to produce 2 because the starting material 9 could not be separated from 10.

Scheme 4. Oxidation using a methyl protecting group

This scheme refined by Sharber involves installing a methyl group at the 4-hydroxyl position to function as a protecting group. Then, the methyl group at the 6-position is to be oxidized to an aldehyde to form product 12, which would then be converted into an allylic alcohol 13. This group would then be brominated and the benzyl group could attach there in a Suzuki coupling reaction to form product 2. This scheme initially featured higher purities and yields, but product 12 still suffered issues of solubility and purity. Despite this, product 13 was formed even though it was in low yield and was fairly impure.

Scheme 5. Addition of a benzyl protecting group

This scheme was proposed by Cross and is identical to Scheme 4, with the only difference being the attachment of a benzyl group to function as a protecting group. Synthesis of 16 was successful and synthesis of 17 showed promise, but had not been successful. The focus of this study will be to replicate synthesis of 16 and to optimize the reaction to create product 17 and subsequent products.
EXPERIMENTAL:

Synthesis of 6-methyl-4-(phenylmethoxy)-2-pyrene

0.9403g (7.45mmol) of commercially available 6-methyl-4-hydroxy-2-pyrene was added to a round bottom flask with 0.940mL (7.90mmol) benzyl bromide, 0.9956g K₂CO₃ and 25mL of acetone. The flask was fitted with a drying tube containing CaCl₂. The reaction was heated in a mantle attached to the Glas-col heating device at 40% heat with stirring for 48 hours. The product was a dark liquid with some solid precipitate in the bottom of the flask. Thin layer chromatography using 1:3 ethyl acetate:hexane as a solvent showed a very small amount of product and a newly formed spot. A vacuum pump was filled with a mixture of dry ice and acetone, and the reaction flask was attached in order to pump the acetone out. A silica gel flash column was run using 70g of silica gel and 1:2 ethyl acetate:hexane as a solvent. The potential product travelled though the gel and was eluted in fractions 7-14. The fractions were combined and concentrated through rotary evaporation to yield 0.7196g of product for a 44.6% yield. Analysis via ¹H-NMR in deuterated chloroform confirmed that the structure was 6-methyl-4-(phenylmethoxy)-2-pyrene.

Oxidation of 6-methyl-4-(phenylmethoxy)-2-pyrene

Attempt 1

0.2014g (0.931mmol) of the synthesized compound was added to a 75 mL pressure flask with 0.789g (7.11mmol) SeO₂ and 40 mL dioxane. The vessel was fitted with a screw top and heated at 130°C in a sand bath with stirring. After four days, the mixture had a slight green tint with a back solid and showed no starting material as determined by TLC. The resulting mixture was filtered through celite and washed with CH₂Cl₂. The product was concentrated through rotary evaporation to yield 0.5001g (4.55mmol) SeO₂ and 20 mL glacial acetic acid. The flask was fixed with a reflux condenser and gently heated. The reaction was allowed to run overnight until TLC showed no evidence of starting material. The reaction mixture was filtered through celite to remove elemental Se, washed with saturated NaHCO₃ and extracted with CH₂Cl₂ three times. The organic layers were combined and concentrated. The resulting solid was dissolved in deuterated chloroform and assessed by ¹H-NMR. This spectrum showed the presence of the expected benzene ring, but no evidence of an aldehyde peak. Therefore, it was determined that glacial acetic acid is not a suitable solvent for the reaction.

Attempt 2

The reaction was attempted again using 0.2136g (0.988mmol) benzyl starting material, 1.5606g (14.1mmol) and 30 mL of dioxane. The reaction was heated at 160°C in a sand bath with stirring for two days until TLC indicated that no starting material was present. Saturated NaHCO₃ was added to the mixture and it was extracted with CH₂Cl₂, discarding the aqueous layers. The resulting mixture was concentrated to a pale brown powder. Methanol and CH₂Cl₂ were added to the powder to dissolve the product and left a considerable amount of solid. The solid was isolated through vacuum filtration and discarded. The resulting liquid was concentrated to 0.1028g of a brown powder for a 47.8% yield. The product was dissolved in deuterated DMSO and assessed with ¹H-NMR. The spectra revealed that although the aldehyde had formed, the benzyl group was not evident. It was hypothesized that the SeO₂ could be destroying the benzyl ring, putting the efficacy of this scheme featuring a benzyl protecting group in great jeopardy.

Attempt 3

0.200g (.925mmol) of benzyl starting material was added to a 50 mL round bottom flask with 0.103g (.928mmol) SeO₂ and 20 mL glacial acetic acid. The flask was fixed with a reflux condenser and gently heated. The reaction was allowed to run overnight until TLC showed no evidence of starting material. The reaction mixture was filtered through celite to remove elemental Se, washed with saturated NaHCO₃ and extracted with CH₂Cl₂ three times. The organic layers were combined and concentrated. The resulting solid was dissolved in deuterated chloroform and assessed by ¹H-NMR. This spectrum showed the presence of the expected benzene ring, but no evidence of an aldehyde peak. Therefore, it was determined that glacial acetic acid is not a suitable solvent for the reaction.

Attempt 4

Following the procedure published by Hach, 0.1139g (0.527mmol) of benzyl starting material was added to a 3-necked flask. 0.5001g (4.55mmol) SeO₂ in 50 mL dioxane and 15 mL H₂O were added dropwise over the course of three hours. The reaction was expected to be considerably exothermic and therefore was run on ice to equilibrate temperature. The reaction was not observed to be exothermic, nor was a color change observed that was expected, after addition of all reactants. Assessment with TLC showed presence of only starting material.

CONCLUSION: The preliminary work of Cross was able to be repeated, and a pure compound 16 was synthesized in good yield. Oxidation of the methyl group at the 6-position proved to be ineffective regardless of heat, solvent used, or technique used to run the reaction. It can be determined that SeO₂ is not an effective oxidizing agent for the purposes of this reaction scheme. If these schemes are to be continued, it is critical to find another compound that is reported to oxidize a methyl group in this way. Alternatively, a different scheme will need to be developed that would install an allylic bromine.

ABBREVIATIONS: NMR, nuclear magnetic resonance spectroscopy; TLC, thin layer chromatography

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REFERENCES:


