PRODUCTION OF DISUBSTITUTED ISOQUINOLINE DERIVATIVES: STEPS TOWARD THE SYNTHESIS OF A PRATOSINE ANALOG

by

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ABSTRACT

The pyrrolophenanthridone alkaloid pratosine is a natural product related to hippadine, which is known to be a powerful but reversible inhibitor of spermatogenesis in rats. Hippadine has also shown cardiovascular as well as anticancer activity. Given the structural similarities between the two molecules, it is expected that pratosine will demonstrate biological activity similar to hippadine. Our work toward a laboratory synthesis of pratosine will facilitate large-scale production thus affording sufficient quantities of the material for a complete study of its pharmacological properties. Although we have a synthetic plan for preparing pratosine based on a model study, several reactions have failed due to solubility problems. This research focuses on using an alternate starting material with a large, lipophilic group to improve the solubility in ethereal solvents. The commercially available compound vanillin, which is extracted from vanilla beans, is a simple and inexpensive aldehyde with an appropriate structure for attaching the specific group(s) to be investigated. The goal is to find an extraneous substituent that will provide the required characteristics but which is sufficiently labile to be removed easily at the end of the synthesis.

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INTRODUCTION

Lycorine-type alkaloids derived from the *Amaryllidaceae* family of plants have gained considerable recognition in the last two decades due to the extraordinary biological activity exhibited by the alkaloid lycorine and related compounds (Figure 1). Lycorine has been shown to target cellular actin cytoskeletons and is thus linked to cytostatic effects as opposed to apoptosis promotion.[1] This relation is promising due to the deadly nature of several cancers that do not respond to traditional pro-apoptotic chemotherapy treatments including gliomas, melanomas, and esophageal and non-small-cell lung cancers. As such, lycorine is a prominent member of a group of alkaloids that possess the pyrrolophenanthridone general structure **1**. The alkaloid hippadine (**2**), whose structure closely resembles that of lycorine, is a related compound that also has anti-cancer activity. However, a more significant property of hippadine that has prompted extensive research is its spermicidal potency and its ability to inhibit spermatogenesis reversibly in rats.[2] This includes significant loss of testicular weight as well as DNA content. Hippadine appears to be functioning at the genetic level due to the lack of anti-mitotic activity.

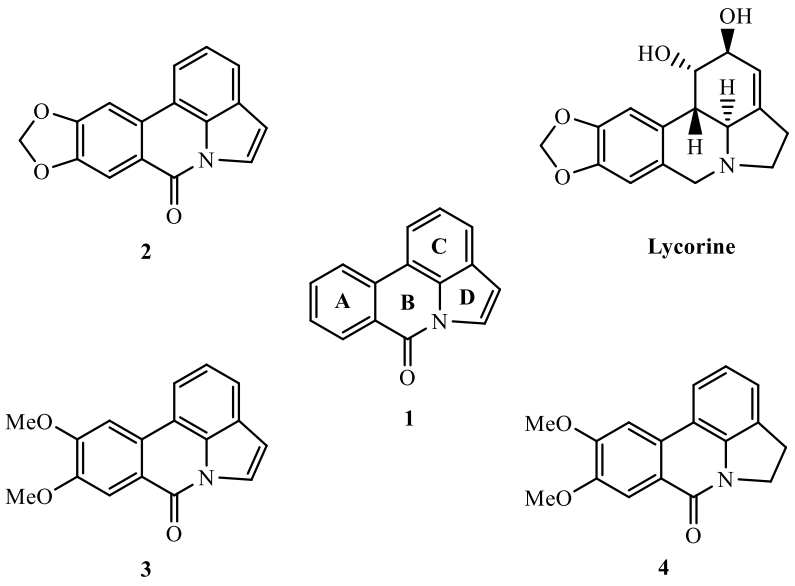


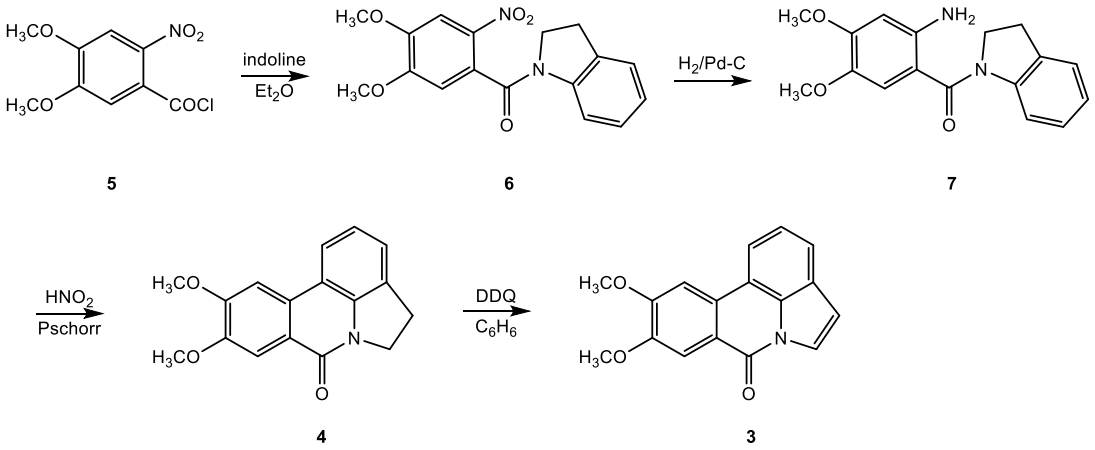
Figure 1. General structural features found in *Amaryllidaceae* alkaloids.

Other pharmacologically-active lycorine-type alkaloids isolated from the *Amaryllidaceae* family possess acetylcholinesterase (AChE) activity that could be used in a possible treatment for Alzheimer's disease. This effect is proposed to be a result of the aromatic C-ring, which gives these alkaloids a degree of planarity. This feature is shared by oxoassoanine (**4**), which has already been shown to have high AChE activity,[3] as well as hippadine (**2**) (See Figure 1).

Because of the history of beneficial pharmaceutical activity shared by these molecules, we have become interested in another alkaloid pratosine (**3**), which has structural features in common with both hippadine (**2**) and oxoassoanine (**4**) that make it a promising candidate for testing. However, pratosine has not been as extensively studied to date due to the extremely low availability from the natural source and the lack of a high-yielding synthetic pathway. The three reported syntheses of pratosine described below provide some insight into the approaches used by others to produce this molecule.

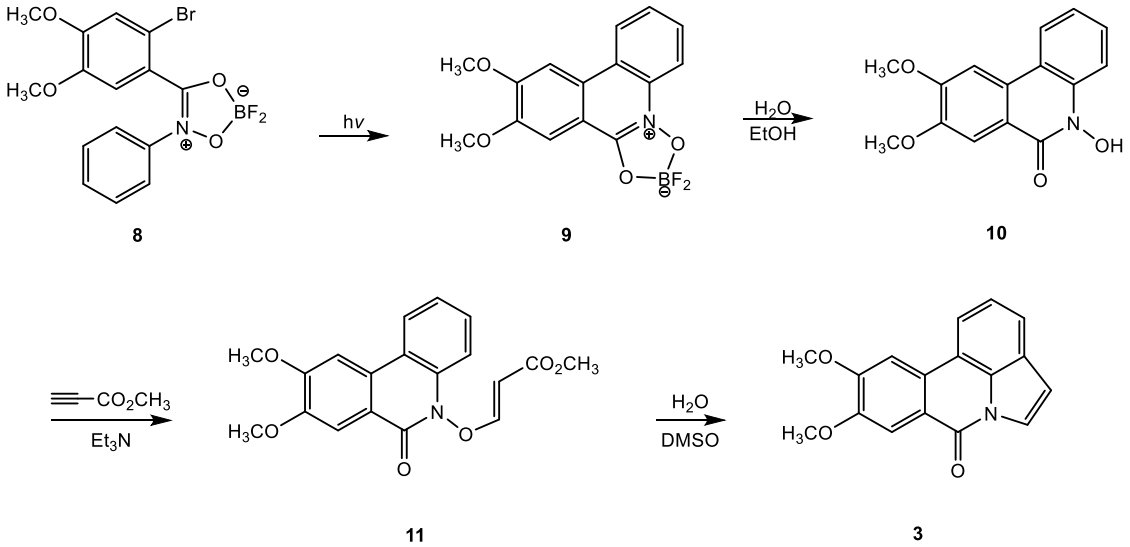
Previous Syntheses of Pratosine

The first documented synthesis of pratosine was published by Goshal[4] in 1983 and was adapted from an original procedure used to synthesize anhydrolycorine. This procedure, illustrated in Scheme 1, begins with the *N*-acylation of indoline with the acid chloride **5** to yield **6** followed by a catalytic reduction of the nitro group to produce **7**. Amide **7** undergoes a Pschorr cyclization to generate **4**, which is then aromatized using DDQ to afford pratosine (**3**). All of the steps except for the Pschorr cyclization reaction resulted in yields of 70-80%, but the yield of this cyclization never exceeded approximately 10% yield.[4] This resulted in an overall yield of less than 5% for the entire synthetic route.



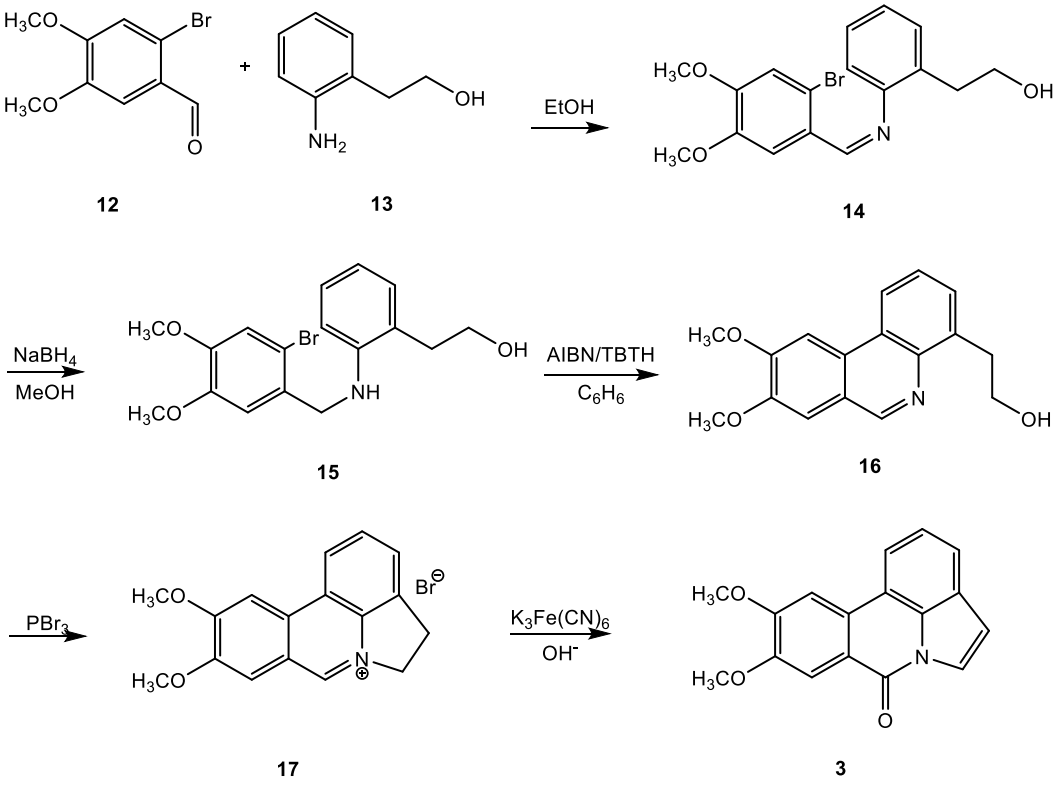
Scheme 1. Ghosal's Synthesis of Pratosine (**3**).

A more recent synthetic procedure for the total synthesis of pratosine was published by Pereira in 1996.[5] As shown in Scheme 2, the sequence begins with a photolysis of the boron-containing compound **8** in benzene to produce the cyclic borate molecule **9**. Through hydrolysis in aqueous ethanol, **9** was converted to the N-hydroxyphenanthridone **10**. A base-catalyzed Michael addition with methyl propiolate yielded the enol ether **11,** which was converted to pratosine (**3**) when refluxed in wet DMSO. The overall yield was not revealed in the manuscript, but the last step was quite poor and gave not only **3** but also two significant side products.



Scheme 2. Pereira's Synthesis of Pratosine (**3**).

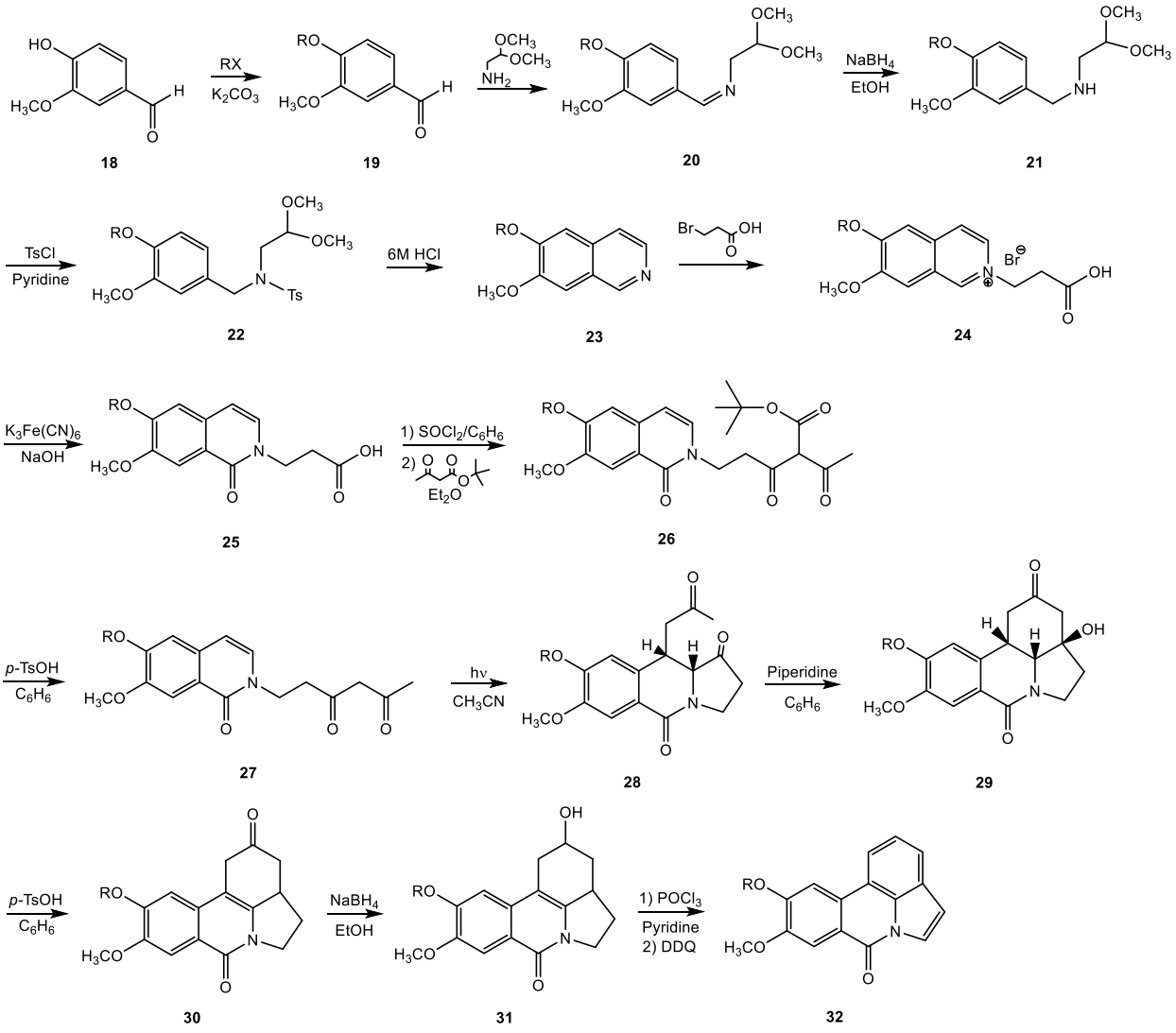
Another interesting synthetic route by Rosa[6] (Scheme 3) involves the condensation of the halogenated dimethoxybenzaldehyde **12** and 2-β-hydroxyethylaniline (**13**) to form aldimine **14,** which was then reduced to the aminoalcohol **15**. Compound **15** was treated with tributyltin hydride and AIBN to cyclize and aromatize the molecule in one step thus forming tricyclic structure **16**. Bromination of **16** with PBr_3 led to the salt **17**, a precursor to pratosine. Finally, oxidation of **17** with $K_3Fe(CN)_6$ and base produced two products including pratosine (**3**) in 21% yield as well as oxoassoanine (**4**) in 59% yield. This procedure suffered from difficulty in the purification of the alcohol **16**, which could be produced in no greater than 27% yield.[6]



Scheme 3. Rosa's Synthesis of Pratosine (**3**).

The Research Plan: Our Approach to the Synthesis of Pratosine (3)

Our work on the total synthesis of pratosine (**3**) has been underway for over two years and is based on research that was completed by a former graduate student in the Minter group. Scheme 4 shows the specific pathway starting with vanillin (**18**), which is a readily available and



Scheme 4. Proposed Novel Synthetic Scheme for Pratosine.

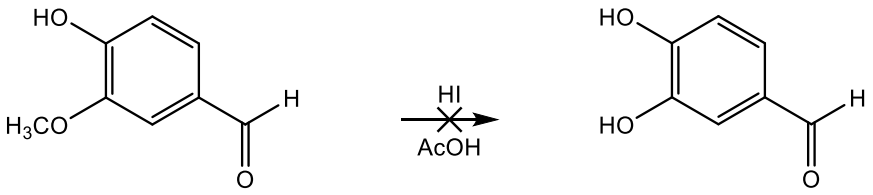
inexpensive material. My role in the research effort began as a result of a solubility problem that occurred when the carboxylic acid **25** was converted into the acid chloride. This material proved

to be insoluble in the ethereal solvents that were required to synthesize **26**. Two possible solutions to the problem were investigated in my project. First, the cleavage of the methyl ether in **18** would give a catechol derivative that could be substituted at the phenolic oxygens with extraneous groups that would improve the solubility in ethereal solvents. Second, the hydroxyl group in **18** could be converted to an ether with a large lipophilic group that could accomplish the same goal. The results of this investigation are described below.

EXPERIMENTAL SECTION

Attempted Preparations of Protocatechualdehyde (36)

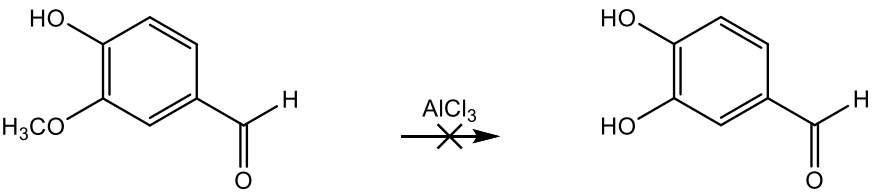
a. Failed cleavage of the methyl ether using HI/HOAc



18 **36**

A mixture containing vanillin (6.80 g, 44.7 mmol), glacial acetic acid (25 mL) and conc. hydroiodic acid (25 mL of 57% solution) was heated at reflux for 4 hours under N_2. The reaction mixture was cooled to room temperature and diluted with water to approximately double the original volume. The resulting solution was extracted with benzene (2 x 50 mL), and the organic layers were combined and washed with 25% $NaHSO_3$ (1 x 25 mL) and 15% NaOH (1 x 25 mL). The NaOH extract was acidified with HCl to a pH of approximately 5 and extracted with ether (2 x 25 mL). After drying the extracts with sodium sulfate, volatiles were removed by rotary evaporation followed by pumping under high vacuum. An NMR analysis of the residue indicated that only the starting material was present.

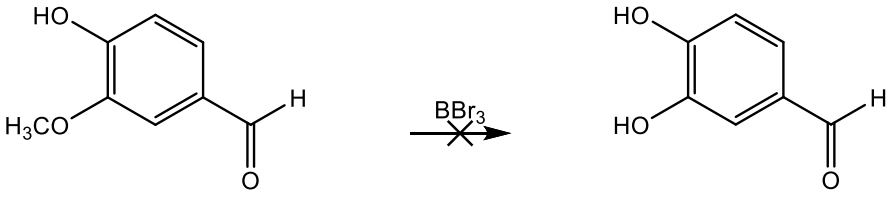
b. Failed cleavage of the methyl ether using $AlCl_3$



18 **36**

Vanillin (2.00 g, 13.1 mmol) was dissolved in 20 mL of CH_2Cl_2 in a 50-mL round-bottomed flask under N_2. $AlCl_3$ (1.98 g, 14.9 mmol) was added with vigorous stirring while the reaction mixture was cooled using a water bath. Pyridine (4.6 mL) was added slowly via an addition funnel and the mixture was heated at reflux for 24 hours. The solution was then cooled to room temperature and acidified using approximately 5 mL of 3 M HCl until acidic to Congo Red indicator paper. The aqueous phase was separated and extracted with ether (1 x 25 mL). This led to an emulsion that was broken up by filtration and the ether layer was separated. The aqueous solution was then extracted twice more with ether (2 x 25 mL) for a total of three extractions. The combined organic layers were concentrated by rotary evaporation, and the resulting pale yellow oil was placed under high vacuum to remove the remaining volatiles. An NMR analysis of the residual solid showed only recovered vanillin.

c. Failed cleavage of the methyl ether using BBr_3



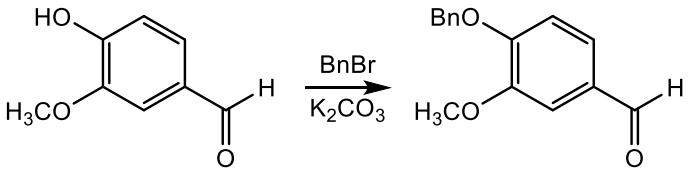
18 **36**

Vanillin (1.41 g, 9.3 mmol) was dissolved in 30 mL of CH_2Cl_2 in a 100-mL round-bottomed flask and the resulting solution was cooled to -78 °C under N_2. BBr_3 (1.51 mL, 15.2 mmol) was added to the stirred solution via syringe through an air condenser and the dry ice bath was removed. After stirring for 24 hours at room temperature, the reaction was quenched with 33 mL of water and the aqueous layer was extracted with ether (1 x 125 mL). The ether extract was

washed with 2 M NaOH (1 x 60 mL), and the aqueous layer was separated and neutralized using 3 M HCl to a pH of approximately 7. The resulting solution was extracted with ether (1 x 75 mL) and the extract was dried over $MgSO_4$. Rotary evaporation followed by removal of residual solvent at high vacuum resulted in a solid that proved to be recovered vanillin by NMR analysis.

Preparation of Vanillin Benzyl Ether (37)

a. Preparation of Vanillin Benzyl Ether using K_2CO_3 and BnBr

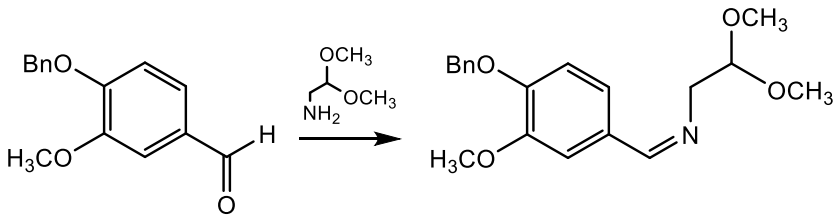


18 **37**

Vanillin (20.0 g, 132 mmol) was dissolved in 660 mL of MeOH in a 1-L round-bottomed flask under N_2. K_2CO_3 (21.8 g, 158 mmol) was added first followed by benzyl bromide (19 mL, 160 mmol) via syringe through the condenser. The reaction mixture was heated at reflux for 2 hours followed by filtration to remove solids. The methanol was removed by rotary evaporation, and CH_2Cl_2 (600 mL) was added to the residue. This solution was extracted with water (3 x 140 mL). The organic layer was dried over $MgSO_4$, concentrated via rotary evaporation, and placed under high vacuum to remove residual volatiles. The resulting yellow solid (26.8 g, 84.7%) was identified by NMR as vanillin benzyl ether (**37**), which was sufficiently pure to be used in the next step without further purification.

Preparation of 6-benzyloxy-7-methoxyisoquinoline (41)

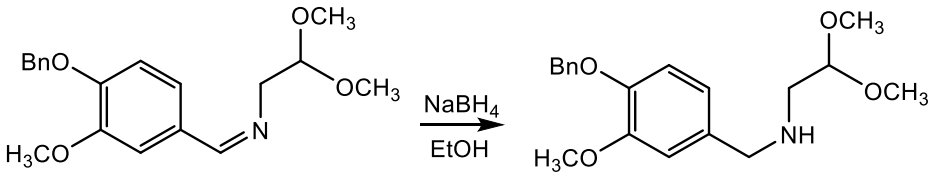
a. Imine Formation using Vanillin Benzyl Ether and Aminoacetaldehyde Dimethyl Acetal



37 **38**

A mixture containing vanillin benzyl ether (26.8 g, 111 mmol) and aminoacetaldehyde dimethyl acetal (12.2 g, 116 mmol) was heated overnight at 70 °C under N_2 in a 250-mL round-bottomed flask fitted with a water condenser. The resulting pale yellow oil was cooled to room temperature and used directly in the next step without further purification. The NMR analysis showed that the oil contained imine **38** and excess starting material.

b. Reduction of Imine 38 with $NaBH_4$

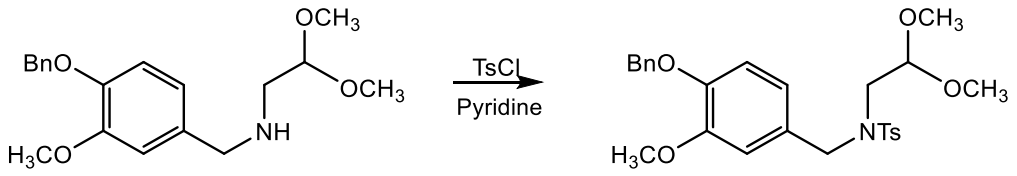


38 **39**

The imine **38** from the previous step was dissolved in 170 mL of 90% ethanol and cooled to 0 °C. Sodium borohydride (8.25 g, 218 mmol) was added over a one-hour period resulting in the evolution of hydrogen gas. The solution was stirred overnight at room temperature under N_2. The reaction was quenched by the addition of 125 mL of water and the mixture was transferred to a 1-L separatory funnel containing an additional 350 mL of water. The solution was extracted

with CH_2Cl_2 (3 x 125 mL) and the combined organic extracts were dried over $MgSO_4$. Removal of the volatiles by rotary evaporation followed by pumping at high vacuum resulted in the formation of a viscous amber oil. NMR analysis showed the oil to be the amine **39** (38.5 g, 95.4% yield over 2 steps), which was used subsequently without further purification.

c. Tosylation of amine 39 with TsCl in Pyridine

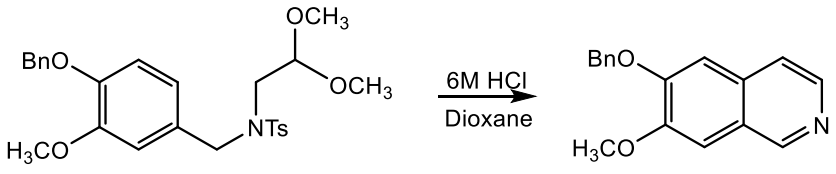


39 **40**

Amine **39** (38.5 g, 116 mmol) was dissolved in 130 mL of dry pyridine in a 1-L round-bottomed flask and cooled to 0 °C using an ice bath. Tosyl chloride (26.0 g, 136 mmol) was added all at once and the mixture was capped and stirred at room temperature for 48 hours under N_2. The reaction mixture was cooled to 0 °C and stirred rapidly as cold sat. $NaHCO_3$ (250 mL) was added. The contents of the flask were transferred to a separatory funnel and extracted with CH_2Cl_2 (2 x 120 mL). The organic extracts were combined and washed sequentially with saturated $CuSO_4$ (2 x 120 mL) and 3 M HCl (1 x 150 mL) to remove most of the pyridine. The organic layer was washed with water (1 x 125 mL) and dried over $MgSO_4$. Removal of volatiles by rotary evaporation followed by pumping at high vacuum resulted in the isolation of a viscous dark brown oil (48.6 g, 86.2% yield). NMR analysis revealed the oil to be a mixture of the crude tosylamide **40** and other impurities. Multiple attempts to use this crude material for the synthesis of the isoquinoline **41** (see the next reaction) resulted in consistently low yields. Finally the

tosylamide **40** was recrystallized from ethyl acetate/hexane to give pure material (m.p. 68-72 °C) that was suitable for the cyclization reaction described below.

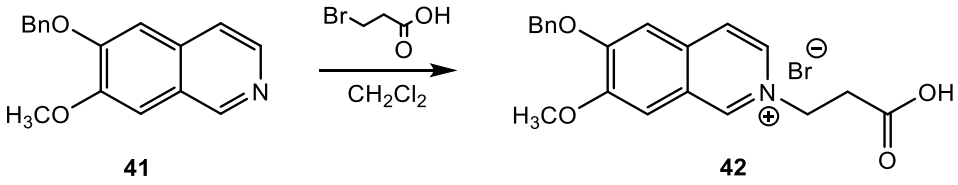
d. Cyclization of Tosylamide 40 with HCl to give 6-Benzyloxy-7-methoxyisoquinoline (41)



40 **41**

A small sample of purified tosylamide **40** (0.99 g, 2.0 mmol) was dissolved in 13 mL of dioxane in a 25-mL round-bottomed flask and stirred as 4 mL of 6 M HCl was added dropwise via an addition funnel. After complete addition, the mixture was heated at reflux for three hours and monitored by TLC before simple distillation was used to remove approximately 6 mL of dioxane. The reaction mixture was poured slowly into 60 mL of cold sat. $NaHCO_3$ resulting in the formation of a brown precipitate. Since this precipitate was known not to contain any of the product, it was removed by filtration before the product was extracted using CH_2Cl_2 (3 x 7 mL). The organic extracts were combined and dried over $MgSO_4$. Removal of the volatiles by rotary evaporation followed by pumping under high vacuum resulted in a light brown powder. The crude product was recrystallized from cyclohexane to give 0.25 g (47.2%) of isoquinoline **41**, which was pure by NMR analysis.

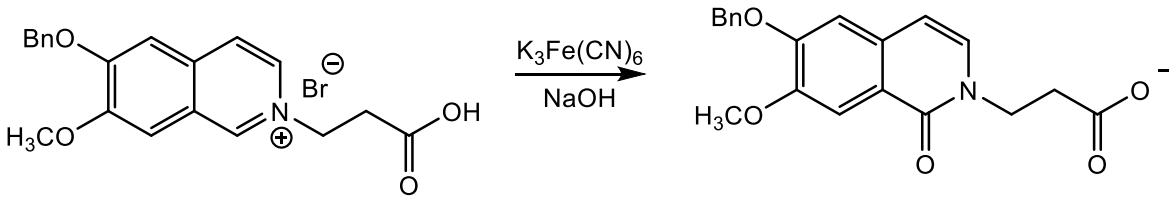
Isoquinolinium Salt Formation



41 42

A solution containing **41** (0.25g, 0.94 mmol) and 3-bromopropionic acid (0.23 g, 1.5 mmol) in 3 mL of CH_2Cl_2 was prepared in a 25-mL round-bottomed flask. A total of 3 drops of triethylamine was added to the solution and the flask was capped and stirred for 72 hours. Typically, the isoquinolinium salts will precipitate due to their insolubility in CH_2Cl_2. However, this was not the case for compound **42**, so NMR analysis was used to confirm the formation of the product. Removal of the volatiles by rotary evaporation followed by pumping under high vacuum resulted in the isolation of 0.46 g of a solid mixture of materials including the product along with 3-bromopropionic acid and trimethylamine.

Oxidation of isoquinolinium Salt 42 using $K_3Fe(CN)_6$ and NaOH



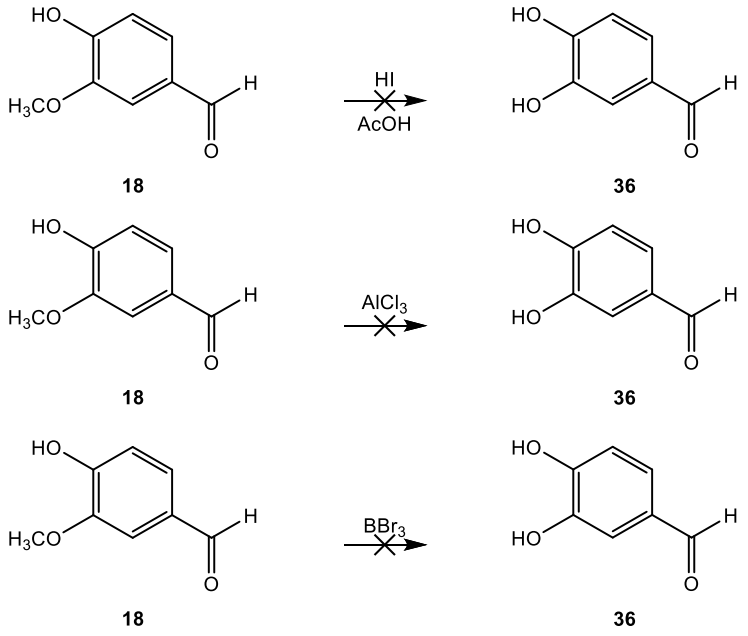
42 43

A solution of the mixture containing of **42** (0.46 g, unknown molar amount) from the previous step in 2 mL of DI water was prepared in a 25-mL round-bottomed flask which was cooled to 0 °C using an ice bath. A solution of $K_3Fe(CN)_6$ (1.57 g, 4.8 mmol) in 7 mL of DI water was added all at once to the flask. A solution of NaOH (0.54 g, 13.5 mmol) in 2 mL of DI water was added slowly to the flask. The ice bath was removed and the reaction mixture was

stirred open to air for 24 hours at room temperature. Dichloromethane (7 mL) was added and the mixture was transferred to a separatory funnel. After vigorous shaking, a precipitate formed so the entire lot was filtered to isolate the solid. The precipitate was dried by placement under high vacuum overnight to give 0.25 g of material. By NMR analysis, the solid appeared at first to be the pure isocarbostyril **43**. However, by careful inspection of the proton NMR spectrum, it was determined that the isolated material was a carboxylate anion—the conjugate base of **43**—rather than **43**. This is not unreasonable since the reaction mixture had not been neutralized prior to attempts to isolate the product.

DISCUSSION AND CONCLUSION

An extensive model study from previous work in the Minter group showed that the tetracyclic ring system in compound **1** was accessible using a 6-step sequence from isoquinoline as a starting material.[14] This work has allowed us to formulate a plan for the total synthesis of pratosine presented earlier as Scheme 4, which incorporates not only the model study but also a number of variations. Accordingly, the isoquinoline **23** (R = OCH_3) was prepared and converted to the acid **25**; but the next step in the sequence failed due to solubility problems with the acid chloride required to generate **26**. This is the origin of my project, which was designed to modify the solubility properties of these intermediates through manipulation of the substituent groups on the aromatic ring in vanillin (**18**).

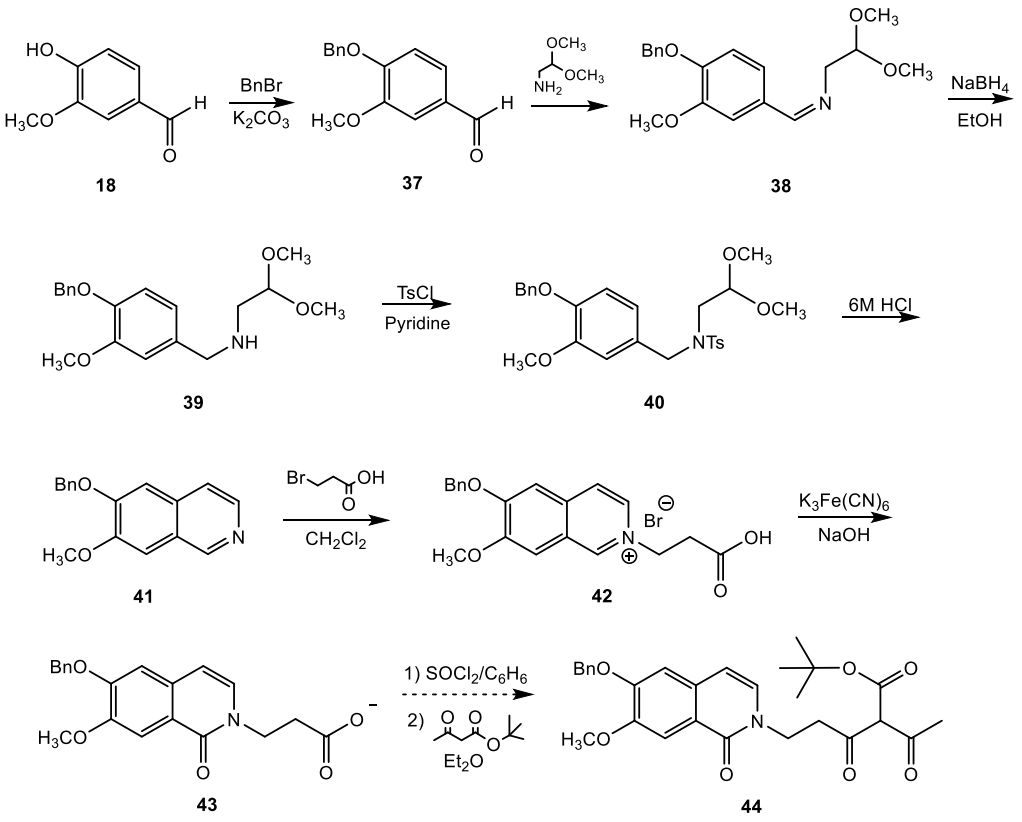


Scheme 5. Various Syntheses of Protocatechualdehyde.[7-9]

In the first part of the project, the aldehyde **36** was to be used to generate a compound with two ether functions containing large lipophilic groups that could improve the solubility in

ethereal solvents. After numerous unsuccessful attempts to obtain **36** from vanillin by cleaving the methyl ether moiety (see Scheme 5), the decision was made to functionalize the hydroxyl substituent on **18** with a bulky hydrophobic group.

Benzylation of vanillin (**18**) to give **37** provided high yields by using a relatively simple procedure indicated in Scheme 6. The next three steps proceeded without incident; but the cyclization of compound **40** to isoquinoline **41** gave a very poor yield.



Scheme 6. Synthetic approach to pratosine using vanillin benzyl ether (**37**).

The problem was traced to the use of a crude material rather than a recrystallized sample. Since the tosylamide **40** had not been synthesized previously, no data on its purification were available. However, the compound was successfully recrystallized using hexanes and ethyl acetate as a solvent system resulting in a yellow, crystalline solid. Moreover, the NMR analysis confirmed the crystalline solid as highly pure tosylamide **40**. Using purified **40** in the subsequent

cyclization reaction gave **41** in 46.9% yield after recrystallization, which was nearly double the original yield. Using the recrystallized isoquinoline **41** to form salt **42** also resulted in high yields and purity.

A notable unexpected consequence of using the benzylated starting material **42** includes the work-up required for the formation of the isocarbostyril **43**. The original procedure calls for extraction of the reaction mixture with CH_2Cl_2 followed by careful acidification using 6 M HCl. However, the product from the reaction using **42** precipitated out of solution upon addition of CH_2Cl_2 and prior to addition of any HCl. It is suspected that this occurred due to the decreased solubility of the carboxylate salt in water as a result of the addition of the benzyl group. This led to the isolation of **43** as the carboxylate rather than as the carboxylic acid.

At this point, the project is one step short of answering the question regarding the use of a benzyl group to improve the solubility properties of compounds leading to intermediate **44**. However, if the benzyl group does not solve the problem, this research can still be used to allow other modifications of the structure to achieve the final goal. If successful, this project provides the groundwork for an inexpensive, high-yielding synthetic scheme to produce pratosine. Ideally, this synthesis will allow for mass production of the alkaloid affording sufficient quantities for pharmacological testing and analysis. Given the structural similarities between pratosine and other notable pyrrolophenanthridone alkaloids, pratosine will almost surely prove to be biologically active.

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