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Determining an Efficient Means of Producing Anabaseine and One of its Derivatives

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SENIOR PROJECT - APPROVAL

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PROJECT TITLE: _Determining an Efficient Means of Producing Anhaloscine and One of its Derivatives_

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.
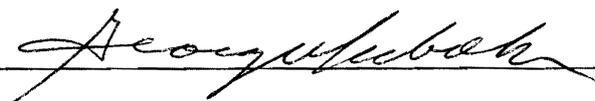
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Date: _12/11/03_

Comments (Optional):

Determining an Efficient Means of Producing Anabaseine and One of its Derivatives

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Fall Term 2003

Abstract: The derivatives of anabaseine have the potential to be imaging agents for small cell lung carcinoma, which is a form of lung cancer. This imaging agent could become an all-important early detection device for the deadly lung cancer. Since much of the anabaseine derivatives will be required for testing and possibly even production as an imaging agent, an efficient way of preparing these derivatives is important to keep production costs down. During this research anabaseine was produced with a 97% yield, while one of its derivatives was prepared with a yield of 74%.

I. Introduction: The purpose of the research performed this semester was to find the most efficient means necessary to produce anabaseine and at lease one or two of its derivatives. The importance of this chemical, which occurs naturally as a toxin in ants and nemertine worms,(1) is found in its similarity to nicotine (both pictured below), even though the two rings of anabaseine are coplanar and the saturated ring of nicotine is twisted 90 degrees out of the plane of the pyridyl ring (3).

Image 1.1: Structures of anabaseine and nicotine

Anabaseine
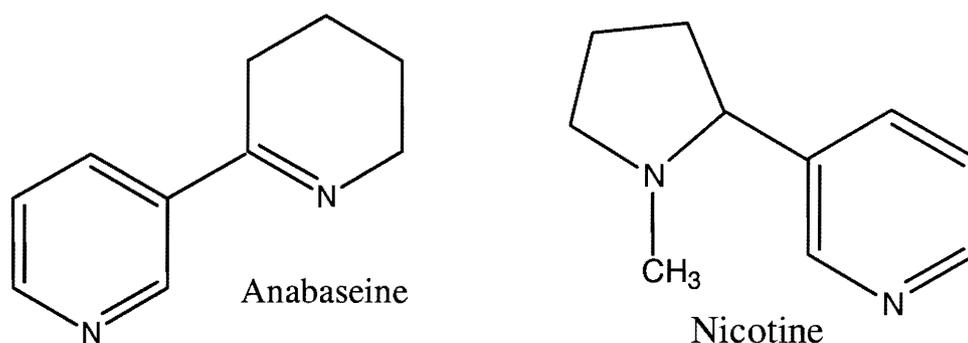
Nicotine

Due to this similarity, both anabaseine and nicotine bind with high affinity to a neuronal nicotinic acetylcholine receptor comprised of α_7 subunits, which is derived from a form of lung cancer known as human small cell lung carcinoma (SCLC). Once this binding occurs, it sets off a chain reaction that results in the increase of the number of small cell carcinoma cells (2). Therefore, the SCLC are strongly associated with the nicotine from cigarettes and represent 20-25% of lung cancers. Lung cancer is the leading cause of cancer death for both men and women world-wide causing an estimated 1.3 million deaths(4). According to the Surgeon General of the United States, lung cancer in women alone has increased 600% since 1950 and has clearly reached epidemic proportions (5). However, if the cancer can be detected early, when it is only 2 cm and can be removed surgically, then there is an 80% survival rate. Yet, the early detection methods for lung cancer have not progressed as well as the methods for other cancers,

such as mammography for breast cancer, so the survival rates are dismal. This is where

the anabaseine potentially comes into play. Since the anabaseine molecule and, more

importantly, its derivatives, such as benzylidene and cinnamylidene derivatives among

others (3), have been proven to preferentially stimulate these α_7 subunits, it may be able

to be radioiodinated and used as an imaging agent for the Positron Emission Tomography

scan. The following derivatives will be prepared in the lab of Dr. George Kabalka, and

tested for their utility in their preparation of radioiodinated anabaseines:

Image 1.2: General structure of derivatives that will be prepared
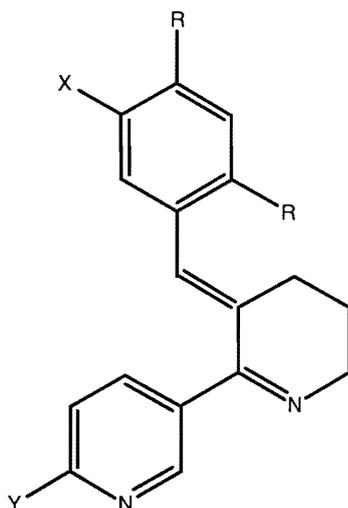
R	X	Y
CH$_3$	^{123}I	H
CH$_3$	H	^{123}I
OCH$_3$	^{123}I	H
OCH$_3$	H	^{123}I

The derivatives will then undergo further testing, so that in the future, one of the

derivatives may be injected into a possible cancer patient. Once inside the patient, the

molecule will clump in the lungs at the α_7 subunits of the cancer and provide an image

of where and how big the tumor is. This would be ideal since the α_7 subunits are

normally not found in the tissue of human lungs but in the central nervous system (1), so the only clustering seen in the lungs would be due to the presence of SCLC. The purpose of finding an efficient means of producing each derivative is so that each can be mass-produced cheaply for possible future use as imaging agents.

II. History: Previously published attempts to prepare anabaseine have yielded 53% and 88%. There are actually two published reports that have yielded 53%, and even though the percent yield is not very good the reactants used in the reaction were far simpler, probably indicating a cheaper price. The reactants in one report were 2-piperidinone (9Cl) and 3-pyridinecarbonyl chloride (7). The other report yielding 53% was a rearrangement of one reactant named 2-piperidinone-1-(3-pyridinylcarbonyl) (9Cl) (7). 1-(3-bromopropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane and 2-Propanamine-N-[1-(3-pyridinyl)-ethylidene] (9Cl) react in three steps to form anabaseine with the 88% yield (6). So the more simple reactions only yield 53% while the more complicated have a higher yield. A middle ground of high yield and easy reaction was a goal of this research.

Another useful molecule in the preparation of anabaseine derivatives is anabaseine dihydrochloride. This can be used to prepare 6-bromoanabaseine dihydrochloride, which is a very important precursor of the derivatives mentioned above. This can best be prepared at a 74% yield by three different methods. The reaction with the fewest steps involves the reconfiguration of 2-piperidinone-1-[(diethylamino)methyl]-3-(hydroxyl-3-pyridinylmethylene), sodium salt (8). This molecule is still rather large and difficult to obtain or produce.

III. Procedural Overview: The reactions carried out to produce one of these derivatives began with 3-(5-chloro-1-pentanone-1-yl) pyridine. This molecule was made previously,

but the reactants to produce it are small, easily obtainable molecules. By means of a

substitution reaction the chlorine was replaced by the azide at the end of the carbon chain.
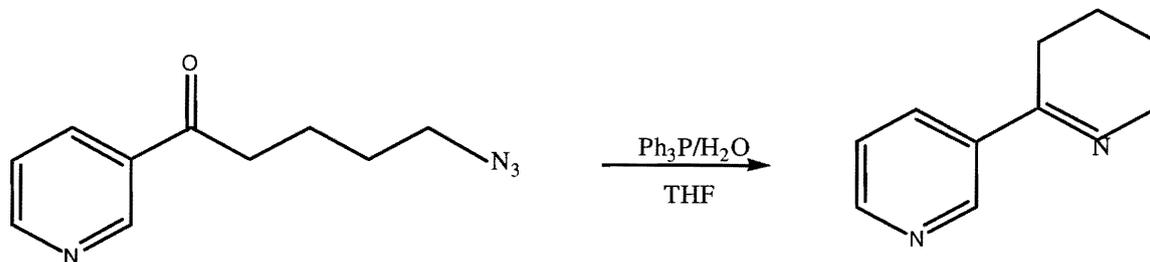
<u>Reaction 1.1</u>: Formation of 3-(5-azido-1-pentanone-1-yl) pyridine from 3-(5-chloro-1-

pentanone) pyridine
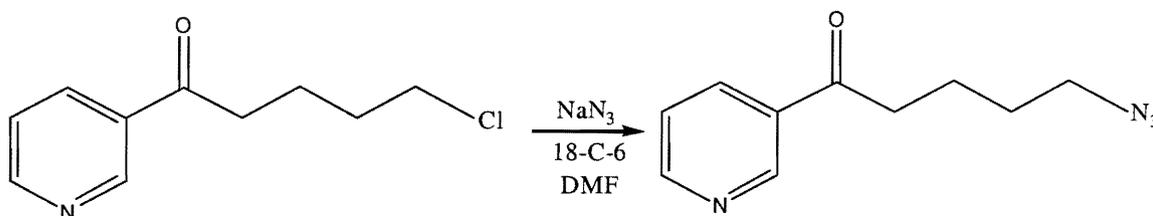
This reaction was completed with a 72.9% yield.

Then the carbon chain was condensed to form the second ring of the anabaseine

compound.

<u>Reaction 1.2</u>: Formation of anabaseine from 3-(5-azido-1-pentanone-1-yl) pyridine

This reaction was an impressive 97% efficient. This yield and simple reaction described

in the next section are two very attractive aspects of this reaction. This seems to be the

middle ground spoken of earlier with a high yield and easy reaction.

This next step produced anabaseine dihydrochloride from the anabaseine. Its

yield (68%) was slightly less than the most successful literature value of 74%. However,

the simplicity of the reaction makes up for this slight lack in yield.

Reaction 1.3: Reaction of anabaseine to produce anabaseine dihydrochloride
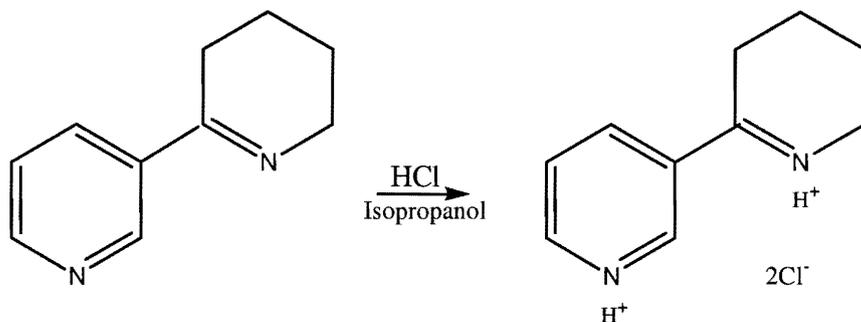
The following reaction skips from the anabaseine dihydrochloride to a sample of 6-bromoanabaseine dihydrochloride produced earlier by another lab student. This sample was added to 2,4-dimethoxy benzaldehyde to produce 6'-Bromo-3-(2,4-dimethoxy-benzylidene)-3,4,5,6-tetrahydro-[2,3']bipyridinyl with a yield of 74%. This is only one or two more steps away from completely producing one of the derivatives described above in the introduction.

Reaction 1.4: Formation of 6'-Bromo-3-(2,4-dimethoxy-benzylidene)-3,4,5,6-tetrahydro-[2,3']bipyridinyl
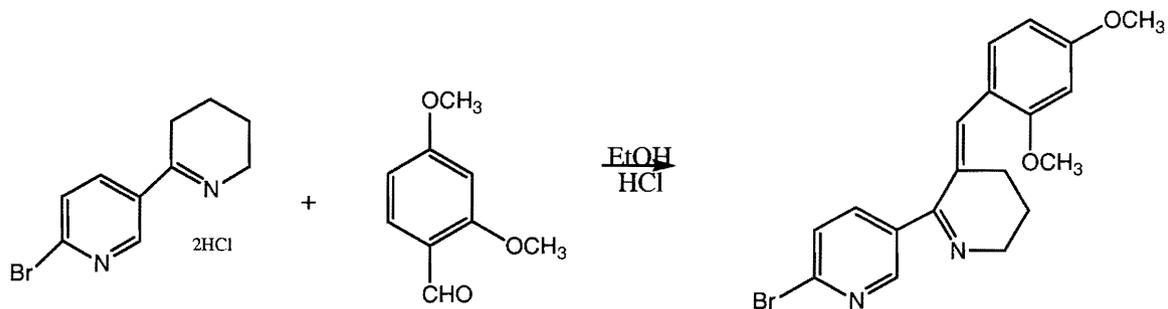

The remaining molecules were produced during off shoot reactions. This following set of reactions shows another possible way to produce the pre-cursor of the 6-bromoanabaseine dihydrochloride. However, the reaction only made it two steps and the final product included some side product.

Reaction 1.5: Reaction producing 6-bromonicotinic acid from 2-bromo-5-methyl-pyridine
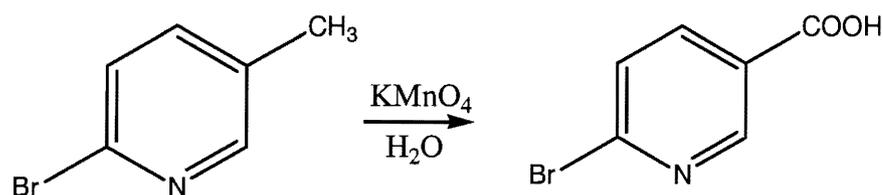
This first reaction had a yield of 83%, which is very successful. Yet there was a flaw in the next reaction allowing for a side product to be formed as well.

Reaction 1.6: Formation of Ethyl 2-bromo-5-pyridinecarboxylate from 6-bromonicotinic acid
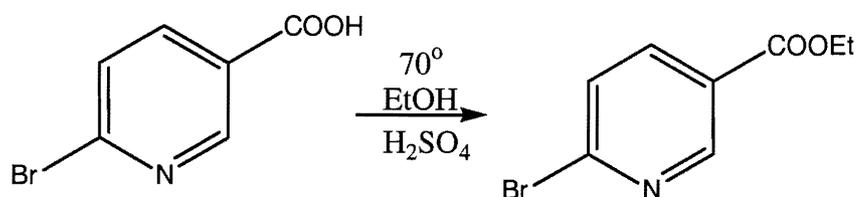
This reaction was deemed unsuccessful after it was analyzed by an NMR.

IV. Experimental:

3-(5-azido-1-pentanone-1-yl) pyridine: 18-crown-6 ether (15.0mg, .055mmol), sodium azide (0.647g, 9.95mmol), and a stir-bar were placed in a 100mL round bottom flask equipped with a pressure regulated addition funnel. The mixture was placed on high vacuum for three hours, backfilled with argon, and diluted with 6.13mL of dimethyl formamide. 3-(5-chloro-1-pentanone-1-yl) pyridine (1.64g, 8.30mmol) was also placed on high vacuum for three hours in a separate flask. This second flask was backfilled with argon, and transferred with 30mL dimethyl formamide to the addition funnel via syringe. The 100mL flask was heated to 60 degrees Celsius and the 3-(5-chloro-1-pentanone-1-yl) pyridine / dimethyl formamide solution was added drop-wise, pausing periodically to allow the mixture to stir. After the entire solution was added, the reaction mixture was left to stir for twenty-four hours at 60 degrees Celsius. The reaction mixture was

quenched with 60mL of water after it had cooled to room temperature and extracted with ethyl acetate (2 rinses of 60mL). Each extraction was washed with water (4 rinses of 60mL) and the combined organic extractions were dried over anhydrous magnesium sulfate. The solid was filtered and the solvent evaporated to give 1.22g (5.97mmol) of product that was pure enough for the next step.

Anabaseine: A 250mL round bottom flask containing 3-(5-azido-1-pentanone-1-yl) pyridine (1.22g, 5.97mmol) and a stir bar was purged with argon and injected with tetrahydrofuran (29mL) and water (145 μl). Triphenylphosphine (2.37g, 9.00mmol) was added in four separate portions in fifteen-minute intervals. The reaction mixture was left to stir for 24 hours or until no starting material was present in the TLC. The tetrahydrofuran was evaporated and 52mL of ethyl acetate was added to dissolve the resulting solid. This was purified directly by column chromatography (Silica gel; Ethyl Acetate, 7.50cm by 1.25cm). The product is eluted after approximately 1500mL of ethyl acetate. The combined fractions were evaporated to give approximately 0.937g of a clear liquid that turns light yellow when exposed to air. To prevent decomposition before the next step, the anabaseine was kept solvated in ethyl acetate at a low temperature. Shown below is an image of what the TLC plate looked like once only trace amounts of the starting material were present in the mixture.

Image 1.3: TLC plate performed in pure ethyl acetate (SM = starting material, P = product, with traces of starting materials remaining).
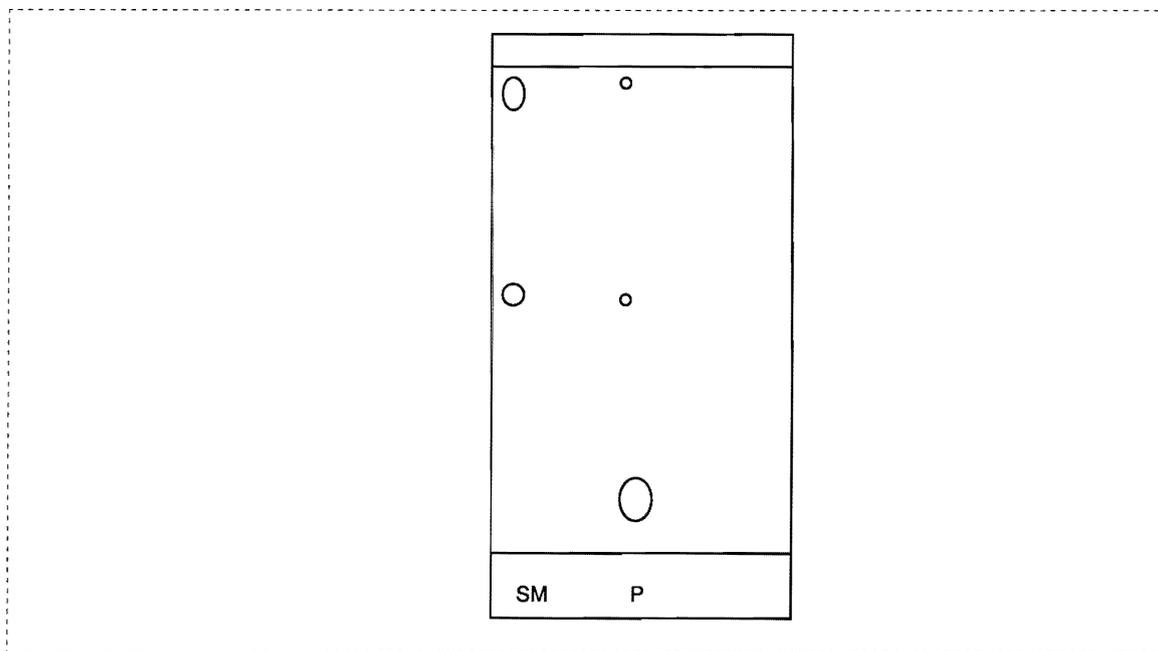
Anabaseine Dihydrochloride: The fresh purified anabaseine (0.937g, 5.85mmol) was diluted with isopropyl alcohol (68mL). Dilute hydrochloric acid (6M, 2mL) was added, followed by concentrated hydrochloric acid (3mL). The mixture was concentrated by evaporation until a whitish-green solid precipitated. The solid was filtered and rinsed with ethyl ether and the filtrate was concentrated to give more of the anabaseine dihydrochloride solid. The total weight of the solid collected was 0.923g (3.95mmol).

6'-Bromo-3-(2,4-dimethoxy-benzylidene)-3,4,5,6-tetrahydro-[2,3']bipyridinyl: 98.06mg (0.314mmol) of 6-bromoanabaseine dihydrochloride and 150mg (0.903mmol) 2,4-dimethoxy benzaldehyde were weighed in a 25mL Erlenmeyer flask. 8.5mL of ethanol, a stir bar, and one drop of concentrated hydrochloric acid were added to the flask. The reaction stirred for one week at a constant temperature of 60 degrees Celsius, while being purged with argon gas. The mixture was then transferred into a 125mL sep.

funnel and an equivalent amount of ethyl acetate and water were added. The organic

layer was washed with sodium bicarbonate solution to neutralize the acid in the mixture

and then the layer was once again washed with fresh water. The original water layer was

saved. The solvent was evaporated. 2mL of water and about 1mL of triethylamine were

added to the organic product by means of a syringe. The triethylamine will extract all

hydrogen atoms from the product salt, therefore making the product a free base. As a

free base the product salt will be able to accept hydrogens. The mixture was extracted

with ethyl acetate and washed with a brine solution composed of concentrated sodium

chloride. The organic layer was evaporated and added to a packed column, with a height

of 24cm. The product was a light yellow band that was pushed through the column with

the help of an ethyl acetate and methanol solution. The 125mL of the product eluted was

evaporated in a strong 250mL flask to give a yellow product with a mass of 80mg.

6-Bromonicotinic Acid: Initially, 66mL of water was heated to 70 degrees Celsius. The

water was then added to 2-bromo-5-methyl-pyridine (2.13g, 12.4mmol) in a 100mL

round bottom flask equipped with a stir bar and reflux condenser. This was followed by

the addition of five separate portions of potassium permanganate, each having a mass of

0.980g. In between each addition the mixture was given time to turn a dark brown before

making the next addition. During the additions the system was flushed with argon gas.

Before the final two additions, the temperature was increased to a constant temperature

between 80 and 90 degrees Celsius. The hot reaction mixture was then filtered with two

200mL portions of hot water. While filtering, each portion was allowed to soak into the

cake without the application of vacuum. Then the vacuum was applied until the cake was

sufficiently dry before the next portion of fresh wash water was added. Next, the

combined filtrate and washings were evaporated down to approximately 200mL, allowed

to cool, and the pH was adjusted to 3.2. The precipitate of nicotinic acid was collected by

means of suction filtration after an hour, washed with three 50mL portions of cold

deionized water and dried. The filtrate was then rotavapped and the solid collected was

added to the solid collected earlier. The total mass of the solid collected was 2.08g.

Ethyl 2-bromo-5-pyridinecarboxylate: The 2.08g (.0103mol) of 6-bromonicotinic acid

were put into a 100mL round bottom flask and then placed on high vacuum for

approximately thirty minutes to remove most of the solvent. Then, 21mL of ethanol was

added along with approximately 2mL of sulfuric acid, which was added in a drop wise

fashion. The mixture was then heated to 70 degrees Celsius for three hours. During the

heating of the mixture, it was purged with argon gas. A TLC was performed with 8 parts

hexanes to 1 part ethyl acetate after three hours and the results are pictured below.

Image 1.4: TLC plate with lone product spot for 6-bromonicotinic acid ethyl ester
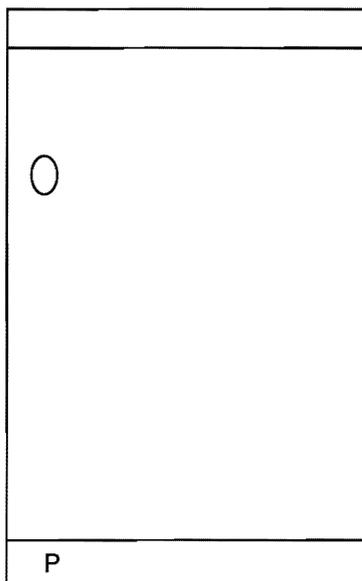
A column chromatography was also performed (Silica gel; 8:1 = Hexane : Ethyl Acetate, 57cm x 7cm). An NMR was later run. The results of the NMR were not satisfactory and possibly a side product was also formed.

V.   Sources:

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