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Progress Toward the Synthesis of a Truncated Ergoline

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Abstract

Many people are diagnosed with thyroid related disorders, and many more are unaware of their existing thyroid problems. T_1AM, a naturally occurring metabolite of the thyroid hormone (TH), has been shown to activate the Trace Amine Associated Receptor 1 (TAAR1) and exhibits effects that oppose those of the TH. It is, therefore, likely that there is regulatory relationship between T_1AM and the TH. In order to better understand this relationship, a compound must be developed that will effectively block TAAR1. Previously our lab has examined the two different mirror images of apomorphine. One of the mirror images inhibited and the other activated TAAR1. The project described herein, is targeted toward the synthesis of a truncated ergoline, which is structurally similar to both T_1AM and apomorphine. Only one mirror image is present in the naturally occurring form of ergolines, and they are known to be activators of TAAR1. To determine if the non-natural mirror image is an effective inhibitor, it is essential that an efficient synthesis be developed that will allow for the formation of both mirror images of the truncated ergoline. Several steps have been optimized using the desired system, and several other steps have been optimized using a model system. Currently the key step for the completion of the truncated ergoline is being examined using data collected from the model system.

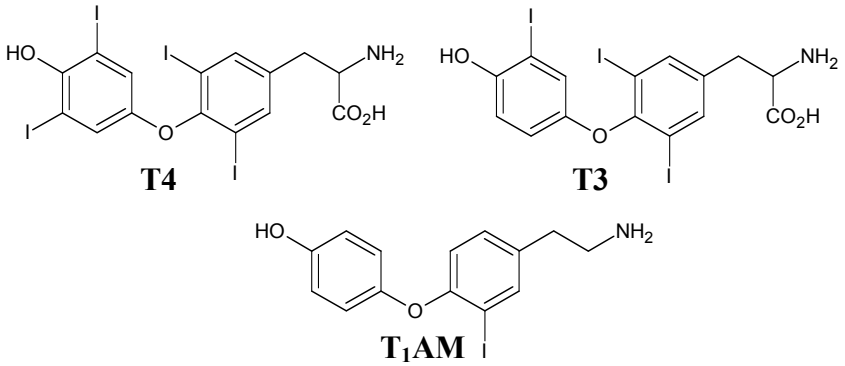
Introduction

Many people each year are diagnosed with thyroid hormone (TH) related disorders, and there are many more who are unaware of their existing condition. The current treatment options are very limited, and provide a non-comprehensive approach to

treating such disorders. Further research into the biochemical regulatory processes of the TH production and post-thyroid metabolism may be the key to unlocking more comprehensive treatment options. This project is targeted toward synthesizing a molecule that will help us to better understand this regulatory process.

Figure 1

Structural Comparison of TH and T_1AM

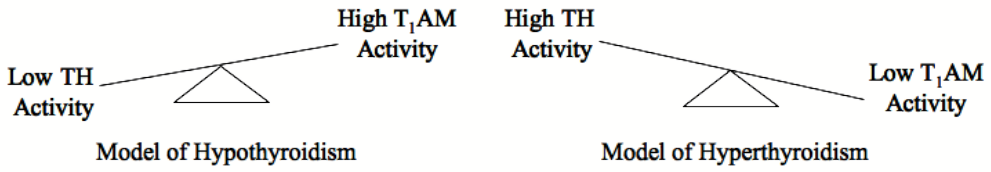


In the body there are two chemical compounds normally associated with the thyroid hormone: Thyroxine (T4) and triiodothyronine (T3). T4 is the most prevalent form of the thyroid hormone in the body, while T3 is the most potent form. T4 acts as a non-potent reservoir of the TH that is converted via enzymes in the liver, kidney, and other tissues to the more potent T3. Hyperthyroidism and Hypothyroidism are the two most common disorders associated with the thyroid. Classically hyperthyroidism was associated with too much TH in the body and was characterized by symptoms including increased core body temp, metabolism, and heart rate (tachycardia). Similarly, hyperthyroidism was classically associated with too little TH in the body and was characterized by symptoms including decreased core body temp, metabolism, and heart rate (bradycardia).

Recently the discovery of a natural metabolite of the TH, 3-iodothyronamine (T_1AM), has begun to shed some light on a possible biochemical regulatory process of the TH[1]. In rats and mice, T_1AM exhibited physiological effects opposite those associated with the TH. An increase in T_1AM levels caused bradycardia with a decrease in core body temp and metabolism. Due to the fact that T_1AM is a natural metabolite of the TH, it has been proposed that it might be a negative regulator of the TH. If this is the case, then the symptoms normally associated with hyperthyroidism may not only be caused by too much TH, but also caused by too little T_1AM. Also, the symptoms normally associated with hypothyroidism, may not only be caused by too little TH, but also caused by too much T_1AM. The symptoms may arise due to an imbalance in these compounds (figure 2). If this is the case, then targeting this imbalance may lead to new treatments for people suffering from hyperthyroidism and hypothyroidism.

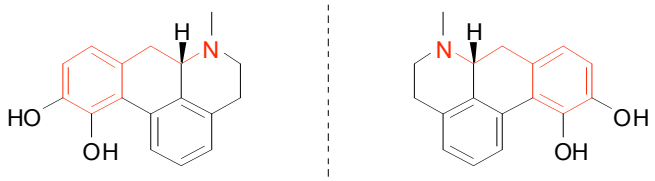
Figure 2

Hypothyroidism vs. Hyperthyroidism



In order to prove this relationship exists, we need to better understand the regulation of the receptor for T_1AM. This receptor is a G-Protein Coupled Receptor (GPCR) called Trace Amine Associated Receptor 1 (TAAR1). Many agonists (activators) of this receptor, like T_1AM, have been tested, but there are very few successful antagonists (inhibitors) of this receptor. Several compounds have been reported; however, these exhibit only modest inhibition at best.

Figure 3
R- and S-Apomorphine

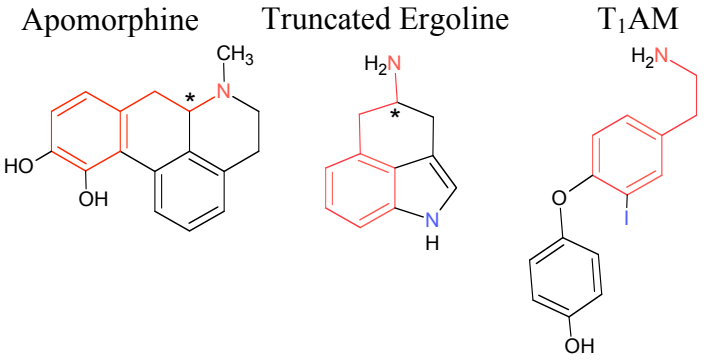


S(+)-Aporophine **R(-)-Aporophine**

Upon the discovery of TAAR 1, the two mirror images of a compound called apomorphine were separated and tested independently[2]. It was noted that the R(-)-apomorphine was an activator while S(+)-apomorphine was not. S(+)-apomorphine, however, was not tested as an inhibitor in this study. Apomorphine is an example of an enantiomer, which is the name given to two compounds with the same bond-to-bond structure but with different special arrangement. The two compounds are non-superimposable mirror images of each other. The Hart lab decided to test S(+)-apomorphine to see if it was an inhibitor, and it was experimentally determined to be the best inhibitor reported thus far. The amount of compound needed to block half of the receptor (the IC50 value) was 835nM. This is the first inhibitor for TAAR1 in the nM range. Using T_1AM as a reference for comparison, the amount of compounded needed to activate half of the receptor (EC50 value) is 24nM. It is intriguing to consider enantiomers with different activity profiles. The focus of this research is to explore this concept in other groups of stereoisomers.

Figure 4

Structural Comparison



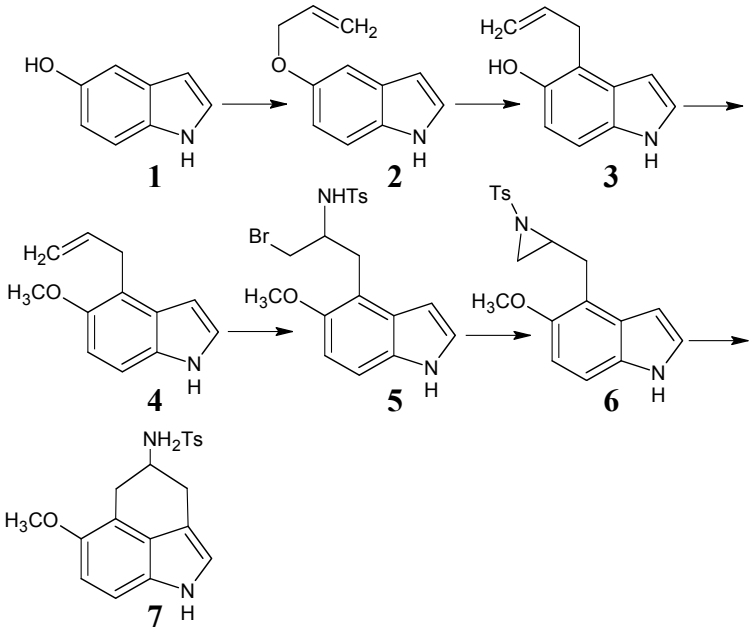
Ergolines were also tested upon the discovery of TAAR1, and they were found to be good activators[2]. Only one stereoisomer of the ergolines occurs in nature, so in order to test the other non-naturally occurring stereoisomer, it must be synthesized. The truncated ergoline is the simplest form of the ergoline structure, and it incorporates a phenethylamine (highlighted in red) that is found in both apomorphine and T_1AM. It also contains a stereocenter like apomorphine, and a secondary amine that may mimic the iodine from T_1AM. The project discussed below is focused on the synthesis of a truncated ergoline.

Results and Discussion

The proposed synthesis in scheme 1 illustrates the current method being examined to make the truncated ergoline, **7**. The aminobromination (**5**), the formation of the aziridine (**6**), and the intramolecular cyclization to form the truncated ergoline (**7**) have not yet been tested on the target system. A styrene test system has been used to test the aminobromination, and the aziridine formation. Data collected from these tests is being applied to the target system.

Scheme 1

Proposed Synthesis of Truncated Ergoline



Scheme 2

O-Alkylation with Allyl Bromide

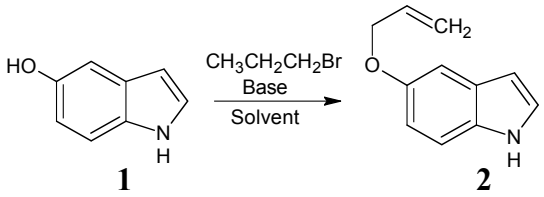


Table 1

Data for O-Alkylation with Allyl Bromide

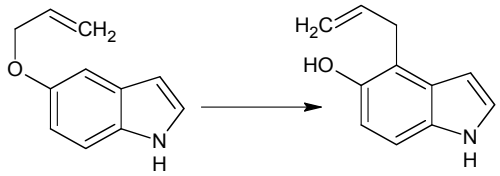
Base	Solvent	Temp.	Rxn Time (Hours)	% Yield
K_2CO_3	THF	Reflux	14.5-26	20-27
K_2CO_3	Acetone	Reflux	23	99
Cs_2CO_3	Acetone	Reflux	22	58
Cs_2CO_3	Acetone	Room Temp	19	100

The first step of the synthesis was an o-alkylation of the hydroxyl on the starting material, 5-hydroxyindole (**1**). In many of the first trials this compound was refluxed, which promoted a small amount of **2** to rearrange to **3**. The claisen rearrangement is the second step in the synthetic process, but the premature rearrangement became

problematic due to that fact that it very difficult to separate from the unreacted starting material. By changing the base to Cs_2CO_3, which is a stronger base, it allowed the reaction to be carried out at room temperature. This allowed us to convert all of the starting material and avoid any of the rearrangement. No separation or purification was needed when Cs_2CO_3 in acetone was used.

Scheme 3

Claisen Rearrangement



2 **3**

Table 2

Data for Claisen Rearrangement

Solvent	Temp. (^oC)	Rxn Time	% Yield
1,2,4,5-Tetramethylbenzene	190-195	3-6 hours	55-86
M-xylene	139	5 days	89
M-xylene (Sealed Tube)	190-195	2 days	100 (with recovery of staring material)

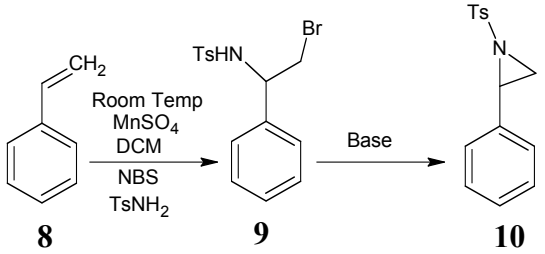
The second step involved the claisen rearrangement of **2**. The literature source for this claisen rearrangement called for the use of 1,2,3,4-tetramethylbenzene as the solvent, but 1,2,4,5-tetramethylbenzene was used instead because it was more readily available and much less expensive[3]. It is noteworthy that both 1,2,3,4-tetramethylbenzene and 1,2,4,5-tetramethylbenzene reflux between 190-195^{o}C, but only 1,2,3,4-tetramethylbenzene is a liquid at room temperature. As a result, it was very difficult to work with the solid 1,2,4,5-tetramethylbenzene as a solvent. This required us to heat the solvent until it was molten and then add **2** to the reaction flask. Purification of the reaction was also quite difficult using column chromatography. The 1,2,4,5-

tetramethylbenzene was eluted using hexane, and the product was eluted using a mixture of hexane and ethyl acetate.

M-xylene was used as an alternative solvent because it is a liquid at room temperature. The lower boiling point of m-xylene compared to tetramethylbenzene caused a much longer reaction time that was impractical. It was proposed that a sealed tube be used with the m-xylene so that the reaction temperature could be increased to a boiling point comparable with tetramethylbenzene. The sealed tube allowed the reaction to run in m-xylene while at a temperature of 190-195^{o}C. This reaction was purified in the same way that the tetramethylbenzene was purified, but recovery of unreacted starting material was now possible. With the recovery of both the product and the small amount of unreacted staring material, an effective yield of 100% was obtained.

Scheme 4

Aminobromination and Aziridine Formation on Styrene



With the rearranged product in hand, we pursued the aziridination of the olefin. Due to the high cost of the 5-hydroxyindole (**1**), a styrene test system (**8**) was used to test the aziridine formation. The original planned synthesis was to go directly from **8** to **10** using dirhodium caprolactamate as a catalyst[4]. This catalyst also had to be synthesized, and when tested it did not work for us[5]. Due to the high cost of rhodium chemistry, an alternative method was used. This method involved a $MnSO_4$ catalyst to aminobrominate the allyl group of styrene[6]. As it turns out, **9** is a proposed intermediate that the rhodium

catalyst uses to form the aziridine. This alternative method added an extra step, but it provided a much cheaper alternative to the rhodium chemistry.

Table 3
Data for Aminobromination of Styrene

Rxn Time (Hours)	% Yield
18.5	27.8
27	48.2 (Not completely pure)

The purification of **9** proved to be challenging when using hexane and ethyl acetate. It was noted that **9** was mostly insoluble in dichloromethane (DCM), and only the p-toluenesulfonamide shared this property. Due to this fact, DCM was added to the reaction flask after the workup. A precipitate formed, which we determined to be pure product. Occasionally there would be some p-toluenesulfonamide contamination. It's anticipated that separation of the target system will be easier.

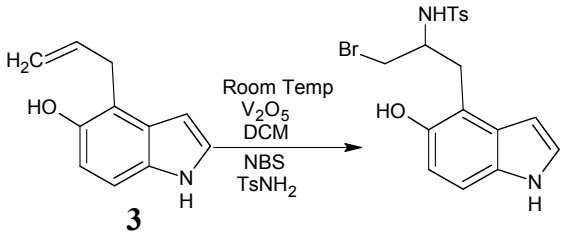
Table 4
Data for Aziridination of Styrene

Base	Rxn Time (Hours)	% Yield
K_2CO_3	20	90.94
Cs_2CO_3	20	100

The intramolecular cyclization of the p-toluenesulfonamide in **9** to form the aziridine on **10** was run in DCM with a base. Cs_2CO_3, which was the better base in the alkylation, turned out to improve the yield of this reaction as well. The complete conversion of **9** to **10** makes the low yield of the aminobromination more tolerable. If a higher yield is obtained for the formation of **5** than what was obtained for **9**, the aziridine formation on the target system could be a very successful reaction.

Scheme 5

Failed Aminobromination

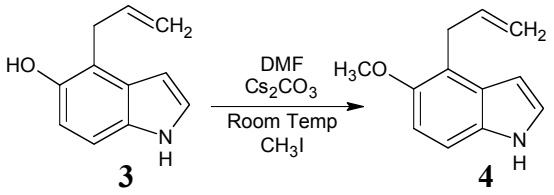


3

The chemistry tested on the styrene system was then implemented using **3** in scheme 5, but the reaction did not go as planned. A black mass developed as soon as the NBS was added to the reaction flask. It was thought that this might be due to an intramolecular cyclization of the aminobrominated product onto the oxygen of the hydroxyl. The indole ring system is also light sensitive, and it is also possible that radicals could have caused some unexpected chemistry with the NBS. The first step in trying to solve this problem was to protect the hydroxyl as an ether.

Scheme 6

O-Alkylation with Methyl Iodide



3 **4**

Table 5

Data for O-Alkylation with Methyl Iodide

Rxn Time (Hours)	% Yield
21	69.5

The conversion of the hydroxyl into a methoxy group was carried out via a second o-alkylation. This reaction has only been attempted once, so there was not time to optimize the yield. This initial trial, however, indicates that the reaction was successful at producing **4** even if low yielding.

Conclusion and Future Work

Much progress has been made toward the synthesis of the truncated ergoline, but there is still much work to be done. To date, the first alkylation and the claisen rearrangement have been optimized for the target system. The second alkylation for the synthesis of **4** has shown to be successful, however, optimization of this reaction has yet to be completed. Some very valuable data was collected from the styrene test system, and this was unsuccessfully tested on **3**. It is hoped that with the protection of the hydroxyl the aminobromination of **4** will be more successful.

On several occasions Cs_2CO_3 instead of K_2CO_3 improved yields and purity. When using Cs_2CO_3 for the first alkylation, complete conversion of the starting material was observed with no need for purification. This was also observed when using Cs_2CO_3 to form the aziridine after the aminobromination. The astonishing improvement to the yield and purity make Cs_2CO_3 much more favorable than K_2CO_3 for future reactions.

Continuing work on progress toward the synthesis of the truncated ergoline will be done in the following semesters. If the aziridine can be obtained on the target system, then testing will be done on the final step of this novel intramolecular cyclization of the aziridine to form a truncated ergoline. If this can be accomplishing, we will not only have been successful at developing the most efficient synthesis of a truncated ergoline, but also developed a compound that will hopefully be an effective agonist for TAAR1.

References

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